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Review Article

# Diminished stress resistance and defective adaptive homeostasis in age-related diseases

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Adaptive homeostasis is defined as the transient expansion or contraction of the homeostatic range following exposure to subtoxic, non-damaging, signaling molecules or events, or the removal or cessation of such molecules or events (*Mol. Aspects Med. (2016) 49, 1–7*). Adaptive homeostasis allows us to transiently adapt (and then de-adapt) to fluctuating levels of internal and external stressors. The ability to cope with transient changes in internal and external environmental stress, however, diminishes with age. Declining adaptive homeostasis may make older people more susceptible to many diseases. Chronic oxidative stress and defective protein homeostasis (proteostasis) are two major factors associated with the etiology of age-related disorders. In the present paper, we review the contribution of impaired responses to oxidative stress and defective adaptive homeostasis in the development of age-associated diseases.

## Introduction

Stress resistance is the phenomenon in which a mild stress enables cells, tissues, or whole organisms to withstand future toxic levels of that stress. The ability to cope with fluctuating levels of stressors such as temperature, pH, oxidative stress, food deprivation, hypoxia, osmolarity, and heavy metals to name a few, is not a fixed characteristic of cells, tissues, or organisms. Cells and organisms make transient and reversible changes to cope with fluctuating internal and external conditions, including many forms of stress. These changes occur through adaptive homeostasis. Adaptive homeostasis is defined as 'the transient expansion or contraction of the homeostatic range in response to exposure to subtoxic, non-damaging, signaling molecules or events, or the removal or cessation of such molecules or events' [1]. Adaptive responses to stress depend on biochemical changes to existing enzymes and significant alterations in patterns of gene expression, such as specific stress response factors, including proteases, molecular chaperones, antioxidants, and heat-shock proteins (HSPs) [2-4]. As will be shown in this review, adaptive homeostasis and stress resistance decline with age (Figure 1).

Cells and organisms are constantly exposed to oxidative stress. Oxidative stress is a major type of stress which is defined on the basis of a pro-oxidizing shift in the thiol-redox state and the resulting dysfunction of redox-sensitive proteins. Oxidative stress occurs when the equilibrium of oxidant/antioxidant balance is disrupted, and there is a shift toward an oxidative status, that is accompanied by detrimental effects on cell survival including lipid peroxidation and oxidative modification of DNA, RNA, and proteins [5]. Free radicals play a vital role in physiological processes in signaling pathways, gene regulation, and cellular differentiation [6].

During periods of high oxidative stress, the ability of cells and organisms to cope with such stress can be transiently altered to meet changing demands through a process called oxidative stress adaptation [7-9]. Studies in *Caenorhabditis elegans* (nematode worms) and *Drosophila melanogaster* (fruit flies)

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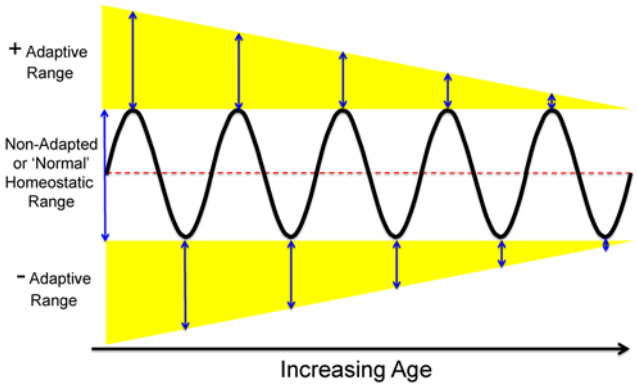


**Figure 1. A graphic representation of the decline in adaptive homeostasis that occurs with increasing age**

The 'normal' or non-adapted range of homeostasis, experienced during unstressful conditions with little or no exposure to toxicants, pollutants, or metabolic disorders, can be expanded or even contracted by a wide variety of signaling agents or conditions. Thus, adaptive homeostasis allows young and healthy individuals to rapidly and transiently modify their defense and repair systems to cope with internal and external stressors. As individuals age, the expansive/contractive ability of the adaptive homeostatic range diminishes; this decline may contribute to the high incidence of disease development amongst the elderly population.

show that adaptation to oxidative stress involves an increase in proteolytic activity, and increased expression of the 20S proteasome mediated by the SKN-1 and CNC-C orthologs of the mammalian nuclear factor erythroid-2-like factor 2 (Nrf2) transcription factor, respectively [10]. However, the ability to mount an adaptive response to oxidative stress declines with age. Defective SKN-1 signaling in aged *C. elegans* impairs 20S proteasome-dependent adaptation to oxidative stress [11]. When proteolytic capacity declines below a critical threshold of activity required to cope with oxidative stress, aggregates of damaged macromolecules impair normal cell function and lead to further complications resulting in disease.

Adaptive responses to oxidative stress decline with ageing. Ageing is characterized by loss of oxidant/antioxidant homeostasis and an impaired ability to mount an adaptive response against oxidative stress, which leads to an increase in protein oxidation and accumulation of protein aggregates [12-14]. An overproduction of reactive oxygen species and a diminished antioxidant capacity is implicated in ageing and age-related diseases. Oxidative damage to mitochondrial enzymes and the mitochondrial genome plays major roles in various age-related degenerative processes. Recent studies in *D. melanogaster* have shown that adaptive responses to oxidative stress are sex- and age-dependent. Increased expression and activity of the 20S proteasome, and resistance to oxidative stress upon exposure to low non-toxic $H_2O_2$ concentrations was lost in aged female flies. In contrast, male flies showed no adaptation to $H_2O_2$, regardless of age [15].

The mitochondrial Lon protease is a key enzyme involved in the degradation of oxidized proteins within the mitochondrial matrix, key regulator of mitochondrial metabolism, and essential factor for maintenance and repair of mtDNA. Under conditions of acute oxidative stress, Lon is up-regulated. However, Lon levels decline in ageing and aberrant Lon expression is involved in age-related diseases [16,17]. Oxidative stress adaptation in *D. melanogaster* flies requires Lon protease expression. However, stress adaptation requires sex-specific Lon isoform expression. Both aged female and male flies exhibited decrease in basal Lon protease activity and proteolytic activity compared with their young counterparts, despite similar Lon protein levels between both age groups [18]. These studies suggest that stress adaptation is sex-biased and declines with age. There are multiple human diseases involving chronic oxidative stress that show a marked sex bias such as cardiovascular disease, neurological disorders, and diabetes, and the results revealed by these studies will aid in understanding these differences.

The etiology of ageing and various disorders is multifactorial; however re-occurring themes linking ageing and disease are compromised response to stress and impaired proteostasis. Our discussion presented here attempts to integrate two major factors that are associated with diminished stress resistance and defective adaptive homeostasis in age-related diseases: oxidative stress and protein turnover. Specifically, we will focus on the role of the Keap1-Nrf2 (Kelch-like erythroid cell derived protein with CNC homology associated protein 1) pathway in regulating an antioxidant response against oxidative stress and the contribution of impaired protein degradation in onset and progression of a subset of age-related disorders, in particular neurological disorders. Discussion on the role of mitochondrial Lon protease in this review will be limited as its role in human disease and ageing has been reviewed [17,19].

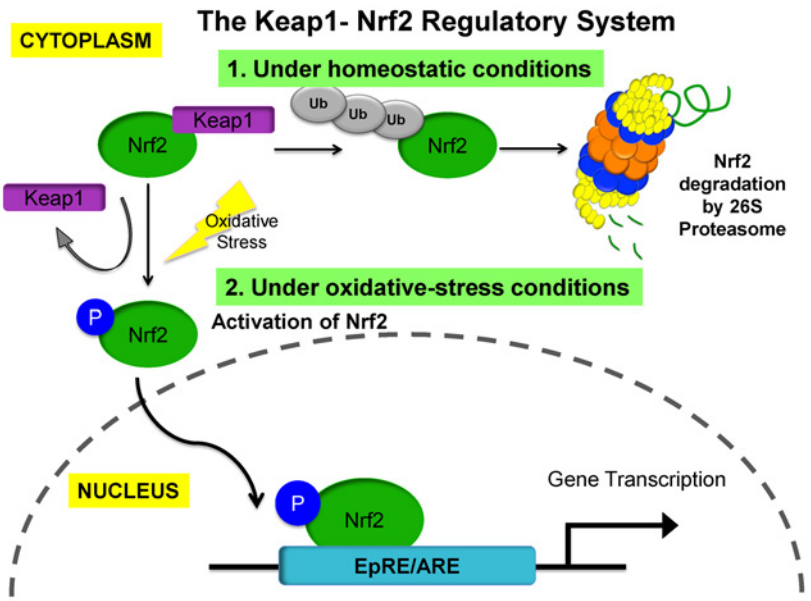


**Figure 2. The Keap1-Nrf2 regulatory system**

Under non-stressful conditions, the Nrf2 protein is maintained in the cytoplasm, bound to a Keap1 complex. The Keap1 complex contains an E3 ubiquitin ligase which 'tags' Nrf2 with a polyubiquitin chain and ensures its rapid turnover by the 26S proteasome, thus keeping normal cytoplasmic Nrf2 levels rather low. Upon receiving a suitable signal or an actual stress, Nrf2 translocates into the nucleus where it binds to EpRE/ARE DNA sequences and activates the transcription of multiple target genes, most of which are involved in cellular protection or the repair/removal of damaged nucleotides, membranes, and proteins.

# Stress resistance and adaptive homeostasis pathways

## Nrf2/electrophile response element signaling

The 'cap'n'collar' (CNC) basic leucine zipper transcription factor Nrf2 is the master regulator of cellular redox homeostasis. Nrf2 is ubiquitously expressed in all the tissues in humans. Under homeostatic conditions, Nrf2 is inhibited from translocating into the nucleus by its negative regulator Keap1. Keap1 plays a role in regulating the localization and degradation of Nrf2 and in sensing oxidative stimuli through its Kelch domain. Keap1 binds Nrf2 and sequesters it to the actin or myosin cytoskeleton. Keap1 also acts as a Cullin-3 ubiquitin ligase E3 complex substrate adaptor, which targets Nrf2 for polyubiquitinylation and subsequent degradation by the 26S proteasome. Lastly, Keap1 also has cysteine residues in the cysteine-rich intervening region ($Cys^{151}$, $Cys^{273}$, and $Cys^{288}$) that are required for Nrf2 binding. Upon oxidative modification of these cysteine residues, Keap1 loses its Nrf2 binding ability and releases Nrf2 [20].

During periods of oxidative stress, Keap1 is also phosphorylated, further enhancing its dissociation from Nrf2. Oxidant-induced dissociation of the 26S proteasome (mediated by Ecm29 and HSP70 [4]) prevents further Nrf2 degradation and increases the Nrf2 pool size. Following phosphorylation by AKT and/or PKCγ, Nrf2 enters the nucleus where it binds to various electrophile response elements (EpREs), also known as the antioxidant response elements (AREs) of target genes, thereby acting as an 'on' switch that increases their transcription [5]. By activating the expression of genes whose products reduce oxidative stress, detoxify and eliminate toxins, Nrf2 regulates the adaptive response to various stressors. The Keap1-Nrf2 regulatory system is graphically described in Figure 2.

Nrf2/EpRE signaling regulates the basal and stress-inducible expression of over 250 cytoprotective genes, including enzymes involved in biosynthesis, utilization, and recycling of reduced glutathione (GSH). These include glutathione reductase (GR) and glutathione peroxidase (GPx), detoxification enzymes such as glutathione *S*-transferase (GST), NADPH:quinone oxidoreductase (NQO1), heme-oxygenase (HO-1), isoforms of superoxide dismutase (SOD) 1–3 (SOD1–3), as well as proteins involved in proteostasis [21,22].

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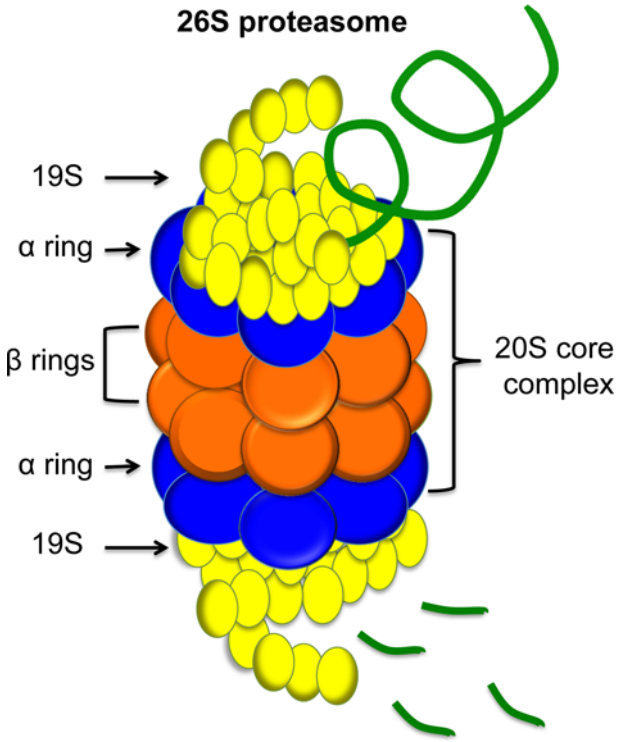


**Figure 3. Structure of the 26S proteasome found in the cytoplasm, nucleus, and endoplasmic reticulum**

At the top of the figure, a coiled target protein is being unfolded and 'fed' into the proteasome. At the bottom of the figure, small peptides and amino acids are released after the target protein has been degraded. The 26S proteasome consists of a core 20S proteasome which itself consists of two α rings and two β rings: each α ring contains seven distinct gene products, as does each β ring. Thus the core 20S proteasome comprises 14 distinct protein subunits. The core 20S proteasome (often 'assisted' by an 11S complex) plays a major role in recognizing and degrading oxidatively damaged proteins in a process that does not involve ubiquitin or ATP. The immunoproteasome, which consists of a core 20S proteasome with three substituted β subunits, is also highly effective in degrading oxidized proteins without ubiquitin or ATP. The addition of two 19S regulatory particles (each made up of multiple subunits) to the core 20S proteasome produces the 26S proteasome which recognizes polyubiquitin 'tagged' proteins for degradation. A system of E1, E2, and E3 ubiquitin conjugating and polymerizing enzymes 'tags' proteins for degradation and delivers them to the 26S proteasome. The 19S regulatory complex binds polyubiquitin 'tagged' proteins and then uses ATP to remove the ubiquitin and unfold the target protein for degradation within the 20S proteasome core. The 26S proteasome, the 20S proteasome (± the 11S regulator), and to a lesser extent the immunoproteasome (also ± the 11S regulator), are all found in the cytoplasm, nucleus, and endoplasmic reticulum (ER).

## The 20S proteasome

The 20S proteasome is one of the cell's primary ATP-independent mechanisms to degrade oxidatively damaged proteins in the cytoplasm, endoplasmic reticulum, and nucleus of eukaryotes [9]. The 20S proteasome is an approximately 670 kDa core tube-like complex, consisting of four stacked rings. The two external rings are made of seven α subunits, and the two internal rings each consist of seven β subunits – three of which are responsible for distinct proteolytic activities including chymotrypsin-like, trypsin-like, and caspase-like activities [23,24]. In the center of the ring, there is a narrow pore where proteins targeted for degradation can enter in an unfolded state.

The 26S proteasome is formed by the addition of a 19S regulatory particle to each of the α rings of the 20S proteasome. The 19S regulatory particle is an approximately 700 kDa multisubunit complex that recognizes, de-ubiquitinylates, and unfolds substrate proteins. The de-ubiquitinylated protein substrate then enters the 20S proteasome core where it is hydrolyzed by the catalytic β subunits of the core, generating peptides that are subsequently degraded to their constituent amino acids. Key structural and functional aspects of the proteasome are shown in Figure 3.

Protein synthesis and protein degradation are important processes involved in cellular homeostasis. During periods of homeostasis, the cell relies on the ubiquitin-proteasome system (UPS), with the 26S proteasome as the predominant

form for protein degradation. As noted above, the 26S proteasome recognizes and degrades substrate proteins tagged with a polyubiquitin recognition signal. The ubiquitinylation of a substrate protein is regulated by a multienzyme system. Ubiqutin (a small protein of 76 amino acids (8.5 kDa)) is activated by the E1 enzyme. The activated ubiquitin is then transferred on to an E2 enzyme, which then associates with E3 ubiquitin ligases. The E3s ligate the ubiquitin to a lysine residue on the target protein. A polyubiquitin chain is then formed through the covalent attachment of a second ubiquitin to the first. The polyubiquitinated protein substrate is then recognized by the 26S proteasome and degraded into small peptides [25].

During conditions of high oxidative stress, critical sulphydryl groups in the 19S regulatory subunits may lose their proteolytic capacity as they are highly susceptible to oxidative damage. Most of the remaining 26S proteasomes are disassembled by Ecm29, and the 19S caps are sequestered by HSP70 [4,5]. 20S core proteasomes are freed to quickly degrade oxidized proteins. Approximately 3–5 h after the initial stress, 26S Proteasome reassembly is catalyzed by HSP70. During periods of high oxidative stress, when the cellular proteome is most susceptible to oxidative damage, it is critical that there is a functioning pool of 20S proteasome. Induction of the 20S proteasome is regulated by the stress-responsive transcription factor Nrf2 [3]. When proteasomal activity is low, the Nrf2 signal transduction pathway is used to increase expression of the 20S proteasome.

### Mitochondrial Lon protease

The ATP-stimulated mitochondrial Lon P1 protease is a major regulator of mitochondrial metabolism and response to free radical damage. Mitochondria contain an electron transport chain (ETC), that transfers high energy electrons to a series of membrane protein complexes, before final acceptance by oxygen. Unfortunately, this process also generates reactive oxygen species as by-products, including sueperoxide and hydrogen peroxide, which can cause damage to surrounding macromolecules. Superoxide is dismutated to hydrogen peroxide by SOD. Hydrogen peroxide is converted into water by GPx with the concomitant oxidation of GSH to GSSG. As mitochondria are one of the major producers of cellular free radicals, removal of mitochondrial oxidatively damaged proteins is crucial for mitochondrial homeostasis and normal cellular function [16,19].

Lon P1 is the primary mitochondrial protease found in the matrix that degrades oxidatively damaged mitochondrial proteins, including aconitase [26]. Oxidized mitochondrial proteins must be rapidly degraded or else they will aggregate, cross-link, and cause subsequent oxidative toxicity. During periods of acute increased cellular stress, Lon is transiently increased to cope with elevated mitochondrial damage [16,27]. However, during ageing and prolonged periods of oxidative stress, Lon protease protein levels are down-regulated, and this Lon deficiency is associated with increased levels of carbonylated proteins that accumulate within the mitochondria [28]. Key functional roles of the Lon protease are shown in Figure 4. Under non-stressful conditions, Lon migrates to the inner surface of the inner mitochondrial membrane where it binds to mtDNA and is crucial for its maintenance and repair as well as for mitochondrial proliferation [17].

## Neurogical disorders

The brain is highly susceptible to damage induced by oxidative stress and reactive oxygen species production, as most of neuronal ATP is generated by mitochondrial oxidative phosphorylation (OXPHOS), only 10% of brain ATP is produced by glycolysis [29]. In addition, its high lipid content makes the brain vulnerable to lipid peroxidation. Mitochondrial dysfunction reduces neuronal capacity to respond to bioenergetic challenges, which impair brain metabolism, and may contribute to neuronal death. Oxidative stress lesions in mtDNA, along with mitochondrial respiratory dysfunction, apoptosis, and impaired antioxidant defense systems are involved in the pathology of neurodegenerative disorders [30].

### Alzheimer's disease

Alzheimer's disease (AD) is the main cause of dementia in older adults, and is currently ranked as the sixth leading cause of death in the United States. Two major hallmarks of AD pathology are the accumulation of amyloid-β (Aβ) plaques and neurofibrillary tangles (NFTs) which comprise the hyperphosphorylated τ protein [31]. Studies of AD rodent models and brains from AD patients have shown that accumulation of Aβ plaques and NFTs causes synaptic dysfunction, oxidative stress, calcium dysregulation, and neurodegeneration [32,33].

Oxidative stress is associated with AD, in which Nrf2/EpRE signaling and proteasomal activity are reduced [34]. The proteasome is essential for the degradation of both Aβ and τ. In studies using the 3xTg murine model of AD, young mice exhibited increased phosphorylation of Nrf2, whereas aged mice show a sex-dependent decrease in Nrf2 phosphorylation [35]. These findings suggest that in the early stages of AD, there is an attempt by the Nrf2 system

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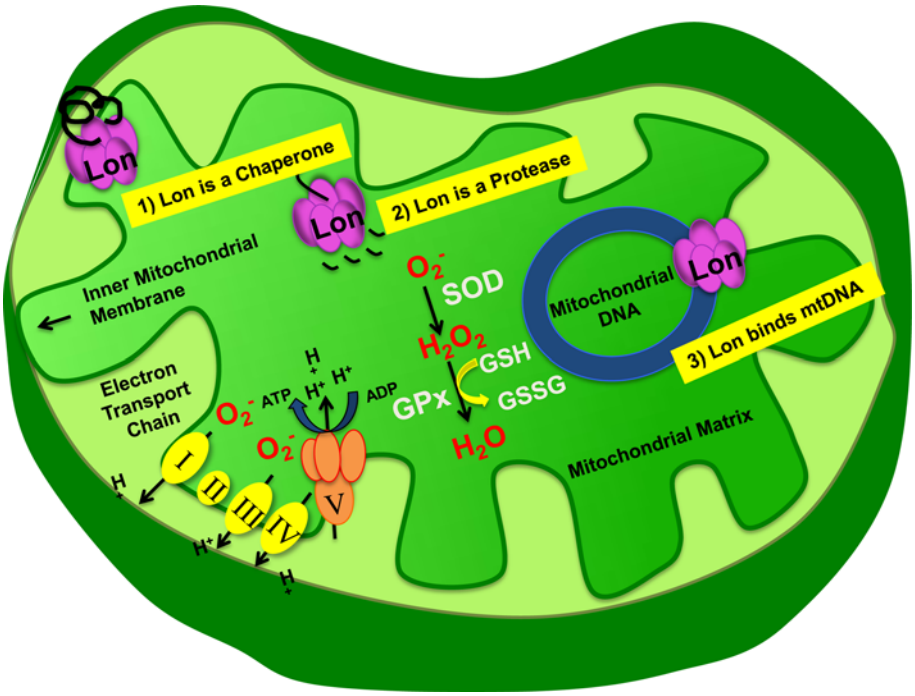


**Figure 4. Schematic representation of antioxidant responses to mitochondrial superoxide and hydrogen peroxide production, and Lon involvement in mitochondrial biology**

The figure shows a mitochondrion with electron transport complexes I, II, III, and IV in the inner membrane, along with the ATP synthase complex V. In many situations, Lon can function as a chaperone within the mitochondrial matrix. The mitochondrial respiratory chain can generate stressful levels of superoxide, however, which is quickly dismutated to hydrogen peroxide by SOD. Despite quite effective removal of hydrogen peroxide, by GPx with the concomitant oxidation of GSH to GSSG some protein damage still occurs. During such oxidative stress conditions, Lon functions as a protease to degrade and remove oxidatively damaged proteins, thus preventing their accumulation, aggregation, and cross-linking. Under non-stressful conditions, Lon migrates to the inner surface of the inner mitochondrial membrane where it binds in the D-loop of the mtDNA: a requirement for mitochondrial proliferation.

to combat oxidative damage. As the disease progresses, however, Nrf2 phosphorylation decreases, and is targeted for degradation by the 26S proteasome. Disruption of Nrf2 signaling in AD prevents up-regulation of many Nrf2 antioxidant targets and up-regulation of the 20S proteasome – two critical defenses against the increased oxidative stress involved in the AD progression [22,35]. Notably, assessment of Nrf2 expression in the frontal cortex and the hippocampus of AD patients has found that Nrf2 is predominantly expressed in the cytoplasm, in contrast with age-matched normal hippocampal and frontal cortex tissues, in which it is expressed in both the nucleus and cytoplasm. In addition, Nrf2 does not co-localize with Aβ plaques or τ in AD [22]. These findings suggest that in AD, Nrf2 is not translocating to the nucleus despite increased oxidative stress and misfolded proteins in these regions, therefore the Nrf2 pathway may be dysfunctional. Additional studies are warranted to understand how the Nrf2 pathway is altered by AD progression.

Aβ is a peptide that is produced by aberrant proteolytic processing of amyloid precursor protein (APP) into two isoforms which are 40 and 42 amino acids in length ($A\beta_{40}$ and $A\beta_{42}$). The $A\beta_{42}$ isoform contains two additional hydrophobic residues, which makes it aggregate readily and is therefore more toxic to neurons than the $A\beta_{40}$ isoform [36]. Under normal physiological conditions, low (picomolar) amounts of Aβ can modulate synaptic activity and act as a metal ion chelator due to its high binding affinity for Cu, Fe, and Zn [37-39]. Aβ clearance and degradation is modulated by astrocytes and microglia [40]. Although the mechanisms involved in the transition from normal physiological function to pathological disease are unclear, studies suggest that dysregulation of protein degradation is involved [13]. As the disease progresses, prolonged and widespread activation of microglia and astrocytes increase neuroinflammation and oxidative stress, and the severity of glial activation correlates with the extent of neuronal death and cognitive decline [41,42]. The inability to maintain a homeostatic balance between amyloid production and its degradation results in Aβ accumulation, which directly inhibits proteasomal activity [43,44], and elevates oxidative

stress, resulting in exacerbated protein malfunction [45,46]. The proteolytic activity of the 20S proteasome and the ATP-ubiquitin-dependent 26S proteasome is greatly reduced in the AD brain [47,48]. It is important to note that the reduced proteolytic activity may be the result of accumulation of damaged, aggregated, and cross-linked cytosolic proteins that impede proteolytic degradation (including Aβ and hyperphosphorylated τ) rather than a decrease in proteasome expression.

Under homeostatic conditions, endogenous τ is turned over by the proteasome [49]. However in AD, accumulation of hyperphosphorylated τ blocks proteolysis by the 20S proteasome *in vitro*. Studies using τ aggregates isolated from human AD brains show that 20S proteasome co-immunoprecipitates with $Ser^{212}/Thr^{214}$ hyperphosporylated τ, and low proteasomal activity correlated with increased τ aggregates in these samples, notably there was no significant difference in proteasomal protein expression between AD and age-matched controls, which suggested that τ aggregates inhibit proteasomal activity in AD [50].

## Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease after AD, and is characterized by a progressive accumulation of α-synuclein, mitochondrial dysfunction, impaired protein degradation in the substantia nigra (SN), and loss of dopaminergic neurons in the SN pars compacta (SNpc) causing severe motor impairments. PD affects over 1% of the population over the age of 60 in the U.S.A., with an average age of diagnosis of 62 years; however PD prevalence reaches 5% in individuals ages over 85 [51].

The SN is particularly susceptible to loss of dopaminergic neurons with increasing age and prominently accumulates oxidative lesions in PD [52]. Interestingly, the SN seems to be more susceptible to neuronal loss with ageing compared with other brain regions. The SN is a highly oxidative environment, partially due to neuronal dopamine metabolism, which generates superoxide and $H_2O_2$ and also reduced reuptake of dopamine into synaptic terminals as expression of the dopamine transporter (DAT) declines with age in the ventral SN [53,54]. α-synuclein increases in the SN during normal ageing, and in PD, α-synuclein accumulates within Lewy bodies and mitochondria [55]. Mutations in the gene encoding α-synuclein *(PARK1*) are associated with familial forms of PD [56]. Numerous genes involved in maintenance of mitochondrial homeostasis have been linked to PD. *PARK1*, Pink1 (*PARK6*), Parkin (*PARK2*), and DJ-1 (*PARK7*) genes have functions linked to maintaining mitochondrial homeostasis, including membrane potential, calcium homeostasis, cristae structure, respiratory activity, mtDNA integrity, and mitophagy – all processes which have been implicated in PD pathogenesis [57]. PTEN-induced kinase 1 (Pink1) is a mitochondria-targeted serine/threonine (Ser/Thr) kinase linked to autosomal recessive familial form and early onset PD. In damaged mitochondria, Pink1 recruits the E3 ubiquitin ligase Parkin from the cytosol to the outer mitochondrial membrane to initiate mitophagy. Decreased expression and activity of mitochondrial ETC complexes I and IV have been found in SN tissue from PD patients [58].

DJ-1 is an oxidative stress-regulated chaperone and transcriptional modulator that translocates from the cytosol to mitochondria and the nucleus upon oxidation. DJ-1 is involved in the Keap1-Nrf2 pathway by stabilizing Nrf2, following its release from Keap1 under oxidative conditions. Loss of DJ-1 function renders mitochondria more sensitive to oxidative stress and complex I inhibitors [57]. DJ-1 inhibits the aggregation and toxicity of α-synuclein. α-synuclein accumulates in mitochondria during PD and is associated with impaired complex I activity, decreased mitochondrial membrane potential, and increased oxidative stress [53]. Mutations in DJ-1 and alterations in DJ-1 levels or isoforms have been documented in early onset of familial and sporadic PD, suggesting a common role in the pathogenesis of PD [59-61].

Under homeostatic conditions, α-synuclein is degraded by the UPS and autophagy, however impaired proteolytic activity of the 20S proteasome has been detected in both familial and sporadic forms of PD within the SN. McNaught et al. [62] detected a selective loss of 20S proteasome α-subunits in dopaminergic neurons of the SN in sporadic PD. Altered Nrf2 expression in the SN has also been implicated in PD. Nrf2 expression patterns in neuromelanin-containing neurons in the SN, as assessed by immunohistochemistry, revealed that Nrf2 predominantly localizes in the cytoplasm whereas a strong nuclear Nrf2 staining is found in age-matched PD brains. Nuclear Nrf2 localization in PD suggests the activation of endogenous antioxidant responses in dopaminergic neurons within the SN, however this response may be insufficient to protect surviving neurons from chronic oxidative insult [22].

## Huntington's disease

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by an autosomal dominant mutation in the *huntingtin* (*HTT*) gene resulting in an expansion of polyglutamine repeats $(CAG)_n$ in the HTT protein. It is

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characterized by chorea, dementia, and neuronal death in the cortex and striatum. Mutant HTT forms intraneuronal inclusion bodies in the nucleus and cytoplasm, especially in the perinuclear area and in dendrites [63].

The onset of HD is inversely correlated with the length of the CAG trinucleotide expansion that codes for polyglutamine repeats; longer CAG repeats are associated with an earlier and more severe presentation of HD [64]. Repeats of 40 or more are associated with disease expression, and on an average HD symptoms typically appear in midlife (40 years). Late-onset HD (age 50 years or more) tends to be associated with 36–39 CAG repeats [64,65]. HD is not an ageing-related disorder *per se*, however age-related changes in proteostasis and oxidative stress may augment the damage caused by the mutant *HTT*. As HD patients age, the ability to cope with the mutant HTT aggregates, UPS inhibition, and mitochondrial dysfunction diminishes, resulting in oxidative damage, neuronal death, and massive degeneration of the cortex and striatum [66]. DNA methylation analysis of HD brain samples revealed accelerated epigenetic ageing in the frontal lobe, parietal lobe, and cingulate gyrus with more broad changes in brain methylation levels [67]. A multivariate model analysis of the DNA methylation data in the present study suggests that HD status increases the biological age by 3.2 years [67].

Mutant HTT expression is associated with increased oxidative stress and reactive oxygen species production. Damage to mtDNA is implicated as a potential mechanism by which mutant HTT leads to mitochondrial dysfunction and contributes to HD progression [68]. Assessment of oxidative stress in postmortem brain tissue samples of HD patients have found increased oxidative mtDNA lesions (8-OHdG) in various regions including the caudate putamen, striatum, and parietal cortex compared with age-matched controls [68-70]. Gene expression studies on animal models and patient tissue samples have provided valuable information regarding the cellular processes and pathways involved in HD pathology [71,72], but the direct effects of mutant HTT may be masked by age-related changes and other secondary processes in the advanced stages of the disease, therefore cell culture HD models have been used to study early HD pathogenesis. Rat pheochromocytoma 12 (PC12) cells induced to express the mutant *HTT* gene have been used to characterize the effects of mutant HTT in the initial stages of HD. Gene expression analysis of PC12 cells, 5 days after induction revealed up-regulation of a subset of Nrf2-responsive transcripts in mutant HTT expressing cells including *NQO1* and *GST*. The present study also identified a few Nrf2-responive genes that were down-regulated in mutant HTT expressing cells: *HO-1*, *Taldo1*, and *Prdx6* [73]. In agreement with the present *in vitro* study, some antioxidant proteins such as GPx, catalase, and SOD (but not all Nrf2/EpRE-driven gene products) are increased in human HD brain [74]. These studies suggest that although the Nrf2 pathway is activated early in HD pathogenesis in response to oxidative stress, Nrf2 signaling may not be fully activated or is impaired. Recently, Quinti et al. found that neural stem cells (NSCs) differentiated from HD patient-derived induced pluripotent stem cells (iPSCs) had impaired Nrf2 signaling, which was ameliorated pharmacologically, by inducing Nrf2 using a selective Keap1-modifying small molecule (MIND4-17) [75,76]. These studies are indicative of an impaired Nrf2/EpRE antioxidant response in HD and highlight Nrf2 as an attractive potential therapeutic target for HD.

## Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is an adult onset, severe neurodegenerative disorder characterized by progressive weakness, atrophy, and spasticity of muscles due to upper and lower motor neuron death in the cortex, brainstem, and spinal cord, which leads to paralysis. As ALS progresses, motor neurons show axonal degeneration and myelin loss. Surviving motor neurons contain cytoplasmic ubiquitinylated protein aggregates and oxidative stress lesions. Three of the commonly found proteins in these inclusions are TAR DNA-binding protein-43 (TDP-43), SOD1, and fused in sarcoma (FUS) [77]. In the later stages, ALS affects the motor neurons controlling the respiratory muscles and throat, which culminates in lethal respiratory failure. ALS is classified as either familial or sporadic, depending on whether it contains an inherited genetic component. The onset for sporadic ALS (sALS) occurs between ages 50–60 years and 40–60 years for familial ALS (fALS) [78].

The first causative mutation to be identified in ALS is mutation in SOD1. Mutations in copper-zinc SOD1 play a causal role in approximately 20% of fALS cases. fALS etiology is characterized by mitochondrial dysfunction, increasing oxidative stress, and accumulation of mutant SOD1 which impairs proteasomal activity and induces endoplasmic reticulum (ER) stress via dysfunction of the ER associated degradation (ERAD) process [79]. The role of mutant SOD1 in ALS has been studied primarily in transgenic rodent models overexpressing mutant SOD1. Overexpression of the mutant *G93A-SOD1* gene in transgenic mice impaired mitochondrial respiration and ATP synthesis, in addition to oxidative damage to mitochondrial proteins and lipids in the brain and spinal cord at the time of disease onset [80]. Mutant SOD1 accumulates in vacuolated mitochondria in G93A-SOD1 mutant mice, where it co-localizes with cytochrome *c*. Notably, mitochondrial vacuolization is preceded by motor neuron death, implicating mitochondrial

dysfunction as a causal factor in fALS [81,82]. Accumulation of mutant SOD1 is associated with impaired proteasomal function. A progressive decrease in proteolytic activity of the 20S proteasome but not a decrease in proteasomal levels in the lumbar spinal cord of mutant G93A-SOD1 transgenic mice was observed prior to significant motor neuron death which suggests that proteasomal dysfunction may be an early event that contributes to ALS progression [83].

Although oxidative stress has been implicated in the pathogenesis of ALS, the role of Nrf2 antioxidant defense in ALS is more complex. *Nrf2* knockout in G93A-SOD1 mice had modest effects on EpRE/ARE-driven gene induction. *NQO1* induction was impaired by $Nrf2^{-/-}$, whereas the induction of other EpRE/ARE-driven genes, such as *HO-1* and genes involved in the GSH pathway (*GCLC* and *GCLM*), and tissue GSH levels do not differ between $Nrf2^{-/-}$ G93A-SOD1 and $Nrf2^{+/+}$ G93A-SOD1 mice [84]. Selective overexpression of Nrf2 in neurons or type II skeletal muscle fibers delayed disease onset, increased EpRE/ARE-driven gene expression and total GSH content, but did not extend survival in G93A-SOD1 mice [85]. These findings suggest that there are additional mediator(s) besides Nrf2 that may be regulating protection against oxidative stress in ALS.

Mutations in sequestrome-1 (*SQSTM1*), which codes for p62, are also linked to ALS and impaired expression/regulation of oxidative stress response genes. Nrf2 regulates *SQSTM1* gene expression via a positive-feedback loop [86], and Nrf2 expression is induced when SQSTM1 binds to Keap1, via its Keap1-interaction region (KIR) [87]. Mutations in the KIR of SQSTM1 are associated with a loss of Keap1 binding, and impaired Nrf2 signaling [88]. p62 deficiency has also been associated with impaired complex 1 mitochondrial respiration, elevated GSH levels, and reactive oxygen species production in patient fibroblasts carrying *SQSTM1* mutations [89].

Approximately 25% of ALS patients also experience subtle cognitive deficits. In particular, up to 15% of ALS patients are diagnosed with frontotemporal dementia (FTD), with up to 50% showing some FTD symptoms [90]. Mutations in the same disease-causing genes have been reported for both disorders, these include the expanded GGGGCC hexanucleotide repeat expansion in chromosome 9 ORF 72 (*C9orf72*) [91] and mutations in the p62 gene (*SQSTM1*). FTD is the second most common type of dementia after AD, and is characterized by degeneration of the frontal and temporal lobes of the brain which results in progressive changes in behavior, personality, and language difficulties while perception and memory remain relatively intact [92,93]. The *C9orf72* mutation is the most common genetic abnormality in both familial FTD (25%) and fALS (30–50%). iPSC-derived motor neurons from patients with the *C9orf72* repeat expansion show an age-dependent increase in DNA damage, reactive oxygen species production, and induction of the p53 pathway. Interactome analysis of the *C9orf72* dipeptide repeat protein indicate that the expanded repeat, $(GR)_{80}$, preferentially binds to ribosomal proteins, two-thirds of which are mitochondrial ribosomal proteins required for translation of the mitochondrial ETC subunits, which compromises mitochondrial respiratory function [94].

## Brain ischemia/stroke

The blood–brain barrier (BBB) maintains the homeostatic environment in the brain, and age-induced changes in the microvasculature increase BBB permeability [95,96]. The incidence of ischemic stroke is higher in the elderly than in younger age groups particularly in females, and is associated with age-related health complications such as type II diabetes, hypertension, and cardiovascular disease. The endothelial cells that form the BBB have greater mitochondrial content than non-barrier forming endothelial cells, and mitochondrial content is decreased with age [97,98]. Reduced mitochondrial respiration and reduced expression of ETC complexes in cerebrovascular endothelial cells is associated with increased blood–brain permeability and worsened outcomes after transient middle cerebral artery occlusion (MCAO) in mice [96]. In ageing, increased production of $H_2O_2$ and superoxide in endothelial cells impairs the vasodilatory activity of nitric oxide [99,100]. Deletion of endothelial nitric oxide synthase in mice is associated with larger brain infarcts after MCAO compared with controls [101]. Endothelial nitric oxide acts as a vasodilator, and its inactivation reduces vasodilating capacity and tissue perfusion, which suggest that oxidative stress-induced vascular deterioration in ageing may contribute to ischemic stroke [102].

Brain GSH levels play a crucial role in protection against ischemia/stroke and preserving the integrity of the BBB. Depletion of GSH is associated with increased BBB permeability, and GSH levels are significantly decreased in response to ischemic injury in rat models of hypoxia-induced BBB breakdown [103,104]. Decreased levels of brain GSH following hypoxia/reperfusion may involve membrane GSH transport mediated by multidrug resistance proteins (Mrps). GSH and GSSG are transport substrates for Mrp1, Mrp2, and Mrp4. Ibbotson et al. [105], recently found increased Nrf2 nuclear translocation following hypoxia/reperfusion in an ischemia rodent model. Nrf2 signaling mediated increased transcription of the genes encoding the GSH-transporting Mrp isoforms (*Abcc1*, *Abbc2*,

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and *Abcc4*) in brain microvessels. These findings indicate that the Mrp transporters may be novel therapeutic targets for modulating GSH transport and restoring redox balance in disorders involving a compromised BBB, such as stroke. Systemic administration of sulphoraphane increased the expression of Nrf2/EpRE-driven genes in rat models of traumatic brain injury [106] and intracerebral hemorrhage [107]. Nrf2 induction and subsequent expression of antioxidant genes, including *GST*, *GPx*, and *HO-1*, attenuated loss of tight junction proteins and endothelial cells after brain injury, thus protecting the integrity of the BBB [106].

Brain ischemia-induced oxidative stress results in production of misfolded and oxidized proteins, and impairs protein degradation pathways, which results in impaired proteasomal function and accumulation of ubiquitin-containing protein aggregates [108]. Transient global ischemia in a gerbil model resulted in a progressive accumulation of ubiquitinylated protein aggregates in neurons, which preceded neuronal death in the CA1 region of the hippocampus [109]. Transient global ischemia impairs 26S proteasome function by promoting proteasome disassembly during the early period of reperfusion in rat and gerbil models [110,111]. The down-regulation of proteasomal activity is associated with increased oxidative stress, and ATP depletion following ischemia prevents 26S proteasome reassembly [111,112]. These results suggest that brain ischemia induces an accumulation of ubiquitinylated protein aggregates and impairs proteasomal activity, which is associated with apoptotic neuronal death.

Hypertension is seen in approximately 70% of elderly stroke patients [113]. In particular, elderly women are at a higher risk for stroke and hypertension, and tend to have worse outcomes than men [113]. Diminished insulin-like growth factor-1 (IGF-1) levels and estrogen–IGF-1 interaction contribute to the development of hypertension in post-menopausal women [114,115]. In addition, estrogen increases cerebral blood flow by enhancing endothelial-derived nitric oxide production, and angiogenesis. Estrogen depletion in post-menopausal women may also contribute to the sex differences in stroke incidence amongst the elderly [116].

## Emotional stress, depression, and anxiety

Stress is a common reaction to adversity, but chronic stress exposure and diminished stress resiliency can lead to major depressive disorder (MDD) and anxiety disorders, amongst other psychiatric illnesses. Depression is prominent amongst older people, with a significant impact on well being and quality of life. Oxidative stress is implicated in the development of anxiety disorders, emotional stress, and depression. A link between oxidative stress and emotional stress is not surprising considering the evidence that depression and anxiety is often experienced by patients with disorders linked to oxidative stress (chemotherapy-related cognitive impairment (CRCI), AD, PD, HD, cancer). A recent review of the research on depression spanning the past 20 years suggest that the prevalence of depression does not increase with age, but depression in older adults is more chronic and more difficult to treat as antidepressants are less effective [117].

Many reports have also found an association between depression and diminished antioxidant levels in human clinical studies and animal models of depression. Analysis of oxidative stress markers in depressed patients revealed an association between depression, higher plasma levels of malondialdehyde (MDA) an end product of lipid peroxidation, diminished antioxidant capacity, and increased SOD activity in red blood cells of patients with MDD [118,119]. Similar results have been reported by meta-analysis studies [120,121]. Antioxidant supplementation, with N-acetylcysteine (NAC) augments antidepressant efficacy in clinical studies for depressive symptoms [122,123].

MDD is associated with alterations in the UPS pathway. A few studies have linked genetic variants in proteasomal subunit proteins and down-regulation of genes involved in ubiquitinylation of synaptic proteins in patients with psychiatric disorders [124-126]. Single-nucleotide polymorphisms (SNPs) in proteasome subunit $\alpha 7$ (PSMA7), proteasome 26S non-ATPase subunit 9 (PSMD9), and proteasome 26S non-ATPase subunit 13 (PSDM13) are associated with poor antidepressant treatment response in depressed patients [124,126,127]. The intronic SNP *PSMD13* rs3817629 is associated with lower *PSMD13* expression in fibroblasts of patients with MDD. *PSMD9* missense SNP rs1043307 is associated with anxiety disorder in type II diabetes and MDD. *PSMD9* mRNA expression is down-regulated in peripheral blood cells of MDD patients, specifically in non-responders to antidepressants. Notably, anxiety in MDD patients is a negative predictor of antidepressant treatment response [126]. Under oxidative conditions, the damage to proteins by reactive oxygen species results in misfolded proteins and altered proteasomal activity. The SNPs in proteasomal subunits described above, and low expression levels proteasomal subunits may result in the inability of the proteasome to effectively degrade damaged proteins in oxidative conditions such as those implicated in MDD, resulting in impaired proteostasis.

Chronic stress exposure can increase susceptibility to depression and is associated with oxidative stress. Bouvier et al. [128], demonstrated that Nrf2 is a key regulator of oxidative stress in the chronic stress (social defeat) rat model. Animals who developed a depression-like phenotype displayed down-regulation of nuclear Nrf2 in the hippocampus,

lipid peroxidation, DNA oxidation, and an increase in the inactive form of peroxiredoxin-2 (Prx-2), an antioxidant enzyme involved in $H_2O_2$ reduction to $H_2O$. In addition to the alterations in hippocampal redox homeostasis, neurons of the CA3 hippocampal region had a significant reduction in apical dendritic length and spine loss. Investigation of the upstream mechanism regulating Nrf2, revealed that brain-derived neurotrophic factor (BDNF) is involved in the nuclear translocation of Nrf2. BDNF down-regulation in rat hippocampi decreased Nrf2 translocation to the nucleus and induced oxidative stress. Rescue of Nrf2 function with the antioxidant *tert*-Butylhydroquinone or BDNF augmentation via 7,8-DHF, a selective TrkB agonist, prevented susceptibility to depression [128]. Separate studies examining the influence of the antidepressant fluoxetine, a commonly prescribed selective serotonin reuptake inhibitor, on Nrf2 signaling in rodent models of stress [129] and anxiety/depression [130] have revealed that fluoxetine up-regulates HO-1 through an Nrf2-dependent pathway, and increases cortical and hippocampal BDNF expression levels. These findings open up the potential for development of oxidative stress targeted therapies for various neurodegenerative and psychiatric disorders that are associated with alteration in redox homeostasis.

Anxiety is an aversive emotional response to a threat or a perceived threat. The feeling of fear is accompanied by emotional stress, and when this emotion is extreme and persistent, it is classified as pathological [131]. Most research on anxiety had focussed on neurotransmitter dysregulation including the γ-aminobutyric acidergic (GABAergic) and serotoninergic systems, until Hovatta et al. [132] identified a correlation between expression of two genes involved in antioxidant response to oxidative stress and anxiety. Gene expression profiling of mice strains expressing anxiety-like behavior identified two genes *glyoxalase 1* (*Glo1*) and *glutathione reductase 1 (GR 1*) to play a causal role in the anxiety-like phenotype [132]. GR catalyzes the reduction of GSSG to the sulphydryl form of glutathione (GSH). Glo1 uses GSH as a cofactor to detoxify methylglyoxal – a highly reactive dicarbonyl compound formed as a glycolysis by-product. This was the first study that demonstrated a link between altered oxidative stress response and anxiety. A separate study examining the oxidative status in the brains of Swiss albino male mice with contrasting levels of anxiety reported elevated reactive oxygen species levels in neuronal and glial cells in the cerebellum, hippocampus, cerebral cortex, and in peripheral leukocytes of anxious mice. Nrf2 knockdown by siRNA injected into the dorsal third ventricle of the brains of adult male Wistar rats induced anxiety-like behavior, increased expression of cleaved caspase-3, and quite unexpectedly increased respiratory activity of the mitochondrial ETC complexes, perhaps as a compensatory mechanism in response to oxidative stress-induced neuronal damage [133]. Together these findings suggest an imbalance of the redox system in anxiety-related disorders [131,134].

## CRCIs

Chemotherapy-related cognitive impairments (CRCIs) are commonly reported during and after completion of chemotherapy treatment. CRCI includes changes across various cognitive domains such as working memory, executive function, and processing speed. While 33% of cancer survivors experience long-term CRCI, older adults may have an increased likelihood of developing CRCI [135-137]. Although studies focussing solely on the prevalence of CRCI in the ageing population are limited, two of the most commonly affected groups by CRCI – the breast cancer and ovarian cancer populations are composed of patients with a median age of 60–63 years [138]. Studies examining cognitive function of women aged 60+ receiving adjuvant chemotherapy for breast cancer have found reductions in performance in neurocognitive testing 6 months after the completion of chemotherapy treatment [139-141]. Notably, cognitive dysfunction in the studies of breast cancer patients correlate with reductions in gray and white matter density [142,143] and reduced hippocampal volume [144]. In addition, Heflin et al. found that elderly cancer survivors have an increased risk for developing persistent long-term cognitive impairments and dementia [145].

The mechanisms by which chemotherapy can cause long-term cognitive changes have not been well characterized; however multiple candidate mechanisms for CRCI have been proposed. Amongst the CRCI candidate mechanisms, DNA damage and oxidative stress have been described for various chemotherapeutic agents including cisplatin, doxorubicin, carmustine, methotrexate, and cyclophosphamide [146-149]. Doxorubicin results in oxidative stress by producing superoxide ions that damage complex I of the ETC, in addition it increases levels of tumor necrosis factor-α (TNF-α) in the plasma, which crosses the BBB and activates apoptotic pathways resulting in neural death [150]. Rodent studies examining the effects of cisplatin on neurotoxicity and cognition have found that cisplatin causes neural free radical production and mtDNA mutations which impair mitochondrial respiratory capacity and result in neuronal dendritic spine loss and apoptotic cell death in hippocampal neurons and NSCs [146,151-153]. Diminished antioxidant capacity and low levels of key antioxidants (GSH, SOD) have been observed in the blood of chemotherapy-treated cancer patients [154,155] as well as in the blood and brain tissue of chemotherapy-treated rodents [156,157].

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## Cancers (a brief overview)

Cancers are an extremely heterogenous group of diseases about which it is hard to make generalizations. In addition, the importance of oxidative stress and proteostasis (or deviations from proteostasis) in various forms of cancer have been widely studied. For these reasons, we have made the topic of oxidative stress, adaptive homeostasis, and proteolysis in various cancers its own review (to be published separately). In the present monograph, therefore, we have limited the discussion of cancers to just a bare minimum.

For most cancer types, incidence increases with age. Adults aged 50–74 account for 53% of all new cancer cases, and elderly people aged 75+ account for 36%, with more cancer cases in males than females in both age groups. The elderly population is more susceptible to developing cancers [158]. A few factors involved in this age discrepancy in cancer incidence include accumulation of genomic damage (mtDNA and nuclear DNA lesions), prolonged exposure to environmental carcinogens, chronic inflammation, weakened immune response, and increased oxidative stress [159].

Mitochondrial dysfunction, oxidative stress, and deregulation of cellular energetics are hallmarks of cancer biology. To fuel uncontrolled cell proliferation under fluxing oxygen conditions, cancer cells must adapt, or reprogram their energy production by limiting their energy production largely to glycolysis (Warburg effect) [160]. Under homeostatic conditions, Nrf2 levels are kept low due to Keap1 inhibition. Nrf2 plays a protective role by regulating the cellular adaptive response to oxidative stress and maintaining cellular redox homeostasis. However in many cancers, aberrant Nrf2/EpRE regulation results in Nrf2 constitutive activation, which accelerates cancer progression in pancreatic cancer and in non-small-cell lung carcinoma, [161,162] and is associated with chemoresistance and radioresistance [163].

Nrf2 activation in cancer cells facilitates metabolic programming by redirecting glucose and glutamine into anabolic pathways through the PI3K/Akt pathway [164]. Nrf2 activation has also been shown to increase the expression of anti-apoptotic genes *Bcl-2*, $Bcl\text{-}X_L$ which enhances cancer cell survival and drug resistance in the Hepa-1 and Hepa-G2 hepatocarcinoma cell lines [165,166]. Pharmacological inhibition of Nrf2 has been shown to reverse the chemoresistance in several human cancer cell lines including human breast adenocarcinoma, myelogenous leukemia, and liver cancer [167-169]. While Nrf2 represents a potential target for novel adjuvant therapies in cancer treatment, careful consideration must be made to possible toxicities and additional diseases associated with oxidative stress and antioxidant depletion.

## Osteoporosis

A dynamic balance between bone resorption by osteoclasts and bone formation by osteoblasts is required for the maintenance, strength, and integrity of the human skeleton. In osteoporosis, bone homeostasis is lost, and bone resorption exceeds bone formation leading to low bone mass and deterioration of bone tissue and an increased susceptibility to fractures, especially of the hip, spine, and wrist. Post-menopausal women are particularly susceptible to osteoporosis as estrogen deficiency negatively affects bone homeostasis [170]. Estrogen deficiency leads to increased levels of TNF and receptor activator of NF-κB ligand (RANKL) [171] and enhanced oxidative stress [172] which promotes osteoclast formation. GPx, the primary antioxidant enzyme expressed by osteoclasts is up-regulated by estrogen [173], and estrogen deficiency in ovariectomized rodent models is associated with impaired antioxidant expression [174].

Keap1-Nrf2 regulates RANKL-dependent osteoclastogenesis through modulation of intracellular reactive oxygen species signaling [175]. Nrf2 deficiency in bone marrow derived macrophages derived from $Nrf2^{-/-}$ mice augments RANKL-induced osteoclast differentiation and bone resorption which is associated with diminished antioxidant production [176,177]. In separate studies, stimulation of mouse osteoclast precursors with RANKL up-regulates Keap1, decreases Keap1-Nrf2 ratio, and down-regulates cytoprotective enzymes including HO-1 and NQO1 [175]. Kanzaki et al. recently found that RANKL attenuates Nrf2-mediated expression of cytoprotective enzymes by inducing the nuclear translocation of BTB and CNC homology 1 (Bach1), a repressor of Nrf2 [178]. Interestingly, pharmacological induction of Bach1 export from the nucleus induced Nrf2 translocation to the nucleus, increased the expression of antioxidant enzymes, and resulted in reduction in osteoclastogenesis and bone damage in a bone destruction mouse model [178].

Furthermore, other groups have shown that reactive oxygen species production via a non-mitochondrial pathway, NADPH oxidases (NOXs) 1–4 (NOX1–4) is implicated in osteoclast differentiation. Although the relative contributions of each NOX isoform to reactive oxygen species production and role in osteoclasts are disputed in the field, the involvement of NOX4 reactive oxygen species production in osteoclastogenesis has been characterized [179]. NOX4 is a constitutively active enzyme, found on intracellular organelle membranes and produces $H_2O_2$. *NOX4* knockout

in mice increased bone density, reduced osteoclast numbers and circulating bone resorption markers. Loss of *NOX4* also down-regulated $H_2O_2$ and $Ca^{2+}$ levels during osteoclast differentiation. Pharmacological inhibition of *NOX4* in an ovariectomy-induced osteoporosis murine model attenuated bone loss [180]. Bone from patients with untreated osteoporosis exhibited increased *NOX4* expression and protein levels compared with age-matched controls [180].

## Osteoarthritis

Osteoarthritis (OA) is one of the most common degenerative diseases of the joints amongst the elderly population. OA is characterized by an age- or time-dependent progressive loss of joint homeostasis due to degeneration of the articular cartilage and elevated chondrocyte death affecting individuals over the age of 50 [181]. Chondrocytes are responsible for the synthesis and breakdown of the cartilaginous extracellular matrix [182]. Under homeostatic conditions, chondrocytes are quiescent cells. However in OA, chondrocytes take on an activated phenotype resulting in increased secretion of proinflammatory cytokines including IL-6, IL-1β, and TNF-α. OA chondrocytes also produce extracellular matrix degrading proteins, such as matrix metalloproteinases (MMPs) [183,184]. The anabolic activity of chondrocytes in articular cartilage diminishes with age. IGF-1 is the main anabolic growth factor for articular cartilage and plays a key role in cartilage homeostasis by balancing proteoglycan synthesis and breakdown by chondrocytes. Chondrocyte responsiveness to IGF-1 decreases with age and in OA, which may contribute to cartilage deterioration in OA [185,186].

Human articular chondrocytes actively produce reactive oxygen species and reactive nitrogen species [187-189]. Enhanced reactive oxygen species and reactive nitrogen species production, mitochondrial dysfunction, and diminished activity of GR and thioredoxin reductase [190] occurs in ageing chondrocytes and contributes to the activated chondrocyte phenotype in OA [183,191]. Increased reactive oxygen species levels may contribute to IGF-1 loss of responsiveness in aged OA chondrocytes by interfering with cartilage matrix synthesis [192,193]. Excessive reactive oxygen species production triggers the activation of signaling pathways leading to apoptosis or necrosis of chondrocytes in OA [194].

Nrf2 activation in human OA chondrocytes suppresses IL-1β-induced *MMP-1*, *MMP-3*, and *MMP-13* mRNA synthesis and protein levels, and induces the expression of antioxidant enzymes NQO1 and HO-1 [195,196]. Studies using the monosodium iodoacetate (MIA) articular injection and destabilization of the medial meniscus (DMM) models of OA have revealed more severe cartilage damage in $Nrf2^{-/-}$ mice compared with wild-type controls. In both, MIA and DMM models, Nrf2 downstream proteins HO-1 and NQO1 are up-regulated [197]. These results suggest that the Nrf2 pathway exerts a protective function in OA development. Recently, Nrf2 has been recognized as a protein acetylation target, and its acetylation increases Nrf2 signaling and EpRE/ARE-driven gene expression [198,199]. Nrf2 acetylation using histone deacetylase (HDAC) inhibitor trichostatin A promotes Nrf2 nuclear translocation, increased expression of HO-1 and NQO1 in chondrocytes, and reduced levels of proinflammatory cytokines in MIA and DMM mouse models of OA [197].

The UPS plays a crucial role in regulating NF-κB activation [200]. The NF-κB pathway is activated and contributes to the proinflammatory environment in rheumatic disorders including OA. NF-κB activation mediates *MMP-1*, *MMP-3*, *MMP-13* mRNA and protein expression induced by the proinflammatory cytokines TNF-α and IL-1β in human OA articular chondrocytes [201]. NF-κB is present in the cytoplasm in most mammalian cells in an inactive state through its association with the inhibitory κB proteins (IκBs). The IκBs are degraded by the 26S proteasome following polyubiquitinylation. Upon degradation of IκB, the NF-κB complex translocates to the nucleus where it regulates inflammatory signaling [202]. UPS inhibition via proteasomal inhibitor MG132 in the DMM OA mouse model protected cartilage from cytokine-mediated resorption and degradation and *MMP-13* expression. Although the molecular mechanism by which proteasomal inhibition exerts a protection against OA cartilage damage in this model remains unclear, it was suggested that it limits IκB degradation by impeding NF-κB signal transduction [203].

## The ageing kidney

A normal kidney contains approximately 1 million nephrons, each of which contributes to the total glomerular filtration rate (GFR). Under homeostatic conditions, the kidney is able to maintain GFR, despite renal injury, as the remaining nephrons adapt through hyperfiltration and compensatory hypertrophy to continue normal clearance of plasma solutes. Kidney function declines with age. Some notable age-related changes in the kidney are decreased number of functional glomeruli and tubules, decreased GFR and renal blood flow, nephron loss, glomerulosclerosis, and tubulointerstitial fibrosis [204]. In addition to age-related decline in kidney function, other factors that affect vascular health such as hypertension, diabetes, cardiovascular disease, and smoking may increase susceptibility to nephrotoxicity in advanced age [205].

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Oxidative stress is a major mediator of age-related loss of renal function and renal diseases such as chronic kidney disease. The prevalence of chronic kidney disease is higher in older people, and it is a major co-morbidity associated with diabetes, hypertension, heart disease, and stroke [206]. In a rodent model of chronic kidney disease, rats subjected to a 5/6 nephrectomy exhibited a progressive increase in oxidative stress, NF-κB activation, decrease in Nrf2 nuclear expression, and down-regulation of Nrf2 target genes including *SOD* isoforms, *GPx*, *NQO1*, and *HO-1* in the remaining kidney. GSH depletion and down-regulation of GSH-related enzymes (GST, GPx, and glutamate cysteine ligase subunits) were also observed in this model, indicating a maladaptive response by the remnant kidney to cope with oxidative stress and inflammation [207]. These changes were associated with marked increase in Keap1, suggesting that Nrf2 activation was repressed, accounting for the impaired antioxidant response. Nrf2 knockout intensifies oxidative stress and renal injury in mice models, including models of ischemia–reperfusion and cisplatin chemotherapy-induced nephrotoxicity [208]. Renal function and survival is significantly worse in $Nrf2^{-/-}$ mice compared with the wild-type control mice. Induction of Nrf2 by antioxidant supplementation with NAC or GSH improves renal function [209].

Several studies suggest that deficiencies in antioxidant capacity may play a role in the ageing kidney. In aged rats, GST activity is decreased. Although renal basal GSH levels were not lower in aged rats compared with younger rats, basal renal GSH levels were depleted more rapidly when aged rats were challenged with acute ischemia exposure [210].

Kidney androgen regulated protein (KAP) is expressed in proximal tubule epithelial cells and is critical for maintaining cardiovascular–renal homeostasis, although the exact function of the KAP protein is unknown [211]. Overexpression of KAP in the kidneys of male transgenic mice induced hypertension and enhanced the excretion of urinary markers of oxidative stress (8-iso-prostaglandin F2α, 8-OHdG), augmented mtDNA damage and MDA levels, and diminished catalase and GPx activity in the kidneys. These observations suggest an association between sex-dependent androgen differences in the development of hypertension and renal damage [211,212].

# Chronic prostatitis

Pathogenic bacteria, leukocyte infiltration, increased levels of proinflammatory cytokines, and elevated reactive oxygen species production are associated with chronic prostatitis, which is persistent inflammation of the prostate gland [213]. The most common type of prostatitis is chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) which accounts for 90% of cases, and affects up to 15% of men [214]. The risk for developing prostatitis increases with age [215], CP/CPPS is characterized by pain or discomfort in the abdomen, pelvis, and genitals, for a period of 3 months, as well as irritation and obstructive lower urinary tract symptoms in the absence of urinary tract infection. The causes for CP/CPPS are not well understood.

Patients with chronic prostatitis have higher seminal oxidative stress and diminished antioxidant levels compared with healthy men [216]. Symptoms in patients with chronic prostatitis may last for several weeks or recur periodically for many years, which can negatively impact sperm function, and result in infertility in some patients. Expression of inflammatory mediators IL-8, IL-1β, and ICAM-1 is associated with endothelial cell damage, and negatively correlates with erectile dysfunction in CP/CPPS patients [217]. In particular, higher levels of reactive oxygen species were detected in chronic prostatitis patients with leukocytospermia [216,218]. Leukocytes are the primary source of reactive oxygen species production in semen [216,219]. A separate study in patients with chronic bacterial prostatitis, found increased nitric oxide and MDA levels, and decreased levels of the antioxidant enzymes SOD, catalase, and GPx in the blood [220]. Oxidative stress in the chronic non-bacterial prostatitis (CNP) rat model is associated with down-regulation of Nrf2 target genes *NQO1*, *GST*, and *HO-1*, and a decrease in the Nrf2 to Keap1 ratio. Antioxidant NAC administration ameliorated prostatitis and increased Nrf2/EpRE-driven gene expression [221].

# Liver diseases

## Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in Western countries. The prevalence rate of NAFLD is between 15% and 30% in adults, and increases to 35.1% in people aged greater than 65 [222]. NAFLD is characterized by defective fatty acid metabolism – an imbalance between lipid uptake and synthesis that exceeds oxidation and removal, resulting in an excess accumulation of fat deposits in hepatocytes [223]. NAFLD is commonly associated with obesity, type II diabetes, and insulin resistance. NAFLD ranges from simple steatosis (fatty infiltration in liver) to non-alcoholic steatohepatitis (NASH), which can progress to fibrosis and ultimately liver cirrhosis [224]; 20%–30% of individuals with NAFLD develop NASH, which consists of lipid buildup and inflammation. Lipid metabolism is increased as a response to the accumulation of fatty acids and triglycerides in the liver, which

causes oxidative stress through overproduction of reactive oxygen species. Oxidative stress also triggers production of inflammatory cytokines, which causes inflammation via activation of NF-κB and c-Jun N-terminal kinase (JNK), ultimately resulting in the development of NASH [224].

Mitochondrial dysfunction plays a central role in the pathogenesis of NAFLD. Depletion of mtDNA, decreased respiratory activity, and impaired mitochondrial β-oxidation are associated with NAFLD [225]. Impairment of β-oxidation increases fatty acid accumulation in hepatocytes which combined with elevated reactive oxygen species levels augments lipid peroxidation which results in a vicious cycle of hepatic oxidative stress-induced damage. Reactive oxygen species and by-products of lipid peroxidation further impair respiratory chain function in hepatocytes and increase generation of proinflammatory cytokines (TNF-α, Fas ligand). Impaired mitochondrial function in hepatocytes resulting in reduced ATP synthesis and increased reactive oxygen species production has been reported in patients with NASH [226-228].

Several studies indicate that antioxidant defense mechanisms are altered in NAFLD. Hepatocyte GSH content and SOD activity was decreased in NAFLD patients, this depletion is further exacerbated in patients with steatohepatitis – inflammation and lipid accumulation in the liver [229]. Hepatic GSH, as well as SOD, catalase, and GPx activity were decreased in the livers of obese *fa/fa* rats fed a high-fat diet [230]. Lipid peroxidation and protein carbonyls and NOX activity were elevated which was associated with fibrosis [230]. In a separate study, $Nrf2^{-/-}$ mice fed a high-fat diet developed more severe NASH with cirrhosis, than wild-type $Nrf2^{+/+}$ mice. The livers of $Nrf2^{-/-}$ mice fed a high-fat diet had increased induction of lipogenesis genes and suppression of β-oxidation genes compared with the wild-type livers, which suggests greater fatty acid synthesis in the $Nrf2^{-/-}$ livers. Administration of the high-fat diet stimulated hepatic oxidative stress as indicated by increased levels of GSSG, MDA, protein carbonyls, and increased levels of apoptosis and DNA damage. Unlike the $Nrf2^{+/+}$ mice, $Nrf2^{-/-}$ mice had decreased expression of EpRE/ARE-driven genes, including genes involved in GSH homeostasis and *NQO1*. This attenuated antioxidant response in $Nrf2^{-/-}$ mice may stimulate inflammation, contributing to the rapid development of NASH [231]. This study suggests that Nrf2 protects the liver against steatosis and NASH by suppressing lipogenesis and promoting fatty acid oxidation, and enabling adaptation to high-fat diet-induced oxidative stress. Notably, studies on aged $Ldlr^{-/-}$ mice, which models human NASH and atherosclerosis, fed high-fat diets show a greater decline in antioxidant gene expression which correlates with a decrease in Nrf2 expression compared with young $Ldlr^{-/-}$ animals [232]. Aged $Ldlr^{-/-}$ mice also displayed more severe steatosis, along with NASH, in contrast with the young $Ldlr^{-/-}$ mice which only developed fatty livers [233].

## Alcoholic liver disease

Alcoholic liver disease (ALD) complications in the elderly are higher due to impaired ethanol metabolism. An elderly person's liver is more susceptible to the toxic effects of alcohol, and prognosis of ALD in the elderly is poor usually due to hepatic damage induced by heavy lifelong consumption of alcohol [234]. The severity of ALD ranges from steatosis to cirrhosis. Hepatic ethanol is processed by two major pathways: alcohol dehydrogenase (ADH), which resides in the cytosol and cytochrome P450 2E1 (CYP2E1) which is a component of the ER. The activity of alcohol metabolizing enzymes ADH, acetaldehyde dehydrogenase (ALDH), and CYP2E1 diminishes with age [235]. In addition, body water content also decreases with age, these two factors lead to elevated concentrations of ethanol in the blood [235].

Acetaldehyde is a highly reactive intermediate of alcohol metabolism that accounts for most of the ethanol-induced toxicity. Acetaldehyde promotes hepatic lipid accumulation. $Nrf2^{-/-}$ mice fed an ethanol diet, displayed increased mortality associated with liver failure at doses that were tolerated by wild-type control animals. Loss of Nrf2 resulted in significantly lower ADH activity and accumulation of acetaldehyde in the liver, leading to steatosis and up-regulation of proinflammatory cytokines TNF-α and IL-6. $Nrf2^{-/-}$ mice also had impaired GSH homeostasis; mitochondrial GSH levels were significantly lower and associated with mitochondrial swelling [236].

In ALD, inclusions of damaged intermediate filaments, known as Mallory–Denk bodies (MDBs) accumulate within hepatocytes. MDBs have been found to contain ubiquitin, HSPs 70, 90, and 25, as well as the β5 catalytic subunit of the 20S proteasome, and Tbp7 (an ATPase subunit of the 26S proteasome) in an ALD mouse model [237]. Serum concentrations of free ubiquitin and polyubiquitin chains are higher in subjects with alcoholic liver cirrhosis than in normal subjects [238]. These studies suggest that the UPS is unable to degrade the protein components of the MDBs. Formation of MDB aggregates in ethanol-exposed HepG2 cells correlates with an ethanol-induced reduction in proteasomal activity [239]. Ethanol administration in rats results in decreased proteolytic activity of the 20S proteasome, which is associated with increased hepatic content of liver peroxides, suggesting that ethanol-induced oxidative stress may impair proteasomal activity [240].

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## Insulin resistance and type II diabetes

Diabetes is characterized by dysfunction in the body's ability to maintain glucose and insulin homeostasis. Diabetes increases the risks for multiple age-related diseases including cancer, stroke, cardiovascular diseases, atherosclerosis, NAFLD, PD, and AD [241,242]. Diabetes is divided into two categories, type I and type II. Type I is a genetic disorder where autoimmune destruction of pancreatic β cells leads to insufficient production of insulin, whereas type II diabetes occurs when insufficient insulin is produced or insulin secretion is impaired resulting in high blood glucose levels. Type II diabetes accounts for more than 90% of individuals diagnosed with diabetes. The elderly are at a higher risk for developing type II diabetes, due to the increase in insulin resistance and pancreatic β cell dysfunction with age. Glucose homeostasis requires fasting blood glucose levels to be maintained between 70 and 100 mg/dl. Higher fasting glucose blood levels between 100 and 125 mg/dl are indicative of insulin resistance, and levels above 126 mg/dl are an indicator of diabetes [243]. Under homeostatic conditions, pancreatic β cells adapt to insulin resistance by increasing mass and insulin secretion. However, failure of β cells to properly compensate results in hyperglycemia [244].

Ageing in humans and rodents is associated with a decline in glucose-stimulated insulin secretion from pancreatic β cells. Impaired insulin secretion and insulin resistance are two hallmarks of type II diabetes [245]. Multiple factors in ageing contribute to the development of diabetes including decrease in β cell mass and impaired function, altered glucose transport as indicated by decreased expression of glucose transporters in skeletal muscle (GLUT4) [246], increased oxidative stress, and mitochondrial dysfunction [247-249]. Oxidative stress impairs insulin signaling, which plays a role in the development of insulin resistance and type II diabetes. Low levels of $H_2O_2$ are required for β-cell insulin secretion in response to glucose, however chronic elevation of reactive oxygen species decreases β-cell mass and function by decreasing transcription factor (*Pdx2* and *MafA*) binding to the insulin gene [250]. Islets from type II diabetes patients' exhibit lower ATP content and diminished glucose-stimulated insulin secretion. In addition, other studies have demonstrated augmented release of proinflammatory cytokines (TNF-α, IL-6) from leukocytes which may further impair insulin signaling and glucose uptake [247,251].

Decreased Nrf2 expression and activity is associated with diabetes and is also involved in diabetic complications. At the onset of diabetes, Nrf2 protein expression and Nrf2-gene products NQO1 and HO-1 are up-regulated due to acute hyperglycemic conditions, but decreased in late stages of type II diabetes in patients and mouse models [252,253].

## Age-related macular degeneration

Age-related macular degeneration (AMD) is the leading cause of blindness in people over the age of 65. AMD is a progressive degeneration of the retina, the retinal pigment epithelium (RPE), Bruch's membrane, and underlying choroid. Macula damage results in loss of central vision. Mitochondria are a major source reactive oxygen species in the photoreceptors and RPE. Increased mtDNA lesions [254,255] and down-regulation of the DNA repair enzymes 8-oxoguanine-DNA glycosylase 1 (OGG1) [255-257] in the macula and RPE correlate with AMD progression. Mice deficient in *SOD1* and *SOD2* developed age-dependent degeneration of the retina, with features typical of AMD in humans [207,258]. Nrf2 knockout mice develop age-dependent degeneration of the retina with similarities with human AMD pathology including drusen deposition, RPE degeneration, and deregulated autophagy [259]. These studies strongly implicate oxidative stress as a causal factor in AMD.

## Cardiac disorders

Ageing is accompanied by a higher incidence of cardiovascular disease due to age-related changes in heart morphology, impaired proteolysis, and mitochondrial dysfunction. In ageing, there is an increase in maladaptive cardiac hypertrophy which contributes to hypertension and development of heart failure. In rodents, cardiomyocyte number decreases and remaining cardiomyocytes become enlarged with age [260]. Nrf2 protects against maladaptive cardiac responses to mechanical stress by suppressing oxidative stress. $Nrf2^{-/-}$ mice in a mouse model of pressure overload after transverse aortic constriction (TAC) developed cardiac hypertrophy, myocardial fibrosis and apoptosis, resulting in overt heart failure and diminished expression of antioxidant genes including *GPx*, *HO-1*, *SOD2*, and *SOD3* [261]. Nrf2 signaling and mitochondrial manganese SOD2 are also depleted in aged vessels, and disruption of Nrf2 signaling impairs angiogenesis in human coronary arterial endothelial cells [262].

The heart is especially prone to oxidative damage with increasing age. Mitochondrial complex III and IV respiratory activity is impaired in interfibrillar mitochondria isolated from aged (24 months) male Fisher rats. This decline in respiratory activity was associated with a significant increase in oxidative stress levels as measured by 4-hydroxy-2-nonenal-modified proteins, protein carbonyls, and MDA [263]. In addition cardiac mitochondria isolated from elderly rats had increased mtDNA damage as assessed by an increased content of 8-OHdG lesions [264]. Cardiomyocyte mitochondrial respiratory dysfunction and impaired protein turnover is associated with heart failure

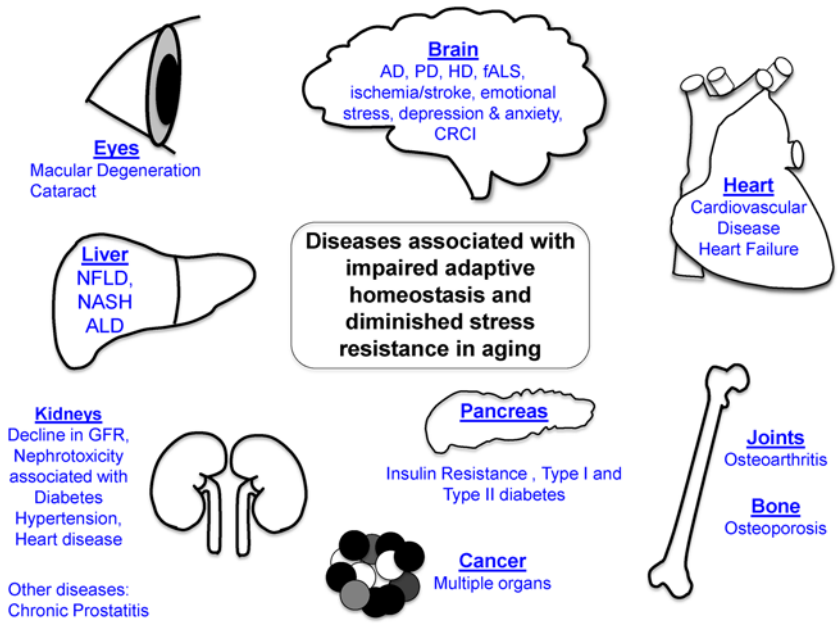


**Figure 5. A graphic representation of age-related diseases associated with diminished stress responses and defective adaptive homeostasis**

A growing number of age-related diseases have now been associated or linked with diminished stress responsiveness and defective adaptive homeostasis. In some cases, e.g. cataract, the association has been shown to be causal.

[265-267]. Accumulation of ubiquitinated proteins has been reported in human heart failure, which suggests impaired UPS function [268,269].

## Summary and conclusion

Ageing is characterized by increase in oxidative stress and diminished adaptive homeostatic responses which lead to an accumulation of oxidatively damaged proteins, DNA, and lipids in cells, especially in the last third of lifespan. Proteasomal dysfunction and accumulation of misfolded proteins further impair protein turnover, also weakening resistance to multiple stressors. Oxidative stress and impaired proteostasis are common amongst various diseases that afflict the ageing population. Multiple age-related human diseases show a significant sex bias including neurodegenerative diseases, kidney diseases, and cardiovascular diseases. Age- and sex-related hormonal changes have been associated with this disparity in disease incidence between male and females. Recent studies have elucidated a potential molecular mechanism for sex bias in adaptation to oxidative stress involving the mitochondrial Lon protease [18]. Here, we reviewed the involvement of impaired response to oxidative stress and defective adaptive homeostasis in a subset of age-associated diseases, a summary of which is presented in Figure 5.

Diminished Nrf2 expression and subsequent impaired Nrf2/EpRE signaling are the key features of ageing that contribute to high levels of oxidative stress in various age-related disorders. In particular, Nrf2/EpRE signaling is impaired or insufficient to attenuate severe oxidative stress in many disorders described in this review. As a master regulator of cellular homeostasis, activation of Nrf2 is widely considered as a potential target for the treatment of various neurodegenerative disorders. Nrf2 overexpression has prevented neuronal pathology in rodent models of AD, PD, HD, and ALS. In clinical trials, antioxidant supplementation via NAC increased dopamine transporter binding in the caudate and putamen in the brains of PD patients [270]. Antioxidant clinical trials in AD, have shown limited therapeutic efficacy in ameliorating cognitive deficits [271]. Limited efficacy or conflicting results of antioxidant supplementation for various disorders may be the result of limited ability to cross the BBB.

Potent activators of Nrf2 have been shown to be neuroprotective, however unregulated Nrf2 expression is detrimental as Nrf2 overexpression may be oncogenic and is associated with resistance to chemotherapy [272]. Recent studies suggest that selective Keap1 inhibition, using a selective Keap1-modifying small molecule (MIND4-17) [75,76], activated Nrf2 signaling and was protective against oxidative damage in NSCs differentiated from HD patient-derived iPSCs.

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Impaired proteostasis is also a common feature of ageing and various age-related disorders discussed in this review. Oxidative stress is often accompanied by impaired proteasomal activity and accumulation of protein aggregates in neurological disorders. Overexpression of the proteasomal catalytic β5 subunit of the 20S core proteasome in *C. elegans* enhances proteasomal activity, resulting in lifespan extension and increased resistance to oxidative stress [273]. Notably, in this same study, β5 subunit overexpression protected against the aggregate-related pathology progression and polyQ or Aβ proteotoxicity in *C. elegans* models of AD and HD [273]. Proteasomal activation using 18α-glycrrhetinic acid, decreased accumulation of Aβ deposits in an AD *C. elegans* model [274]. These studies suggest that modulation of proteolytic activity in neurodegenerative disorders may be a considerable therapeutic option for the future.

An understanding of the role of defective adaptive homeostasis in ageing and disease is critical for the development of new therapeutic approaches to treat various disorders, especially those in which oxidative stress and impaired proteostasis contribute to the pathology.

## Competing interests

The authors declare that there are no competing interests associated with the manuscript.

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## Author contribution

N.L., D.A.B., and K.J.A.D. contributed equally to the conception, initial writing, and revisions of this review paper.

## Abbreviations

AD, Alzheimer's disease; ADH, alcohol dehydrogenase; Akt, serine/threonine kinase 1; ALD, alcoholic liver disease; ALS, amyotrophic lateral sclerosis; AMD, age-related macular degeneration; ARE, antioxidant response element; Aβ, amyloid-β; Bach1, BTB and CNC homology 1; Bcl-2, B-cell lymphoma 2; Bcl-$X_L$, B-cell lymphoma-extra large; BDNF, brain-derived neurotrophic factor; BTB, Broad-complex, Tramtrack, and Bric-à-brac; CAG, polyglutamine repeat; CNC, cap'n'collar; CP/CPPS, chronic prostatitis/chronic pelvic pain syndrome; CRCI, chemotherapy-related cognitive impairment; CYP2E1, cytochrome P450 2E1; C9orf72, chromosome 9 ORF 72; DMM, destabilization of the medial meniscus; EpRE, electrophile response element; ER, endoplasmic reticulum; ETC, electron transport chain; Ecm29, Extracellular mutant 29; fALS, familial ALS; FTD, frontotemporal dementia; GFR, glomerular filtration rate; Glo1, glyoxalase 1; GPx, glutathione peroxidase; GR, glutathione reductase; HD, Huntington's disease; HO-1, heme-oxygenase; HSP, heat-shock protein; HTT, huntingtin; IGF-1, insulin-like growth factor 1; iPSC, induced pluripotent stem cell; IκB, inhibitory κB protein; ICAM-1, intracellular adhesion molecule 1; IL-1β, interleukin 1 beta; IL-6, interleukin 6; KAP, kidney androgen regulated protein; Keap1, Kelch-like erythroid cell derived protein with CNC homology associated protein 1; KIR, Keap1-interaction region; MCAO, middle cerebral artery occlusion; MDA, malondialdehyde; MDB, Mallory–Denk body; MDD, major depressive disorder; MIA, monosodium iodoacetate; MMP, matrix metalloproteinase; MIND4-17, 5-nitro-2-{[5-(phenoxymethyl)-4-phenyl-4H-1,2,4-triazol-3-yl]thio}pyridine; NAC, N-acetylcysteine; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NFT, neurofibrillary tangle; NOX, NADPH oxidase; NQO1, NADPH:quinone oxidoreductase; Nrf2, nuclear factor erythroid-2-like factor 2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NSC, neural stem cell; OA, osteoarthritis; PC12, pheochromocytoma 12; PD, Parkinson's disease; Pink1, PTEN-induced kinase 1; PKCγ, protein kinase C gamma; Prx-2, peroxiredoxin 2; RANKL, receptor activator of NF-κB ligand; RPE, retinal pigment epithelium; SKN-1, skinhead 1; SN, substantia nigra; SNP, single-nucleotide polymorphism; SOD, superoxide dismutase; SQSTM1, sequestrom 1; TNF-α, tumor necrosis factor α; TrkB, tropomyosin receptor kinase B; UPS, ubiquitin-proteasome system; 7,8-DHF, 7,8-Dihydroxyflavone.

# References

1 Davies, K.J. (2016) Adaptive homeostasis. *Mol. Aspects Med.* **49**, 1–7

2 Wiese, A.G., Pacifici, R.E. and Davies, K.J. (1995) Transient adaptation of oxidative stress in mammalian cells. *Arch. Biochem. Biophys.* **318**, 231–240

3 Pickering, A.M., Linder, R.A., Zhang, H., Forman, H.J. and Davies, K.J. (2012) Nrf2-dependent induction of proteasome and Pa28alphabeta regulator are required for adaptation to oxidative stress. *J. Biol. Chem.* **287**, 10021–10031

4 Grune, T., Catalgol, B., Licht, A., Ermak, G., Pickering, A.M., Ngo, J.K. et al. (2011) HSP70 mediates dissociation and reassociation of the 26S proteasome during adaptation to oxidative stress. *Free Radic. Biol. Med.* **51**, 1355–1364
5 Zhang, H., Davies, K.J. and Forman, H.J. (2015) Oxidative stress response and Nrf2 signaling in aging. *Free Radic. Biol. Med.* **88**, 314–336
6 Trachootham, D., Lu, W., Ogasawara, M.A., Nilsa, R.D. and Huang, P. (2008) Redox regulation of cell survival. *Antioxid. Redox Signal.* **10**, 1343–1374
7 Davies, K.J. (2000) Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. *IUBMB Life* **50**, 279–289
8 Pickering, A.M., Koop, A.L., Teoh, C.Y., Ermak, G., Grune, T. and Davies, K.J. (2010) The immunoproteasome, the 20S proteasome and the PA28alphabeta proteasome regulator are oxidative-stress-adaptive proteolytic complexes. *Biochem. J.* **432**, 585–594
9 Pickering, A.M. and Davies, K.J. (2012) Degradation of damaged proteins: the main function of the 20S proteasome. *Prog. Mol. Biol. Transl. Sci.* **109**, 227–248
10 Pickering, A.M., Staab, T.A., Tower, J., Sieburth, D. and Davies, K.J. (2013) A conserved role for the 20S proteasome and Nrf2 transcription factor in oxidative stress adaptation in mammals, *Caenorhabditis elegans* and *Drosophila melanogaster*. *J. Exp. Biol.* **216**, 543–553
11 Raynes, R., Juarez, C., Pomatto, L.C., Sieburth, D. and Davies, K.J. (2017) Aging and SKN-1-dependent loss of 20S proteasome adaptation to oxidative stress in *C. elegans*. *J. Gerontol. A Biol. Sci. Med. Sci.* **72**, 143–151
12 Rattan, S.I. (2008) Hormesis in aging. *Ageing Res. Rev.* **7**, 63–78
13 Morawe, T., Hiebel, C., Kern, A. and Behl, C. (2012) Protein homeostasis, aging and Alzheimer's disease. *Mol. Neurobiol.* **46**, 41–54
14 Calderwood, S.K., Murshid, A. and Prince, T. (2009) The shock of aging: molecular chaperones and the heat shock response in longevity and aging–a mini-review. *Gerontology* **55**, 550–558
15 Pomatto, L.C., Wong, S., Carney, C., Shen, B., Tower, J. and Davies, K.J. (2017) The age- and sex-specific decline of the 20s proteasome and the Nrf2/CncC signal transduction pathway in adaption and resistance to oxidative stress in *Drosophila melanogaster*. *Aging (Albany N.Y.)* **9**, 1153–1185
16 Ngo, J.K. and Davies, K.J. (2009) Mitochondrial Lon protease is a human stress protein. *Free Radic. Biol. Med.* **46**, 1042–1048
17 Bota, D.A. and Davies, K.J. (2016) Mitochondrial Lon protease in human disease and aging: Including an etiologic classification of Lon-related diseases and disorders. *Free Radic. Biol. Med.* **100**, 188–198
18 Pomatto, L.C., Carney, C., Shen, B., Wong, S., Halaszynski, K., Salomon, M.P. et al. (2017) The Mitochondrial lon protease is required for age-specific and sex-specific adaptation to oxidative stress. *Curr. Biol.* **27**, 1–15
19 Ngo, J.K., Pomatto, L.C. and Davies, K.J. (2013) Upregulation of the mitochondrial Lon Protease allows adaptation to acute oxidative stress but dysregulation is associated with chronic stress, disease, and aging. *Redox Biol.* **1**, 258–264
20 Kobayashi, A., Kang, M.I., Okawa, H., Ohtsuji, M., Zenke, Y., Chiba, T. et al. (2004) Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol. Cell. Biol.* **24**, 7130–7139
21 Nguyen, T., Nioi, P. and Pickett, C.B. (2009) The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *J. Biol. Chem.* **284**, 13291–13295
22 Ramsey, C.P., Glass, C.A., Montgomery, M.B., Lindl, K.A., Ritson, G.P., Chia, L.A. et al. (2007) Expression of Nrf2 in neurodegenerative diseases. *J. Neuropathol. Exp. Neurol.* **66**, 75–85
23 Coux, O., Tanaka, K. and Goldberg, A.L. (1996) Structure and functions of the 20S and 26S proteasomes. *Annu. Rev. Biochem.* **65**, 801–847
24 Bhattacharyya, S., Yu, H., Mim, C. and Matouschek, A. (2014) Regulated protein turnover: snapshots of the proteasome in action. *Nat. Rev. Mol. Cell Biol.* **15**, 122–133
25 Hegde, A.N. and DiAntonio, A. (2002) Ubiquitin and the synapse. *Nat. Rev. Neurosci.* **3**, 854–861
26 Bota, D.A. and Davies, K.J. (2002) Lon protease preferentially degrades oxidized mitochondrial aconitase by an ATP-stimulated mechanism. *Nat. Cell Biol.* **4**, 674–680
27 Hori, O., Ichinoda, F., Tamatani, T., Yamaguchi, A., Sato, N., Ozawa, K. et al. (2002) Transmission of cell stress from endoplasmic reticulum to mitochondria: enhanced expression of Lon protease. *J. Cell Biol.* **157**, 1151–1160
28 Bota, D.A., Van Remmen, H. and Davies, K.J. (2002) Modulation of Lon protease activity and aconitase turnover during aging and oxidative stress. *FEBS Lett.* **532**, 103–106
29 Rolfe, D.F. and Brown, G.C. (1997) Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol. Rev.* **77**, 731–758
30 Raefsky, S.M. and Mattson, M.P. (2017) Adaptive responses of neuronal mitochondria to bioenergetic challenges: Roles in neuroplasticity and disease resistance. *Free Radic. Biol. Med.* **102**, 203–216
31 Alzheimer, A., Stelzmann, R.A., Schnitzlein, H.N. and Murtagh, F.R. (1995) An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde". *Clin. Anat.* **8**, 429–431
32 Selkoe, D.J. (2001) Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein. *J. Alzheimers Dis.* **3**, 75–80
33 Mattson, M.P. (2004) Pathways towards and away from Alzheimer's disease. *Nature* **430**, 631–639
34 Bonet-Costa, V., Pomatto, L.C. and Davies, K.J. (2016) The proteasome and oxidative stress in Alzheimer's disease. *Antioxid. Redox Signal.* **25**, 886–901
35 Mota, S.I., Costa, R.O., Ferreira, I.L., Santana, I., Caldeira, G.L., Padovano, C. et al. (2015) Oxidative stress involving changes in Nrf2 and ER stress in early stages of Alzheimer's disease. *Biochim. Biophys. Acta* **1852**, 1428–1441
36 Yankner, B.A., Dawes, L.R., Fisher, S., Villa-Komaroff, L., Oster-Granite, M.L. and Neve, R.L. (1989) Neurotoxicity of a fragment of the amyloid precursor associated with Alzheimer's disease. *Science* **245**, 417–420
37 Plant, L.D., Boyle, J.P., Smith, I.F., Peers, C. and Pearson, H.A. (2003) The production of amyloid beta peptide is a critical requirement for the viability of central neurons. *J. Neurosci.* **23**, 5531–5535
38 Kontush, A. (2001) Amyloid-beta: an antioxidant that becomes a pro-oxidant and critically contributes to Alzheimer's disease. *Free Radic. Biol. Med.* **31**, 1120–1131

*Clinical Science* (2017) **131** 2573–2599
https://doi.org/10.1042/CS20160982


39 Atwood, C.S., Obrenovich, M.E., Liu, T., Chan, H., Perry, G., Smith, M.A. et al. (2003) Amyloid-beta: a chameleon walking in two worlds: a review of the trophic and toxic properties of amyloid-beta. *Brain Res. Brain Res. Rev.* **43**, 1–16

40 Ries, M. and Sastre, M. (2016) Mechanisms of Abeta clearance and degradation by glial cells. *Front. Aging Neurosci.* **8**, 160

41 Garwood, C.J., Pooler, A.M., Atherton, J., Hanger, D.P. and Noble, W. (2011) Astrocytes are important mediators of Abeta-induced neurotoxicity and tau phosphorylation in primary culture. *Cell Death Dis.* **2**, e167

42 Agostinho, P., Cunha, R.A. and Oliveira, C. (2010) Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. *Curr. Pharm. Des.* **16**, 2766–2778

43 Tseng, B.P., Green, K.N., Chan, J.L., Blurton-Jones, M. and LaFerla, F.M. (2008) Abeta inhibits the proteasome and enhances amyloid and tau accumulation. *Neurobiol. Aging* **29**, 1607–1618

44 Oh, S., Hong, H.S., Hwang, E., Sim, H.J., Lee, W., Shin, S.J. et al. (2005) Amyloid peptide attenuates the proteasome activity in neuronal cells. *Mech. Ageing Dev.* **126**, 1292–1299

45 Lustbader, J.W., Cirilli, M., Lin, C., Xu, H.W., Takuma, K., Wang, N. et al. (2004) ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease. *Science* **304**, 448–452

46 Vina, J., Lloret, A., Valles, S.L., Borras, C., Badia, M.C., Pallardo, F.V. et al. (2007) Mitochondrial oxidant signalling in Alzheimer's disease. *J. Alzheimers Dis.* **11**, 175–181

47 Reinheckel, T., Sitte, N., Ullrich, O., Kuckelkorn, U., Davies, K.J. and Grune, T. (1998) Comparative resistance of the 20S and 26S proteasome to oxidative stress. *Biochem. J.* **335**, 637–642

48 Keller, J.N., Hanni, K.B. and Markesbery, W.R. (2000) Impaired proteasome function in Alzheimer's disease. *J. Neurochem.* **75**, 436–439

49 David, D.C., Layfield, R., Serpell, L., Narain, Y., Goedert, M. and Spillantini, M.G. (2002) Proteasomal degradation of tau protein. *J. Neurochem.* **83**, 176–185

50 Keck, S., Nitsch, R., Grune, T. and Ullrich, O. (2003) Proteasome inhibition by paired helical filament-tau in brains of patients with Alzheimer's disease. *J. Neurochem.* **85**, 115–122

51 de Lau, L.M. and Breteler, M.M. (2006) Epidemiology of Parkinson's disease. *Lancet Neurol.* **5**, 525–535

52 Castellani, R.J., Perry, G., Siedlak, S.L., Nunomura, A., Shimohama, S., Zhang, J. et al. (2002) Hydroxynonenal adducts indicate a role for lipid peroxidation in neocortical and brainstem Lewy bodies in humans. *Neurosci. Lett.* **319**, 25–28

53 Reeve, A., Simcox, E. and Turnbull, D. (2014) Ageing and Parkinson's disease: why is advancing age the biggest risk factor? *Ageing Res. Rev.* **14**, 19–30

54 Ma, S.Y., Ciliax, B.J., Stebbins, G., Jaffar, S., Joyce, J.N., Cochran, E.J. et al. (1999) Dopamine transporter-immunoreactive neurons decrease with age in the human substantia nigra. *J. Comp. Neurol.* **409**, 25–37

55 Chu, Y. and Kordower, J.H. (2007) Age-associated increases of alpha-synuclein in monkeys and humans are associated with nigrostriatal dopamine depletion: is this the target for Parkinson's disease? *Neurobiol. Dis.* **25**, 134–149

56 Ross, O.A., Braithwaite, A.T., Skipper, L.M., Kachergus, J., Hulihan, M.M., Middleton, F.A. et al. (2008) Genomic investigation of alpha-synuclein multiplication and parkinsonism. *Ann. Neurol.* **63**, 743–750

57 Bueler, H. (2009) Impaired mitochondrial dynamics and function in the pathogenesis of Parkinson's disease. *Exp. Neurol.* **218**, 235–246

58 Schapira, A.H., Cooper, J.M., Dexter, D., Jenner, P., Clark, J.B. and Marsden, C.D. (1989) Mitochondrial complex I deficiency in Parkinson's disease. *Lancet* **1**, 1269

59 Bonifati, V., Rizzu, P., van Baren, M.J., Schaap, O., Breedveld, G.J., Krieger, E. et al. (2003) Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science* **299**, 256–259

60 Hague, S., Rogaeva, E., Hernandez, D., Gulick, C., Singleton, A., Hanson, M. et al. (2003) Early-onset Parkinson's disease caused by a compound heterozygous DJ-1 mutation. *Ann. Neurol.* **54**, 271–274

61 Choi, J., Sullards, M.C., Olzmann, J.A., Rees, H.D., Weintraub, S.T., Bostwick, D.E. et al. (2006) Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer diseases. *J. Biol. Chem.* **281**, 10816–10824

62 McNaught, K.S., Belizaire, R., Jenner, P., Olanow, C.W. and Isacson, O. (2002) Selective loss of 20S proteasome alpha-subunits in the substantia nigra pars compacta in Parkinson's disease. *Neurosci. Lett.* **326**, 155–158

63 DiFiglia, M., Sapp, E., Chase, K.O., Davies, S.W., Bates, G.P., Vonsattel, J.P. et al. (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science* **277**, 1990–1993

64 Finkbeiner, S. (2011) Huntington's disease. *Cold Spring Harb. Perspect. Biol.* **3**, doi:10.1101/cshperspect.a007476

65 Myers, R.H. (2004) Huntington's disease genetics. *NeuroRx* **1**, 255–262

66 Browne, S.E. and Beal, M.F. (2006) Oxidative damage in Huntington's disease pathogenesis. *Antioxid. Redox Signal.* **8**, 2061–2073

67 Horvath, S., Langfelder, P., Kwak, S., Aaronson, J., Rosinski, J., Vogt, T.F. et al. (2016) Huntington's disease accelerates epigenetic aging of human brain and disrupts DNA methylation levels. *Aging (Albany N.Y.)* **8**, 1485–1512

68 Siddiqui, A., Rivera-Sanchez, S., Castro Mdel, R., Acevedo-Torres, K., Rane, A., Torres-Ramos, C.A. et al. (2012) Mitochondrial DNA damage is associated with reduced mitochondrial bioenergetics in Huntington's disease. *Free Radic. Biol. Med.* **53**, 1478–1488

69 Browne, S.E., Bowling, A.C., MacGarvey, U., Baik, M.J., Berger, S.C., Muqit, M.M. et al. (1997) Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. *Ann. Neurol.* **41**, 646–653

70 Polidori, M.C., Mecocci, P., Browne, S.E., Senin, U. and Beal, M.F. (1999) Oxidative damage to mitochondrial DNA in Huntington's disease parietal cortex. *Neurosci. Lett.* **272**, 53–56

71 Hodges, A., Strand, A.D., Aragaki, A.K., Kuhn, A., Sengstag, T., Hughes, G. et al. (2006) Regional and cellular gene expression changes in human Huntington's disease brain. *Hum. Mol. Genet.* **15**, 965–977

72 Kuhn, A., Goldstein, D.R., Hodges, A., Strand, A.D., Sengstag, T., Kooperberg, C. et al. (2007) Mutant huntingtin's effects on striatal gene expression in mice recapitulate changes observed in human Huntington's disease brain and do not differ with mutant huntingtin length or wild-type huntingtin dosage. *Hum. Mol. Genet.* **16**, 1845–1861
73 van Roon-Mom, W.M., Pepers, B.A., t Hoen, P.A., Verwijmeren, C.A., den Dunnen, J.T., Dorsman, J.C. et al. (2008) Mutant huntingtin activates Nrf2-responsive genes and impairs dopamine synthesis in a PC12 model of Huntington's disease. *BMC Mol. Biol.* **9**, 84
74 Sorolla, M.A., Reverter-Branchat, G., Tamarit, J., Ferrer, I., Ros, J. and Cabiscol, E. (2008) Proteomic and oxidative stress analysis in human brain samples of Huntington disease. *Free Radic. Biol. Med.* **45**, 667–678
75 Quinti, L., Casale, M., Moniot, S., Pais, T.F., Van Kanegan, M.J., Kaltenbach , L.S. et al. (2016) SIRT2- and NRF2-targeting thiazole-containing compound with therapeutic activity in Huntington's disease models. *Cell Chem. Biol.* **23**, 849–861
76 Quinti, L., Dayalan Naidu, S., Trager, U., Chen, X., Kegel-Gleason, K., Lleres, D. et al. (2017) KEAP1-modifying small molecule reveals muted NRF2 signaling responses in neural stem cells from Huntington's disease patients. *Proc. Natl. Acad. Sci. U.S.A.* **114**, E4676–E4685
77 Blokhuis, A.M., Groen, EJN, Koppers, M., van den Berg, L.H. and Pasterkamp, R.J. (2013) Protein aggregation in amyotrophic lateral sclerosis. *Acta Neuropathol.* **125**, 777–794
78 Ingre, C., Roos, P.M., Piehl, F., Kamel, F. and Fang, F. (2015) Risk factors for amyotrophic lateral sclerosis. *Clin. Epidemiol.* **7**, 181–193
79 Karademir, B., Corek, C. and Ozer, N.K. (2015) Endoplasmic reticulum stress and proteasomal system in amyotrophic lateral sclerosis. *Free Radic. Biol. Med.* **88**, 42–50
80 Mattiazzi, M., D'Aurelio, M., Gajewski, C.D., Martushova, K., Kiaei, M., Beal, M.F. et al. (2002) Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice. *J. Biol. Chem.* **277**, 29626–29633
81 Jaarsma, D., Rognoni, F., van Duijn, W., Verspaget, H.W., Haasdijk, E.D. and Holstege, J.C. (2001) CuZn superoxide dismutase (SOD1) accumulates in vacuolated mitochondria in transgenic mice expressing amyotrophic lateral sclerosis-linked SOD1 mutations. *Acta Neuropathol.* **102**, 293–305
82 Kong, J. and Xu, Z. (1998) Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1. *J. Neurosci.* **18**, 3241–3250
83 Kabashi, E., Agar, J.N., Taylor, D.M., Minotti, S. and Durham, H.D. (2004) Focal dysfunction of the proteasome: a pathogenic factor in a mouse model of amyotrophic lateral sclerosis. *J. Neurochem.* **89**, 1325–1335
84 Guo, Y., Zhang, Y., Wen, D., Duan, W., An, T., Shi, P. et al. (2013) The modest impact of transcription factor Nrf2 on the course of disease in an ALS animal model. *Lab. Invest.* **93**, 825–833
85 Vargas, M.R., Burton, N.C., Kutzke, J., Gan, L., Johnson, D.A., Schafer, M. et al. (2013) Absence of Nrf2 or its selective overexpression in neurons and muscle does not affect survival in ALS-linked mutant hSOD1 mouse models. *PLoS ONE* **8**, e56625
86 Jain, A., Lamark, T., Sjottem, E., Larsen, K.B., Awuh, J.A., Overvatn, A. et al. (2010) p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. *J. Biol. Chem.* **285**, 22576–22591
87 Komatsu, M., Kurokawa, H., Waguri, S., Taguchi, K., Kobayashi, A., Ichimura, Y. et al. (2010) The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat. Cell Biol.* **12**, 213–223
88 Goode, A., Rea, S., Sultana, M., Shaw, B., Searle, M.S. and Layfield, R. (2016) ALS-FTLD associated mutations of SQSTM1 impact on Keap1-Nrf2 signalling. *Mol. Cell. Neurosci.* **76**, 52–58
89 Bartolome, F., Esteras, N., Martin-Requero, A., Boutoleau-Bretonniere, C., Vercelletto, M., Gabelle, A. et al. (2017) Pathogenic p62/SQSTM1 mutations impair energy metabolism through limitation of mitochondrial substrates. *Sci. Rep.* **7**, 1666
90 Ringholz, G.M., Appel, S.H., Bradshaw, M., Cooke, N.A., Mosnik, D.M. and Schulz, P.E. (2005) Prevalence and patterns of cognitive impairment in sporadic ALS. *Neurology* **65**, 586–590
91 DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J. et al. (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* **72**, 245–256
92 Ji, A.-L., Zhang, X., Chen, W.-W. and Huang, W.-J. (2017) Genetics insight into the amyotrophic lateral sclerosis/frontotemporal dementia spectrum. *J. Med. Genet.* **54**, 145–154
93 Boeve, B.F., Boylan, K.B., Graff-Radford, N.R., DeJesus-Hernandez, M., Knopman, D.S., Pedraza, O. et al. (2012) Characterization of frontotemporal dementia and/or amyotrophic lateral sclerosis associated with the GGGGCC repeat expansion in C9ORF72. *Brain* **135**, 765–783
94 Lopez-Gonzalez, R., Lu, Y., Gendron, T.F., Karydas, A., Tran, H., Yang, D. et al. (2016) Poly(GR) in C9ORF72-related ALS/FTD Compromises mitochondrial function and increases oxidative stress and DNA damage in iPSC-derived motor neurons. *Neuron* **92**, 383–391
95 Farrall, A.J. and Wardlaw, J.M. (2009) Blood-brain barrier: ageing and microvascular disease–systematic review and meta-analysis. *Neurobiol. Aging* **30**, 337–352
96 Doll, D.N., Hu, H., Sun, J., Lewis, S.E., Simpkins, J.W. and Ren, X. (2015) Mitochondrial crisis in cerebrovascular endothelial cells opens the blood-brain barrier. *Stroke* **46**, 1681–1689
97 Oldendorf, W.H., Cornford, M.E. and Brown, W.J. (1977) The large apparent work capability of the blood-brain barrier: a study of the mitochondrial content of capillary endothelial cells in brain and other tissues of the rat. *Ann. Neurol.* **1**, 409–417
98 Burns, E.M., Kruckeberg, T.W. and Gaetano, P.K. (1981) Changes with age in cerebral capillary morphology. *Neurobiol. Aging* **2**, 283–291
99 Vaziri, N.D., Liang, K. and Ding, Y. (1999) Increased nitric oxide inactivation by reactive oxygen species in lead-induced hypertension. *Kidney Int.* **56**, 1492–1498
100 El Assar, M., Angulo, J. and Rodriguez-Manas, L. (2013) Oxidative stress and vascular inflammation in aging. *Free Radic. Biol. Med.* **65**, 380–401
101 Huang, Z., Huang, P.L., Ma, J., Meng, W., Ayata, C., Fishman, M.C. et al. (1996) Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by nitro-L-arginine. *J. Cereb. Blood Flow Metab.* **16**, 981–987
102 Cai, H. and Harrison, D.G. (2000) Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ. Res.* **87**, 840–844

*Clinical Science* (2017) **131** 2573–2599
https://doi.org/10.1042/CS20160982

103 Agarwal, R. and Shukla, G.S. (1999) Potential role of cerebral glutathione in the maintenance of blood-brain barrier integrity in rat. *Neurochem. Res.* **24**, 1507–1514
104 Al Ahmad, A., Gassmann, M. and Ogunshola, O.O. (2012) Involvement of oxidative stress in hypoxia-induced blood-brain barrier breakdown. *Microvasc. Res.* **84**, 222–225
105 Ibbotson, K., Yell, J. and Ronaldson, P.T. (2017) Nrf2 signaling increases expression of ATP-binding cassette subfamily C mRNA transcripts at the blood–brain barrier following hypoxia-reoxygenation stress. *Fluids Barriers CNS* **14**, 6
106 Zhao, J., Moore, A.N., Redell, J.B. and Dash, P.K. (2007) Enhancing expression of Nrf2-driven genes protects the blood brain barrier after brain injury. *J. Neurosci.* **27**, 10240–10248
107 Zhao, X., Sun, G., Zhang, J., Strong, R., Dash, P.K., Kan, Y.W. et al. (2007) Transcription factor Nrf2 protects the brain from damage produced by intracerebral hemorrhage. *Stroke* **38**, 3280–3286
108 Ge, P., Zhang, F., Zhao, J., Liu, C., Sun, L. and Hu, B. (2012) Protein degradation pathways after brain ischemia. *Curr. Drug Targets* **13**, 159–165
109 Yamashiro, K., Liu, R., Maeda, M., Hattori, N. and Urabe, T. (2007) Induction and selective accumulation of mutant ubiquitin in CA1 pyramidal neurons after transient global ischemia. *Neuroscience* **147**, 71–79
110 Ge, P., Luo, Y., Liu, C.L. and Hu, B. (2007) Protein aggregation and proteasome dysfunction after brain ischemia. *Stroke* **38**, 3230–3236
111 Asai, A., Tanahashi, N., Qiu, J.H., Saito, N., Chi, S., Kawahara, N. et al. (2002) Selective proteasomal dysfunction in the hippocampal CA1 region after transient forebrain ischemia. *J. Cereb. Blood Flow Metab.* **22**, 705–710
112 Keller, J.N., Huang, F.F., Zhu, H., Yu, J., Ho, Y.S. and Kindy, T.S. (2000) Oxidative stress-associated impairment of proteasome activity during ischemia-reperfusion injury. *J. Cereb. Blood Flow Metab.* **20**, 1467–1473
113 Benjamin, E.J., Blaha, M.J., Chiuve, S.E., Cushman, M., Das, S.R., Deo, R. et al. (2017) Heart disease and stroke statistics—2017 update: a report from the American Heart Association. *Circulation* **136**, e196
114 Denti, L., Annoni, V., Cattadori, E., Salvagnini, M.A., Visioli, S., Merli, M.F. et al. (2004) Insulin-like growth factor 1 as a predictor of ischemic stroke outcome in the elderly. *Am. J. Med.* **117**, 312–317
115 Delafontaine, P., Song, Y.H. and Li, Y. (2004) Expression, regulation, and function of IGF-1, IGF-1R, and IGF-1 binding proteins in blood vessels. *Arterioscler. Thromb. Vasc. Biol.* **24**, 435–444
116 Krause, D.N., Duckles, S.P. and Pelligrino, D.A. (2006) Influence of sex steroid hormones on cerebrovascular function. *J. Appl. Physiol. (1985)* **101**, 1252–1261
117 Haigh, EAP, Bogucki, O.E., Sigmon, S.T. and Blazer, D.G. (2017) Depression among older adults: a 20-year update on five common myths and misconceptions. *Am. J. Geriatr. Psychiatry*, doi:10.1016/j.jagp.2017.06.011
118 Maes, M., Galecki, P., Chang, Y.S. and Berk, M. (2011) A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **35**, 676–692
119 Sarandol, A., Sarandol, E., Eker, S.S., Erdinc, S., Vatansever, E. and Kirli, S. (2007) Major depressive disorder is accompanied with oxidative stress: short-term antidepressant treatment does not alter oxidative-antioxidative systems. *Hum. Psychopharmacol.* **22**, 67–73
120 Liu, T., Zhong, S., Liao, X., Chen, J., He, T., Lai, S. et al. (2015) A meta-analysis of oxidative stress markers in depression. *PLoS ONE* **10**, e0138904
121 Jimenez-Fernandez, S., Gurpegui, M., Diaz-Atienza, F., Perez-Costillas, L., Gerstenberg, M. and Correll, C.U. (2015) Oxidative stress and antioxidant parameters in patients with major depressive disorder compared to healthy controls before and after antidepressant treatment: results from a meta-analysis. *J. Clin. Psychiatry* **76**, 1658–1667
122 Berk, M., Copolov, D.L., Dean, O., Lu, K., Jeavons, S., Schapkaitz, I. et al. (2008) N-acetyl cysteine for depressive symptoms in bipolar disorder–a double-blind randomized placebo-controlled trial. *Biol. Psychiatry* **64**, 468–475
123 Hasebe, K., Gray, L., Bortolasci, C., Panizzutti, B., Mohebbi, M., Kidnapillai, S. et al. (2017) Adjunctive N-acetylcysteine in depression: exploration of interleukin-6, C-reactive protein and brain-derived neurotrophic factor. *Acta Neuropsychiatr.* 1–10, doi:10.1017/neu.2017.2
124 Gragnoli, C. (2014) Proteasome modulator 9 gene SNPs, responsible for anti-depressant response, are in linkage with generalized anxiety disorder. *J. Cell. Physiol.* **229**, 1157–1159
125 Ryan, M.M., Lockstone, H.E., Huffaker, S.J., Wayland, M.T., Webster, M.J. and Bahn, S. (2006) Gene expression analysis of bipolar disorder reveals downregulation of the ubiquitin cycle and alterations in synaptic genes. *Mol. Psychiatry* **11**, 965–978
126 Minelli, A., Magri, C., Barbon, A., Bonvicini, C., Segala, M., Congiu, C. et al. (2015) Proteasome system dysregulation and treatment resistance mechanisms in major depressive disorder. *Transl. Psychiatry* **5**, e687
127 Wong, M.L., Dong, C., Maestre-Mesa, J. and Licinio, J. (2008) Polymorphisms in inflammation-related genes are associated with susceptibility to major depression and antidepressant response. *Mol. Psychiatry* **13**, 800–812
128 Bouvier, E., Brouillard, F., Molet, J., Claverie, D., Cabungcal, J.H., Cresto, N. et al. (2016) Nrf2-dependent persistent oxidative stress results in stress-induced vulnerability to depression. *Mol. Psychiatry*, doi:10.1038/mp.2016.211
129 Omar, N.N. and Tash, R.F. (2017) Fluoxetine coupled with zinc in a chronic mild stress model of depression: providing a reservoir for optimum zinc signaling and neuronal remodeling. *Pharmacol. Biochem. Behav.* **160**, 30–38
130 Mendez-David, I., Tritschler, L., Ali, Z.E., Damiens, M.H., Pallardy, M., David, D.J. et al. (2015) Nrf2-signaling and BDNF: a new target for the antidepressant-like activity of chronic fluoxetine treatment in a mouse model of anxiety/depression. *Neurosci. Lett.* **597**, 121–126
131 Bouayed, J., Rammal, H. and Soulimani, R. (2009) Oxidative stress and anxiety: relationship and cellular pathways. *Oxid. Med. Cell Longev.* **2**, 63–67
132 Hovatta, I., Tennant, R.S., Helton, R., Marr, R.A., Singer, O., Redwine, J.M. et al. (2005) Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. *Nature* **438**, 662–666
133 Khalifeh, S., Oryan, S., Digaleh, H., Shaerzadeh, F., Khodagholi, F., Maghsoudi, N. et al. (2015) Involvement of Nrf2 in development of anxiety-like behavior by linking Bcl2 to oxidative phosphorylation: estimation in rat hippocampus, amygdala, and prefrontal cortex. *J. Mol. Neurosci.* **55**, 492–499

134 Rammal, H., Bouayed, J., Younos, C. and Soulimani, R. (2008) Evidence that oxidative stress is linked to anxiety-related behaviour in mice. *Brain Behav. Immun.* **22**, 1156–1159
135 Ahles, T.A. and Saykin, A.J. (2007) Candidate mechanisms for chemotherapy-induced cognitive changes. *Nat. Rev. Cancer* **7**, 192–201
136 Janelsins, M.C., Kesler, S.R., Ahles, T.A. and Morrow, G.R. (2014) Prevalence, mechanisms, and management of cancer-related cognitive impairment. *Int. Rev. Psychiatry* **26**, 102–113
137 Magnuson, A., Mohile, S. and Janelsins, M. (2016) Cognition and cognitive impairment in older adults with cancer. *Curr. Geriatr. Rep.* **5**, 213–219
138 Loh, K.P., Janelsins, M.C., Mohile, S.G., Holmes, H.M., Hsu, T., Inouye, S.K. et al. (2016) Chemotherapy-related cognitive impairment in older patients with cancer. *J. Geriatr. Oncol.* **7**, 270–280
139 Ahles, T.A., Saykin, A.J., McDonald, B.C., Li, Y., Furstenberg, C.T., Hanscom, B.S. et al. (2010) Longitudinal assessment of cognitive changes associated with adjuvant treatment for breast cancer: impact of age and cognitive reserve. *J. Clin. Oncol.* **28**, 4434–4440
140 Hurria, A., Rosen, C., Hudis, C., Zuckerman, E., Panageas, K.S., Lachs, M.S. et al. (2006) Cognitive function of older patients receiving adjuvant chemotherapy for breast cancer: a pilot prospective longitudinal study. *J. Am. Geriatr. Soc.* **54**, 925–931
141 Craig, C.D., Monk, B.J., Farley, J.H. and Chase, D.M. (2014) Cognitive impairment in gynecologic cancers: a systematic review of current approaches to diagnosis and treatment. *Support. Care Cancer* **22**, 279–287
142 McDonald, B.C., Conroy, S.K., Ahles, T.A., West, J.D. and Saykin, A.J. (2010) Gray matter reduction associated with systemic chemotherapy for breast cancer: a prospective MRI study. *Breast Cancer Res. Treat.* **123**, 819–828
143 de Ruiter MB, R.L., Boogerd, W., Veltman, D.J., Caan, M., Douaud, G. et al. (2012) Late effects of high-dose adjuvant chemotherapy on white and gray matter in breast cancer survivors: converging results from multimodal magnetic resonance imaging. *Hum. Brain Mapp.* **33**, 2971–2983
144 Bergouignan, L., Lefranc, J.P., Chupin, M., Morel, N., Spano, J.P. and Fossati, P. (2011) Breast cancer affects both the hippocampus volume and the episodic autobiographical memory retrieval. *PLoS ONE* **6**, e25349
145 Heflin, L.H., Meyerowitz, B.E., Hall, P., Lichtenstein, P., Johansson, B., Pedersen, N.L. et al. (2005) Cancer as a risk factor for long-term cognitive deficits and dementia. *J. Natl. Cancer Inst.* **97**, 854–856
146 Lomeli, N., Di, K., Czerniawski, J., Guzowski, J.F. and Bota, D.A. (2017) Cisplatin-induced mitochondrial dysfunction is associated with impaired cognitive function in rats. *Free Radic. Biol. Med.* **102**, 274–286
147 Gaman, A.M., Uzoni, A., Popa-Wagner, A., Andrei, A. and Petcu, E.B. (2016) The role of oxidative stress in etiopathogenesis of chemotherapy induced cognitive impairment (CICI)-"Chemobrain". *Aging Dis.* **7**, 307–317
148 Joshi, G., Sultana, R., Tangpong, J., Cole, M.P., St Clair, D.K., Vore, M. et al. (2005) Free radical mediated oxidative stress and toxic side effects in brain induced by the anti cancer drug adriamycin: insight into chemobrain. *Free Radic. Res.* **39**, 1147–1154
149 Konat, G.W., Kraszpulski, M., James, I., Zhang, H.T. and Abraham, J. (2008) Cognitive dysfunction induced by chronic administration of common cancer chemotherapeutics in rats. *Metab. Brain Dis.* **23**, 325–333
150 Tangpong, J., Cole, M.P., Sultana, R., Joshi, G., Estus, S., Vore, M. et al. (2006) Adriamycin-induced, TNF-alpha-mediated central nervous system toxicity. *Neurobiol. Dis.* **23**, 127–139
151 Podratz, J.L., Knight, A.M., Ta, L.E., Staff, N.P., Gass, J.M., Genelin, K. et al. (2011) Cisplatin induced mitochondrial DNA damage in dorsal root ganglion neurons. *Neurobiol. Dis.* **41**, 661–668
152 Chiu, G.S., Maj, M.A., Rizvi, S., Dantzer, R., Vichaya, E.G., Laumet, G. et al. (2017) Pifithrin-mu prevents cisplatin-induced chemobrain by preserving neuronal mitochondrial function. *Cancer Res.* **77**, 742–752
153 Zhou, W., Kavelaars, A. and Heijnen, C.J. (2016) Metformin prevents cisplatin-induced cognitive impairment and brain damage in mice. *PLoS ONE* **11**, e0151890
154 Kaya, E., Keskin, L., Aydogdu, I., Kuku, I., Bayraktar, N. and Erkut, M.A. (2005) Oxidant/antioxidant parameters and their relationship with chemotherapy in Hodgkin's lymphoma. *J. Int. Med. Res.* **33**, 687–692
155 Kasapovic, J., Pejic, S., Stojiljkovic, V., Todorovic, A., Radosevic-Jelic, L., Saicic, Z.S. et al. (2010) Antioxidant status and lipid peroxidation in the blood of breast cancer patients of different ages after chemotherapy with 5-fluorouracil, doxorubicin and cyclophosphamide. *Clin. Biochem.* **43**, 1287–1293
156 Joshi, G., Aluise, C.D., Cole, M.P., Sultana, R., Pierce, W.M., Vore, M. et al. (2010) Alterations in brain antioxidant enzymes and redox proteomic identification of oxidized brain proteins induced by the anti-cancer drug adriamycin: implications for oxidative stress-mediated chemobrain. *Neuroscience* **166**, 796–807
157 Joshi, G., Hardas, S., Sultana, R., St Clair, D.K., Vore, M. and Butterfield, D.A. (2007) Glutathione elevation by gamma-glutamyl cysteine ethyl ester as a potential therapeutic strategy for preventing oxidative stress in brain mediated by *in vivo* administration of adriamycin: implication for chemobrain. *J. Neurosci. Res.* **85**, 497–503
158 Siegel, R.L., Miller, K.D. and Jemal, A. (2016) Cancer statistics, 2016. *CA Cancer J. Clin.* **66**, 7–30
159 Finkel, T., Serrano, M. and Blasco, M.A. (2007) The common biology of cancer and ageing. *Nature* **448**, 767–774
160 Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. *Cell* **144**, 646–674
161 Lister, A., Nedjadi, T., Kitteringham, N.R., Campbell, F., Costello, E., Lloyd, B. et al. (2011) Nrf2 is overexpressed in pancreatic cancer: implications for cell proliferation and therapy. *Mol. Cancer* **10**, 37
162 Singh, A., Misra, V., Thimmulappa, R.K., Lee, H., Ames, S., Hoque, M.O. et al. (2006) Dysfunctional KEAP1-NRF2 interaction in non-small-cell lung cancer. *PLoS Med.* **3**, e420
163 Zhu, J., Wang, H., Chen, F., Fu, J., Xu, Y., Hou, Y. et al. (2016) An overview of chemical inhibitors of the Nrf2-ARE signaling pathway and their potential applications in cancer therapy. *Free Radic. Biol. Med.* **99**, 544–556
164 Mitsuishi, Y., Taguchi, K., Kawatani, Y., Shibata, T., Nukiwa, T., Aburatani, H. et al. (2012) Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. *Cancer Cell* **22**, 66–79

*Clinical Science* (2017) **131** 2573–2599
https://doi.org/10.1042/CS20160982

165 Niture, S.K. and Jaiswal, A.K. (2013) Nrf2-induced antiapoptotic Bcl-xL protein enhances cell survival and drug resistance. *Free Radic. Biol. Med.* **57**, 119–131
166 Niture, S.K. and Jaiswal, A.K. (2012) Nrf2 protein up-regulates antiapoptotic protein Bcl-2 and prevents cellular apoptosis. *J. Biol. Chem.* **287**, 9873–9886
167 Xu, X., Zhang, Y., Li, W., Miao, H., Zhang, H., Zhou, Y. et al. (2014) Wogonin reverses multi-drug resistance of human myelogenous leukemia K562/A02 cells via downregulation of MRP1 expression by inhibiting Nrf2/ARE signaling pathway. *Biochem. Pharmacol.* **92**, 220–234
168 Zhong, Y., Zhang, F., Sun, Z., Zhou, W., Li, Z.Y., You, Q.D. et al. (2013) Drug resistance associates with activation of Nrf2 in MCF-7/DOX cells, and wogonin reverses it by down-regulating Nrf2-mediated cellular defense response. *Mol. Carcinog.* **52**, 824–834
169 Qian, C., Wang, Y., Zhong, Y., Tang, J., Zhang, J., Li, Z. et al. (2014) Wogonin-enhanced reactive oxygen species-induced apoptosis and potentiated cytotoxic effects of chemotherapeutic agents by suppression Nrf2-mediated signaling in HepG2 cells. *Free Radic. Res.* **48**, 607–621
170 Riggs, B.L., Khosla, S. and Melton, III, L.J. (1998) A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. *J. Bone Miner. Res.* **13**, 763–773
171 Weitzmann, M.N., Cenci, S., Rifas, L., Haug, J., Dipersio, J. and Pacifici, R. (2001) T cell activation induces human osteoclast formation via receptor activator of nuclear factor kappaB ligand-dependent and -independent mechanisms. *J. Bone Miner. Res.* **16**, 328–337
172 Basu, S., Michaelsson, K., Olofsson, H., Johansson, S. and Melhus, H. (2001) Association between oxidative stress and bone mineral density. *Biochem. Biophys. Res. Commun.* **288**, 275–279
173 Lean, J.M., Jagger, C.J., Kirstein, B., Fuller, K. and Chambers, T.J. (2005) Hydrogen peroxide is essential for estrogen-deficiency bone loss and osteoclast formation. *Endocrinology* **146**, 728–735
174 Muthusami, S., Ramachandran, I., Muthusamy, B., Vasudevan, G., Prabhu, V., Subramaniam, V. et al. (2005) Ovariectomy induces oxidative stress and impairs bone antioxidant system in adult rats. *Clin. Chim. Acta* **360**, 81–86
175 Kanzaki, H., Shinohara, F., Kajiya, M. and Kodama, T. (2013) The Keap1/Nrf2 protein axis plays a role in osteoclast differentiation by regulating intracellular reactive oxygen species signaling. *J. Biol. Chem.* **288**, 23009–23020
176 Hyeon, S., Lee, H., Yang, Y. and Jeong, W. (2013) Nrf2 deficiency induces oxidative stress and promotes RANKL-induced osteoclast differentiation. *Free Radic. Biol. Med.* **65**, 789–799
177 Ibanez, L., Ferrandiz, M.L., Brines, R., Guede, D., Cuadrado, A. and Alcaraz, M.J. (2014) Effects of Nrf2 deficiency on bone microarchitecture in an experimental model of osteoporosis. *Oxid. Med. Cell Longev.* **2014**, 726590
178 Kanzaki, H., Shinohara, F., Itohiya, K., Yamaguchi, Y., Katsumata, Y., Matsuzawa, M. et al. (2017) RANKL induces Bach1 nuclear import and attenuates Nrf2-mediated antioxidant enzymes, thereby augmenting intracellular reactive oxygen species signaling and osteoclastogenesis in mice. *FASEB J.* **31**, 781–792
179 Callaway, D.A. and Jiang, J.X. (2015) Reactive oxygen species and oxidative stress in osteoclastogenesis, skeletal aging and bone diseases. *J. Bone Miner. Metab.* **33**, 359–370
180 Goettsch, C., Babelova, A., Trummer, O., Erben, R.G., Rauner, M., Rammelt, S. et al. (2013) NADPH oxidase 4 limits bone mass by promoting osteoclastogenesis. *J. Clin. Invest.* **123**, 4731–4738
181 Anderson, A.S. and Loeser, R.F. (2010) Why is osteoarthritis an age-related disease. *Best Pract. Res. Clin. Rheumatol.* **24**, 15
182 Goldring, M.B. and Goldring, S.R. (2007) Osteoarthritis. *J. Cell. Physiol.* **213**, 626–634
183 Loeser, R.F., Goldring, S.R., Scanzello, C.R. and Goldring, M.B. (2012) Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum.* **64**, 1697–1707
184 Reference deleted
185 Martin, J.A., Ellerbroek, S.M. and Buckwalter, J.A. (1997) Age-related decline in chondrocyte response to insulin-like growth factor-I: the role of growth factor binding proteins. *J. Orthop. Res.* **15**, 491–498
186 Trippel, S.B. (1995) Growth factor actions on articular cartilage. *J. Rheumatol. Suppl.* **43**, 129–132
187 Henrotin, Y., Deby-Dupont, G., Deby, C., De Bruyn, M., Lamy, M. and Franchimont, P. (1993) Production of active oxygen species by isolated human chondrocytes. *Br. J. Rheumatol.* **32**, 562–567
188 Henrotin, Y., Kurz, B. and Aigner, T. (2005) Oxygen and reactive oxygen species in cartilage degradation: friends or foes? *Osteoarthritis Cartilage* **13**, 643–654
189 Hayashi, T., Abe, E., Yamate, T., Taguchi, Y. and Jasin, H.E. (1997) Nitric oxide production by superficial and deep articular chondrocytes. *Arthritis Rheum.* **40**, 261–269
190 Carlo, Jr, M.D. and Loeser, R.F. (2003) Increased oxidative stress with aging reduces chondrocyte survival: correlation with intracellular glutathione levels. *Arthritis Rheum.* **48**, 3419–3430
191 Blanco, F.J., Rego, I. and Ruiz-Romero, C. (2011) The role of mitochondria in osteoarthritis. *Nat. Rev. Rheumatol.* **7**, 161–169
192 Yin, W., Park, J.I. and Loeser, R.F. (2009) Oxidative stress inhibits insulin-like growth factor-I induction of chondrocyte proteoglycan synthesis through differential regulation of phosphatidylinositol 3-Kinase-Akt and MEK-ERK MAPK signaling pathways. *J. Biol. Chem.* **284**, 31972–31981
193 Brandl, A., Hartmann, A., Bechmann, V., Graf, B., Nerlich, M. and Angele, P. (2011) Oxidative stress induces senescence in chondrocytes. *J. Orthop. Res.* **29**, 1114–1120
194 Hashimoto, S., Takahashi, K., Amiel, D., Coutts, R.D. and Lotz, M. (1998) Chondrocyte apoptosis and nitric oxide production during experimentally induced osteoarthritis. *Arthritis Rheum.* **41**, 1266–1274
195 Kong, P., Chen, G., Jiang, A., Wang, Y., Song, C., Zhuang, J. et al. (2016) Sesamin inhibits IL-1beta-stimulated inflammatory response in human osteoarthritis chondrocytes by activating Nrf2 signaling pathway. *Oncotarget* **7**, 83720–83726
196 Marchev, A.S., Dimitrova, P.A., Burns, A.J., Kostov, R.V., Dinkova-Kostova, A.T. and Georgiev, M.I. (2017) Oxidative stress and chronic inflammation in osteoarthritis: can NRF2 counteract these partners in crime? *Ann. N.Y. Acad. Sci.* **1401**, 114–135

197 Cai, D., Yin, S., Yang, J., Jiang, Q. and Cao, W. (2015) Histone deacetylase inhibition activates Nrf2 and protects against osteoarthritis. *Arthritis Res. Ther.* **17**, 269
198 Sun, Z., Chin, Y.E. and Zhang, D.D. (2009) Acetylation of Nrf2 by p300/CBP augments promoter-specific DNA binding of Nrf2 during the antioxidant response. *Mol. Cell. Biol.* **29**, 2658–2672
199 Kawai, Y., Garduno, L., Theodore, M., Yang, J. and Arinze, I.J. (2011) Acetylation-deacetylation of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) regulates its transcriptional activity and nucleocytoplasmic localization. *J. Biol. Chem.* **286**, 7629–7640
200 Chen, Z.J. (2005) Ubiquitin signaling in the NF-κB pathway. *Nat. Cell Biol.* **7**, 758–765
201 Liacini, A., Sylvester, J., Li, W.Q., Huang, W., Dehnade, F., Ahmad, M. et al. (2003) Induction of matrix metalloproteinase-13 gene expression by TNF-alpha is mediated by MAP kinases, AP-1, and NF-kappaB transcription factors in articular chondrocytes. *Exp. Cell Res.* **288**, 208–217
202 Palombella, V.J., Rando, O.J., Goldberg, A.L. and Maniatis, T. (1994) The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. *Cell* **78**, 773–785
203 Radwan, M., Wilkinson, D.J., Hui, W., Destrument, A.P.M., Charlton, S.H., Barter, M.J. et al. (2014) Protection against murine osteoarthritis by inhibition of the 26S proteasome and lysine-48 linked ubiquitination. *Ann. Rheum. Dis.* **74**, 1580–1587
204 Denic, A., Glassock, R.J. and Rule, A.D. (2016) Structural and functional changes with the aging kidney. *Adv. Chronic Kidney Dis.* **23**, 19–28
205 Weinstein, J.R. (2010) The aging kidney: physiological changes. *Adv. Chronic Kidney Dis.* **17**, 302–307
206 Tonelli, M. and Riella, M. (2014) Chronic kidney disease and the aging population. *Nephrol. Dial. Transplant.* **29**, 221–224
207 Justilien, V., Pang, J.J., Renganathan, K., Zhan, X., Crabb, J.W., Kim, S.R. et al. (2007) SOD2 knockdown mouse model of early AMD. *Invest. Ophthalmol. Vis. Sci.* **48**, 4407–4420
208 Yoh, K., Itoh, K., Enomoto, A., Hirayama, A., Yamaguchi, N., Kobayashi, M. et al. (2001) Nrf2-deficient female mice develop lupus-like autoimmune nephritis. *Kidney Int.* **60**, 1343–1353
209 Liu, M., Grigoryev, D.N., Crow, M.T., Haas, M., Yamamoto, M., Reddy, S.P. et al. (2009) Transcription factor Nrf2 is protective during ischemic and nephrotoxic acute kidney injury in mice. *Kidney Int.* **76**, 277–285
210 Chen, G., Bridenbaugh, E.A., Akintola, A.D., Catania, J.M., Vaidya, V.S., Bonventre, J.V. et al. (2007) Increased susceptibility of aging kidney to ischemic injury: identification of candidate genes changed during aging, but corrected by caloric restriction. *Am. J. Physiol. Renal. Physiol.* **293**, F1272–F1281
211 Tornavaca, O., Pascual, G., Barreiro, M.L., Grande, M.T., Carretero, A., Riera, M. et al. (2009) Kidney androgen-regulated protein transgenic mice show hypertension and renal alterations mediated by oxidative stress. *Circulation* **119**, 1908–1917
212 Grande, M.T., Pascual, G., Riolobos, A.S., Clemente-Lorenzo, M., Bardaji, B., Barreiro, L. et al. (2011) Increased oxidative stress, the renin–angiotensin system, and sympathetic overactivation induce hypertension in kidney androgen-regulated protein transgenic mice. *Free Radic. Biol. Med.* **51**, 1831–1841
213 Everaert, K., Mahmoud, A., Depuydt, C., Maeyaert, M. and Comhaire, F. (2003) Chronic prostatitis and male accessory gland infection–is there an impact on male infertility (diagnosis and therapy)? *Andrologia* **35**, 325–330
214 Schaeffer, A.J., Datta, N.S., Fowler, Jr, J.E., Krieger, J.N., Litwin, M.S., Nadler, R.B. et al. (2002) Overview summary statement. Diagnosis and management of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). *Urology* **60** (6 Suppl.), 1–4
215 Krieger, J.N., Lee, S.W.H., Jeon, J., Cheah, P.Y., Liong, M.L. and Riley, D.E. (2008) Epidemiology of prostatitis. *Int. J. Antimicrob. Agents* **31**, S85–S90
216 Potts, J.M. and Pasqualotto, F.F. (2003) Seminal oxidative stress in patients with chronic prostatitis. *Andrologia* **35**, 304–308
217 Huang, T.R., Li, W. and Peng, B. (2017) Correlation of inflammatory mediators in prostatic secretion with chronic prostatitis and chronic pelvic pain syndrome. *Andrologia*, doi:10.1111/and.12860
218 Pasqualotto, F.F., Sharma, R.K., Potts, J.M., Nelson, D.R., Thomas, A.J. and Agarwal, A. (2000) Seminal oxidative stress in patients with chronic prostatitis. *Urology* **55**, 881–885
219 Wolff, H. (1995) The biologic significance of white blood cells in semen. *Fertil. Steril.* **63**, 1143–1157
220 Zhou, J.F., Xiao, W.Q., Zheng, Y.C., Dong, J. and Zhang, S.M. (2006) Increased oxidative stress and oxidative damage associated with chronic bacterial prostatitis. *Asian J. Androl.* **8**, 317–323
221 Wang, L.L., Huang, Y.H., Yan, C.Y., Wei, X.D., Hou, J.Q., Pu, J.X. et al. (2016) N-acetylcysteine ameliorates prostatitis via *miR-141* regulating Keap1/Nrf2 signaling. *Inflammation* **39**, 938–947
222 Kim, I.H., Kisseleva, T. and Brenner, D.A. (2015) Aging and liver disease. *Curr. Opin. Gastroenterol.* **31**, 184–191
223 Serviddio, G., Bellanti, F. and Vendemiale, G. (2013) Free radical biology for medicine: learning from nonalcoholic fatty liver disease. *Free Radic. Biol. Med.* **65**, 952–968
224 Farrell, G.C. and Larter, C.Z. (2006) Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* **43**, S99–S112
225 Wei, Y., Rector, R.S., Thyfault, J.P. and Ibdah, J.A. (2008) Nonalcoholic fatty liver disease and mitochondrial dysfunction. *World J. Gastroenterol.* **14**, 193–199
226 Begriche, K., Igoudjil, A., Pessayre, D. and Fromenty, B. (2006) Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it. *Mitochondrion* **6**, 1–28
227 Grattagliano, I., de Bari, O., Bernardo, T.C., Oliveira, P.J., Wang, D.Q. and Portincasa, P. (2012) Role of mitochondria in nonalcoholic fatty liver disease–from origin to propagation. *Clin. Biochem.* **45**, 610–618
228 Pessayre, D. and Fromenty, B. (2005) NASH: a mitochondrial disease. *J. Hepatol.* **42**, 928–940
229 Videla, L.A., Rodrigo, R., Orellana, M., Fernandez, V., Tapia, G., Quiñones, L. et al. (2004) Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. *Clin. Sci.* **106**, 261–268
230 Carmiel-Haggai, M., Cederbaum, A.I. and Nieto, N. (2005) A high-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. *FASEB J.* **19**, 136–138

*Clinical Science* (2017) **131** 2573–2599
https://doi.org/10.1042/CS20160982

231 Meakin, P.J. (2014) Susceptibility of Nrf2-null mice to steatohepatitis and cirrhosis upon consumption of a high-fat diet is associated with oxidative stress, perturbation of the unfolded protein response, and disturbance in the expression of metabolic enzymes but not with insulin resistance. *Mol. Cell. Biol.* **34**, 3305–3320
232 Collins, A.R., Lyon, C.J., Xia, X., Liu, J.Z., Tangirala, R.K., Yin, F. et al. (2009) Age-accelerated atherosclerosis correlates with failure to upregulate antioxidant genes. *Circ. Res.* **104**, e42–e54
233 Gupte, A.A., Lyon, C.J. and Hsueh, W.A. (2013) Nuclear factor (erythroid-derived 2)-like-2 factor (Nrf2), a key regulator of the antioxidant response to protect against atherosclerosis and nonalcoholic steatohepatitis. *Curr. Diab. Rep.* **13**, 362–371
234 Seitz, H.K. and Stickel, F. (2007) Alcoholic liver disease in the elderly. *Clin. Geriatr. Med.* **23**, 905–921
235 Meier, P. and Seitz, H.K. (2008) Age, alcohol metabolism and liver disease. *Curr. Opin. Clin. Nutr. Metab. Care* **11**, 21–26
236 Lamle, J., Marhenke, S., Borlak, J., von Wasielewski, R., Eriksson, C.J., Geffers, R. et al. (2008) Nuclear factor-eythroid 2-related factor 2 prevents alcohol-induced fulminant liver injury. *Gastroenterology* **134**, 1159–1168
237 Bardag-Gorce, F., van Leeuwen, F.W., Nguyen, V., French, B.A., Li, J., Riley, N. et al. (2002) The role of the ubiquitin–proteasome pathway in the formation of mallory bodies. *Exp. Mol. Pathol.* **73**, 75–83
238 Takagi, M., Yamauchi, M., Takada, K. and Ohkawa, K. (2002) Serum ubiquitin-protein conjugates in normal subjects and patients with alcoholic liver diseases: immunoaffinity isolation and electrophoretic mobility. *Alcohol Clin. Exp. Res.* **26**, 1692–1696
239 Bardag-Gorce, F., French, B.A., Nan, L., Song, H., Nguyen, S.K., Yong, H. et al. (2006) CYP2E1 induced by ethanol causes oxidative stress, proteasome inhibition and cytokeratin aggresome (Mallory body-like) formation. *Exp. Mol. Pathol.* **81**, 191–201
240 Donohue, Jr, T.M., Kharbanda, K.K., Casey, C.A. and Nanji, A.A. (2004) Decreased proteasome activity is associated with increased severity of liver pathology and oxidative stress in experimental alcoholic liver disease. *Alcohol Clin. Exp. Res.* **28**, 1257–1263
241 Dineley, K.T., Jahrling, J.B. and Denner, L. (2014) Insulin resistance in Alzheimer's disease. *Neurobiol. Dis.* **72**, 92–103
242 Reaven, G.M. (1988) Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* **37**, 1595–1607
243 (2014) Diagnosis and classification of diabetes mellitus. *Diabetes Care* **37**, S81–S90
244 Alejandro, E.U., Gregg, B., Blandino-Rosano, M., Cras-Meneur, C. and Bernal-Mizrachi, E. (2015) Natural history of beta-cell adaptation and failure in type 2 diabetes. *Mol. Aspects Med.* **42**, 19–41
245 Wang, S.Y., Halban, P.A. and Rowe, J.W. (1988) Effects of aging on insulin synthesis and secretion. Differential effects on preproinsulin messenger RNA levels, proinsulin biosynthesis, and secretion of newly made and preformed insulin in the rat. *J. Clin. Invest.* **81**, 176–184
246 Houmard, J.A., Weidner, M.D., Dolan, P.L., Leggett-Frazier, N., Gavigan, K.E., Hickey, M.S. et al. (1995) Skeletal muscle GLUT4 protein concentration and aging in humans. *Diabetes* **44**, 555–560
247 Hernandez-Mijares, A., Rocha, M., Apostolova, N., Borras, C., Jover, A., Banuls, C. et al. (2011) Mitochondrial complex I impairment in leukocytes from type 2 diabetic patients. *Free Radic. Biol. Med.* **50**, 1215–1221
248 Matschinsky, F.M., Pagliara, A.S., Stillings, S.N. and Hover, B.A. (1976) Glucose and ATP levels in pancreatic islet tissue of normal and diabetic rats. *J. Clin. Invest.* **58**, 1193–1200
249 Ma, Z.A., Zhao, Z. and Turk, J. (2012) Mitochondrial dysfunction and β-cell failure in type 2 diabetes mellitus. *Exp. Diabetes Res.* **2012**, 703538
250 Poitout, V. and Robertson, R.P. (2008) Glucolipotoxicity: fuel excess and beta-cell dysfunction. *Endocr. Rev.* **29**, 351–366
251 Wieser, V., Moschen, A.R. and Tilg, H. (2013) Inflammation, cytokines and insulin resistance: a clinical perspective. *Arch. Immunol. Ther. Exp. (Warsz.)* **61**, 119–125
252 Tan, Y., Ichikawa, T., Li, J., Si, Q., Yang, H., Chen, X. et al. (2011) Diabetic downregulation of Nrf2 activity via ERK contributes to oxidative stress-induced insulin resistance in cardiac cells *in vitro* and *in vivo*. *Diabetes* **60**, 625–633
253 Bai, Y., Cui, W., Xin, Y., Miao, X., Barati, M.T., Zhang, C. et al. (2013) Prevention by sulforaphane of diabetic cardiomyopathy is associated with up-regulation of Nrf2 expression and transcription activation. *J. Mol. Cell Cardiol.* **57**, 82–95
254 Kenney, M.C., Atilano, S.R., Boyer, D., Chwa, M., Chak, G., Chinichian, S. et al. (2010) Characterization of retinal and blood mitochondrial DNA from age-related macular degeneration patients. *Invest. Ophthalmol. Vis. Sci.* **51**, 4289–4297
255 Karunadharma, P.P., Nordgaard, C.L., Olsen, T.W. and Ferrington, D.A. (2010) Mitochondrial DNA damage as a potential mechanism for age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* **51**, 5470–5479
256 Wang, A.L., Lukas, T.J., Yuan, M. and Neufeld, A.H. (2008) Increased mitochondrial DNA damage and down-regulation of DNA repair enzymes in aged rodent retinal pigment epithelium and choroid. *Mol. Vis.* **14**, 644–651
257 Lin, H., Xu, H., Liang, F.Q., Liang, H., Gupta, P., Havey, A.N. et al. (2011) Mitochondrial DNA damage and repair in RPE associated with aging and age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* **52**, 3521–3529
258 Imamura, Y., Noda, S., Hashizume, K., Shinoda, K., Yamaguchi, M., Uchiyama, S. et al. (2006) Drusen, choroidal neovascularization, and retinal pigment epithelium dysfunction in SOD1-deficient mice: a model of age-related macular degeneration. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 11282–11287
259 Zhao, Z., Chen, Y., Wang, J., Sternberg, P., Freeman, M.L., Grossniklaus, H.E. et al. (2011) Age-related retinopathy in NRF2-deficient mice. *PLoS ONE* **6**, e19456
260 Anversa, P., Palackal, T., Sonnenblick, E.H., Olivetti, G., Meggs, L.G. and Capasso, J.M. (1990) Myocyte cell loss and myocyte cellular hyperplasia in the hypertrophied aging rat heart. *Circ. Res.* **67**, 871–885
261 Li, J., Ichikawa, T., Villacorta, L., Janicki, J.S., Brower, G.L., Yamamoto, M. et al. (2009) Nrf2 protects against maladaptive cardiac responses to hemodynamic stress. *Arterioscler. Thromb. Vasc. Biol.* **29**, 1843–1850
262 Valcarcel-Ares, M.N., Gautam, T., Warrington, J.P., Bailey-Downs, L., Sosnowska, D., de Cabo, R. et al. (2012) Disruption of Nrf2 signaling impairs angiogenic capacity of endothelial cells: implications for microvascular aging. *J. Gerontol. A Biol. Sci. Med. Sci.* **67**, 821–829

263 Judge, S., Jang, Y.M., Smith, A., Hagen, T. and Leeuwenburgh, C. (2005) Age-associated increases in oxidative stress and antioxidant enzyme activities in cardiac interfibrillar mitochondria: implications for the mitochondrial theory of aging. *FASEB J.* **19**, 419–421
264 Wallace, D.C. (2000) Mitochondrial defects in cardiomyopathy and neuromuscular disease. *Am. Heart J.* **139** (Suppl. 2), S70–S85
265 Kakinuma, Y., Miyauchi, T., Yuki, K., Murakoshi, N., Goto, K. and Yamaguchi, I. (2000) Mitochondrial dysfunction of cardiomyocytes causing impairment of cellular energy metabolism induces apoptosis, and concomitant increase in cardiac endothelin-1 expression. *J. Cardiovasc. Pharmacol.* **36**, S201–S204
266 Lauritzen, K.H., Kleppa, L., Aronsen, J.M., Eide, L., Carlsen, H., Haugen, O.P. et al. (2015) Impaired dynamics and function of mitochondria caused by mtDNA toxicity leads to heart failure. *Am. J. Physiol. Heart Circ. Physiol.* **309**, H434–H449
267 Herrmann, J., Wohlert, C., Saguner, A.M., Flores, A., Nesbitt, L.L., Chade, A. et al. (2013) Primary proteasome inhibition results in cardiac dysfunction. *Eur. J. Heart Fail.* **15**, 614–623
268 Birks, E.J., Latif, N., Enesa, K., Folkvang, T., Luong le, A., Sarathchandra, P. et al. (2008) Elevated p53 expression is associated with dysregulation of the ubiquitin-proteasome system in dilated cardiomyopathy. *Cardiovasc. Res.* **79**, 472–480
269 Weekes, J., Morrison, K., Mullen, A., Wait, R., Barton, P. and Dunn, M.J. (2003) Hyperubiquitination of proteins in dilated cardiomyopathy. *Proteomics* **3**, 208–216
270 Monti, D.A., Zabrecky, G., Kremens, D., Liang, T.W., Wintering, N.A., Cai, J. et al. (2016) N-Acetyl Cysteine May Support Dopamine Neurons in Parkinson's Disease: Preliminary Clinical and Cell Line Data. *PLoS ONE* **11**, e0157602
271 Mecocci, P. and Polidori, M.C. (2012) Antioxidant clinical trials in mild cognitive impairment and Alzheimer's disease. *Biochim. Biophys. Acta* **1822**, 631–638
272 Sporn, M.B. and Liby, K.T. (2012) NRF2 and cancer: the good, the bad and the importance of context. *Nat. Rev. Cancer* **12**, 564–571
273 Chondrogianni, N., Georgila, K., Kourtis, N., Tavernarakis, N. and Gonos, E.S. (2015) 20S proteasome activation promotes life span extension and resistance to proteotoxicity in Caenorhabditis elegans. *FASEB J.* **29**, 611–622
274 Papaevgeniou, N., Sakellari, M., Jha, S., Tavernarakis, N., Holmberg, C.I., Gonos, E.S. et al. (2016) 18alpha-glycyrrhetinic acid proteasome activator decelerates aging and Alzheimer's disease progression in *Caenorhabditis elegans* and neuronal cultures. *Antioxid. Redox Signal.* **25**, 855–869