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Review

Interventions for age-related diseases: Shifting the paradigm

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ABSTRACT

Over 60% of people aged over 65 are affected by multiple morbidities, which are more difficult to treat, generate increased healthcare costs and lead to poor quality of life compared to individual diseases. With the number of older people steadily increasing this presents a societal challenge. Age is the major risk factor for age-related diseases and recent research developments have led to the proposal that pharmacological interventions targeting common mechanisms of ageing may be able to delay the onset of multimorbidity. Here we review the state of the knowledge of multimorbidity, appraise the available evidence supporting the role of mechanisms of ageing in the development of the most common age-related diseases and assess potential molecules that may successfully target those key mechanisms.

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1. Introduction

The number of people over the age of 65 is predicted to double in the next 50 years with consequent increase in the incidence of age-related chronic diseases such as arthritis, type 2 diabetes, cancer, osteoporosis, cardiovascular and neurodegenerative disorders (http://europa.eu/epc/pdf/ageing_report_2015_en.pdf). As a result the costs in long term care are set to sharply increase (http://europa.eu/epc/pdf/ageing_report_2015_en.pdf). Up until now, research has focused predominantly on single diseases often with a focus on mortality as the main endpoint. However, this approach may no longer serve patients and society well (Tinetti et al., 2012; Smith et al., 2012). Approximately 60% of people over 65 s have been shown to have multimorbidity (Vogeli et al., 2007) *i.e.* the presence of more than 1 condition at the same time, and this is the main factor responsible for decreased quality of life and increased healthcare costs (Fortin et al., 2007; Fortin et al., 2004; Wolff et al., 2002). In addition, there is evidence that treatments, which are effective when patients present only one disease, become far less so in the presence of multimorbidity and there is an increasing risk of serious side effects associated with polypharmacy (Marengoni et al., 2014; Tinetti et al., 2004). Recently studies commissioned by the population level commissioning for the future in the UK were set up to assess the burden of multimorbidity and ways to improve clinical outcomes (http://www.nhsiq.nhs.uk/news-events/news/population-level-commissioning-for-the-future.aspx). In agreement with other healthcare providers worldwide, the outcome was the urgent need to identify interventions which are holistic and move away from the single disease model (Tinetti et al., 2012).

In an attempt to fulfil this need, a new paradigm has been proposed to devise interventions, which target common mechanisms of ageing to delay the onset of more than one age-related disease at the same time and focus not on mortality as endpoint *per se* but rather on contraction of the period of morbidity before death, or in other words, healthspan (Riera and Dillin, 2015). In this review we aim to give a brief overview of the available evidences that this may be a sound approach. We will consider the understanding of multimorbidity in terms of clustering, predictive risks and common mechanisms of ageing and discuss priority areas for the discovery of new targets.

2. Multimorbidity

At present it is unknown whether multimorbidity is the result of random chance, common risk factors, common mechanisms or the result of side effects of particular treatments. Defining the etiology of multimorbidity is particularly important in the decision of what mechanisms to target and for which group of diseases. What is clear is that the prevalence of multimorbidity increases dramatically with age and ageing is by far the strongest risk factor for many chronic diseases (Barnett et al., 2012; Fabbri et al., 2015a). Moreover, patients with one disease have a higher risk of developing multimorbidity than older individuals with no disease (Melis et al., 2014). Fabbri et al., (2015c) proposed that ageing brings a dysregulation of multiple organ systems and when a certain threshold of impairment is reached this is manifested in the onset of diseases and their accumulation. They suggest that the onset of multimorbidity is a landmark for loss of resilience and homeostasis. If this is the case, in order to implement strategies that slow the progression of ageing and the burden of multimorbidity it is necessary to identify markers or risk factors which predict the onset of multimorbidity clusters (Fig. 1).

However, such approaches are still in their infancy. Attempts to identify patterns of associated multimorbidity, where a group of diseases occur together with a higher frequency than by chance alone, have yielded inconsistent results due to the absence of standardization (van den Akker et al., 1998; Prados-Torres et al., 2014; Marengoni et al., 2009; Schäfer et al., 2010). In particular, the lack of a definition of what constitutes multimorbidity, in particular how many conditions and which ones should be included in the definition, has made it difficult to compare findings across studies and reach conclusion even on simple parameters such as incidence (Le Reste et al., 2015; Fortin et al., 2012).

In an attempt to identify risk factors that predict the onset of multimorbidity the InCHIANTI study and the Baltimore longitudinal study of ageing have found that age-related proinflammatory state such as elevated level of Interleukin-6 (IL-6), Tumor Necrosis factor alpha receptor 2 (TNFAR2), Interleukin-1 Receptor Antagonist (IL-1RA) and decline in dehidroepiandrosterones (DHEAS) were found to be associated with higher multimorbidity, relative to participants with normal level of the same markers. In addition higher baseline IL-6 and steeper increase of IL-6 were associated with increasing number of chronic diseases (Fabbri et al., 2015a,b). Although encouraging, there is a pressing need for more studies such as these in order to identify risks which are predictive in individual patients rather than simply at the population level.

As there are no specific clusters of diseases that have been shown to be tightly associated in age-related morbidity, we have considered the most prevalent diseases in the ageing population. We focus on cardiovascular (atherosclerosis), musculoskeletal (osteoporosis and osteoarthritis), metabolic (Type 2 diabetes) and neurodegenerative (Alzheimer's and Parkinson disease) disorders and review the evidence for ageing mechanisms and key nodes common to all these diseases. We find evidence that processes related to inflammation, autophagy, DNA damage and senescence are consistently altered across all these disorders.

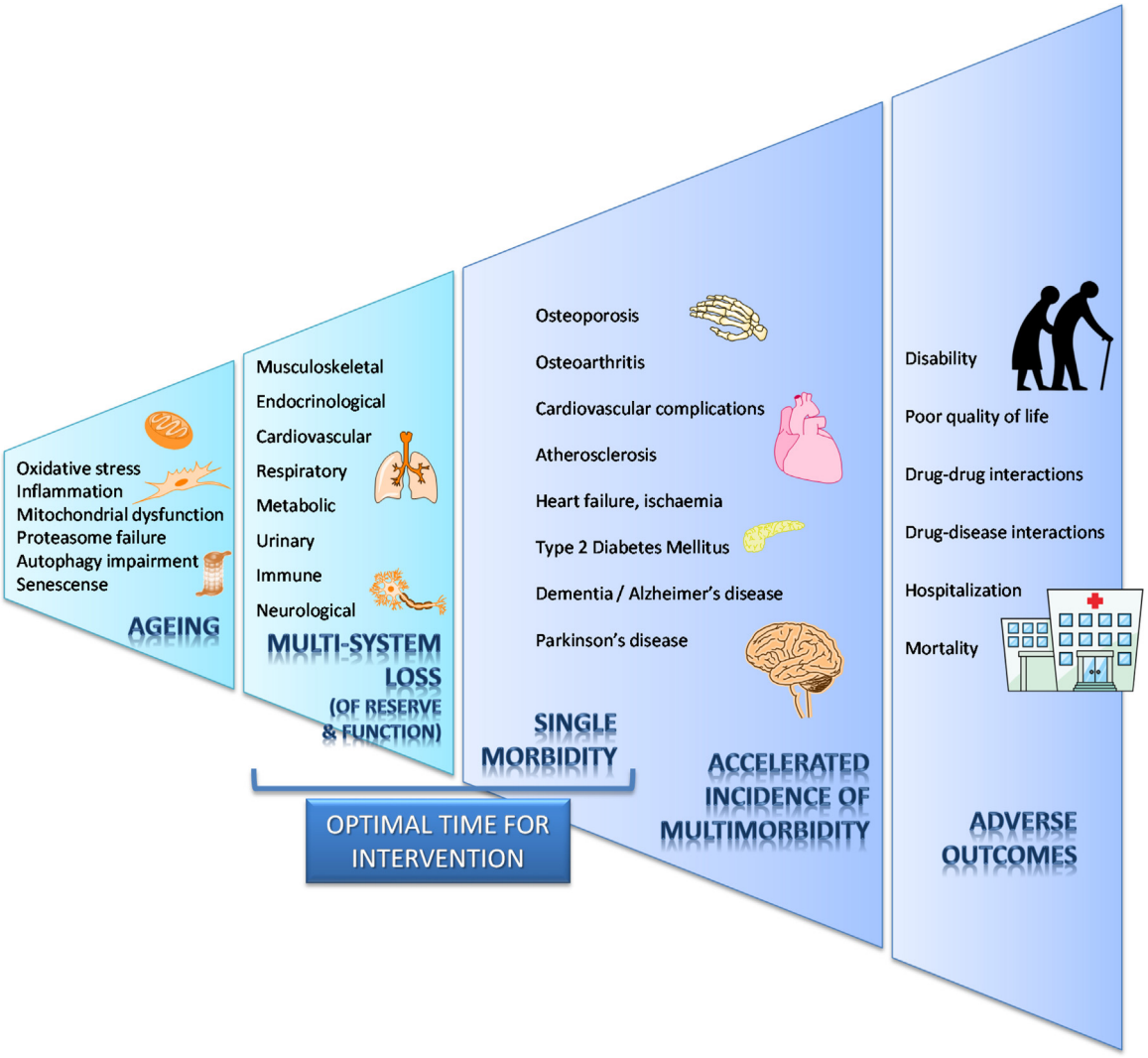

Fig. 1. Schematic representation of the proposed sequence of events moving from age-related alterations to multimorbidity. The multi-system loss of reserve and function has its origin in the biological determinants of the ageing process, and increases susceptibility to chronic diseases. This phenomenon manifests clinically as multimorbidity when a certain threshold of impairment has been reached. The proposed optimal time for intervention is between multi-system loss of reserve and single morbidity (adapted from Fabbri et al., 2015c).

3. Mechanisms of ageing

3.1. Inflammation and senescence

Inflammageing has been described as a chronic state of low-grade, inflammation that ensues with ageing (Franceschi and Campisi, 2014). This persistent, non-resolved inflammation contributes to tissue damage, which contrasts with the acute, transient, natural response to pathogens that is required for the maintenance of tissue homeostasis. There are multiple potential sources of inflammageing, which are all potentiated by the concomitant impairment of the immune system with ageing, termed "immunosenescence" (Frasca and Blomberg, 2015; Campos et al., 2014; Linton and Thoman, 2014; Macaulay et al., 2013). Immunosenescence is associated with an increased susceptibility to infectious disease, cancer and decreased response to vaccination (Chebel et al., 2009; Clambey et al., 2005; Plunkett et al., 2005, 2007; Reed et al., 2004; Weng, 2008), and is mainly thought to be a consequence of repeated antigen exposure throughout life (Akbar and Henson, 2011; Franceschi et al., 2007). Immunosenescence affects both the innate and the adaptive immune system (Frasca and Blomberg, 2015; Akbar and Henson, 2011).

This repeated mild, chronic activation of the immune system leads to a chronic secretion of inflammatory factors, which is ultimately a hallmark of inflammageing itself. For example, chronic exposure to viruses such as cytomegalovirus (CMV), namely B-herpes virus HHV5, as well as endogenous, host-derived damaged macromolecules, reactive oxidative species (ROS) and cell debris lead to re-current activation and re-modelling of inflammatory networks (Franceschi and Campisi, 2014; Freeman, 2009; Pawelec et al., 2010; Salminen et al., 2012). The inflammatory networks have been shown to be mediated *in vitro* by activation of NF-κB-mediated signalling networks, including transcription of proinflammatory TNF-α (Prosch et al., 1995). Interestingly, NF-κB signalling has both positive and negative effects as it is also required as host defence mechanisms against CMV replication (Eickhoff and Cotten, 2005). Another source of inflammageing comes from microbial components that reside in oral and gut cavities, which due to immunosenescence and compromised integrity of the intestinal barrier (Mabbott et al., 2015; Saffrey, 2014) leak into other tissues and activate inflammatory networks, which will differ depending on the pathogen. Furthermore, the microbiome itself has been shown to change dramatically with ageing, which may also contribute to inflammation (Biagi et al., 2010; Rampelli et al., 2013).

Over-time persistent sources of low-grade inflammation are thought to lead to immune dysfunction, as both innate and adaptive ageing immune cells become incompetent in responding to acute stimuli, including viral infections and decreased antibody production in response to vaccination (McElhaney et al., 2012). Therefore, even though there is a chronic activation of the immune and inflammatory networks in the elderly, acute inflammatory responses and consequent immune protection are reduced (Frasca and Blomberg, 2015). The balance between pro-inflammatory and anti-inflammatory cytokines is shifted during ageing. In particular, inflammageing is characterised by an increase in proinflammatory cytokines in the blood of aged people, namely IL-6 (Wikby et al., 2006; Giacconi et al., 2004), IL-8 (Zanni et al., 2003), IL-15 (Zanni et al., 2003) and TNF-α (Bruunsgaard et al., 1999) and other inflammatory markers such as C-reactive protein (CRP), combined with decreased anti-inflammatory cytokines such as IL-10 (Frasca and Blomberg, 2015; Franceschi et al., 2007).

In addition, several lines of evidence now point to the accumulation of senescent cells in aged tissues as a significant factor contributing to inflammageing (Freund et al., 2010). Cellular senescence is a distinct state, initially identified by Hayflick in the 1960's (Hayflick, 1965), by showing that *in vitro* cultivated human fibroblasts reached a replicative limit after approximately 50 cell population doublings- termed "Hayflick's limit". This replicative limit is caused by telomere shortening and eventual cellular dysfunction, since human cells lose about 100 bp of telomere sequence per cell doubling, in the absence of compensatory mechanisms such as activation of the enzyme telomerase (Bodnar et al., 1998; Karlseder et al., 2002). Critically short and dysfunctional telomeres cause cells to stop dividing, resulting in either apoptosis or an irreversible state of "dormancy" termed replicative, telomere-dependent senescence. However, cellular senescence can also be induced by telomere-independent mechanisms, such as by oncogene-induced reactive oxidative stress (Chandeck and Mooi, 2010), mitochondria dysfunction (Correia-Melo and Passos, 2015) or through exogenous factors such as exposure to ionising radiation, as is the case for radiotherapy treatments in cancer (Mirzayans et al., 2013). Senescent cells do not divide, which means they can no longer take part in the proliferative requirements of the tissue, but they are metabolically active. Such cells secrete several tissue remodelling factors including matrix metalloproteinases (MMPs), proinflammatory cytokines and chemokines, termed the Senescence-Associated Secretory Phenotype (SASP) (Coppe et al., 2010).

In young, healthy tissues, senescent cells play a normal role in wound healing, by recruiting tissue-resident immune cells that contribute to tissue healing and clearance of senescent and apoptotic cells (Adams, 2009; Sagiv and Krizhanovsky, 2013). In ageing, however, this balance is somehow tipped, and senescent cells accumulate in tissues of different organisms including zebrafish (Henriques et al., 2013; Kishi, 2004), mice (Krishnamurthy et al., 2004), primates (Jeyapalan et al., 2007) and humans (Dimri et al., 1995). This means that aberrant accumulation of senescent cells negatively impacts on tissue repair in both a cell autonomous, by impaired proliferation, and in a non-cell-autonomous manner *via* SASP positively re-enforcing inflammageing (Ohtani and Hara, 2013). Moreover, adding to the complexity of this non-cell autonomous positively re-enforcing network, recent data shows that senescent cells can induce senescence in adjacent cells in a paracrine manner (Acosta et al., 2013). In particular, chronic inflammation caused by NF-κB activation has been shown to lead to telomere dysfunction and ageing phenotypes that were underlined by an accumulation of senescence (Bernal et al., 2014; Jurk et al., 2014).

All factors contributing to inflammageing are further modulated by the individual's genetic background, where different single-nucleotide polymorphisms (SNPs) in both the promoter of the proinflammatory cytokine IL-6 (Giacconi et al., 2004; Fishman et al., 1998; Olivieri et al., 2002) and IFN-ɣ (Lio et al., 2002a) as well as anti-inflammatory IL-10 (Lio et al., 2002b), regulate the individual's susceptibility to inflammageing and morbidity. More importantly inhibition of NF-κB as well selective removal of senescence in premature ageing mouse models that suffer from chronic inflammation (Osorio et al., 2012) or accumulation of senescence, respectively (Baker et al., 2016), can significantly reduce ageing phenotypes and improve health span (Baker et al., 2011), suggesting a causal relationship between inflammation and ageing.

3.2. Oxidative stress

DNA damage (or genomic instability) is accumulated in our tissues during ageing, contributing to loss of functionality and regenerative capacity. During DNA replication errors may occur as a result of misincorporation of nucleotides opposite modified DNA bases or by incorporation of modified nucleotides. Furthermore, DNA is constantly exposed to damaging agents from both endogenous and exogenous sources. If these lesions are not repaired they can lead to mutations and result in cellular dysfunction including uncontrolled cell proliferation, apoptosis or senescence. Thus, in order to maintain the integrity of the genome, a complicated integrated system of DNA repair pathways remove the vast majority of the excess of deleterious lesions (Harper and Elledge, 2007; White RyanÂ and Vijg, 2016; Hoeijmakers, 2009). However, DNA repair may occasionally fail or become limited due to an excess of DNA damage resulting in DNA damage accumulation. In such situations DNA damage is pathogenic and one of the most serious sequelae of DNA repair deficiency is carcinogenesis.

Aerobic cellular metabolism is the primary source of endogenous reactive oxygen species (ROS). Pathways and events that produce ROS include mitochondrial and peroxisomal metabolism, enzymatic synthesis of nitric oxide (NO), phagocytic leukocytes, heat, ultraviolet (UV) light, therapeutic drugs, oxidizing agents, ionizing radiation, and redox-cycling compounds. The reaction of ROS with pyrimidines and purines produce a variety of different DNA lesions (Cooke et al., 2003). Superoxide radicals are normally dismutated by superoxide dismutase (SOD), to form the less reactive hydrogen peroxide (H_2O_2) and O_2. H_2O_2 is further converted to H_2O and O_2 by catalase. SOD is present within mitochondria (SOD2, Mn-SOD), cytoplasm (SOD1, CuZn-SOD) and extracellularly (SOD3, EC-SOD) (Zelko et al., 2002). A large number of other factors also contribute to cellular defence against ROS, for example arginine, vitamins A, C, and E, thiols (glutathione), polyphenols (tea), enzyme-bound minerals (selenium and zinc), and enzymes such as glutathione reductase and glutathione peroxidases. All these are important for preventing damage of amino acids, proteins, and lipids (Fang et al., 2002).

Mitochondrial respiration is the major source of endogenous ROS. Under normal physiological conditions electrons leak from the electron transport chain converting about 1–2% of oxygen molecules into O_2^- (Boveris and Cadenas, 1975; Boveris, 1977; Loft and Poulsen, 1996; Papa, 1996). Mitochondrial dysfunction can lead to increased ROS production (Bai et al., 1999; Esposito et al., 1999; Raha and Robinson, 2000) and the correct mitochondrial function is important for to avoid accumulation of DNA mutation in the mitochondrial DNA (Rasmussen et al., 2003; Karthikeyan et al., 2002; Mandavilli et al., 2002). However, mitochondria are not only involved in generation of oxidative damage they also have an effect on repair of DNA lesions. It was shown that a human cell line depleted of the mitochondrial genome showed impaired repair of H_2O_2-induced DNA damage (Delsite et al., 2003). Pre-exposure of human cells to H_2O_2 also suppresses DNA repair of alkylation damage (Hu et al., 1995) suggesting that extensive oxidative dam-

age inhibits cellular repair systems. One possible target could be the mitochondrial DNA polymerase γ since it was shown that this enzyme is a target of oxidative damage, which might result in reduced replication of the mitochondrial genome as well as attenuated repair capacity (Graziewicz et al., 2002). Overall, these results indicate that optimal mitochondrial function is important for both optimal repair of oxidative DNA damage and prevention of oxidative damage.

3.3. Proteasome and lysosome

Proteins are continuously damaged by various intrinsic and extrinsic factors. Aggregation of damaged proteins depends on the balance between their generation and their elimination by protein degradation (Chondrogianni et al., 2014; Morimoto and Cuervo, 2014). This aggregation affects several intracellular pathways, results in the eventual failure of organism homeostasis, which, in turn, associates with the appearance of several degenerative diseases and ageing (López-Otín et al., 2013). The Ubiquitin-Proteasome-System (UPS) is responsible for the degradation of normal as well as damaged proteins. Specifically, the 26S proteasome consists of the catalytic 20S core and the 19S regulatory complex. Whereas the 20S complex confers the proteolytic activities of the proteasome, the documented role of 19S is to recognize, unfold, de-ubiquitinate and control the entry of multi-ubiquitinated substrates into the 20S proteasome (Finley, 2009; Weissman et al., 2011).

Many studies have shown a general decline of proteasome activities in different aged tissues (Chondrogianni et al., 2015). Senescent human cells resulted in decreased levels of the β-catalytic subunits that, in turn, result in lower proteasome content and activities in these cells (Chondrogianni et al., 2003). These *in vitro* findings are supported by *in vivo* evidence. Specifically, Kasahara and colleagues have shown that transgenic mice engineered with decreased levels of chymotrypsin-like (CT-L) proteasome activity exhibit an accelerated age-related phenotype (Tomaru et al., 2012). In addition, proteasome activation obtained by overexpressing the β5 subunit in different human cell lines resulted in increased rates of proteolysis and cell survival following treatment with various cytotoxic agents (Catalgol et al., 2009; Chondrogianni et al., 2005). Importantly, overexpression of the β5 subunit significantly extended lifespan in human primary cultures (Chondrogianni et al., 2005). Moreover, recent comparative studies have reported that longer lived species have greater proteasome levels in immune cells and enhanced proteostasis relative to shorter-lived species (Pickering et al., 2015; Pride et al., 2015).

In contrast to the substrate degradation specificity of the proteasome, lysosomal degradation has a nearly unlimited degradation capacity. Autophagy is a lysosomal bulk degradation process involved in the clearance of long-lived proteins and organelles. The mechanism for the delivery of cargo to the lysosomes gives rise to the different forms of autophagy, namely macroautophagy, microautophagy and chaperone-mediated autophagy (Guo et al., 2012; Mijaljica et al., 2011; Orenstein and Cuervo, 2010). Levels of autophagy are under the tight control of multiple signal transduction pathways that are highly altered by various environmental signals (Chen and Klionsky, 2011; Levine and Kroemer, 2008). The best characterised regulator of autophagy is the (mammalian) target of rapamycin (mTOR (Jung et al., 2010, 2009)). mTOR is, in turn, regulated by various pathways. For instance, upon binding of insulin (or growth factors) to the insulin receptor, tyrosine kinases activate insulin substrate receptors that lead to subsequent AKT activation and stimulation of mTOR activity (Lionaki et al., 2013). Specifically, AKT promotes mTORC1 activity through multiple phosphorylation of TSC2, an upstream negative regulator of mTORC1, and in turn mTORC1 downstream kinase S6K1 blocks PI3K *via* phosphorylation of IRS1 to complete the negative feed-back loop. Another regulator is p53. Following activation by genotoxic or oncogenic stimuli, p53 transactivates several autophagy inducers including DRAM1 (which operates through JNK1 activation) and SESTRIN2 (which binds to the ternary complex TSC1/TSC2-AMPK, inducing phosphorylation and activation of TSC2 by AMPK (Kroemer et al., 2010)). In this case, TSC2 is activated *via* AMPK which means that mTORC1 signaling pathway is ultimately involved. These intriguing data propose that there is a mechanistic overlap between mTORC1 and p53 (Hay, 2008). Importantly, genetic or chemical inhibition of p53 also activates autophagy (Fleming et al., 2011). An additional level of autophagy control occurs *via* the Beclin 1/VPS34 complex (Simonsen and Tooze, 2009; Sinha and Levine, 2008). Notably the apoptosis-related proteins BCL-2 or BCL-XL can bind Beclin 1 and inhibit autophagy (Lalaoui et al., 2015). In contrast, serum levels of Beclin 1 are increased in healthy centenarians (Emanuele et al., 2014).

Several studies suggest that the decline of lysosomal and autophagic proteolytic activity during ageing correlates with the accumulation of damaged proteins and organelles (Rajawat et al., 2009; Rubinsztein et al., 2011). As a consequence, the loading of lysosomes with lipofuscin during ageing, interferes with their ability to fuse with autophagosomes and degrade their cargo (Terman et al., 2007). Moreover a recent study highlights that loss of chaperone-mediated autophagy accelerates proteostasis failure during ageing (Schneider et al., 2015). Other work has documented the reduced expression of essential components of the autophagic machinery in different tissues during ageing and age-related pathologies (Rubinsztein et al., 2011). For instance in mice, the depletion of essential ATG proteins (key components of autophagosomes) during the early postnatal period is lethal (Levine and Kroemer, 2008). The age-associated phenotypes that were observed in tissue-specific ATG deficient mice (*i.e.* increased levels of oxidized proteins, accumulation of age-pigments into lysosomes, ubiquitin-containing aggregates and malfunctioning mitochondria) highlight the important role of autophagy during ageing (Komatsu et al., 2005; Nakai et al., 2007).

Autophagy is also required for lifespan extension induced by activation of sirtuins (Morselli et al., 2010). Several autophagy proteins, such as ATG5, ATG7, and ATG8 that are known to be major regulators of autophagy, are deacetylated by SIRT1 in a NAD-dependent manner, while overexpression of sirt1 induces autophagy (Lee et al., 2008). Moreover, overexpression of ATG5 not only activates autophagy but also directly increases lifespan in mice (Pyo et al., 2013). Conversely, knock-down or deficiency of autophagy components reverses these effects, (Alvers et al., 2009; Bjedov et al., 2010; Eisenberg et al., 2009; Morselli et al., 2009). These data indicate that autophagy is a common downstream effector in various life-prolonging signalling pathways (Folick et al., 2015).

4. Mechanisms of ageing and chronic age-related diseases

4.1. Musculoskeletal diseases

Musculoskeletal conditions cause more functional limitations in the adult population in the western world than any other group of disorders and are the main cause of disability among older age groups (Woolf and Pfleger, 2003). Osteoarthritis, back pain and osteoporosis are the 3 leading cause for medical consultation (Woolf and Pfleger, 2003). Whilst there is little understanding of the causes leading to back pain, intense research efforts are on-going to understand the causes of osteoarthritis and osteoporosis.

4.1.1. Osteoarthritis (OA)

Increased age is the most important risk factor for the initiation and progression of osteoarthritis a condition which typically affects the joints (Bijlsma et al., 2011). The articular cartilage is progressively degraded leading to chronic pain and stiffness. Although the defects in cartilage structure are the main feature of the disease, other joint structures are affected during OA including subchondral bone, menisci, ligaments, synovial membrane and muscle surrounding the affected joints (Bijlsma et al., 2011). Cartilage is maintained by chondrocytes mainly through production of extracellular matrix (Goldring, 2000). The onset of OA is characterised by increased chondrocyte proliferation which leads to formation of chondrocyte clusters and increased synthesis of irregular matrix components such as proteoglycans and collagen (Rothwell and Bentley, 1973). This is an attempt to repair the damaged extracellular matrix. With OA progression increased catabolic activity causes excessive cartilage breakdown. The catabolic events are largely mediated by proinflammatory cytokines and mediators such as metalloproteinases (Burrage et al., 2006). Current pharmacological management of OA is limited to symptom's alleviation and there are no approved drugs which modify the course of the disease (Bijlsma et al., 2011). Therefore there is a great need to identify new interventions.

There is evidence that chondrocytes undergo several mechanisms of ageing including telomere shortening and senescence and this influences their ability to produce extracellular matrix (Loeser, 2009). However, most studies are on chondrocytes cultured *in vitro* in normoxia and under proliferative stress (Loeser, 2009). It is known that *in vivo* chondrocytes rarely proliferate (Aigner et al., 2001), and are exposed to low oxygen tension (Brighton and Heppenstall, 1971), consequently questioning how many of the phenomenon described *in vitro* actually apply *in vivo*.

Focusing on the evidence that supports the presence of ageing mechanisms *in vivo*, the overproduction of ROS in the cartilage of patients affected by OA has been well documented. Accumulation of nitrotyrosine was observed in chondrocytes and degenerate articular cartilage compared to non-degenerate areas in the same sample, suggesting that degenerated cartilage may exhibit more oxidative damage than an intact region from the same OA cartilage (Yudoh et al., 2005; Loeser et al., 2002). Moreover, decreased expression of ROS scavengers has also been described. In a study on the expression of superoxide dismutase (SOD) family expression of all 3 forms of SOD were decreased at the transcriptional level in patients affected by OA (Scott et al., 2010). Immunofluorescence studies revealed that mitochondrial superoxide dismutase 2 (SOD2) was largely missing in OA but was present consistently in the superficial layer of normal cartilage (Ruiz-Romero et al., 2009). In addition the decreased expression of SOD2 was related to increased methylation of the SOD2 promoter suggesting an epigenetic regulation of its expression (Scott et al., 2010). In line with increased production of ROS and defects in the mitochondrial antioxidant system is the increased accumulation of mtDNA mutations in the cartilage of patients affected by OA as compared to age matched controls (Grishko et al., 2009).

Increased ROS is responsible for the presence of oxidative damage in DNA (Section 3.2.), which can directly contribute to apoptosis or senescence. Whilst evidence of increased apoptosis and cell death in the cartilage of OA patients is controversial (Aigner et al., 2001; Sharif et al., 2004) evidence of senescence was found *in vivo* in terms of greater expression of p16-INK4A and β-galactosidase in OA chondrocytes as compared to age-matched controls (Zhou et al., 2004; Price et al., 2002). Inhibition of p16-INK4A expression led to increased ability of the cells to proliferate and increased matrix gene expression when examined *in vitro*, suggesting a possible mechanism to explain the reduction in the ability of chondrocytes to repair matrix in OA patients (Zhou et al., 2004).

It is well established that OA is associated with increased local production of proinflammatory signals such as nitric oxide and cytokines such as IL1, IL6 and TNF-alpha. These are overexpressed in chondrocytes, stromal cells and synovial macrophages in OA joint; these signals negatively affect the balance of cartilage matrix degradation and repair (Goldring and Otero, 2011). It is unclear how the proinflammatory process is initiated. Cytotoxic effects and oxidative stress can both compromise chondrocyte viability and activate inflammatory signals as demonstrated by the activation of NF-kB and mitogen activated protein kinases in surviving chondrocytes (Goldring et al., 2011).

Mitochondrial dysfunction and inflammation have been linked to decreased autophagy (Section 3.3). A decrease in autophagy genes Beclin-1, ULK1 and LC3 was observed in the superficial and deep cartilage zone of patients with mild OA (Caramés et al., 2010). In more advanced stages of OA these genes were downregulated in all 3 zones (superficial, middle and deep zone) compared to cartilage from age-matched controls (Caramés et al., 2010). In contrast these genes were highly expressed in the middle zone and deep zone in the early phase of OA when chondrocytes clusters were formed as an attempt to repair cartilage (Caramés et al., 2010). This was also reproduced in models of OA in mice (Caramés et al., 2010). These data suggest that during the development of OA increased autophagy may reflect an adaptive response. When this response fails, decreased autophagy may lead to further degeneration. The fact that autophagy decreases with age in human joint cartilage, and precedes cartilage structural damage suggests that it may play a role in the establishment of the disease. More importantly decreased autophagy activity was shown to lead to OA-like changes in the joint cartilage in ATG5 KO mice (Bouderlique et al., 2015). Reduced autophagy leads to premature chondrocytes senescence, increased matrix degradation and increase of metalloproteinases and ROS (Bouderlique et al., 2015).

4.1.2. Osteoporosis

The adult skeleton is continuously remodelled by osteoclasts, which resorb bone and osteoblasts, which form new bone. Imbalance between bone formation and resorption leads to osteoporosis. Increased age has long been associated with reduced bone mass, which is largely thought to be due to hormonal deficiency, mainly oestrogen due to menopause. However, age-associated bone loss occurs even in individuals with normal levels of sex steroids (Riggs et al., 2008). In addition, rodents do not experience a significant decline in sex steroids with age but bone mass declines and long lived mice are resistant to age-related bone loss suggesting that other mechanisms can contribute to reduced bone mass with age (Almeida et al., 2007; Selman and Withers, 2011).

The RANKL/RANK signalling pathway is central to the process of osteoclastogenesis and provides evidence of the interconnection between immune and skeletal systems. Osteoblasts and their precursors express RANKL, which binds to the transmembrane receptor RANK (receptor activator of nuclear factor κ-B) expressed on the surface of osteoclasts and their precursors. It promotes proliferation and differentiation of osteoclast precursors, and the maturation and activity of osteoclasts. The osteoclastogenic activity induced by RANKL-RANK binding is inhibited by another member of the TNF receptor superfamily 'osteoprotegerin' (OPG) produced by osteoblasts. Although RANKL and macrophage colony stimulating factor (M-CSF) are essential for osteoclastogenesis, additional cytokines such as TNF-alpha and IL-1 are likely to contribute to the regulation of osteoclast formation both in physiological and pathological condition such as oestrogen deficiency and inflammation. This is based largely on animal studies of bone loss following ovariectomy (Kimble et al., 1995; Lorenzo et al., 1998) and lim-

ited evidence in postmenopausal women affected by osteoporosis. Indeed epidemiologic studies report an increase in the risk of developing osteoporosis in various inflammatory conditions (Mitra et al., 2000; Haugeberg et al., 2004). Gene polymorphism in IL-1, IL-6 and TNF-alpha and their receptors has been shown to correlate with different levels of bone mass in humans (Fontova et al., 2002; Tasker et al., 2004; Chung et al., 2003). Similarly, IL-1α and IL-1 receptor antagonist (IL-1Ra) gene polymorphisms have been associated with reduced bone mineral density and osteoporosis at the lumbar spine (Chen et al., 2003) In addition mRNA for IL-1, IL-6 and TNF-alpha was increased in a higher percentage of patients with fractures as compared with patients without fractures (Ralston, 1994). Anti-TNF drugs, currently used in the therapy of several immunological disorders, are also useful in preventing and/or reversing systemic bone loss associated with the disease, targeting both the bone and the inflammatory processes (Roux, 2011). The transcription factor NF-κB, activated in the presence of inflammation has a well described role in osteoclastogenesis (Ruocco et al., 2005). More recently suppression of NF-κB activity in differentiated osteoblasts was shown to prevent bone loss in an ovariectomy mouse model by maintaining osteoblast function (Chang et al., 2009). Although no data are currently available to confirm that this mechanism is relevant to patients affected by osteoporosis, the oestrogen receptor has been found to directly inhibit NK-κB transcription (Harnish, 2006) and is expressed in osteoblasts suggesting that oestrogen may regulate NF-κB activities under physiological conditions.

The functional role of oxidative stress and autophagy in osteoporosis is still largely unexplored in patients and most of the data derive from functional studies in mice. Suppression of autophagy by deletion of ATG7 expression in osteocytes has been shown to be sufficient to mimic many of the skeletal changes associated with advanced age in young adult mice (Onal et al., 2013). Although it is unknown whether autophagy does indeed decline with age in osteocytes or in cells at any stage of osteoblast differentiation, the relationship between the autophagic pathway and osteoporosis was highlighted in a genome wide association study of wrist bone mineral density in human subjects (Zhang et al., 2010). This analysis showed significant association of wrist bone mineral density with regulation of autophagy genes including ATG7 and Beclin1 (Zhang et al., 2010).

An increased production of ROS, and associated decreases in antioxidants was demonstrated subsequent to the depletion of oestrogen in ovariectomised (OVX) mice (Almeida et al., 2007; Lean et al., 2003). The levels of antioxidant enzymes superoxide dismutase, glutathione peroxidase and glutathione-s transferase were decreased in the femur of ovariectomized rats (Muthusami et al., 2005). The same enzymes were found to be lower in the plasma of osteoporotic patients (Maggio et al., 2003). In addition mice lacking mitochondrial SOD2 in osteocytes showed enhanced production of cellular superoxide *in vivo*. A bone morphological analysis demonstrated that the SOD1 and SOD2-deficient femurs showed remarkable bone loss in an age-dependent manner (Nojiri et al., 2011; Kobayashi et al., 2015). In particular, SOD2 deletion led to disorganized osteocytic canalicular networks and decreased number of live osteocytes (Kobayashi et al., 2015). Furthermore, SOD2 deficiency significantly suppressed bone formation and increased bone resorption concomitant with the upregulation of sclerostin and RANKL (Kobayashi et al., 2015). Goettsch et al. (Goettsch et al., 2013) found that intracellular NADPH oxidase 4 (NOX4), an enzymatic source of ROS, was increased following ovariectomy in female mice, and NOX4 deletion reduced bone loss. More importantly in middle-aged women, NOX4 mutation was associated with altered parameters of bone metabolism, and conversely, there was an increased expression of NOX4 in the bones of patients with untreated osteoporosis as compared to age-matched controls (Goettsch et al., 2013).

4.2. Cardiovascular disease

Age is the single biggest risk factor for cardiovascular disease (CVD). The annual incidence of CVD, defined as coronary artery disease, stroke, heart failure or claudication, at age 85–94y is 10-fold higher than at age 45–54y (Mozaffarian et al., 2015). Ageing itself is accompanied by an increase in the prevalence of other CVD risk factors, particularly hypertension, which affects 7% of adults <40y and two-thirds of the over 60y (Ong et al., 2007). Lifetime risks of CVD and hypertension for CVD-free 40y-old are 50% and 85% respectively, whilst over 30% of global mortality is attributable to CVD (Lakatta, 2015). These disorders thus constitute a huge disease burden in the ageing population and a major limiting factor on health span. Distinguishing the role of ageing *per se* in CVD pathogenesis *versus* cumulative exposure to other risk factors is complex. Ageing animal models generally do not manifest overt CVD unless exposed to other CVD precipitants, such as high fat diet, emphasising the importance of the interaction between ageing and other environmental factors.

Nevertheless, evidence that molecular mechanisms of ageing are potentially modifiable contributors to CVD comes from several observations: Firstly, known cellular and molecular antecedents of CVD are upregulated with age in animals and in apparently healthy humans (Lakatta, 2015). Secondly, diseased human vessels manifest 'ageing-related' phenomena (*e.g.* telomere shortening (Ogami et al., 2004), senescence (Minamino et al., 2002) and DNA damage (Matthews et al., 2006)) implying that CVD pathologies are in some respects a form of accelerated ageing. Thirdly, genetic or pharmacological manipulation of some ageing mechanisms modulates markers of vascular health in animal models (LaRocca et al., 2013; Csiszar et al., 2007).

At the functional level, ageing leads to arterial stiffening and impaired vasodilatory responses, phenomena that progress from age 40–50y and contribute to the onset of hypertension (Harvey et al., 2015; Sun, 2015). Hypertension is a major risk factor for atherosclerosis and cardiac failure, but ageing is accompanied by increased risks of these pathologies independently of blood pressure. The biggest single contributor to ageing-associated cardiovascular morbidity and mortality is atherosclerosis, responsible for myocardial infarction, angina, ischaemic stroke and peripheral vascular disease (Mozaffarian et al., 2015). The roles of ageing mechanisms in atherosclerosis are consequently considered here.

At the cellular level, ageing-induced changes in endothelium seem particularly important for promoting atherosclerosis. Atherosclerosis consists of the accumulation of lipid-laden macrophages and inflammatory cells in the arterial wall, leading to vessel stenosis, superimposed thrombosis and occlusion. Healthy endothelium maintains a vasodilatory, antithrombotic, anti-inflammatory arterial luminal surface that retards this process (Seals et al., 2014). Production of the vasodilator nitric oxide (NO) by endothelial cells is key to the atheroprotective phenotype and impaired endothelial-dependent vasodilation is an independent predictor of atherosclerotic CVD (Lerman and Zeiher, 2005). Ageing is accompanied by a reduction in endothelial NO-dependent vasodilatation in animals (Csiszar et al., 2007; Tschudi et al., 1996) and healthy non-hypertensive humans (Celermajer et al., 1994; Donato et al., 2007), implying that ageing *per se* increases endothelial vulnerability to atherosclerosis.

Of the molecular ageing mechanisms implicated in the development of CVD and atherosclerosis in particular, oxidative stress appears to play a central role. An extensive body of literature describes the contribution of oxidative stress to atherosclerotic plaque development *via* endothelial injury, inflammation, leuco-

cyte recruitment and lipid modification, as reviewed elsewhere (Madamanchi et al., 2005). Ageing is accompanied by elevated oxidative stress in the arteries of rodents (Csiszar et al., 2007; Ungvari et al., 2007) and primates (Ungvari et al., 2011a). Although clinical measurement of vascular oxidative stress *in vivo* is not possible, endothelial cells isolated from arteries of healthy older men have increased concentrations of nitrotyrosine, a marker of oxidative stress, compared to younger men (Donato et al., 2007). Contributors to increased vascular ROS generation identified in ageing animals include vascular NADPH oxidases (NOX) (Trott et al., 2011) and mitochondrial dysfunction (Ungvari et al., 2007), as well as insufficient activation of protective antioxidant pathways such as the antioxidant transcription factor Nrf2 (Ungvari et al., 2011b). Since Nrf2 is involved in mitochondrial biogenesis insufficient activation of this transcription factor could have negative consequences for mitochondrial function and increase ROS production (Wan et al., 2012). Studies of endothelial cells isolated from older men without vascular disease, diabetes or hypertension confirm that vascular NOX expression also increases in human ageing (Donato et al., 2007).

In endothelial cells oxidation of tetrahydrobiopterin, the cofactor for endothelial nitric oxide synthase (eNOS), causes uncoupling of eNOS activity resulting in NO deficiency and further superoxide generation (Seals et al., 2014). Superoxide in turn reacts with NO, reducing NO bioavailability. A role for oxidative stress in ageing-associated endothelial dysfunction is demonstrated by the finding that ROS scavengers restore endothelial vasodilatory responses in ageing rats (Tatchum-Talom and Martin, 2004).

Hypertension, hyperglycaemia and other proatherosclerotic insults increase vascular cell oxidative stress *in vitro* and in animals (Touyz, 2004), thus at the molecular level may mimic and summate with the effects of ageing. Although clinical trials of antioxidants have found no benefit in patients with CVD, this may be because established CVD is too late a stage at which to intervene, or because the antioxidants used were ineffective (Seals et al., 2014; Touyz, 2004; Dai et al., 2012).

Inflammation and oxidative stress are closely related in age-related vascular disease. Ageing rodents and primates have increased vascular NF-κB expression (Ungvari et al., 2011a; Csiszar et al., 2008), accompanied by upregulation of proatherosclerotic NF-κB target molecules such as the leukocyte adhesion molecule ICAM-1 (Csiszar et al., 2007). *Ex vivo* endothelial cells isolated from healthy older men also manifest increased NF-κB and inflammatory cytokine (IL6, TNFα and monocyte chemoattractant protein-1) expression, correlating with impaired vasodilator function (Donato et al., 2008). Oxidative stress increases NF-κB expression and antioxidants reverse the upregulation of NF-κB in arteries of ageing rats (Ungvari et al., 2007). However, vascular inflammation is a cause as well as a consequence of oxidative stress since TNFα upregulates vascular NOX expression (Csiszar et al., 2007). Inflammation is thus a potentially important therapeutic target for prevention of adverse vascular ageing. Indeed, administration of the TNFα antagonist etanercept attenuated the adverse effects of ageing on endothelial dilatory function, ROS production and ICAM expression in arteries of rats (Csiszar et al., 2007). Improvements in endothelial vasodilator function have also been reported with etanercept and salsalate (an NF-κB antagonist) in some, but not all studies of middle-aged and elderly participants (Seals et al., 2014).

Oxidative DNA damage (8-oxodG), telomere shortening, activation of the DNA damage response and senescence are evident in atherosclerotic lesions from clinical specimens (Minamino et al., 2002; Matthews et al., 2006; Wang and Bennett, 2012). Endothelial telomere length also declined more rapidly with age in human iliac arteries compared to less atherosclerotic-prone internal thoracic arteries (Chang and Harley, 1995). Whether these molecular ageing phenomena play a major role in accelerating atherosclerosis or are simply acting as markers of vascular stress/injury is currently unclear. However, vascular cell senescence is considered a proatherosclerotic phenotype; in addition to the presumed detrimental effects of senescence on vascular repair capacity, *in vitro* senescent endothelial cells and vascular smooth muscle cells upregulate expression of inflammatory cytokines (IL-6, MCP1) and adhesion molecules (ICAM1) known to be important in atherosclerosis (Minamino et al., 2002; Wang and Bennett, 2012).

Impaired autophagy is another ageing-associated process implicated in vascular disease; arteries from ageing mice and endothelial cells isolated from older humans have lower expression of Beclin1 and accumulation of the autophagy-cleared protein p62 (LaRocca et al., 2012). Inhibition and stimulation of autophagy in cultured endothelial cells respectively inhibit and improve nitric oxide bioavailability (Nussenzweig et al., 2015). Furthermore, treatment of ageing mice with the autophagy stimulators trehalose or spermidine restored endothelial function, attenuated vascular oxidative stress and prevented arterial stiffening (LaRocca et al., 2013, 2012).

4.3. Type 2 diabetes mellitus (T2DM)

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder with an estimated global prevalence that has increased from 4.7% of the adult population in 1980 to 8.5% in 2014 (World Health Organisation diabetes fact sheet 2016). Similarly, there has been a significant increase in the incidence of T2DM in both children and adolescents (Amutha and Mohan, 2016). T2DM is a complex, multifactorial disease although the primary driver in the observed global increase in T2DM across all age-classes is obesity, primarily central visceral adiposity In addition, the risk of developing metabolic syndrome and T2DM also increases significantly with advancing age (Twito et al., 2015; Gatineau et al., 2014). T2DM is characterized by insulin resistance across multiple tissues, an inability to regulate hepatic glucose production effectively, and an impairment in insulin secretion due to beta cell dysfunction and ultimately to beta cell failure (Mahler and Adler, 1999). These pathological changes ultimately result in an inability to regulate blood glucose levels, leading to a chronic elevation in blood glucose levels, termed hyperglycaemia (Mahler and Adler, 1999; Scheen, 2003). Hyperglycaemia can induce widespread damage to blood vessels, particularly the microvascular leading to nephropathy, neuropathy, retinopathy and significantly increases the risk of developing stroke, coronary heart disease, peripheral and arterial disease (Adler et al., 2003; Cade, 2008; Dyck et al., 1993; Orchard et al., 1990). Insulin resistance has also been implicated in increased risk of non-alcoholic fatty-liver disease (Wild et al., 2016), and in the development of various cancers, including some colon, liver, pancreatic and breast cancers (reviewed in (Tsugane and Inoue, 2010; O'Neill and O'Driscoll, 2015)). In addition, patients suffering from T2DM have a greater incidence of mild cognitive impairment (MCI) relative to people without T2DM, and accelerated development of AD (reviewed in (Barbagallo and Dominguez, 2014)). This may have a significantly higher risk of developing AD (Whitmer, 2007; Mittal and Katare, 2016).

While the precise molecular mechanisms underlying T2DM are not completely understood, several mechanisms associated with ageing also appear intimately linked to the development of T2DM. Ageing and obesity are both considered to play major roles in the development of T2DM, through the development of systemic inflammation. Chronic inflammation and the infiltration of inflammatory cells into the pancreatic islets can reduce insulin secretion through beta cell dysfunction and ultimately loss of insulin producing beta cells through apoptosis (Keane et al., 2015). Pancreatic β cell dysfunction can also result in increased production of various cytokines by beta cells, thus further exacerbating the inflammatory state. For example, activation of inflammatory signalling pathways,

such as IKKκ/NF-κB (Inhibitor of nuclear factor kappa-B kinase subunit beta/nuclear factor κB) in within the CNS can result in both insulin resistance and impaired insulin release from beta cells (Cai, 2009; Lumeng and Saltiel, 2011; Kang et al., 2009; Calegari et al., 2011; Purkayastha et al., 2011). The activity of JNK (c-Jun N-terminal kinase) and NF-κB-mediated inflammatory pathways are up-regulated in obese individuals, in association with increased levels of expression of downstream cytokines, such as TNF-α and IL-6. Consequently, both IL-6 and CRP (C-reactive protein), a protein produced by the liver in response to systemic inflammation, have been employed as robust predictors for T2DM risk (Pradhan et al., 2001; Spranger et al., 2003; Wang et al., 2013). These data are further supported by studies in genetic and dietary mouse models of obesity, which demonstrate that obesity can induce inflammation within adipose tissue and liver leading to the production of inflammatory mediators such as IL-6 and MCP-1 (chemokine monocyte chemotactic protein-1). Furthermore, adipocytes produce TNF-α which can further contribute to insulin resistance (see review (Jin and Patti, 2009)). In a clinical trial with T2DM patients, salicylate, an IKKβ inhibitor, was shown to be beneficial and to improve glycaemia in patients, further demonstrating the role of inflammation in T2DM (Goldfine et al., 2013).

Cellular senescence within the beta cells has also recently been implicated as a potential factor underlying the pathogenesis of diabetes in various mouse models. The cell cycle inhibitor p27 (*Cdkn1b*), a marker of senescence, increased in pancreatic β-cells in genetic mouse models of T2DM, genetic activation of p27 within the pancreas induced diabetes in mice, and p27 deletion increased insulin secretion and islet mass through an increase in beta cell number in mouse models of T2DM (Uchida et al., 2005). Similarly, in mice harbouring a combination of non-homologous end joining deficiency with a hypomorphic p53 mutation and abrogation of apoptosis, the burden of senescent cells increases rapidly and is associated with β-cell dysfunction and an overt diabetic phenotype by 3–5 months of age (Tavana et al., 2009). This appears to be an accelerated model of age-associated diabetes and is consistent with the hypothesis that cellular senescence of beta cells may be an important factor in beta cell dysfunction. In addition, $p16^{INK4A}$ which increases in expression in mice during ageing was shown to impair both proliferation and regeneration potential of islets in mice, possibly by inducing senescence (Krishnamurthy et al., 2006). However, although these mouse models suggest that cellular senescence may be important in the pathology of T2DM, a role for pancreatic islet senescence in human T2DM is currently unproven. There is widespread support for ROS-induced oxidative damage being an important pathogenic process in the development of insulin resistance, beta cell dysfunction and ultimately in the development of T2DM and many of its pathological sequela (for review see (Wright et al., 2006)).In diabetic patients, fasting plasma levels of nitrotyrosine were elevated when compared to non-diabetics and nitrotyrosine levels were correlated with post-prandial hyperglycaemia (Ceriello et al., 2002). In addition, the beta cell dysfunction has been linked to oxidative damage to mitochondrial membranes, which may in turn induce mitochondrial dysfunction and ultimately apoptosis within the beta cells (Ma et al., 2011). In addition, the accumulation of human amylin seen in T2DM patients has been shown to induce beta cell loss apparently by inducing mitochondrial dysfunction and increasing ROS production (Lim et al., 2010). In elderly humans subjects, with insulin resistance in muscle, mitochondrial oxidative activity and mitochondrial adenosine triphosphate (ATP) synthesis were decreased, implicating mitochondrial dysfunction in T2DM (Petersen et al., 2003). Insulin resistance, in patients with metabolic syndrome, has also been linked to a decrease in the number of mitochondrial DNA (mtDNA) copy number, implicating dysfunctional mitochondrial biogenesis in this disease (Gianotti et al., 2008). Skeletal muscle biopsies have provided evidence that various transcriptional factors (*e.g.* PPAR gamma coactivator 1-alpha and-beta (PGC1-alpha/PPARGC1 and PGC1-beta/PERC), coactivators of NRF-1 (nuclear respiratory factor-1) and PPAR gamma-dependent) are decreased in diabetic patients ((Patti et al., 2003), reviewed in (Jin and Patti, 2009)). Given that all are associated with mitochondrial maintenance and biogenesis (Perez-Schindler and Philp, 2015), suggests that reduced nuclear-encoded mitochondrial gene expression may be a factor in T2DM. The transcriptional coactivators PGC1α and PGC1β are also decreased by age, further linking ageing to the mitochondrial dysfunction that occurs in T2DM (Ling et al., 2004). In addition, muscle mitochondrial from diabetic patients tend to be relatively smaller and found at a lower density (reduced number per unit volume (Kelley et al., 2002)). Moreover, in a mouse model of T2DM (the *db/db* mice) heart mitochondrial uncoupling was shown to occur, leading to decreased oxidative phosphorylation capacity and increased ROS production and lipid peroxidation (Boudina et al., 2007). The non-obese diabetic Goto-Kakizaki rat has also been reported to have increased skeletal muscle oxidative stress and mitochondrial dysfunction (Armour et al., 2009). During ageing the accumulation of mitochondrial DNA mutations and deletions may impair the electron respiratory chain, further increasing ROS production. Mitochondrial dysfunction has also been implicated in age-related insulin resistance, thus promoting a vicious metabolic cycle and increasing the risk of T2DM (Petersen et al., 2003; Ye, 2013; Reznick et al., 2007). These processes do not act in isolation, and many inflammatory processes may also be activated, with several pro-inflammatory cytokines (as stated above) expressed, further impairing insulin signalling, beta cell function and the development of age-related insulin resistance and T2DM (Styskal et al., 2012; Park et al., 2014).

The evidence that a loss in proteostasis during ageing has been gaining momentum in the recent past (Labbadia and Morimoto, 2014). Similarly, a disruption in various components of the proteasomal machinery has been reported in T2DM. For example, the build-up of toxic amyloid polypeptides in the beta cells of T2DM patients is associated with elevated levels of polyubiquitinated proteins and the deficiency of an enzyme (ubiquitin carboxyl-terminal esterase L1; UCHL1) involved in the deubiquitination of proteins (Costes et al., 2014). The loss of *Uchl1* with beta cells of transgenic mice overexpressing human islet amyloid polypeptide increased the onset of overt diabetes relative to mice wild-type for Uchl1 (Costes et al., 2014). Interestingly, the loss of *Uchl1* in transgenic mice further intensified the defective autophagy/lysosomal phenotype already observed in the transgenic mice. Importantly, mitochondrial dysfunction and endoplasmic reticulum (ER) stress promote an increase in autophagy, a cellular mechanism important to restore intracellular homeostasis (Butler and Bahr, 2006; Jung and Lee, 2010). In turn, autophagy blockade leads to the accumulation of mitochondria with excessive ROS production, which promotes NLRP3 (NOD-like receptor family, pyrin domain containing 3) inflammasome activation (Zhou et al., 2011). When ER stress and mitochondrial dysfunction, alongside the consequent production of ROS, are prolonged, failure of the autophagy machinery may occur, further promoting the development of the metabolic syndrome and diabetes (Cai and Liu, 2012; Gonzalez et al., 2011; Muriach et al., 2014). Supporting this hypothesis, there is a positive correlation between ROS levels, ER stress and autophagy markers in leukocytes from T2DM patients (Rovira-Llopis et al., 2015). In addition, in mice fed with a high-fat diet, autophagic flux was shown to be increased in pancreatic β cells as a reaction to the induction of ER stress (Chu et al., 2015). As further evidence that autophagy may play an important role in the development of T2DM, studies in β-cells from diabetic *db/db* and C57BL/6 mice fed with high-fat diet, showed the active formation of autophagosomes (Gonzalez et al.,

2011). In addition, a β cell-specific deletion of Atg7 in human islet amyloid polypeptide (hIAPP) knock-in mice significantly impaired glucose tolerance on a high fat diet (Shigihara et al., 2014). Moreover, pancreatic samples from subjects with T2DM demonstrated the presence of a high number of dead β-cells in diabetic islets with massive vacuole overload, suggesting autophagy-associated cell death (Marchetti and Masini, 2009).

4.4. Neurodegenerative diseases

Neurodegenerative disorders such as Alzheimer's and Parkinson's disease are also increased with age (Szewczyk-Krolikowski et al., 2014; Riedel et al., 2016). As neurons age, they show signs of increased oxidative stress, disturbances in mitochondrial function, and accumulation of misfolded proteins, which are exacerbated in Alzheimer's disease (AD), and in Parkinson's disease (PD). However, a direct link between mechanisms of ageing and the onset of such neurodegenerative disorders is still missing.

4.4.1. Alzheimer's disease

Alzheimer disease (AD) is the neurodegenerative disorder most usually associated to age-related dementia and is etiologically multifactorial (Talwar et al., 2015). Histologically, AD is characterized by extensive neurodegeneration, extracellular deposition of amyloid-β peptide (Aβ) forming senile plaques, and intraneuronal accumulations of hyperphosphorylated microtubule-associated protein tau, the neurofibrillary tangles (NFTs) (Dickson et al., 1988). Aβ is a small protein formed upon cleavage of amyloid precursor protein (APP) by β-site APP cleaving enzyme 1 (BACE1) and γ-secretase, a protease complex containing presenilins 1 and 2 (PS1, PS2) (Cole and Vassar, 2007; Selkoe, 1998). Accumulation and misfolding of Aβ both intracellularly in neurons and extracellularly as oligomers or Aβ aggregates lead to the pathological cascade of AD (Cerasoli et al., 2015). Indeed, oligomeric and fibrillary Aβ, usually cleared by myeloid cells, also activate these cells leading to a neuroinflammatory response (Manocha et al., 2016), which may contribute to neurodegeneration as stated below. Although Aβ has been considered the origin of the disease for many years, accumulating evidence has demonstrated that hyperphosphorylated tau as well as the close relationship between tau and Aβ abnormal metabolism may also have a role during the onset and progression of the disease (reviewed by (Llorens-Marítin et al., 2014)). One common molecule involved in these two main hallmarks, senile plaques/Aβ and neurofibrillary tangles/tau, is GSK-3β. Several canonical substrates of GSK-3β are involved in Aβ production and PS1 function both in rodent models and *in vitro* (Llorens-Marítin et al., 2014), and inhibition of GSK-3β has been reported to reduce Aβ pathology in mice (Ly et al., 2013). In parallel, the action of GSK-3β, together with other tau kinases, is necessary for the phosphorylation of tau in the pre-tangle stage of Aβ in rodents (reviewed by (Llorens-Marítin et al., 2014)). Thus, it is not surprising that the activity of GSK-3β is a key factor widely used to model AD in rodents (Gómez-Sintes et al., 2011). Even more interesting, GSK-3β dysregulation has been found in ageing-related inflammation establishing an important link between ageing and AD (Zhou et al., 2013). Hyperphosphorylation of tau takes place both in AD and in neuroimflammation due to GSK3 hyperactivity (Lucas et al., 2001). GSK3 promotes secretion of proimmflamatory cytokines (such as IL-6) together with the hyperphosphorilation of tau, leading to increased cell death and generating further neuroinflammation (Fuster-Matanzo et al., 2013). However, all these data are in murine models of AD. In human subjects increased activity of neuronal GSK-3β has been found in AD brains (DaRocha-Souto et al., 2012). In addition, although the overexpression of total GSK-3β in frontal cortex of AD human brains compared to healthy age-matched controls did not reach statistical significance, the phosphorylated and active form of GSK-3β (pTyr216), showed to be markedly increased over control subjects and co-localized with several somatodendritic phosphor-tau epitopes (Leroy et al., 2007). This co-localization was described only into discrete cellular compartments, such as the autophagosomes (Taelman et al., 2010).

Inflammation has long been considered a hallmark of AD and highly involved in the etiopathology of the disease (Meraz-Ríos et al., 2013). Pro-inflammatory and immune markers are aberrantly expressed in the brain of healthy elderly (Schuitemaker et al., 2012; Cribbs et al., 2012). Within the CNS, microglial cells, also called brain macrophages, are responsible for the constantly immune surveillance of brain and spinal cord parenchyma, and become activated upon challenge. They are the main producers of inflammatory molecules to fight pathogenic agents or clear damage (for review see (Fernandes et al., 2014)). There is evidence that microglia is primed in the aged brain, thereby developing exacerbated and prolonged neuroinflammatory response after stimulation (Perry et al., 2010; Norden and Godbout, 2013). In line with this microglia over-activation has been reported in AD brains (Mandrekar and Landreth, 2010). In addition the involvement of inflammation in cognitive decline is supported by studies in AD animal models showing that blockade of IL-1 (Kitazawa et al., 2011) or deletion of TNFR1 gene (McAlpine et al., 2009) rescued animal cognitive deficits associated with AD progression. However, this seems a rather early event since the use of anti-inflammatory drugs showed a beneficial effect only if administered in very recent stages of disease or even before disease onset (Stewart et al., 1997). Moreover, inflammation has also been implicated in Aβ accumulation through modulation of BACE activity. Both deletion of Tenascin (Xie et al., 2013), an extracellular matrix protein that is upregulated in inflammation, or of TNRF1 (He et al., 2007) genes were shown to decrease BACE activity in *in vivo* models of AD. Furthermore, expression of p38 MAPK, a common inflammatory cascade, regulates BACE fate. While ROS-mediated BACE activation occurs downstream p38 MAPK activation (Tamagno et al., 2005), the reduction of p38 MAPK expression facilitate BACE lysosomal degradation (Schnöder et al., 2016).

In parallel with the over-activated microglia the presence of microglia showing abnormal morphology typical of senescent cells has also been described in the ageing human brain with fragmented cytoplasmic processes (*e.g.* cytorrhexis) and spheroidal swellings in their ramifications (Streit et al., 2009). Similar alterations have also been reported in samples from AD patients (Streit et al., 2009). In addition rodent microglia aged in culture showed loss of ability to migrate and phagocyte (Caldeira et al., 2014), similarly to what observed with other innate immune cells with age (Hearps et al., 2012) and this may be the case in AD patients. *In vivo* studies using animal models of AD showed that microglia from old mice, but not from young ones, have decreased expression of both Aβ-binding scavenger receptors and Aβ degrading enzymes when compared with their littermate controls, but higher levels of cytokine release (Hickman et al., 2008). While fibrillar Aβ promoted microglia phagocytosis, oligomeric Aβ reduced this ability, while enhancing a higher inflammatory response (Pan et al., 2011), further corroborating that microglial phagocytosis is negatively correlated with inflammatory reactivity. Examination of 3 patients affected by AD showed some clearance of plaques by microglia phagocytosis only following stimulation with Aβ immunization (Nicoll et al., 2006). Interestingly, AD patients subjected to immunization for aggregated Aβ (Rampelli et al., 2013) showed reduced cognitive impairment (Hock et al., 2003), corroborating that microglia may undergo loss of function in AD progression.

Several lines of evidence showed that mutations in mitochondrial DNA and net production of reactive oxygen species (ROS) have a central role not only in the process of brain ageing but also in the pathogenesis of neurodegenerative disorders including AD

(Lin and Beal, 2006). AD patients showed reduced levels of anti-oxidant defence mechanisms (Andersen, 2004). In mouse models, AD neuronal oxidative damage occurs earlier before Aβ deposition and plaque formation (Pratico et al., 2001), and is associated with up-regulation of genes related to mitochondrial metabolism and apoptosis (Reddy et al., 2004). Indeed, oxidative stress was reported as a cause of increased Aβ levels and plaque deposition in both *in vitro* and *in vivo* AD-models (Velliquette et al., 2005; Busciglio et al., 2002). Moreover, oxidative stress also was shown to increase the expression of BACE through activation of JNK and p38 MAPK (Tamagno et al., 2005), and lead to aberrant tau hyperphosphorylation by activation of GSK-3β (Lovell et al., 2004). More importantly a higher number of mutations were found in mitochondrial DNA from AD patients compared to controls (Coskun et al., 2004), and related with Aβ deposition within damaged mitochondria of AD patients (Hirai et al., 2001). It has been described that Aβ not only potentiates NO synthesis but also that inhibits key mitochondrial enzymes, namely complex IV and cytochrome *c*, while Tau inhibits complex I (reviewed in (Querfurth and LaFerla, 2010)), further increasing oxidative stress and mitochondria failure. In accordance, in AD patients structural changes caused by Aβ in mitochondria resulted in increased mitochondrial fragmentation, decreased mitochondrial fusion, mitochondrial dysfunction, and synaptic damage (Reddy et al., 2010; Manczak et al., 2006).

Autophagy-related pathology has been noted in late-onset neurodegenerative diseases including AD (Nixon and Cataldo, 2006). It has been postulated that the age-dependent onset of neurodegenerative diseases most likely correlates with the age-dependent decline of autophagic activity. Recent reports in mice deficient for Atg5 or Atg7 confirmed that impairment of autophagy promoted neuronal loss following accumulation of cytoplasmic inclusion bodies in neurons (Hara et al., 2006; Komatsu et al., 2006). Impairment of lysosomes function in cortical neurons of AD patients was first described in early 90 s (Cataldo et al., 1991). More recently these have been described as endosome or autophagosomal anomalies and impaired lysosome biogenesis (Nixon and Cataldo, 2006) with accumulation of autophagosome vacuoles in swollen dystrophic neurites of affected neurons due to impaired axonal transport of autophagy/lysosomal-related compartments as described *in vitro* in primary mouse cortical neurons (Lee et al., 2011). Moreover, Beclin1 has been shown to be deficient in brain samples from AD patients (Jaeger et al., 2010). Interestingly, mutations of PS1, a common cause for early-onset of familiar AD, led to markedly defective lysosomal acidification and autolysosomal maturation potentiating the autophagic/lysosomal, amyloid, and tau pathologies observed in animal model and AD patients (Cataldo et al., 2004). Also changes in the degradation of specific AD-related proteins have been described, namely by the presence of ubiquitinated forms of tau and Aβ as the major components of their aggregates in brain samples of AD patients (Perry et al., 1987). Interestingly, the use of rapamycin, known to enhance autophagy, before AD development, delayed and reduced AD phenotype, while rapamycin treatment after AD emergence had no significant effect (Majumder et al., 2011), suggesting that altered autophagy may have a major role in AD development.

4.4.2. Parkinson's disease (PD)

PD is a chronic, incurable disorder, whose incidence increase with age and affects 1–3% of the elderly population worldwide (Szewczyk-Krolikowski et al., 2014; Johnson and Bobrovskaya, 2015). Histopathologically, PD is characterized by the loss of dopaminergic neurons of the *substantia nigra* and *locus coeruleus* in parallel with astrocytosis and microgliosis. These events have been associated with the presence of proteinaceous intracellular aggregates called Lewy bodies, comprised primarily of α-synuclein (Syme et al., 2002). A new definition of PD should consider it as a multisystem synucleinopathy with pathology extending beyond the confines of the central nervous system (CNS) and clinical manifestations concerning dopamine cell loss (Stern et al., 2011). Non-dopaminergic and non-motor symptoms of PD are sometimes present before diagnosis and almost inevitably arise and strengthen with disease progression. Indeed, non-motor symptoms dominate the clinical picture of advanced PD and contribute to severe disability, impaired quality of life, and shortened life expectancy. By contrast with the dopaminergic symptoms of the disease, for which treatment is available, non-motor symptoms are often poorly recognised and inadequately treated (Chaudhuri et al., 2006).

Ageing and PD share several physiological changes as well as numerous cellular and molecular mechanisms, including oxidative stress and mitochondria dysfunction, proteasome failure and impaired autophagy. However, it is unknown whether the pathways underpinning these alterations are the same. Involvement of mitochondria dysfunction in PD was first addressed when MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), whose metabolite MPP+ inhibits complex-I of the mitochondrial electron-transport chain, caused a parkinsonism syndrome in adulterated drug abusers (Lee et al., 2012). Further research showed mitochondria complex-I deficiency and glutathione depletion in the substantia nigra of patients with idiopathic or pre-symptomatic PD (Schapira et al., 1989), suggesting an increased mitochondrial dysfunction and reduced anti-oxidant capacity in these patients. In addition, several PD-related genes have been associated with mitochondria/oxidative stress damage. Abnormal α-synuclein accumulation directly alters mitochondria morphology and increases superoxide formation in SH-SY5Y neuroblastoma cell line (Perfeito et al., 2014), also boosting MPTP-induced nigral pathology in human α-synuclein transgenic mice (Song et al., 2004), while mutant α-synuclein co-localizes with degenerating mitochondria also in α-synuclein transgenic mice suggesting a possible direct damaging effect (Martin et al., 2006).

The proteasome activity is also altered in PD. Expression of mutant α-synuclein promotes the formation of filaments which interact directly with the 20S core of the proteasome and decrease its proteolytic activity (Lindersson et al., 2004), which is counteracted by the expression of the E3 ligase Parkin (Petrucelli et al., 2002). While mutations in α-synuclein cause autosomal dominant PD, mutations in Parkin, which reduces α-synuclein ubiquitination and promotes Lewi bodies formation, cause autosomal recessive PD (Hardy, 2003). Furthermore, Parkin also modulates the pro-survival signalling through EGFR-Akt-mTOR pathway (Fallon et al., 2006), which is decreased in disease-related brain regions of PD patients (Iwakura et al., 2005), and has a major role in mitochondria number, maintenance and mitophagy (Bertolin et al., 2015). Interestingly, a recent study on adult *Drosophila melanogaster* showed that ubiquitous or neuron-specific up-regulation of Parkin extends lifespan (Rana et al., 2013).

Once more, autophagosome-like structures are also increased in PD (Stefanis, 2005), suggestive of defective autophagy both at the early stages and in later lysosomal clearance and linking PD with ageing. Mitophagy failure is a hallmark of PD and justified by mutations of Parkin and PINK1, which are associated to autosomal recessive cases of PD (Gasser, 2009). PINK1, a sensor of mitochondrial membrane polarization, is constitutively cleaved but becomes stabilized in the outer mitochondria membrane when mitochondria function is comprised (Abeliovich, 2010). This enable Parkin to ubiquitinate exposed membrane proteins, recruiting LC3 and initiating mitophagy of damaged mitocondria (Narendra et al., 2010). PD-related mutations in Parkin and PINK1 impair mitophagy causing accumulation of damaged mitochondria which signals for apoptotic events. Also mutations in α-synuclein (Cuervo et al., 2004), in familial PD, or changes of α-synuclein by dopamine

(Xilouri et al., 2009), in sporadic PD, impairs chaperone-mediated autophagy blocking not only its own uptake into lysosomes but that of other substrates. Autophagy induction by beclin 1 gene transfer or rapamycin ameliorated pathology in some α-synuclein PD models (Dadakhujaev et al., 2010) but not in others (Zhu et al., 2007), suggesting that further attention should be given to autophagy defects in PD.

Inflammatory-associated features are also observed in PD and related to its ageing increased susceptibility. Several reports using PET imaging studies revealed increased microglial activation in *in vivo* PD patients (Surendranathan et al., 2015), being correlated with midbrain and dopaminergic loss in the early stages of PD (Ouchi et al., 2005). Further studies using *in vivo* models of PD revealed that α-synuclein was the main trigger of microglia activation through induction of MHC class II expression (Harms et al., 2013) and Toll-like receptor-2 (TLR2) engagement in these cells (Kim et al., 2013). This activation was further corroborated by the presence of increased levels of inflammatory cytokines in brain samples of post-mortem PD brains (Mogi et al., 1994), as well as in colony stimulating factors and serum samples of PD patients (Hu et al., 2015).

5. Interventions

The first evidence that it may be possible to delay ageing in more than one tissue simultaneously comes from studies employing dietary restriction (DR); DR is defined here as a measured decrease in calories, macronutrients or micronutrients compared to that eaten by animals maintained on an *ad libitum* (AL) control diet. There is variety in terminology and experimental paradigms explored, but the reduction of calories to 30–50% below *ad libitum* (AL) levels is most frequently used (Speakman and Mitchell, 2011; Chung et al., 2013). The positive effects of DR on both lifespan and health span have been recognised for nearly a century (Osborne et al., 1917). DR is undoubtedly the most widely used experimental intervention in ageing research, demonstrating pleiotropic beneficial effects on several biological systems. Probably the most spectacular effect within this expanding research area is DR-induced increase of median and maximum lifespan observed in a wide number of organisms (Fontana and Partridge, 2015). However, what is also becoming clear is that the effects of DR on longevity may not actually be universal, with studies in a number of organisms not demonstrating an increase in lifespan on DR (reviewed in (Mulvey et al., 2014; Swindell, 2012)). For example, it is now well established that genetic background can influence the extent of DR-induced longevity (Mulvey et al., 2014), with 40% DR in recombinant inbred ILXISS mice leading to lifespan extension in some lines to lifespan shortening in other lines (Liao et al., 2010; Rikke et al., 2010).

Similarly, the recent DR study in rhesus monkeys performed at the National Institute of Ageing (NIA) revealed that DR did not improve survival outcomes (Mattison et al., 2012; Austad, 2012), in contrast with the findings of the study undertaken by the Wisconsin National Primate Research Center (WNPRC) by Coman and colleagues (Colman et al., 2014, 2009). Several differences exist in terms of experimental design, husbandry and dietary composition between the NIA and WNPRC studies that may help explain the discrepancies in terms the ability of DR to impact on lifespan (Partridge, 2012; Selman, 2014). DR both ameliorates and delays a number of age-associated pathologies in a wide range of organisms, including protection against metabolic dysfunction (*e.g.* insulin resistance, glucose intolerance, obesity), neurodegerative disease, sarcopenia, osteoporosis and immune dysfunction (Speakman and Mitchell, 2011; Selman, 2014; Masoro, 2005). In addition, DR significantly decreases the incidence and progression of both spontaneous age-associated and experimentally-induced cancers (Selman, 2014). Excitingly, despite the ambiguous effects of DR on lifespan in non-human primates (discussed above), DR induced protection in non-human primates against a number of age-associated pathologies, including T2DM, cardiovascular disease and cancer, and has also been shown to produce a number of favourable metabolic effects in humans (Fontana and Partridge, 2015; Mattison et al., 2012; Colman et al., 2014, 2009).

Notwithstanding the effects of DR on lifespan and health span being established for many decades now, the precise mechanisms driving these effects are still far from clear (Fontana and Partridge, 2015). Indeed, exactly how DR elicits its beneficial affects is likely to be highly complex, with different dietary interventions capable of exerting their effects through different mechanisms even within a single organism (Walker et al., 2005). DR modulates several signalling pathways and molecules known to modulate lifespan and health span: members of sirtuin family, insulin/insulin growth factor-1 and TOR, peroxisome proliferator activated receptor G coactivator-1 and adenosine monophosphate activated protein kinase (Lamming, 2014; Testa et al., 2014; Ramis et al., 2015). In addition, DR also tends to reduce oxidative damage, preserve mitochondrial function during ageing, and enhance proteostasis and stem cell function, which are all implicated in extended health span and all affected by the various signalling pathways and molecules described above (Fontana and Partridge, 2015).

However, despite the overall effectiveness of DR on lifespan and health span in the laboratory, the translation of such intervention to humans is likely to be confounded the obvious difficulties in individuals all complying to a DR diet over a protracted period of life and by the increased heterogeneity of the population. Work is ongoing to better understand the interaction between DR and, for example, genetic interventions that also modulate lifespan, what components of the diet can be restricted to modulate lifespan and health span (*e.g.* amino acids), without reductions in calorie intake. Perhaps DR in combination with nutritional geometry-type approaches (Piper et al., 2011) may help identify optimal dietary interventions for humans, without unwanted side-effects and the need for life-long restriction. One other potential route to better understand how DR acts mechanistically is through a comparative approach by studying those animals that show some deviation away from the 'DR norm' in terms of lifespan and health span (Mulvey et al., 2014). That is, by studying what changes or does not change under DR in these animals, compared to 'positive controls', may help give additional insights in to the mechanistic nature of DR, and particularly relevant to humans, help understand better the potential confounding nature of genetics on the DR response.

Pharmacological interventions are gaining place as a potential easier alternative for compliance than DR. A number of molecules targeting oxidative stress, autophagy, inflammation or the effects of accumulation of senescence cells are emerging together with evidence that they can have positive effects on more than one organ system in delaying the ageing phenotype (Table 2) and reviewed in (Riera and Dillin, 2015). In addition a new database of life span studies has recently been announced, to be hosted at geroprotectors.org. There is an existing catalogue of lifespan studies in animals at http://lifespandb.sageweb.org.

Very few of these molecules have been thoroughly assessed and testing poses considerable challenges. We consider here two of the most studied candidates, rapamycin and metformin to illustrate some of the challenges. Rapamycin targets the mTOR signalling pathway, an important and evolutionarily conserved player in longevity regulation and is the most extensively tested among the molecules listed in Table 1. It has been shown to be able to delay cancer formation in aged mice and extend their lifespan (Miller et al., 2007; Harrison et al., 2009; Neff et al., 2013). Testing of the compound's effects on a wide range of functional parameters used

Table 1
Chronic age-related diseases with corresponding disease hallmarks and mechanisms of ageing.

	Tissue/cellular alterations	Disease hallmarks	Mechanisms of ageing
Osteoarthritis (OA)	Damaged extracellular matrix Chondrocyte senescence (Bjedov et al., 2010)	Cartilage degeneration Subchondral bone loss Joint inflammation Joint pain (Pyo et al., 2013)	↑ oxidative stress (Bijlsma et al., 2011; Goldring, 2000) ↑ senescence (Brighton and Heppenstall, 1971; Yudoh et al., 2005) ↑ mitochondrial dysfunction ↑ inflammation (IL-1, IL-6, TNF-α) (Eisenberg et al., 2009; Loeser et al., 2002) ↓ autophagy (Ruiz-Romero et al., 2009; Grishko et al., 2009)
Osteoporosis	Imbalance between bone formation and resorption (Sharif et al., 2004)	↓ bone mass (Sharif et al., 2004)	↑ oxidative stress (Sod1, Sod2, GPx, GST, NOX4) (Chang et al., 2009; Onal et al., 2013) ↑ inflammation (NFkB, IL-1, IL-6, TNF-α) (Kimble et al., 1995; Lorenzo et al., 1998) ↓ autophagy (ATG7, Beclin1) (Chen et al., 2003)
Cardiovascular diseases (CVD) Atherosclerosis	Arterial stiffening Compromised vasodilaton (Ong et al., 2007; Lakatta, 2015) Accumulation of lipid-laden macrophages + inflammatory cells (Ogami et al., 2004)	Atherosclerosis (Zhang et al., 2010) ↑ risk of • Myocardial infarction • angina • ischemic stroke • heart failure (Zhang et al., 2010)	↑ oxidative stress (nitrotyrosine, NOX, mitochondrial respiration, ↓Nrf2) (Mozaffarian et al., 2015; Sun, 2015; Seals et al., 2014; Tschudi et al., 1996; Celermajer et al., 1994) ↑ inflammation (NFkB, IL-1, IL-6, TNF-α) (Sun, 2015; Trott et al., 2011) ↑ senescence (IL-6, MCP1, ICAM1) (Nojiri et al., 2011) ↓ NO vasodilation (Mozaffarian et al., 2015; Matthews et al., 2006) ↓ autophagy (Beclin1, p62 accumulation) (Goettsch et al., 2013; Tatchum-Talom and Martin, 2004; Touyz, 2004)
Type 2 Diabetes Melitus (T2DM)	Inefficient insulin secretion Abnormal insulin response (Wang and Bennett, 2012)	Hyperglycemia (Wang and Bennett, 2012; Chang and Harley, 1995) Hemoglobin glycation ↑ risk of • vascular complications (*i.e.* stroke, coronary heart disease, retinopathy) • to develop dementia (206–209; 213–215)	↑ oxidative stress (Goldfine et al., 2013) ↑ inflammation (NFkB, IL-1, IL-6, TNF-α, CRP, MCP-1) (Wild et al., 2016; Tsugane and Inoue, 2010; O'Neill and O'Driscoll, 2015; Barbagallo and Dominguez, 2014; Whitmer, 2007; Mittal and Katare, 2016; Keane et al., 2015; Cai, 2009; Lumeng and Saltiel, 2011; Kang et al., 2009; Calegari et al., 2011; Purkayastha et al., 2011) ↑ mitochondrial dysfunction (ATP, mtDNA, PPAR) (Goldfine et al., 2013; Uchida et al., 2005; Tavana et al., 2009; Krishnamurthy et al., 2006) ↑ senescence (Calegari et al., 2011; Pradhan et al., 2001) ↓ autophagy (Armour et al., 2009; Ye, 2013; Park et al., 2014; Zhou et al., 2011)
Alzheimer's disease (AD)	Extensive neurodegeneration Aβ deposition (senile plaques) Tau accumulation (neurofibrillary tangles) (Rovira-Llopis et al., 2015; Chu et al., 2015; Shigihara et al., 2014; Marchetti and Masini, 2009)	Dementia (Muriach et al., 2014)	↑ oxidative stress (McAlpine et al., 2009) ↑ inflammation (Ly et al., 2013) ↑ mitochondrial dysfunction (Xie et al., 2013) ↑ senescence (Cribbs et al., 2012; Fernandes et al., 2014; Perry et al., 2010; Norden and Godbout, 2013; Mandrekar and Landreth, 2010) ↑ proteasome alterations and autophagy (Pan et al., 2011; Nicoll et al., 2006; Hock et al., 2003; Lin and Beal, 2006; Andersen, 2004; Pratico et al., 2001; Reddy et al., 2004; Velliquette et al., 2005)
Parkinson's disease (PD)	Loss of dopaminergic neurons, astrocytes and microgliosis Lewy bodies (α-syn aggregates) (Coskun et al., 2004; Hirai et al., 2001)	Severe motor disability Impaired quality of life Shortened life expectancy (Querfurth and LaFerla, 2010)	↑ oxidative stress (Reddy et al., 2010; Manczak et al., 2006; Nixon and Cataldo, 2006; Hara et al., 2006; Komatsu et al., 2006) ↑ inflammation (Lindersson et al., 2004; Petrucelli et al., 2002; Hardy, 2003; Fallon et al., 2006; Iwakura et al., 2005; Bertolin et al., 2015) ↑ mitochondrial dysfunction (α-syn, DJ-1, parkin, PINK1) (Reddy et al., 2010; Manczak et al., 2006; Nixon and Cataldo, 2006; Hara et al., 2006; Komatsu et al., 2006) ↑ proteasome failure (Cataldo et al., 1991) ↓ autophagy (LC3, Beclin-1) (Syme et al., 2002; Stern et al., 2011; Chaudhuri et al., 2006; Lee et al., 2012; Schapira et al., 1989; Perfeito et al., 2014)

to assess health status has shown positive age-dependent improvement only on immune function in the T cell compartment both in mice following rapamycin treatment (Neff et al., 2013) and in the elderly when treated for six weeks with a rapamycin analogue (RAD001) (Mannick et al., 2014). Rapamycin has been shown to have stimulatory effects on locomotor behaviour (Neff et al., 2013; Miller et al., 2011; Wilkinson et al., 2012; Flynn et al., 2013) and improves learning and memory (Neff et al., 2013; Majumder et al., 2012; Halloran et al., 2012) across several studies using different mouse strains and in both males and females. However, similar effects were observed in young mice questioning whether the effects observed were modulation of ageing *per se* (Neff et al., 2013). More importantly some of the effects have not been reproducible. For example no significant improvement on index of cardiac function was found in the study by Neff et al. (Neff et al., 2013) in contrast to Flynn et al. (2013) where mice showed a significant improvement in the ejection fraction and significantly less hypertrophy than the control group. The discrepancies may be due to the small size of the effect, which put into question whether the effect is of clinical value, differences in study design (longitudi-

Table 2
Pharmacological interventions as potential alternatives for compliance to dietary restriction (DR). A number of molecules targeting oxidative stress, autophagy, inflammation or the effects of accumulation of senescence cells are summarized, together with evidence that support their positive effects on more than one organ system in delaying the ageing phenotype.

Molecule	Target	Modified ageing mechanisms	Lifespan extension	Healthspan with age/disease improvement	Human testing
Rapamycin	mTORC1	Autophagy (Berger et al., 2006) Oxidative stress (Rotte et al., 2012; Kofman et al., 2012; Miwa et al., 2014)	Yes (reviewed in (Kaeberlein, 2014))	Cancer prevention Increase cognition, cardiac function, insulin sensitivity, mobility, decreased immune dysfunction (Neff et al., 2013; Wilkinson et al., 2012; Flynn et al., 2013; Fang et al., 2013)	Immunosuppression Cancer treatment (Hidalgo and Rowinsky, 2000) Prevention of immunosenescence (Mannick et al., 2014)
Metformin	Reduce hepatic glucose production AMPK activation (Zhou et al., 2001) Respiratory chain complex I (El-Mir et al., 2000)	Autophagy Oxidative stress Chronic inflammation (Martin-Montalvo et al., 2013)	Yes (Martin-Montalvo et al., 2013) and in patients with diabetes (Bannister et al., 2014)	Improved mobility, insulin sensitivity, decreased cataract formation, cancer (Martin-Montalvo et al., 2013; Yin et al., 2011; Anisimov et al., 2005)	Type 2 diabetes (Bosi, 2009) Decrease in cancer risk (Bodmer et al., 2010)
17αestradiol	Estrogen receptor	Oxidative stress (Gelinas et al., 2004) Inflammation (Stout et al., 2016)	Yes, in male mice (Harrison et al., 2014)	Neuroprotective (Simpkins et al., 1997), Based on 17βestradiol activity Alzheimer's disease, Parkinson, CVD	Tested as neuroprotector with conflicting outcomes (Moos et al., 2009)
Acarbose	αglucosidases inhibitor in the intestine (Harrison et al., 2014) Reduce release of glucose	Oxidative stress (Rosen and Osmers, 2006)	Yes (Harrison et al., 2014)	Diabetes Improved voluntary activity in female mice (Harrison et al., 2014) Decrease Myocardial infarct size (Minatoguchi et al., 2009)	Used for Diabetes, reduce risk of CVD (Standl et al., 2014)
NDGA	arachidonic acid 5-lipoxygenase inhibitor	Inflammation (West et al., 2004) Oxidative stress (Shishido et al., 2001)	Yes (Harrison et al., 2014; Strong et al., 2008)	Neuroprotective (Shishido et al., 2001) Increase insulin sensitivity (Reed et al., 1999) Anti-cancer *in vitro* (Youngren et al., 2005)	Used in prostate cancer patients with no effects
Aspirin	COX inhibitor	Inflammation (Kopp and Ghosh, 1994) Oxidative stress (Podhaisky et al., 1997)	Yes male mice only (Strong et al., 2008)	Reduce CVD, anti-inflammatory	Non steroidal anti-inflammatory and anti-thrombotic (prevention of CVD and stroke) (Group et al., 1997)
ACE inhibitors	Angiotensin II inhibitors	Oxidative stress, mitochondria survival (Benigni et al., 2009)	Yes in Agtr1a-/- (Benigni et al., 2009); polymorphism associated with longevity in humans (Benigni et al., 2013)	Decreased cardiac vascular injury (Benigni et al., 2009)	High blood pressure
Quercetin	Inhibit PI3 K, mTOR, serpines (Bruning, 2013)	Eliminate senescent cells (Zhu et al., 2015)	N/A	Given in combination with Quercetin Improved CVD, mobility Osteoporosis, frailty (Zhu et al., 2015)	Approved for human use but little evidence of efficacy in any application
Dasatinib	Tyrosine kinase inhibitor (Montero et al., 2011)	Induce apoptosis(Bannister et al., 2014), eliminate senescence cells (Zhu et al., 2015)	N/A	Given in combination with quercetin, improved CVD, mobility Osteoporosis, frailty (Zhu et al., 2015)	Cancer treatment (Talpaz et al., 2006)
ABT263	BCL-2 and BCL-xL inhibitor (Tse et al., 2008)	Induce apoptosis (Tse et al., 2008), eliminate senescent cells (Chang et al., 2016)	N/A	Recover fitness of hematopoietic and muscle stem cells (Chang et al., 2016)	Cancer treatment (Gandhi et al., 2011)
Zoledronate	FPP synthase inhibitor	Enhance DNA damage repair (Misra et al., 2016)	Yes in combination with statin in HGPS (Varela et al., 2008) & patients with osteoporosis (Colón-Emeric et al., 2010)	N/A	Osteoporosis, cancer bone loss (Russell, 2011)
Methylene Blue	Increases activity of mitochondria complex IV (Atamna et al., 2008)	Senescence, mitochondrial activity (Atamna et al., 2008), oxidative stress	Yes, maximal lifespan in female (Harrison et al., 2014)	Neurodegenerative diseases (AD, PD) (Yang et al., 2015)	Ifosfamide induced encephalopathy (Pelgrims et al., 1999)

Table 2 (*Continued*)

Molecule	Target	Modified ageing mechanisms	Lifespan extension	Healthspan with age/disease improvement	Human testing
PAI-1 Inhibitors	Inhibitors of plasminogen activator inhibitor-1 (PAI-1)	Induce fibrinolysis, delay senescence (Eren et al., 2014)	Yes in klotho-/- mice (Eren et al., 2014)	Improves renal, lung structure in klotho-/- mice (Eren et al., 2014), reduce hypertension, vascular senescence (Boe et al., 2013), thrombotic disorders (Hennan et al., 2005)	Molecules have been patented but none has been trialled in humans (Fortenberry, 2013)
Mitochondria targeted anti-oxidant peptides (MitoQ and SS-31)	Increase mitochondrial biogenesis, anti-oxidant (Kelso et al., 2001)	Oxidative stress (Kelso et al., 2001)	Yes in *C. elegans* (MitoQ) (Ng et al., 2014)	Reduce AD progression (McManus et al., 2011), PD (Yang et al., 2009), Improve muscle weakness (Siegel et al., 2013) CVD (Dai et al., 2014), diabetes (Anderson et al., 2009)	SS-31 Ischemia reperfusion injury (Chakrabarti et al., 2013), MitoQ clinical trial in PD (Snow et al., 2010) and liver damage (Gane et al., 2010)
Minocycline	Broad spectrum Tetracycline antibiotic, inhibits Kynurenine formation from Tryptophan	Inhibit inflammation (Kelly et al., 2004), oxidative stress (Kraus et al., 2005; Morimoto et al., 2005)	Yes in Drosophila (Oxenkrug et al., 2012), *C. elegans* (Ye et al., 2014)	Attenuates neurodenegerative disease (Blum et al., 2004)	Acne vulgaris (Strauss et al., 2007)
JAK inhibitors	JAK1 and JAK2	Inhibition of SASP (Xu et al., 2015)	N/A	Improve muscle weakness (Xu et al., 2015)	Myelofibrosis (Harrison et al., 2012; Pardanani et al., 2013; O'Shea et al., 2015; Verstovsek et al., 2012)

nal *vs* cross-sectional), group size, gender (male *versus* female), genetic background of the mice, route of delivery and treatment duration. In this respect prolonged rapamycin treatment resulted in improved metabolic profiles, increased oxygen consumption and ketogenesis and markedly enhancing insulin sensitivity (Fang et al., 2013). In contrast, mice exposed to a more acute rapamycin treatment showed impaired glucose tolerance (Lamming et al., 2012). More importantly signs of nephro- and gonadotoxicity have been found following prolonged rapamycin treatment (Neff et al., 2013) putting into question whether rapamycin's beneficial effects can be translated to humans. One major caveat to all the studies is the use of a single dose of rapamycin and a single regime. Different systems may be sensitive to different doses or length of time of treatment. More work is required to identify the correct dose, time and length of administration so that this is suitable to correct the many signs of ageing and reduce the side effects (Kaeberlein, 2014). Significant research effort is currently being undertaken to identify safer drugs, termed rapalogs, which can provide all the benefits of rapamycin on lifespan and healthspan without the unwanted side-effects (Lamming et al., 2013).

More recently metformin has taken central stage. It is a clinically approved drug commonly prescribed as an anti-hyperglycaemic agent in the treatment of type 2 diabetes (Campbell et al., 1996). Long-term treatment with low-dose metformin starting in middle age has been shown to promote healthy ageing and longevity in male mice by 4–6%, although a higher dose shortens longevity (Martin-Montalvo et al., 2013). A similar small but significant increase in survival was observed in patients with diabetes treated with metformin compared to patients without diabetes (Bannister et al., 2014). Metformin has also been shown to improve global metabolic fitness similarly to dietary restriction in aged mice, resulting in improved endurance, insulin sensitivity, reduced oxidative damage and chronic inflammation, reducing tumorigenesis (Martin-Montalvo et al., 2013; Anisimov, 2015) and this was mirrored by a decreased cancer risk in patients with diabetes (Noto et al., 2012). In addition a recent study linked this antineoplastic activity of metformin to inhibition of the SASP by interfering with proinflammatory NF-κB signalling (Moiseeva et al., 2013). These evidences led to the FDA approval of a clinical trial to investigate the action of metformin on human ageing in the Targeting/Taming Ageing With Metformin (TAME) study. This is to generate data as proof of principle that the ageing process can be viewed as an indication for pharmacological intervention. Metformin success will be judged by whether it can delay the development of several diseases whose incidence increases dramatically with age: cardiovascular disease, cancer, and cognitive decline, along with mortality. Whilst a success will fast forward research into such interventions, there are concerns that it is too early for such a large trial and more work should be performed to thoroughly assess the effects of these molecules on multiple systems, their reproducibility and their safety profile for prolonged doses in healthy individuals in a heterogeneous population as this is largely unknown. Particular consideration should be given to the age-group and some of the problems related to drug dosing and pharmacokinetics. For example metformin is renally excreted and renal impairment is more common in the elderly. In addition such trials are very expensive and cannot represent the way these drugs will be tested. More work needs to be performed to identify clinical situations and biomarkers that allow testing over a shorter period of time and design of preclinical intervention programmes that are standardised and reflect clinical endpoints.

6. Conclusions

The ultimate goal of geroscience, the field that investigate the relationship between ageing and age-related disorders, is to devise intervention strategies to prolong health span rather than treating individual diseases. Whilst proof-of-concept studies are available in support of such a strategy, there are great challenges ahead. A more systematic investigation of the mechanisms of ageing leading to multiple diseases is required to understand the key nodes to target for intervention and to identify biomarkers for both stratification of patients requiring interventions and monitoring of their efficacy. Pharmacological interventions need to be thoroughly tested in preclinical studies using multiple models, exposed to clinically relevant stresses according to standardised methodologies with endpoints that are clinically meaningful. Large infrastructures are required to support the size of the studies that will involve multiple dosing, regimens, models and interdisciplinary expertise.

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