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TOPICAL REVIEW

Mitochondrial function in ageing: coordination with signalling and transcriptional pathways

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Abstract Mitochondrial dysfunction entailing decreased energy-transducing capacity and perturbed redox homeostasis is an early and sometimes initiating event in ageing and age-related disorders involving tissues with high metabolic rate such as brain, liver and heart. In the central nervous system (CNS), recent findings from our and other groups suggest that the mitochondrion-centred hypometabolism is a key feature of ageing brains and Alzheimer's disease. This hypometabolic state is manifested by lowered neuronal glucose uptake, metabolic shift in the astrocytes, and alternations in mitochondrial tricarboxylic acid cycle function. Similarly, in liver and adipose tissue, mitochondrial capacity around glucose and fatty acid metabolism and thermogenesis is found to decline with age and is implicated in age-related metabolic disorders such as obesity and type 2 diabetes mellitus. These mitochondrion-related disorders in peripheral tissues can impact on brain functions through metabolic, hormonal and inflammatory signals. At the cellular level, studies in CNS and non-CNS tissues support the notion that instead of being viewed as autonomous organelles, mitochondria are part of a dynamic network with close interactions with other cellular components through energy- or redox-sensitive cytosolic kinase signalling and transcriptional pathways. Hence, it would be critical to further understand the molecular mechanisms involved in the communication between mitochondria and the rest of the cell. Therapeutic strategies that effectively preserves or improve mitochondrial function by targeting key component of these signalling cascades could represent a novel direction for numerous mitochondrion-implicated, age-related disorders.

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Abbreviations Aβ, β-amyloid; Akt, protein kinase B; AMPK, 5'-adenosine monophosphate-activated protein kinase; AP-1, activator protein-1; BAT, brown adipose tissue; ER, endoplasmic reticulum; ERRα, oestrogen-related receptor α; GABA, γ-aminobutyric acid; GSK-3β, glycogen synthase kinase-3β; IGF1, insulin-like growth factor 1; IIS, insulin/IGF1 signalling; IRS, insulin receptor substrate; JNK, c-Jun N-terminal kinase; mTORc1, mammalian target of rapamycin complex 1; NAA, *N*-acetylaspartate; NFκB, nuclear factor κ-light-chain-enhancer of activated B cells; NMR, nuclear magnetic resonance; NRF, nuclear respiratory factor; Nrf2, nuclear factor (erythroid-derived 2)-like 2; OXPHOS, oxidative phosphorylation; PDH, pyruvate dehydrogenase; PET, positron emission tomography; PGC1α, peroxisome-proliferator-activated receptor γ coactivator-1α; PI3K, phosphatidylinositide 3-kinase; PTEN, phosphatase and tensin homologue; SCOT, succinyl-CoA transferase; Sirt1, sirtuin 1; TCA, tricarboxylic acid; TRE, tetradecanoyl phorbol acetate response element; UCP, uncoupling protein; WAT, white adipose tissue.

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Energy metabolism in brain ageing and Alzheimer's disease

Brain ageing. Brain utilizes 25% of the total body glucose to meet its energy demands; hence, maintenance of glucose homeostasis is critical for brain function, for glucose is the primary fuel meeting the energy demands of neurons and glial cells. Ketone bodies constitute a secondary fuel, especially during long fasting periods and starvation. Pronounced energy deficits are a feature of the ageing brain that are accompanied by neuronal loss, impaired cognition and memory, and an increased risk for neurodegenerative disorders. The gradual decline in energy metabolism during brain ageing and some neurodegenerative disorders results in a hypometabolic state, which is a function of deficits in (a) substrate supply, (b) mitochondrial catalysis and energy transduction, and (c) cytosolic metabolic and signalling pathways. Mitochondria play a central role for they integrate several signalling pathways and generate molecules that coordinate cytosolic signalling and transcriptional pathways (Fig. 1).

Dynamic micro-positron emission tomography (PET) scanning using ^{18}F-labelled fluorodeoxyglucose (FDG) as a tracer showed a significant decline in glucose uptake during brain ageing in several rodent models. The decrease in glucose uptake (Fig. 2) is paralleled by a decrease in expression and translocation to the membrane of the insulin-sensitive glucose transporters, GLUT4 and GLUT3, as well as the vascular endothelium glucose transporter GLUT1 (55 kDa); the expression of the glial glucose transporter GLUT1 (45 kDa) did not change or was increased as a function of age in Fischer 344 rats (Jiang *et al.* 2013). The increase in astrocytic GLUT1 (45 kDa) may account for the age-dependent astrocytic metabolic shift (Jiang & Cadenas, 2014). Anaerobic glycolysis in astrocytes yields lactate from pyruvate reduction, and lactate released from astrocytes is utilized by neurons as an energy source (Bolaños *et al.* 2010; Bélanger *et al.* 2011). The age-dependent astrocytic metabolic shift consists of an increase in their mitochondrial oxidative metabolism (Jiang & Cadenas, 2014) thereby depriving neurons of energy substrates (lactate) and exacerbating the inherent hypometabolic state in brain. Astrocytes are generally considered neurotrophic inasmuch as they provide neurons with energy substrates and recycle neurotransmitters (Jiang & Cadenas, 2014). Of note, the decline in glucose transport and metabolism was preceded by a shift to a ketogenic system in the female mouse brain during ageing as well as in a triple transgenic mouse model of Alzheimer's disease (Ding *et al.* 2013*a*,*b*).

A decline in the mitochondrial catalytic machinery, in terms of deficits in expression and activity of respiratory chain complexes I and IV and an increase in mtDNA mutations, contributes further to the hypometabolic state (Drew & Leeuwenburgh, 2004; Navarro & Boveris, 2007; Boveris & Navarro, 2008). The age-dependent phosphorylation of the E1α subunit of the pyruvate dehydrogenase complex results in its inactivation and, consequently, a decrease of acetyl-CoA delivery to the tricarboxylic acid (TCA) cycle, increase reduction of pyruvate to lactate, and decrease in ATP production (Zhou *et al.* 2008, 2009). In addition, post-translational modifications can impair mitochondrial function: the age-dependent increase in neuronal nitric oxide synthase expression leads to nitration of mitochondrial proteins, such as succinyl-CoA transferase (SCOT) and F_1-ATPase, thus resulting in a moderately impaired mitochondrial function (Lam *et al.* 2009). Mitochondria are highly dynamic organelles and undergo fusion and fission continuously, which regulates not only mitochondrial morphology, but also their biogenesis, trafficking and localization, quality control and degradation (Twig & Shirihai, 2011; Chan, 2012). Mitochondrial fusion and fission is found to be diminished or imbalanced in tissue ageing and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease (Seo *et al.* 2010). Mitochondrial dynamics has some undefined role in organelle turnover, supposedly affecting the degradation pathways; in this regard, lysosomal autophagy declines in several tissues with age (Seo *et al.* 2010).

Alzheimer's disease. Alzheimer's disease is a progressive neurodegenerative disease involving biochemical (Gottfries *et al.* 1983), metabolic (Mosconi, 2005) and physiological (Farkas & Luiten, 2001) changes that result in impairments of memory, thinking and behaviour. Alzheimer's disease is associated with β-amyloid (Aβ) plaques and neurofibrillary tangles (hyperphosphorylated tau), the detection of which in post-mortem tissue validates a definite diagnosis (Dubois *et al.* 2007). The aetiology of Alzheimer's disease has been hypothesized by several theories such as the β-amyloid hypothesis (Tanzi & Bertram, 2005), cholinergic hypothesis (Francis *et al.* 1999), tau hypothesis (Maccioni *et al.* 2010), oxidative stress hypothesis (Markesbery, 1997), and mitochondrial cascade hypothesis (Swerdlow & Khan, 2009).

There is growing evidence for an early mitochondrial dysfunction preceding the classical Alzheimer's disease pathological hallmarks, i.e. Aβ plaques and neurofibrillary tangles (Brinton, 2008; Mosconi *et al.* 2011; Swerdlow, 2011). Post-mortem tissues of individuals with Alzheimer's disease have been identified to have disruptions of mitochondrial functions in terms of altered morphology, compromised electron transfer chain complexes, and tricarboxylic acid cycle deficiencies (Perry *et al.* 1980; Blass *et al.* 2000). The mitochondrial cascade hypothesis proposes a late-onset, sporadic Alzheimer's disease that reconciles the histopathological

and pathophysiological features. This hypothesis proposes that the genetic makeup of an individual's electron transport train sets the basal rates for production of reactive oxygen species and thus sets the tone for oxidative damage. The cells respond to this oxidative stress by generating pathological features like β-amyloid; this sets a cycle that results in aneuploidy, tau phosphorylation, and neurofibrillary tangle formation. The intrinsic need of brain for high energy dictates its dependence on functional mitochondria and also renders it sensitive to changes in

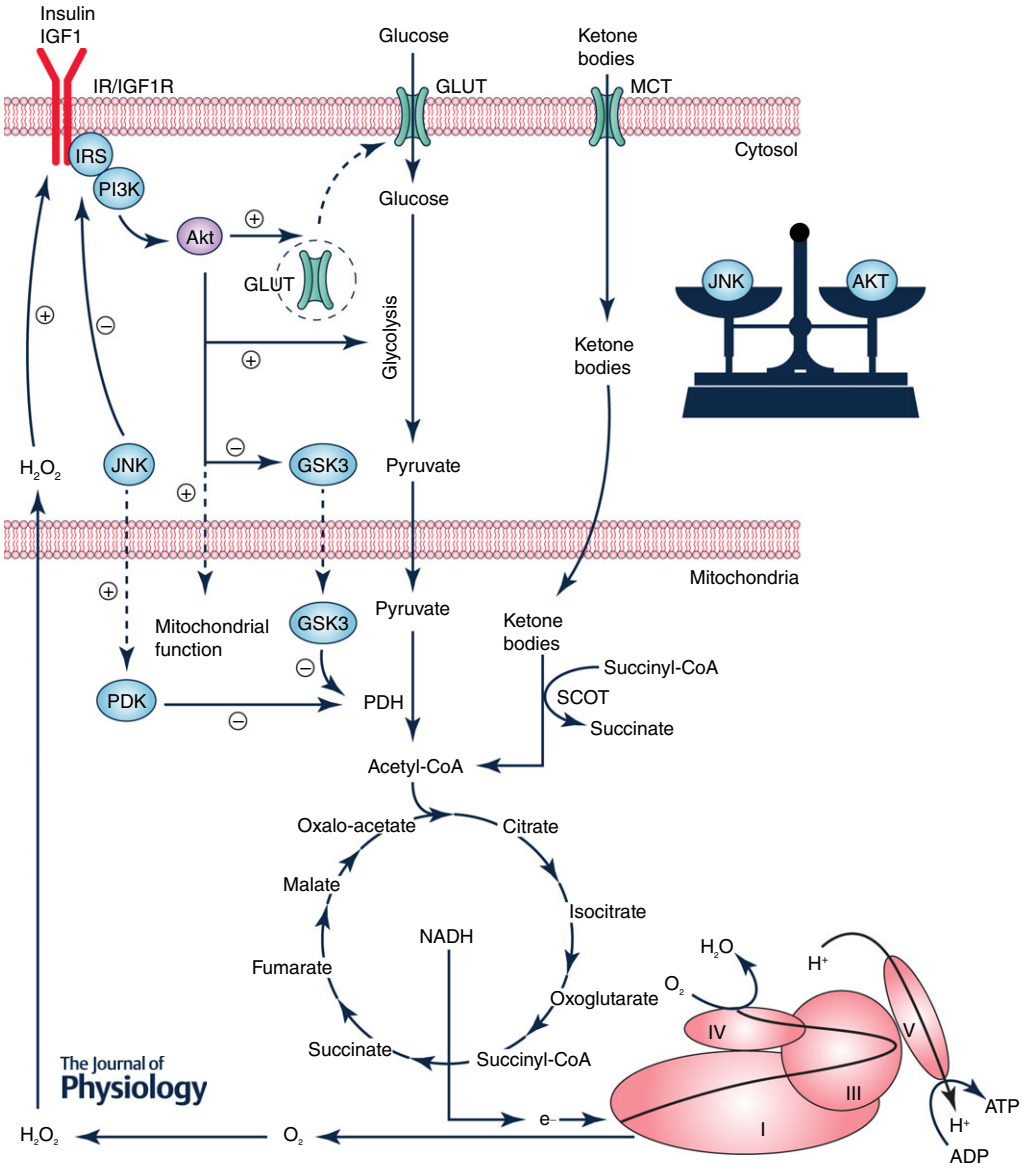

Figure 1. Coordination of insulin/IGF1 signalling and JNK signalling with substrate availability and mitochondrial catalytic machinery in brain
Pyruvate, generated from glucose by glycolysis in cytosol, undergoes oxidative decarboxylation by pyruvate dehydrogenase (PDH) to yield acetyl-CoA. Ketone body metabolism is regulated by succinyl-CoA transferase (SCOT) to yield acetyl-CoA. Acetyl-CoA generated by these pathways enters the TCA cycle to produce primarily NADH, which provides electrons to the electron transport chain to build up the proton motive force for ATP synthesis by complex V. Binding of insulin and IGF1 to their receptors activates the insulin receptor substrate (IRS) and the downstream phosphatidylinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway, which (1) facilitate the translocation of glucose transporters (GLUT3/4 in brain) to plasma membrane, (2) promote glycolytic reactions, and (3) enhance mitochondrial function through the translocation of Akt to mitochondria and the inhibition of glycogen synthase kinase-3β (GSK3β, an inhibitor of PDH). $O_2.^-$ generated by the electron transport chain at complex I and III is reduced to H_2O_2, which is released to the cytosol where it modulates the redox-sensitive insulin/IGF1 signalling (IIS) and c-Jun N-terminal kinase (JNK) signalling. JNK can negatively regulate IIS by phosphorylating IRS at $Ser^{307/312}$.

mitochondrial function (Kann & Kovács, 2007). Because mitochondria also play an important role in cell signalling, changes in mitochondria are relayed to the entire cell and beyond. Several early pieces of evidence demonstrated the role of oxidative damage in Alzheimer's disease (Christen, 2000).

While oxidative stress and pathological manifestations clearly exist in Alzheimer's disease, there is growing research pointing towards the disturbances in energy metabolism being closely associated with this disease. Perturbations of glucose metabolism and mitochondrial bioenergetics apparently precede the development of Alzheimer's disease pathology (Gibson *et al.* 1998; Hauptmann *et al.* 2009; Yao *et al.* 2009; Galindo *et al.* 2010). Multiple clinical studies have demonstrated that decreased brain glucose uptake is a common condition in Alzheimer's disease and mild cognitive impairment (Mosconi, 2005; Mosconi *et al.* 2008, 2009). However, these disturbances also extend to the glycolytic pathway and intermediates of the tricarboxylic acid cycle and the several neurotransmitters derived from it, such as glutamate, glutamine, γ-aminobutyric acid (GABA), and *N*-acetylaspartate (NAA) (Moats *et al.* 1994; Lin *et al.* 2003; Sancheti *et al.* 2014*a*).

Ninety per cent of glucose entering the brain is oxidized to CO_2, primarily by mitochondrial metabolism (Mangialasche *et al.* 2010). The majority of this energy is utilized in maintaining neurotransmission and neuronal potential, and preventing excitotoxicity (Magistretti & Allaman, 2013). Thus, any disturbances in glucose uptake or metabolism would affect neurotransmission and neuronal function, and ultimately impinge on cognition, learning and memory. Long-term potentiation is widely believed to be the cellular biochemical mechanism underlying synaptic plasticity (Bliss & Collingridge, 1993). Decreased brain glucose uptake has been demonstrated to be associated with substantially decreased long-term potentiation in the 3xTg-AD mouse model of Alzheimer's disease (Sancheti *et al.* 2013) and in a rat model of female perimenopausal ageing (Yin *et al.* 2015). The decline in glucose metabolism in 12-month-old 3xTg-AD mice was also reflected by an approximately 50% decrease of glucose supported TCA cycle-related metabolites including glutamate, glutamine, GABA and NAA (Sancheti *et al.* 2014*a*). This led to a decrease in the flux of glucose being converted into TCA cycle metabolites, a process critical to generating neurotransmitters and maintaining synaptic plasticity. In fact, metabolic alterations in these mitochondrial TCA cycle metabolites have been demonstrated in different rodent models of Alzheimer's disease (Dedeoglu *et al.* 2004; Marjanska *et al.* 2005; Salek *et al.* 2010; Esteras *et al.* 2012; Tiwari & Patel, 2012; Haris *et al.* 2013; Nilsen *et al.* 2013; van Duijn *et al.* 2013; Doert *et al.* 2015) and clinical cases (Lin *et al.* 2003). These studies suggest that both lowered brain glucose uptake and alternations in mitochondrial TCA cycle function contribute to the hypometabolic state observed in ageing and Alzheimer's disease.

Although a hypometabolic state of mitochondrial TCA cycle metabolites is well studied in older rodent models of Alzheimer's disease, a relatively unexplored area is the presence of a hypermetabolic state that perhaps precedes this hypometabolic state of the mitochondrial TCA cycle metabolites. A hypermetabolic state was observed in the 7-month-old 3xTg-AD rodent model of Alzheimer's disease at variance with the hypometabolic state that was reported in the 13-month-old 3xTg-AD mice (Sancheti *et al.* 2014*b*). This hypermetabolic state was hypothesized to be linked with the presence of Aβ plaques at 7 months of age in the 3xTg-AD mouse model. Other Aβ-based rodent models of Alzheimer's disease have also shown signs of hypermetabolism (Busche *et al.* 2008; Puzzo *et al.* 2008; Luo *et al.* 2012; Poisnel *et al.* 2012; Nilsen *et al.* 2013; Rojas *et al.* 2013); these studies provide several pieces of evidence that link hypermetabolism with Aβ plaques. This raises a question about the possible presence of a hypermetabolic state (encompassing mitochondrial hypermetabolism) in very early stages of Alzheimer's disease that have not been studied thoroughly. Overall, the mitochondrial perturbations in metabolism

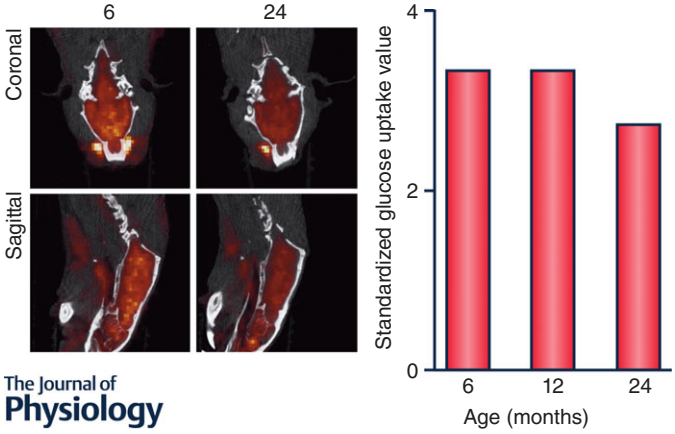

Figure 2. Brain glucose uptake decreases as a function of age
Dynamic microPET scanning using ^{18}F-labelled fluorodeoxyglucose (FDG)-PET as a tracer in Fischer 344 rats demonstrated a significant decline in glucose uptake and metabolism in 24-month-old rats as compared to 6-month-old rats. Calculation of selective uptake values (SUV), which measures the kinetics of glucose uptake, demonstrates that the SUV for the 24-month rat is lower than the 6-month rat (at end of scan, 2.72 as compared to 3.34).

of glycolytic substrates (into the TCA cycle related metabolites) highlight the importance of metabolism in the coordination of pathology and cognitive decline associated with Alzheimer's disease.

Energy metabolism in adipose tissue ageing

Adipose tissue stores energy in the form of triglycerides and supplies energy in the process of fatty acid β-oxidation under conditions of fasting or lowered liver glycogen levels (Girard & Lafontan, 2008). Mammals have two types of adipose tissue, the white and the brown adipose tissue (WAT and BAT, respectively), which can be distinguished by their morphology, metabolic activities, and cellular density of mitochondria (Saely *et al.* 2012). The WAT, with lower density of mitochondria, represents about 10% of body weight in lean humans as visceral and subcutaneous fat. WAT participates in the regulation of energy storage, insulin sensitivity and glucose metabolism in liver and muscle. BAT, with a higher numbers of mitochondria, dissipates energy as heat through adaptive thermogenesis (Virtanen *et al.* 2009).

Mitochondria in WAT ageing. Although their abundance is lower, mitochondria play essential roles in WAT function. Firstly, mitochondria in WAT provide substrates for fatty acids synthesis and fatty acid esterification, in the forms of acetyl-CoA and glycerol-3-phosphate, respectively (Nye *et al.* 2008). Secondly, WAT mitochondria generate ATP to support lipogenic processes in differentiating pre-adipocytes and adipocyte maturation (Lu *et al.* 2010). Moreover, mitochondrial H_2O_2 and enhanced biogenesis are causal factors that promote adipocyte differentiation in a mammalian target of rapamycin complex 1 (mTORc-1)-dependent manner (Tormos *et al.* 2011). Thirdly, mitochondria are involved in the synthesis of WAT-generated adipokines (Trayhurn & Wood, 2004). For instance, mitochondrial dysfunction decreases adiponectin synthesis via the activation of a series of pathways that involve endoplasmic reticulum (ER) stress, c-Jun N-terminal kinase (JNK) and activating transcription factor 3 (ATF3) (Koh *et al.* 2007).

Mitochondrial dysfunction is associated with adipose tissue ageing. In parallel with the decline in lipolysis (Dax *et al.* 1981; Klein *et al.* 1986), both mtDNA content and mitochondrial oxidative phosphorylation (OXPHOS) proteins in WAT decrease with ageing and age-related disorders, such as obesity and type 2 diabetes (Patti & Corvera, 2010; Donato *et al.* 2014). Calorie restriction is the only known reproducible experimental paradigm that extends maximal lifespan and delays the onset of many age-related diseases (Masoro, 2005). Long-term calorie restriction shifts WAT toward the activation of energy metabolism by upregulating genes required for glycolysis, lipogenesis, amino acid metabolism and mitochondrial energy metabolism including those involved in the TCA cycle, β-oxidation, electron transport and OXPHOS (Higami *et al.* 2004). It is proposed that upon calorie restriction, WAT functions as an energy transducer that converts glucose to the high energy-dense lipids (Okita *et al.* 2012). Long-term calorie restriction also downregulates the expression of more than 50 pro-inflammatory genes in mouse epididymal WAT (Higami *et al.* 2004; Higami *et al.* 2006). Fat-specific insulin receptor knock-out (FIRKO) mice exhibit reduced body weight and increased lifespan, despite normal or increased food intake and their WAT has higher expression of genes involved in glycolysis, the TCA cycle, β-oxidation and OXPHOS, which are correlated with increased mitochondrial biogenesis (Katic *et al.* 2007). Data on caloric restriction and FIRKO mouse models suggest a close relationship between WAT ageing and mitochondrial function.

Mitochondria in BAT ageing. BAT was considered to wane fast after birth in humans; however, recent studies using positron emission tomography demonstrated that BAT remains present during adulthood (Zingaretti *et al.* 2009). In mammals, BAT plays an important role in thermogenesis with mitochondria at the centre stage burning fatty acids to generate heat to maintain body temperature in cold environments, a process driven by uncoupling protein 1 (UCP1) by stimulating H^+ leak across the mitochondrial inner membrane without ATP production (Rousset *et al.* 2004). UCP1 is regulated by mitochondrion-associated histone deacetylase SIRT3 through peroxisome-proliferator-activated receptor γ coactivator-1α (PGC1α) and transcription factor CREB (Shi *et al.* 2005). During cold exposure, mitochondrial dynamin-related protein 1 is activated, promoting fission and sensitizing the mitochondria to free fatty acids (Wikstrom *et al.* 2014). In addition to heat generation, BAT thermogenesis is capable of protecting against diet-induced obesity (Hamann *et al.* 1998; Kontani *et al.* 2005)

The mass and thermogenesis capability of BAT declines with age in humans (Saito *et al.* 2009; Pfannenberg *et al.* 2010). Ageing reduces mitochondrial biogenesis, which, in turn, impairs the formation of thermogenic brown adipocytes (Graja & Schulz, 2014). As seen in WAT, caloric restriction is also effective in preventing BAT activity loss during ageing by preserving mitochondrial function. Long-term caloric restriction delays the age-related decline in mitochondrial mass, complex IV activity, uncoupling levels and mitochondrial transcription factor A (Tfam) in BAT of rats (Valle *et al.* 2008). Caloric restriction also increased fatty acid biosynthesis but not mitochondrial respiratory capacity (Okita *et al.* 2012). Exercise is

another potent inducer of BAT mass and adrenergic brown recruitment of adipocytes in aged animals, without changing the mRNA or protein levels of mitochondrial UCPs (Scarpace *et al.* 1994; Oh-ishi *et al.* 1996; De Matteis *et al.* 2013).

Liver energy metabolism and metabolic diseases

These age-related changes in liver modulate digestion, metabolism, immunity, storage of nutrients and clearance of drugs (Le Couteur & McLean, 1998). Ageing exhibits a significant negative correlation with liver volume, ascribed to the age-dependent decrease in hepatic blood flow (Wynne *et al.* 1989). The gross appearance of liver from elderly subjects is similar to younger individuals with malnutrition and cachexia. It has a brown colour due to the accumulation of lipofuscin within hepatocytes. There is also the presence of macrohepatocytes and polyploidy along with an increase in nuclei and nucleoli (Schmucker, 1998; Anantharaju *et al.* 2002). Liver triglycerides and cholesterol levels increase with age and are correlated with declining metabolism of low-density lipoprotein and decrease in their receptors (Aaronson & Woo, 1981).

During ageing, liver mitochondria show increased size (Sastre *et al.* 1996), decreased matrical density and decreased number (Schmucker, 1998). Additionally, there is a decrease in membrane potential (Sastre *et al.* 1996) and respiratory chain enzymes (Muller-Hocker *et al.* 1997). Around 87% of those above 50 years of age were found to have defects in the respiratory chain caused by a loss of enzyme proteins involving both nuclear and mitochondrial coded subunits. The majority of these subjects (94%) had a defect in the complex IV subunit, whereas 4% had defects in the complex III subunit (Muller-Hocker *et al.* 1997). Additionally, the content of cytochrome oxidase also declined with age along with age-related decline in the mtRNA synthesis in heart, lungs, brain, liver and skeletal muscle (Anantharaju *et al.* 2002). These decreases in respiratory chain enzymes are correlated with a decrease in mitochondrial respiratory capacity. A significant negative correlation between age and respiratory control ratio was observed in Chinese populations of various ages (Yen *et al.* 1989).

Ageing liver mitochondria are also accompanied by increased oxidative modifications that negatively impact their function (Richter, 1995; Sastre *et al.* 2000; Navarro & Boveris, 2004; Castro *et al.* 2012). Multiple mitochondrial proteins also undergo oxidative damage in an age-related manner (Kolosova *et al.* 2003). Interestingly, Lon protease, a key enzyme in the degradation of oxidized proteins within the mitochondrial matrix and typically highly induced under stress, declines with age. Thus, Lon protease has been suggested to be a significant factor in age and age-related diseases (Ngo *et al.* 2013). Mice expressing proofreading-deficient version of the mitochondrial DNA polymerase γ accumulated mtDNA mutations that resulted in accelerated ageing and correlated with the induction and increase of apoptotic markers in an age-related manner (Kujoth *et al.* 2005). There is also an age-related decline of mtRNA synthesis in brain, liver, heart, lungs and skeletal muscle (Anantharaju *et al.* 2002). Additionally, rat liver Kupffer cells show decreased function (Brouwer *et al.* 1985) and efficiency to phagocytose and degrade radiolabelled mitochondria (Martin *et al.* 1994). This perhaps leads to more severely damaged mitochondria that accumulate with age. Thus, the age-related macro changes in liver are accompanied by several subcellular micro changes in the liver mitochondria.

The liver–brain axis. The liver senses blood glucose levels adequately to control utilization of glucose by regulating glycogenesis and glycogenolysis; when the liver glycogen reserves are running low, hepatocytes maintain an adequate supply of glucose to the brain by activating gluconeogenesis from non-carbohydrate carbon sources. Importantly, mitochondria take centre-stage in this liver-centric energy homeostasis and a multi-level regulation ensures a constant supply of energy to the brain, thus forming the core of a metabolic 'liver–brain axis'.

The neurotoxic role of liver-generated ceramides is an example of an impaired 'liver–brain axis' with implications for Alzheimer's disease (de la Monte *et al.* 2009*a*, 2010; de la Monte, 2012). On the one hand, ceramides contribute to cell membrane structure and have roles in growth, proliferation, motility, apoptosis, differentiation, senescence (Zheng *et al.* 2006; de la Monte, 2012) and maintenance of the skin barrier (Wartewig & Neubert, 2007). On the other hand, ceramides function as a lipid signals that can cause insulin resistance (Teruel *et al.* 2001; Chavez *et al.* 2005; Summers, 2006; Delarue & Magnan, 2007), cytotoxicity and inflammation (de Mello *et al.* 2009; Gill & Sattar, 2009). These effects of ceramides have been hypothesized to cause a 'triangulated mal-signalling in Alzheimer's disease' (de la Monte, 2012): toxic ceramides generated from extra-CNS tissues (e.g. liver) are released into the blood, bypass the blood–brain barrier, and cause brain insulin resistance, inflammation and cell death, all of which impair synaptic plasticity. In support of this hypothesis, Long–Evans rat pups administered ceramide analogues by intraperitoneal injection developed hyperglycaemia, hyperlipidaemia, mild steatohepatitis, reduced brain lipid content, increased ceramide levels in liver, brain and serum, and significant abnormalities in spatial learning and memory (de la Monte *et al.* 2010). Impairment of the phosphatidylinositide 3-kinase (PI3K)/protein kinase B (Akt) signalling cascade by

ceramides led to cognitive and motor dysfunctions (de la Monte *et al.* 2012). A similar pattern is also observed with alcohol-associated neurodegeneration: chronic alcohol consumption produced steatohepatitis, promoting insulin resistance and pro-inflammatory cytokines that lead to an increased release of toxic lipids such as ceramides (de la Monte *et al.* 2009*a*). Other compounds directly affecting the liver, i.e. nitrosamines (commonly found in fried fast foods), induced insulin resistance, type 2 diabetes (in liver and brain), non-alcoholic steatohepatitis, deficits in spatial learning and neurodegeneration (de la Monte *et al.* 2009*b*).

Several studies have examined the role of insulin resistance in Alzheimer's disease; however, the mechanistic/pathological aspects of the disease can be modulated by agents that combat insulin resistance (McClean *et al.* 2011; Businaro *et al.* 2012; Sancheti *et al.* 2014*a*). Thus, the signalling pathways involved in the liver–brain axis form an intricate communication network that ensures adequate supply of energy substrates to maintain a healthy brain; pathological or chemical intrusions to the liver seem to play an important role in initiation and progression of neurodegeneration.

What is the role of mitochondria in the liver–brain axis? It is well known that the mitochondrial function is impaired during insulin resistance (Petersen *et al.* 2004; Lowell & Shulman, 2005; Parish & Petersen, 2005; Højlund *et al.* 2008). Insulin resistance is mainly characterized by the inability of insulin to stimulate glucose uptake by peripheral tissues and/or control the synthesis of glucose by liver, the actions of which manifest as hyperglycaemia, hyperinsulinaemia and dyslipidaemia. This dysregulation leads to obesity, type 2 diabetes, cardiovascular disease, and neurodegeneration (Saltiel & Kahn, 2001; White, 2003; Cheng *et al.* 2010). From the point-of-view of the liver–brain axis, mitochondrial functions connected with maintenance of energy metabolism and redox control seem to be of the outmost importance.

Owing to these pivotal roles of mitochondria, it is not surprising that perturbation of mitochondrial function is involved in metabolic disorders like type 2 diabetes, insulin resistance, cardiovascular complications and obesity. Subjects with obesity or type 2 diabetes have mitochondria with an impaired bioenergetic capacity (Kelley *et al.* 2002). The linkage between mitochondrial dysfunction and type 2 diabetes has been reviewed earlier (Lowell & Shulman, 2005). Nuclear magnetic resonance (NMR) studies showed that elderly subjects had a 40% decrease in mitochondrial oxidative phosphorylation capacity (Petersen *et al.* 2003) and insulin-resistant subjects had a 60% decrease in insulin-stimulated rate of glucose uptake and a 30% reduction in mitochondrial oxidative phosphorylation (Petersen *et al.* 2004). Another major link between these metabolic conditions is their association with dysfunctional liver mitochondria and/or increased fat accumulation in the liver tissue (Petersen *et al.* 2003). In fact, liver mitochondrial dysfunction has been shown to precede hepatic steatosis and insulin resistance (Rector *et al.* 2010). Transgenic mice with liver-specific overexpression of lipoprotein lipase were insulin resistant with twofold increased liver triglyceride content. They were also associated with an impaired ability of insulin to suppress endogenous glucose production due to inactivated insulin receptor substrate-2 and PI3K activity (Kim *et al.* 2001*b*). Interestingly, chronic leptin treatment reversed insulin resistance and hepatic steatosis in patients with severe lipodystrophy. Thus, modulation of energy homeostasis presents an interesting target against metabolic conditions like insulin resistance, obesity and type 2 diabetes (Petersen *et al.* 2002). Overall, mitochondrial activity is a prime modulator of the liver–brain axis in maintaining adequate substrate supply for the brain and its dysfunction is associated with the pathologies that impinge on the liver–brain axis.

Metformin, widely used for the treatment of type 2 diabetes, reduces hepatic gluconeogenesis and enhances peripheral insulin sensitivity; its mechanism of action entails inhibition of complex I of the mitochondrial respiratory chain (Owen *et al.* 2000) and this may account partly for activation of 5'-adenosine monophosphate-activated protein kinase (AMPK) (Zhou *et al.* 2001). Inhibition of complex I results in decreased ATP levels and increased AMP, which binds to the γ subunit of AMPK, thereby activating it. However, inhibition of the mitochondrial glycerol-P dehydrogenase activity (a component of the glycerol-P shuttle) by metformin is another mechanism to augment the cytosol reducing environment leading to the overproduction of lactate and inhibition of gluconeogenesis; this together with other metformin-sensitive processes, such as suppression of glucagon signalling, activation of autophagy and lessening of inflammasome-dependent cytokine production, may suggest new target pathways for the treatment of type 2 diabetes (Hur & Lee, 2015).

Mitochondrial signalling molecules and redox-sensitive kinase signalling

Mitochondrial H_2O_2 as a signalling molecule. The redox-regulating capacity of the mitochondria generates second messengers such as H_2O_2 that regulate multiple cell signalling pathways and a range of cell functions (Fig. 3) (Ghafourifar & Cadenas 2005; Yin *et al.* 2012*a*, 2014). H_2O_2 acts as an efficient redox molecule since it can easily pass the mitochondrial membranes. Additionally, H_2O_2 produced by one mitochondrion can diffuse to other mitochondria, thus relaying signals among mitochondria (Murphy, 2009). Mitochondrial $O_2^{\cdot -}$ (more likely a long-lived species) was proposed

as the critical response of cells to hyperglycaemia (Brownlee, 2005) leading to the activation of the four major pathways of hyperglycaemic damage: inhibition of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) by the $O_2^{\cdot-}$-mediated activation of poly ADP ribose polymerase (PARP) seems to be the triggering event. It was emphasized that the cells damaged by hyperglycaemia are those that cannot decrease the transport of glucose inside the cell when exposed to a hyperglycaemic environment (i.e. the cell types involved in diabetic complications). However, the mitochondrial formation of ROS seems to be of importance in the pathogenesis of diabetes mellitus and its complications through modification of various cellular events in many tissues, including vessels, kidney, pancreatic β cells and liver (Piconi *et al.* 2006; Nishikawa & Araki, 2007; Palmeira *et al.* 2007). There is some correlative evidence that certain cell types that depend on dehydroascorbate uptake through GLUT have their ability to counteract oxidative stress impaired (Root-Bernstein *et al.* 2002). This decrease in cellular transport of dehydroascorbate is implicated in worsening the hyperglycaemia-induced oxidative stress. Of course, this would be applicable to those cell types that rely on dehydroascorbate uptake through GLUT.

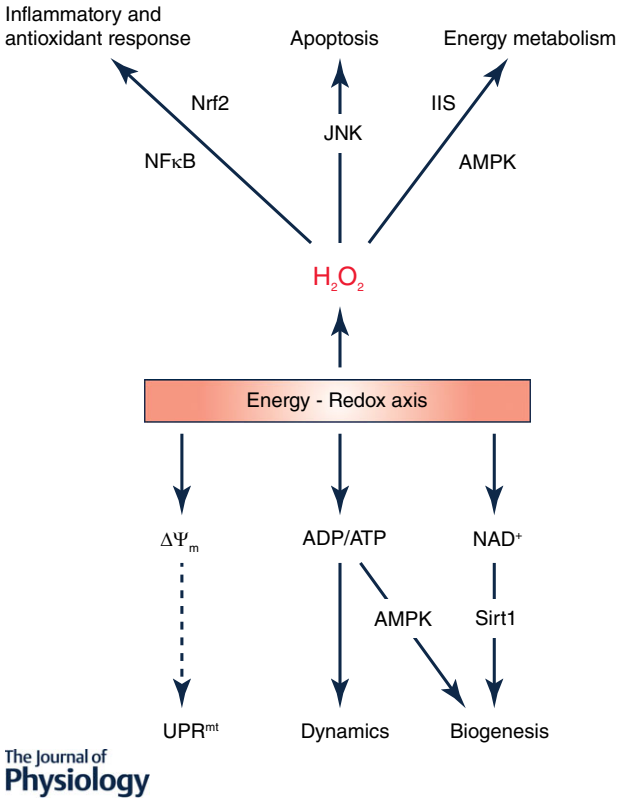

Figure 3. Mitochondrion-derived energy and redox signals regulate multiple cytosolic and nuclear pathways
Redox signals, primarily H_2O_2, modulate cellular energetic pathways through IIS and AMPK signalling; high levels of H_2O_2 activate apoptotic pathways through JNK. H_2O_2 also induces multiple inflammatory and antioxidants pathways in the nucleus via transcription factors such as nuclear factor κ-light-chain-enhancer of activated B cells (NFκB) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2). On the other hand, energy signals transduced from ADP/ATP and NAD^+ to AMPK and sirtuin 1 (Sirt1) control the biogenesis and dynamic remodelling of mitochondria. The mitochondrial unfolded protein response (UPR^{mt}) represents another mechanism through which mitochondria communicate with the nucleus monitoring the organelles' protein import efficiency, a process dependent on mitochondrial inner membrane potential ($\Delta\Psi_m$).

Insulin/IGF1 signalling. Insulin/IGF1 signalling (IIS) is responsive to H_2O_2 owing to the presence of several redox-sensitive cysteine residues on the insulin receptor and IGF-1 receptor (Fig. 1). Oxidation of these cysteine residues to cystine by H_2O_2 promotes their tyrosine autophosphorylation and activates downstream signalling cascades that promote metabolic pathways (Loh *et al.* 2009). Additionally, H_2O_2 inhibits tyrosine phosphatase (e.g. PTP1B) and lipid phosphatase (PTEN), which are both negative regulators of IIS through the dephosphorylation of insulin/IGF1 receptors and phosphatidylinositol-3,4,5-trisphosphate (PIP_3), respectively (Elchebly *et al.* 1999). In cerebellar granule neurons, the mitochondrial respiratory chain-generated H_2O_2 was responsible for insulin receptor activation (Storozhevykh *et al.* 2007); similarly, in hepatocytes, H_2O_2 activates insulin signalling, demonstrated by increased phosphorylation of insulin receptor (on tyrosine), Akt and glycogen synthase kinase-3β (GSK-3β). It is noteworthy that while lower doses of H_2O_2 (5–10 μm) led to activation of insulin signalling, higher doses of H_2O_2 (25–50 μm) led to its inactivation (Iwakami *et al.* 2011). This is likely to be due to the activation of JNK by higher H_2O_2 concentration, considering JNK as a negative regulator of the IIS (Karpac & Jasper, 2009; Yin *et al.* 2013).

JNK. Another signalling pathway critically regulated by H_2O_2 is the stress-activated JNK signalling. JNKs are multifunctional kinases involved in a variety of pathological conditions due to their role of inducing apoptosis (Cui *et al.* 2007). H_2O_2 was shown to activate JNK and decrease cell viability in primary neurons (Zhou *et al.* 2008) and in hepatocytes (Iwakami *et al.* 2011). Importantly, we have shown that JNK can be specifically activated by mitochondrially originated H_2O_2 in nicotinamide nucleotide transhydrogenase (NNT)-silenced PC12 cells (Yin *et al.* 2012*b*).

AMPK. Interestingly, H_2O_2 also seems to have a role in the activation of the energy sensor and regulator AMPK, which is typically activated by increased AMP/ATP ratio. The AMPK pathway was activated by increased H_2O_2 concentrations in HEK cells and in mice (Zmijewski

et al. 2010). Under hypoxic conditions, an increase in mitochondrial H_2O_2 also leads to AMPK activation that is not dependent on the AMP/ATP ratio (Emerling *et al.* 2009). In early diabetic nephropathy, a decrease in mitochondrial ROS resulted in decreased AMPK activity with downstream consequences such as decrease in PGC1α and mitochondrial biogenesis (Dugan *et al.* 2013); an alternative explanation (Nishikawa *et al.* 2015) that considers the substantial increase of ROS in diabetes suggests that a decrease in AMPK activity can account for the increase in mitochondrial ROS.

Regulation of mitochondrial function by cytosolic signalling. Mitochondria modulate cytosolic components through redox-sensitive signalling; on the other hand, mitochondria are also recipients of cytosolic signalling that in turn regulates mitochondrial metabolic and redox functions.

IIS. The cytosolic modulation of mitochondrial bioenergetic functions is primarily carried out by components of the IIS. It is well known that the mitochondrial function is impaired during insulin resistance, an indicator of compromised insulin signalling (Lowell & Shulman, 2005). In addition to its role in regulating glucose metabolism, in the central nervous system, IIS has also been shown to influence neuronal survival and synaptic plasticity (van der Heide *et al.* 2006). Recent studies in our laboratory have shown that α-lipoic acid, an insulin mimetic nutriceutical, is able to rescue the brain metabolic deficits and mitochondrial dysfunction that occur in brain ageing (Jiang *et al.* 2013) and in a mouse model of Alzheimer's disease (Sancheti *et al.* 2013).

An important downstream component of the IIS that facilitates energy metabolism is Akt; Akt has been shown to directly translocate to mitochondria and enhance mitochondrial function in hepatocytes (Li *et al.* 2013). In neuroblastoma cells, insulin stimulates the translocation of phosphorylated Akt to the mitochondria within minutes. Two mitochondrial proteins, GSK-3β and the β-subunit of ATP synthase, are phosphorylated as a result of Akt translocation (Bijur & Jope, 2003). Activated GSK-3β also phosphorylates pyruvate dehydrogenase (PDH) and inhibits its activity (Hoshi *et al.* 1996). Another prominent feature of IIS is its promotion of survival by directly inactivating components of the mitochondrial dependent intrinsic apoptosis. This entails phosphorylation and inactivation of the pro-apoptotic members of the Bcl-2 family (Linseman *et al.* 2002). The prevention of neuronal death could thus have implications for brain ageing and neurodegeneration which are characterized by significant neuronal loss (Kanazawa, 2001).

JNK. JNK is a negative regulator of both mitochondrial metabolic function and the IIS pathway. Anisomycin- or H_2O_2-activated JNK translocates to mitochondria in primary cortical neurons; JNK associated with the outer mitochondrial membrane initiates a cascade that leads to the inhibitory phosphorylation of the E1α subunit of the PDH complex, which results in a decrease in cellular ATP levels and a metabolic shift toward anaerobic glycolysis (Zhou *et al.* 2008). The inactivation of IIS by JNK is due to the inhibitory phosphorylation of the IRS at Ser^{307} (Ser^{312} in human IRS) by JNK, which prevents the insulin/IGF1 mediated tyrosine phosphorylation of IRS (Karpac & Jasper, 2009). Intriguingly, the IIS also inhibits JNK activation through multiple mechanisms including the phosphorylation of MLK3 (Barthwal *et al.* 2003) and the suppression of ASK1 (Kim *et al.* 2001*a*). This is consistent with our findings that aged rats fed with α-lipoic acid to enhance IIS also exhibit decreased JNK activation compared to age-matched controls (Jiang *et al.* 2013).

Coordination of the energy-redox axis with nuclear transcription.

Energy charge and mitochondrial biogenesis. Mitochondrial energy charge is linked to the nuclear transcription pathways and modulates mitochondrial biogenesis (Fig. 3) (Yin & Cadenas, 2015). Mitochondrial biogenesis entails the replication of mtDNA, as well as the synthesis, transport and integration of proteins and lipids to the existing mitochondrial population (Attardi & Schatz, 1988). Most of the 1500 mitochondrial proteins are encoded by the nuclear genome (Calvo *et al.* 2006). Transcription factors such as nuclear respiratory factor-1 and -2 (NRF-1 and NRF-2) and oestrogen-related receptors (ERR) (Scarpulla, 2002) regulate the transcription of these genes, directly or indirectly. These transcriptional pathways are coordinated by members of the peroxisome-proliferator-activated receptor γ coactivator-1 (PGC-1) family, primarily PGC-1α (Handschin & Spiegelman, 2006). As the master regulator of mitochondrial biogenesis, PGC1-α activity is regulated by its transcription, post-translational modification, and degradation (Puigserver & Spiegelman, 2003). PGC1α activity is highly regulated by energy charge-related signals from mitochondria. These regulations involve energy messengers such as NAD^+ and AMP/ADP, and energy sensors including sirtuin 1 (Sirt1) and AMPK (Fig. 3) (Fernandez-Marcos & Auwerx, 2011).

NAD^+–Sirt1. PGC1α is inactivated by acetylation and activated by Sirt1-mediated deacetylation. The

deacetylation is required for sequestering PGC1α to the nucleus and for activating the above-mentioned transcription factors (NRF-1, NRF2, ERRα) (Gerhart-Hines *et al.* 2007). Sirt1 removes the acetyl group on lysine residues using NAD^+ as a substrate and generates *O*-acetyl-ADP-ribose, and nicotinamide (Houtkooper *et al.* 2010). As the major domain of NAD^+/NADH metabolism, the mitochondrial energy component is thus capable of regulating the NAD^+-dependent sirtuin pathways and the activity of PGC1α.

AMP-AMPK. PGC1α expression and activity are also regulated by AMPK. AMPK, an energy sensor in cells, is activated when the cellular AMP/ATP or ADP/ATP ratio is high (Oakhill *et al.* 2011; Xiao *et al.* 2011). The cytosolic ADP/ATP ratio is determined by the consumption of ATP and the synthesis of ATP as a function of mitochondrial bioenergetic status. It is known that activation of AMPK leads to an increase in PGC1α transcription. More importantly, AMPK enhances mitochondrial biogenesis by activating PGC1α through the phosphorylation of threonine^{177} and serine^{538}, which impacts the ability of PGC1α to dock on certain transcription factors and affects the binding or function of other cofactors in the PGC1α coactivator complex (Jäger *et al.* 2007). Whereas AMPK directly enhances PGC-1α expression and activation, another indirect way that AMPK modulates PGC-1α is to increases NAD^+ levels by upregulating fatty acid oxidation, thereby enhancing Sirt1 activity and PGC1α deacetylation (Cantó *et al.* 2009).

Redox-sensitive transcription factors. In addition to the energy charge-sensitive transcriptional pathways that induce mitochondrial biogenesis, a variety of transcriptional pathways are redox sensitive and can be activated upon intracellular redox changes (Fig. 4).

Nrf2. Transcriptional regulation of antioxidant or detoxifying genes is predominantly mediated by a redox-sensitive transcription factor nuclear factor-erythroid derived 2 (NF-E2) related factor-2 (Nrf2) (Kensler *et al.* 2007). Oxidants released from mitochondria induce activation of Nrf2, and this process can be inhibited by the mitochondrion-specific redox enzyme Trx2) (Imhoff & Hansen, 2009). Under basal conditions, Nrf2 interacts with Kelch-like ECH-associated protein 1 (Keap1) in the cytosol where it undergoes ubiquitin-mediated

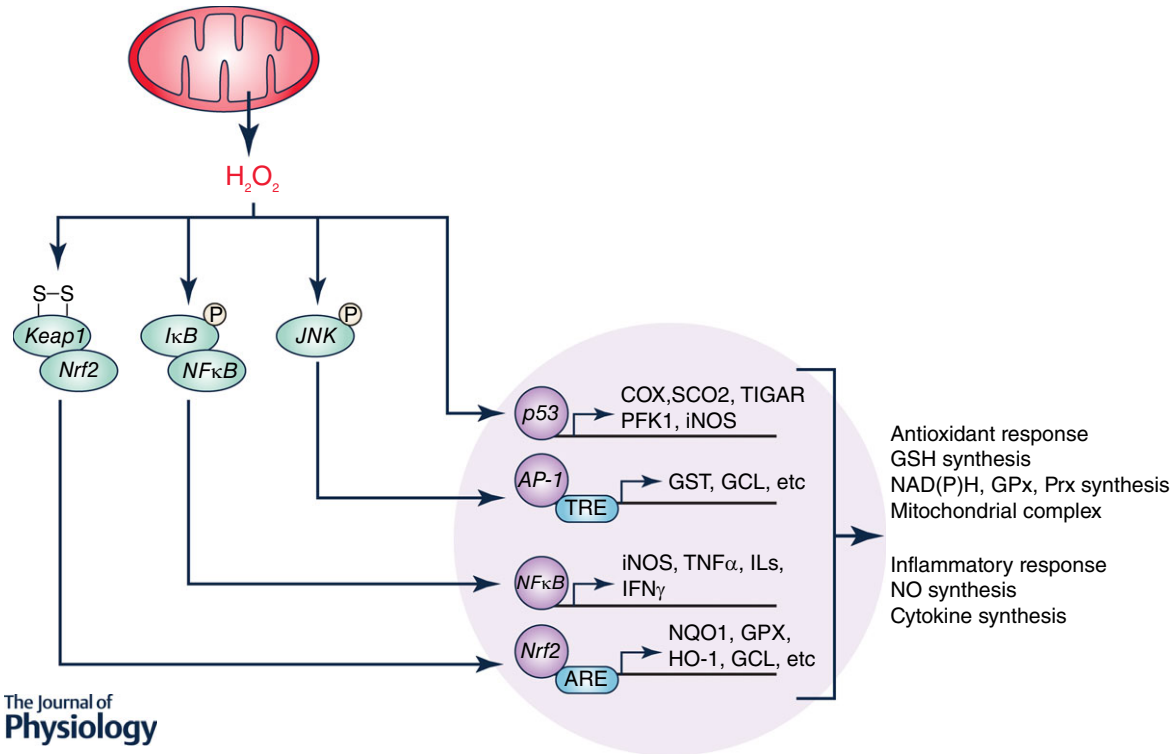

Figure 4. Mitochondrial regulation of redox-sensitive transcription factors
Redox-sensitive transcription factors such as NFκB, AP-1 and Nrf2 can be activated by H_2O_2 generated from mitochondria. H_2O_2 also inhibits p53 DNA binding activity. These transcription factors, in turn, master the synthesis of glutathione (GSH), NAD(P)H, glutathione peroxidase (GPx), peroxiredoxin (Prx), and mitochondrial complex subunits, and thus regulate the cellular redox status. These factors also manage inflammatory response by controlling the transcription of iNOS and a wide-range of cytokines.

degradation. Upon oxidative modification of its cysteine residues, Keap1 dissociates from Nrf2, allowing the translocation of Nrf2 into the nucleus. By binding to the antioxidant response elements (AREs) of a range of phase II antioxidant defense genes, Nrf2 induces their expression such as peroxiredoxins, thioredoxins, glutathione *S*-transferase (GST), NAD(P)H: quinone oxidoreductase (NQO1), haem oxygenase-1 (HO-1), glutathione peroxidase (GPX), and glutamate-cysteine ligase (GCL) (Fig. 4). These genes play major roles in the removal of cytotoxic oxidants or electrophiles (Kensler *et al.* 2007). The Nrf–ARE pathway has also been found to be involved in the attenuation of inflammation-associated conditions, such as rheumatoid arthritis, asthma, emphysema, gastritis, colitis, autoimmune diseases and atherosclerosis (Kim *et al.* 2010). There is also a cross-talk between nuclear factor κ-light-chain-enhancer of activated B cells (NFκB)-mediated inflammatory- and Nrf2-driven antioxidant response pathways. For instance, Nrf2 deficiency leads to disrupted cellular redox balance and increased susceptibility to NFκB activation when the cells are challenged with inflammatory stimuli (Kensler *et al.* 2007). In endothelial cells, it was also reported that overexpression of Nrf2 abolished TNF-α mediated p38 MAPK activation and the downstream VCAM-1 expression (Chen *et al.* 2006).

NFκB. The transcription factor NFκB family comprises five well-characterized proteins, namely p50 (NFκB1), p52 (NFκB2), p65 (RelA), c-Rel and RelB, which form a variety of homo- and heterodimeric combinations under different circumstances (Baeuerle & Baltimore, 1996). NFκB plays a central role in immune and inflammatory responses, through the transcriptional regulation of a large number of cytokines and other immune response genes (Fig. 4) (Janssen-Heininger *et al.* 2000). NFκB is redox-sensitive. Oxidants including $O_2^{\cdot-}$, H_2O_2 and the hydroxyl radical ($OH^{\cdot}$) can positively or negatively modulate NFκB activation. Mitochondria-derived H_2O_2 plays a critical role in the activation of NFκB (Csiszar *et al.* 2008). Under basal conditions, NFκB is localized in the cytoplasm in an inactive form binding with the inhibitor of NFκB (IκB); in response to stimuli, NFκB disassociates from the complex and translocates into the nucleus where it induces the transcription of its target genes (Kabe *et al.* 2005). In the cytosol, oxidative stress can stimulate phosphorylation (serine or tyrosine) of IκB and MAPKs, which in-turn induce NFκB activation.

AP-1. Activator protein-1 (AP-1) is another redox-sensitive transcription factor. AP-1 can be formed by the dimeric combinations of basic leucine zipper proteins that belong the Jun or Fos families (Gius *et al.* 1999). AP-1 protein binds to the tetradecanoyl phorbol acetate response elements (TREs), which are within the regulatory sequence of target genes and control their basal and inducible expression (Fig. 4) (Rahman *et al.* 1999). AP-1 is activated in response to oxidative and pro-inflammatory stimuli, via the MAPK signalling pathways. Mitochondrial oxidant-mediated JNK activation mediates the activation of the c-Jun component of AP-1, which then combines with the c-Fos subunit. The resulting AP-1 heterodimer induces the production of various inflammatory mediators (Sandireddy *et al.* 2014). Interestingly, while some studies show that AP-1 is activated by oxidants, other work also showed that antioxidants such as pyrrolidine dithiocarbamate and *N*-acetyl-cysteine stimulate the activation of AP-1 (Meyer *et al.* 1993; Janssen *et al.* 1995).

p53. p53 is the key factor that maintains genomic stability by regulating the cell cycle and DNA repair process. p53 promotes aerobic metabolism by targeting mitochondria. It has been found that p53 directly regulates mitochondrial oxygen consumption through transcriptional regulation of an assembly factor for the cytochrome *c* oxidase complex (Complex IV), synthesis of cytochrome *c* oxidase 2 (SCO2) (Zhuang *et al.* 2012). p53 activity is redox sensitive, due to the 10 cysteine residues (human) existing exclusively in its DNA-binding domain. Oxidation of these cysteine residues to disulfide bonds suppresses tetramerization and its DNA binding activity of p53. Mitochondrial function thus regulates p53 activity by modulating cellular redox status (Sun *et al.* 2003). p53 also regulates the expression of inducible nitric oxide synthase (iNOS), which produces •NO and promotes inflammation in tissues including hepatocytes (Ambs *et al.* 1998).

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Additional information

Competing interests

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