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Mouse models of ageing and their relevance to disease

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ABSTRACT

Ageing is a process that gradually increases the organism's vulnerability to death. It affects different biological pathways, and the underlying cellular mechanisms are complex. In view of the growing disease burden of ageing populations, increasing efforts are being invested in understanding the pathways and mechanisms of ageing. We review some mouse models commonly used in studies on ageing, highlight the advantages and disadvantages of the different strategies, and discuss their relevance to disease susceptibility. In addition to addressing the genetics and phenotypic analysis of mice, we discuss examples of models of delayed or accelerated ageing and their modulation by caloric restriction.

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1. Introduction

The simplest and best model of ageing is an old organism. This approach has been successful when using lower organisms such as *C. elegans* and *Drosophila*, but studies on mice are hampered by cost and time. However, what makes the mouse an attractive model for studying mammalian biology is the genetic manageability of its genome, ease of breeding, and the large amount of available baseline phenotypic data. Similarities and differences between mouse and man in relation to studies on ageing have been reviewed (Vanhooren and Libert, 2013). Here, we examine some recent mouse studies on ageing to highlight the advantages and disadvantages of various strategies, with a particular emphasis on genetics and phenotypic analysis. We also discuss examples of modulating ageing in mice genetically or by caloric restriction (CR).

2. Importance and usefulness of genetic background

Inbred mice are widely used in research. The advantages of these strains are defined genomes, stable phenotypes, and extensive data that are freely available from published studies and from databases such as mouse genome informatics (MGI) and mouse phenome database (MPD) (Eppig et al., 2015; Bogue et al., 2016). However, though the phenotypes have often been characterized in detail, phenotypes resulting from specific allelic variants in inbred strains can often be overlooked. As we examine the mammalian genome in more detail with ever increasing detailed phenotyping, we will no doubt discover other important variants. The most commonly used inbred strain is probably C57BL/6J, which carries several known allelic variants that could influence phenotypic outcomes of manipulations or treatments. Notably, an allelic variant of the *Cdh23* mutation delays the onset of hearing loss (Henry and Chole, 1980; Johnson et al., 2006). As hearing loss affects various behaviors, this strain is unsuitable for mutational studies on age-related hearing loss. More importantly, C57BL/6J mice carry a deletion in nicotinamide nucleotide transhydrogenase (*Nnt*) (Toye et al., 2005) that impairs glucose tolerance (Freeman et al., 2006). This is particularly important because of the close relationship between metabolism and ageing (Newgard and Pessin, 2014). Other strain-specific differences are in bone mineral density (Beamer et al., 1996), tumor susceptibility, and atherosclerosis (Paigen et al., 1987a, 1987b), all of which are relevant to age-related disease. Such allelic variation can result in phenotypic differences even between closely related strains (Simon et al., 2013; Vanden Berghe et al., 2015). The variation between inbred strains is not restricted to primary phenotypic differences but can influence the response to interventions. The response to CR is clearly strain specific (Liao et al., 2010a), demonstrating the influence of genetic background on the outcomes of such studies. Elucidation of this phenomenon may enhance our understanding of how dietary restriction pathways regulate ageing.

Strain-specific differences and the need for models that reflect the genetic diversity of human populations has led to the development of strategies to generate genetically diverse mouse populations. These strategies include recombinant inbred (RI) mice (Williams et al., 2004), collaborative cross (CC) mice (Churchill et al., 2004), and now the diversity outbred (DO) (Churchill et al., 2012; Svenson et al., 2012) strains derived from CC mice. The CC lines are multiple inbred lines with a wide genetic diversity, thus negating the need for mapping, but each DO mouse is genetically unique. Inbred Long Sleep (ILS) ILSXISS RI strains have been used to demonstrate that the effect of dietary restriction (DR) is under genetic control (Liao et al., 2010a); lifespan was extended in some strains but shortened in others. These strains can be used to study the genetic basis of complex traits, but such studies rely on extensive genetic variation already present in the founder strains. While the introduction of genetic variations to study specific pathways or allelic variants provides a better model of the genetic diversity in humans, it can present logistical problems. The advent of CRISPR technologies (Wang et al., 2013) has made it possible to introduce mutations in the CC strains, but this would involve making mutations in multiple strains, which is possible but expensive. For mixed strains, such as the UM-HET3 derived from four strains used in the Intervention Testing Program (ITP) (Nadon et al., 2008; Miller and Chrisp, 1999), a mutation can be introduced into one of the strains, followed by normal breeding. However, this will fix the region around the mutation to that of a single founder strain and reduce the overall genetic variation. Furthermore, introduction of recessive variants requires the introduction of the genetic variant in more than one strain or a more extensive breeding protocol. This is possible but more expensive in terms of breeding. Depending on how breeding is managed, even outbred mouse colonies can suffer from reduced genetic variance (Yalcin et al., 2010).

Combining genomes randomly can generate unexpected results, as exemplified by the study of Bygraves et al. These authors demonstrated that sequence variants between 129 and C57BL/6J mice in a chromosomal region linked to the development of systemic lupus erythematosus (SLE) resulted in disease on a mixed genetic background but not in the inbred strains, thus confounding the phenotypic interpretation of various knock out lines generated in this region because of the retention of flanking DNA (Bygrave et al., 2004). The importance of sequence variants is further supported by the substantial influence of passenger mutations in congenic mice on interpreting phenotypic data (Vanden Berghe et al., 2015). Thus, while a mixed background may be a more relevant model of the outbred human population, complications could arise. The influence of such variations can be examined by sufficiently powered experimental design (Miller et al., 2007). In particular, inter-strain differences should be taken into consideration when introducing a mutation and thereby fixing a region of the genome in that population of mice. Modifiers of phenotypes are useful in revealing genetic control of phenotypes by QTL studies. In ageing studies, genetic modifiers have proved useful in demonstrating the genetic control of phenotypes associated with immune ageing (Jackson et al., 1999) and with lifespan (Jackson et al., 2002).

In addition to considering the range of strains available, the phenotyping process should also be considered. There is an increasing number of phenotypic tests that can be used in mice to study the effects of perturbations such as ageing on physiological systems. The use of more detailed phenotyping of parameters reflecting health status has shifted the emphasis from lifespan to health-span. A large-scale analysis of ageing in 31 inbred strains was undertaken at the Jackson Laboratories as part of the Ageing Phenome Project. The study provided an insight into the phenotypic variation that occurs in these strains as they age (Yuan et al., 2009). Cohorts of 32 male and 32 female mice were examined in a longitudinal study and phenotyped at different times throughout their life. Phenotyping of the cohorts included lifespan, hormone levels (including insulin-like growth factor 1; IGF-1), clinical chemistry, peripheral blood leucocyte populations, electrophysiology, and hematological, metabolic and sleep analysis. This study demonstrated a link of IGF-1 levels with longevity, as well as with several other important age-related conditions, such as changes in renal function (Tsaih et al., 2010), immune cell populations (Petkova et al., 2008), gait (Wooley et al., 2009), and cardiac function (Xing et al., 2009). Such analyses yield important reference data (Bogue et al., 2016) for researchers and help to build a picture of ageing and disease in these strains. These analyses also make it possible to identify the optimum age for studying a particular strain. Nevertheless, further

in depth phenotypic analysis is needed to understand the ageing of inbred strains.

Phenotypic data are being increasingly incorporated into ageing studies. However, there is no agreement on the range of phenotypic tests, study time points and other parameters. Standard operating procedures (SOPs) have been employed successfully in large scale phenotyping programs (Ayadi et al., 2012; de Angelis et al., 2015; White et al., 2013; Brown and Moore, 2012; Brown et al., 2006, 2005). SOPs are needed to reduce inter-laboratory variations, such as the differences in the reported effects of CR in primates (Mattison et al., 2012; Colman et al., 2014). The ITP uses standardized protocols at three sites (Nadon et al., 2008) to unify the diet and environment in order to ensure reproducibility. However, despite strict SOPs and efforts to reduce variability, differences between sites have been identified; most probably due to environmental differences. There was a difference in survival of males across the three sites of the ITP (Nadon et al., 2008), and the EUMODIC program identified site-specific differences in some phenotypic parameters. This highlights the difficulty of developing robust protocols, which may have to include even metadata such as diet and age of the mice used for breeding because these factors might have long term effects on the offspring (Martin-Gronert and Ozanne, 2013).

Detailed phenotyping is now being employed in studies on ageing to investigate comorbidities associated with ageing and to investigate longevity. Additionally, emerging, defined phenotyping pipelines (Ackert-Bicknell et al., 2015) take into consideration the differences between longitudinal and cross sectional designs and the effect of previous tests, which are particularly important in behavioral testing. The importance of comprehensive phenotyping is underlined by the known side effect of rapamycin treatment (testicular degeneration), which may hint at other unknown effects. Detailed phenotypic analysis will also help elucidate the effects of rapamycin treatment and dietary restriction, both of which lengthen lifespan and health-span but have different effects on metabolism (Miller et al., 2014).

Neff et al. demonstrated the value of using a wide range of phenotyping, and in particular phenotyping over the lifespan of a mouse. They found that rapamycin treatment had early effects on cognitive function but little effect on many ageing phenotypes, and that lifespan extension was primarily due to reduction of tumor burden. This led to the conclusion that rapamycin has limited effects on ageing. This interpretation has been challenged (Blagosklonny, 2013). Nevertheless, this study does demonstrate the importance of detailed phenotyping throughout the life of a mouse.

Data from studies on DO strains demonstrate that complex, time consuming phenotypic tests that are highly relevant to human ageing can be fruitful; they show that preservation of neuronal structures in old mice is correlated with better memory (Koh et al., 2014).

3. Mouse models of ageing

Ageing research on mice is expensive and time-consuming. Therefore, it is tempting to study ageing using mice with accelerated ageing and/or reduced lifespan (Table 1). However, these studies should be interpreted with care. Those mice might show typical features of ageing, but they often exhibit features not seen in normal old mice. A parallel to this is seen in patients with Hutchinson-Gilford progeria syndrome, a genetic condition characterized by the rapid appearance of ageing beginning in childhood. Although these patients display some characteristics of normal ageing, they also present many characteristics not seen among the elderly. Consequently, all available mouse models of accelerated ageing do not fully represent normal ageing in mice (Miller, 2004). Nevertheless, mouse models of accelerated ageing can provide insight into the mechanisms of deterioration seen in normal physiological ageing and into the premature ageing process in progeroid syndromes, as long as these differences are kept in mind (Vanhooren and Libert, 2013). Research on mice with delayed ageing might also provide insight into how ageing rate is controlled. This could be a first step towards development of drugs to slow ageing and subsequently to delay disease progression (Miller et al., 2011).

3.1. Progeroid syndromes

Progeroid syndromes are extremely rare human diseases characterized by premature ageing and shortened lifespan. Progeroid syndromes include laminopathies (such as the Hutchinson-Gilford progeria syndrome; HGPS), which affect nuclear envelope homeostasis, and diseases affecting DNA repair mechanisms and telomere length, such as Cockayne syndrome, Werner syndrome (WS), ataxia telangiectasia, and trichothiodystrophy (Liao and Kennedy, 2014). These conditions exhibit many features of human normal ageing physiology, and identification of the mutations causing them has allowed the generation of mouse models of premature ageing. HGPS and WS are the best characterized progeria mouse models. Wolfram syndrome (WFS) is a very rare genetic disorder caused by mutations in the *WFS1* gene and characterized by diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) (Wolfram and Wagener, 1938; Collier et al., 1996). Lifespan is reduced in WFS and average life expectancy is 30 years. Although the specific function of WFS1 is not known, Wolframin, the transmembrane protein encoded by the *WFS1* gene, is thought to be involved in regulation of endoplasmic reticulum (ER) stress and calcium homeostasis.

3.1.1. Mouse models of Hutchinson-Gilford progeria syndrome

HGPS begins within the first two years of life and average life expectancy is 13.5 years. Patients display growth retardation, lipodystrophy, scleroderma, bone abnormalities (such as joint stiffening and impaired mobility due to osteodysplasia with osteolysis), alopecia, and midface hypoplasia. Cardiovascular complications, such as myocardial infarction or stroke due to premature atherosclerosis, are the most frequent cause of death. HGPS is a progeroid laminopathy caused mainly by *de novo* point mutations in the nuclear lamina A (*LAMNA*) (De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003). The gene encodes A-type lamins (lamin A/C), which form the nuclear envelope. Mutations alter the nuclear morphology and function, cause genomic instability, and provoke aberrant cell proliferation. The most frequent HGPS mutation is a deletion in the C-terminal domain that eliminates a farnesylated proteolytic cleavage site for the zinc metalloproteinase ZMPSTE24. The result is an accumulation of a farnesylated aberrant protein called progerin, which also occurs during normal ageing.

Mouse models of HGPS are models of laminopathies based on modification of the *Lamna* gene or its processing enzyme (Zhang et al., 2013a). $Lmna^{HG}$ knock-in mice (Yang et al., 2005, 2008) express only progerin (the farnesylated uncleaved form of lamin A), the accumulation of which leads to alteration of the nuclear envelope. These mice lose subcutaneous fat and hair, develop osteoporosis, and die prematurely without cardiovascular conditions. $Lmna^{G609G}$ mice (mutation in 1827C>T; Gly609Gly) also accumulate progerin due to defective splicing of the gene and develop abnormal nuclear morphology (Osorio et al., 2011). At three weeks of age, they exhibit growth retardation, reduced weight gain, curvature of the spine and vascular calcification (Villa-Bellosta et al., 2013), and they die at about 3.5 months (Osorio et al., 2011). They also develop hypoglycemia and decreased level

Table 1
Mouse models of accelerated ageing.

Mouse model	Gene defect	Physiologic alterations[a]	Age of disease onset	Human disease[b]	Relevant References[c]
$Lmna^{HG}$ knock-in mice	Farnesylated uncleaved form of LMNA	Loss of subcutaneous fat, hair loss and osteoporosis	6–8 weeks	HGPS	(Yang et al., 2005, 2008)
$Lmna^{G609G}$ knock-in mice	Mutation in *Lmna* gene (1827C > T)	Growth retardation, curvature in the spine, vascular calcification, infertility, decreased insulin-like growth factor levels	3 weeks	HGPS	(Osorio et al., 2011; Villa-Bellosta et al., 2013)
$Zmpste24^{-/-}$	Deficiency in metallopeptidase *Zmpste24* gene	Growth retardation, alopecia, muscle weakness, bone fractures, dilated cardiomyopathy, muscular dystrophy and lipodistrophy	6–8 weeks	HGPS	(Bergo et al., 2002; Pendas et al., 2002)
$Wrn^{-/-}$	WRN protein deficiency	Hypertriglyceridemia, insulin resistance, elevated levels ROS, DNA damage and increased incidence of cancer	5 months	WS	(Lebel and Leder, 1998; Massip et al., 2006)
$Wrn^{-/-}$ $Terc^{-/-}$	Double deficiency in *Wrn* and *Terc* genes	Shortening of telomeres, hair graying, alopecia, osteoporosis, type 2 diabetes, cataracts and cancer	late-generation mice	WS	(Chang et al., 2004)
$Wfs1^{-/-}$	*Wfs1* gene deficiency	Growth retardation, reduced number of pancreatic islets, diabetes, decreased fertility	16 weeks	WFS	(Koks et al., 2009; Noormets et al., 2009; Ishihara et al., 2004)
β-cell specific $Wfs1^{-/-}$	*β-cell specific Wfs1* gene deficiency	Reduced body weight and low insulin levels diabetes	12 weeks	WFS	(Riggs et al., 2005)
$Wfs1^{-/-}$	*Wfs1-LacZ fusion* gene	Psychiatric symptoms, diabetes and growth retardation	3–4 months	WFS	(Noormets et al., 2011; Luuk et al., 2009)
$Cisd2^{-/-}$	Cisd2 deficiency	Early-onset degeneration of central and peripheral nerves, opaque eyes and blindness, decrease in bone density, muscle atrophy, mitochondrial degeneration, impaired glucose tolerance and premature death	8 weeks	WFS2	(Chen et al., 2009)
PolG	Mutation in mtDNA *Pol* γ gene	Kyphosis, alopecia, anemia, weight loss, reduction in median lifespan, sarcopenia, hearing loss, reduced fat mass and bone mineral density, cardiomyopathy.	Onset strain dependent	Early ageing phenotype	(Trifunovic et al., 2004; Kujoth et al., 2005)
$PolgA^{mut/mut}$	Mutations in mtDNA PolgA	Reduced lifespan and premature onset of ageing-related phenotypes such as weight loss, reduced subcutaneous fat, alopecia, kyphosis, osteoporosis, anemia, reduced fertility and heart enlargement	~25 weeks	Early ageing phenotype	(Trifunovic et al., 2004)
$Nfkb1^{-/-}$ mice	*Nfkb1*deficiency	Decrease in lifespan, premature age-related skeletal changes, increased tissue inflammation and central nervous system gliosis, reduced apoptosis and increased cellular senescence	Middle-aged mice	Early ageing phenotype	(Bernal et al., 2014)
$IL\text{-}10^{tm/tm}$	IL-10 deficiency	Anemic, extensive enterocolitis, growth retarded, increased lethality, iron depletion, faster muscle strength decline	3–4 weeks	Human frailty syndrome	(Kuhn et al., 1993; Walston et al., 2008)
$Klotho^{-/-}$	*Klotho* gene deficiency	Infertility, arteriosclerosis, skin atrophy, osteoporosis and emphysema	4 weeks	Early ageing phenotype	(Kuro-o et al., 1997)
XPD	Mutations in the DNA helicase *Xpd* gene	Osteoporosis, osteosclerosis, early graying, cachexia and infertility	3 months	Trichothiodystrophy	(de Boer et al., 2002)
$ERCC1^{-/-\text{or}\Delta/-}$	Mutation and/or deletion in the *Ercc1* gene.	Growth and liver failure, nuclear anomalies, increased mortality, spleen hypoplasia, altered isotype switching, B cell hypoproliferation, dystonia, ataxia, renal failure, sarcopenia, kyphosis, early replicative ageing, increased sensitivity to oxidative stress, poor coordination, loss of visual acuity, uraemic encephalopathy and proteinuria	4 weeks	Premature ageing phenotype	(Weeda et al., 1997; Lawrence et al., 2008)
$Terc^{-/-}$	*Terc* deficiency	Shortened lifespan, overall frailty, decreased fertility and tissue atrophy with impaired organ function	Late-generation	Premature ageing phenotype	(Lee et al., 1998)

(HGPS, Hutchinson-Gilford progeria syndrome; ERCC1, excision repair cross complementing 1; IL-10, interleukin-10; Lmna, Lamin A; PolG, Polymerase γ; Terc, Telomerase RNA component; Wfs, Wolfram syndrome; Wrn, Werner syndrome ATP-dependent helicase; WS, Werner syndrome; XPD, xeroderma pigmentosum, complementation group F; Zmpste24, zinc metalloproteinase Ste24).

[a] Observed physiological alterations.
[b] Corresponding human condition.
[c] Relevant references listed.

of insulin-like growth factor. Two independently generated mouse models deficient in the *Zmpste24* gene (*Zmpste24*$^{-/-}$ mice) showed accumulation of the farnesylated prelamin A protein (Bergo et al., 2002; Pendas et al., 2002). Although lack of the *ZMPSTE24* gene in humans causes perinatal death, *Zmpste24*$^{-/-}$ mice were viable and displayed a phenotype similar to that of HGPS patients. One of the *Zmpste24*$^{-/-}$ mouse models developed growth retardation, alopecia, muscle weakness and bone fractures (Bergo et al., 2002) while the other displayed dilated cardiomyopathy, muscular dystrophy and lipodystrophy, and died prematurely (Pendas et al., 2002).

3.1.2. Mouse models of Werner syndrome

WS, also known as 'progeria of the adult,' is manifested during adolescence (10–20 years) and with an average median lifespan of 54 years (Cox and Faragher, 2007). WS patients have cataract, growth retardation, early graying, hair loss, scleroderma-like skin changes, osteoporosis, diabetes, atherosclerosis and high incidence of cancer (Burtner and Kennedy, 2010). The condition is caused by mutations in Werner syndrome ATP-dependent helicase (WRN), an enzyme with helicase and exonuclease activity involved in DNA metabolism and in telomere homeostasis. The gene defect produces genomic instability, including impaired DNA damage repair and aberrant proliferation (Burtner and Kennedy, 2010).

Several mouse models of WS have been generated (Liao and Kennedy, 2014). Mice deficient in the helicase unit of WRN (*Wrn*$^{-/-}$ mice) display hypertriglyceridemia, insulin resistance, elevated levels of reactive oxygen species (ROS), oxidative DNA damage, increased incidence of cancer, and a shortened lifespan (Lebel and Leder, 1998; Massip et al., 2006). Although *Wrn*$^{-/-}$ mice display molecular and cellular alterations similar to those in WS patients, they do not develop symptoms of accelerated ageing (Lombard et al., 2000). However, double-mutant mice lacking both *Wrn* and Telomerase RNA component (also known as *Terc*) genes recapitulate the progeroid symptoms closely related to WS (Chang et al., 2004). These mice exhibit shortening of telomeres, premature death, hair graying, alopecia, osteoporosis, type II diabetes, cataracts, increased chromosomal instability and cancer. These studies indicate that telomere attrition is a key factor in the manifestation of WS deficiencies. Similarly, Wrn$^{-/-}$ p53$^{-/-}$ double-deficient mice have a higher mortality rate than Wrn$^{+/-}$ p53$^{-/-}$ mice, possibly due to increased genome instability or to acceleration of the cancer phenotype by WRN deficiency (Lombard et al., 2000).

3.1.3. Mouse models of Wolfram syndrome

The function of the *WFS1* gene is still unknown, but there is evidence for its involvement in cell survival and degeneration (Koks et al., 2009). A mouse model with WFS1 deficiency has shown that a non-functional *Wfs1* gene causes growth retardation and shortens life expectancy (Koks et al., 2009). Moreover, these mice display activation of degenerative biological processes, resulting in a reduced number of pancreatic islets, body weight and fertility (Noormets et al., 2009). WFS1-deficient mice have permanently activated ER stress, which leads to degeneration of several tissues.

Three different groups have developed WFS1-deficient mice. The first WFS1 mutant mouse was developed by inserting a Neomycin (Neo) resistance cassette into exon 2 of the *Wfs1* gene, resulting in altered splicing of transcripts in the brain, heart and islets (Ishihara et al., 2004). The authors speculate that the Neo insertion generates amino-terminus truncated WFS1 protein due to translation from one of the internal start codons. At least three transcripts are annotated in the Ensembl database. Therefore, this kind of Neo cassette knock-in might cause truncation of the WFS1 protein without disturbing the reading frame. The non-fasted blood glucose levels in these WFS1 mutant mice started to rise at the age of about 16 weeks, and by 36 weeks of age more than half of the mice had overt diabetes (Ishihara et al., 2004). Interestingly, this effect was dependent on the genetic background of mice, and diabetes was not observed in the C57BL/6J background. Again, this emphasizes the influence of the genetic background on the phenotype. In addition, significantly reduced insulin secretion and increased apoptosis were found in these mice. Decreased insulin secretion was caused by impaired coupling of stimulus to secretion in WFS1-deficient β-cells. Interestingly, the authors did not see reduced weight gain, blindness or any other features of WFS.

Another WFS1 mutant mouse was made by using conditional gene targeting to target WFS1 specifically in pancreatic islets. Exon 8 of the *Wfs1* gene was floxed and mutant mice were crossed with rat insulin promoter (RIP-) Cre mice, resulting in deletion of exon 8 in the *Wfs1* gene specifically in β-cells (Riggs et al., 2005). As expected, these mice had pronounced diabetes, low blood insulin levels, and impaired glucose tolerance. Interestingly, while the WFS1 deficiency was islet-specific, these mice exhibited lower body weight starting from the age of 24 weeks. This difference in body weight was small but clearly significant. In addition, studies on these mice verified that WFS1 deficiency causes apoptosis and increases the unfolded protein response (UPR) (Riggs et al., 2005).

Finally, the third available mouse model for WFS was developed using a more conventional approach and deleting exon 8 almost entirely (Koks et al., 2009), resulting in generation of a small N-terminal part of WFS fused to LacZ (Koks et al., 2009). This strategy was chosen to obtain a model with maximal similarity to WFS. Indeed, these WFS1-deficient mice display a severe phenotypic abnormality that resembles WFS in all the features studied so far. The most significant finding is that these mice stop growing at eight weeks of age and that they are 30% smaller than wild type littermates (Koks et al., 2009). Despite being small, the growth hormone (GH) pathway is activated in these mice and they have increased IGF-1 levels (Koks et al., 2009). Diabetes is caused by gradual degeneration of pancreatic islets and develops progressively (Noormets et al., 2011). Moreover, fertility is reduced in WFS1-deficient mice (Noormets et al., 2009), which has also been observed in WFS patients. Finally, psychiatric symptoms are characteristic in WFS patients, and WFS1-deficient mice also display changes in the physiology of emotions and behavioral adaptation (Luuk et al., 2009).

CDGSH iron sulfur domain (CISD2) gene has been identified as a second causative gene associated with Wolfram syndrome (Amr et al., 2007) and Cisd2 deficiency causes mitochondria-mediated phenotypic defects in mice (Chen et al., 2009). Cisd2$^{-/-}$ mice exhibit many clinical manifestations of WFS patients including early-onset degeneration of central and peripheral nerves and premature death, as well as impaired glucose tolerance (Chen et al., 2009).

Collectively, these findings show that the different mouse models of WS are good models for studying age-related processes and degeneration.

3.2. Mitochondrial mutations and ageing

There is a correlation between age and the accumulation of mitochondrial mutations and attenuation of mitochondrial function (Kennedy et al., 2013; Kazachkova et al., 2013). Hence, it has been postulated that there may also be a causative link between the changes in mitochondria and the development of age-related disease (Lee and Wei, 2012; Sun et al., 2016). As with nuclear DNA there are specific mechanisms to maintain the integrity of the mitochondrial genome during replication. There is a single DNA polymerase in eukaryotes that is known to act within mitochondria; mtDNA polymerase-γ (Polγ). This holoenzyme consists of a catalytic subunit encoded by *PolgA* and dimeric subunit encoded

by *Polg2* (reviewed in (Copeland and Longley, 2014)). The proof reading activity of pol γ can be reduced drastically by the substitution of critical residues, such as Asp198 and Glu200 (Longley et al., 1998), thus increasing the inherent error rate of polγ. In wild type mice it is this inherent error rate which is thought to be the major cause of mutation accumulation in the mitochondrial genome during ageing (Kennedy et al., 2013; Michikawa et al., 1999; Cortopassi et al., 1992; Cortopassi and Arnheim, 1990). Thus, by reducing the efficiency of the mitochondrial polγ and thereby the accumulation of mitochondrial mutations, the relationship between mitochondrial dysfunction and age-related phenotypes can be investigated in these mice.

Inactivation of polγ proof reading in mice by mutating the critical Asp257 resulted in several phenotypes related to ageing developing in young mice in two separate studies (Trifunovic et al., 2004; Kujoth et al., 2005). Both sets of mutant mice (referred to as PolG mice) exhibited kyphosis, alopecia, anemia, and weight loss and there was a significant reduction in median lifespan to 48 weeks (Trifunovic et al., 2004) and 59 weeks (Kujoth et al., 2005) in the separate studies. Further to this there was reduced fat mass and reduced bone mineral density by 40 weeks and cardiomyopathy in the PolG mice. Sarcopenia and hearing loss were detected in the study by Kujoth *et al.* (Kujoth et al., 2005), both age-related phenotypes. Estimates of the increased rate of mutation in different tissues from both strains were between 3 and 8 fold higher than in wild type mice. There are slight variations in the onset of these phenotypes between the two mutant strains which may reflect the influence of background strain or environment but both lines display a similar accelerated development of multiple phenotypes associated with ageing. PolG mice exhibited the same levels of ROS damage and oxidative stress as wild type mice in tissue homogenates and MEFs from PolG mice did not exhibit accelerated cellular senescence *in vitro* (Kujoth et al., 2005). There was, however, evidence of increased levels of apoptosis observed in the mutant mice (Kujoth et al., 2005). The reduced assembly of mitochondrial encoded components of the electron transport chain in muscle is thought to result in a reduced mitochondrial membrane potential and thus induces apoptosis (Hiona et al., 2010). Thus, the causal link between the increased mutation load and accelerated phenotypes in these mice is thought to be a result of increased apoptosis. In more recent investigations assessing the mitochondrial fraction from PolG mice, rather than whole tissue homogenates did detect increased ROS production over that observed in wild types (Safdar et al., 2016; Dai et al., 2010; Logan et al., 2014), suggesting ROS production may indeed play a role in the development of the phenotypes observed in PolG mice. Further to the phenotypes observed in the PolG mice, tissue specific mutants of mtDNA polγ also developed phenotypes relevant to accelerated ageing (Kasahara et al., 2006; Zhang et al., 2000).

Mutant mice exhibit significantly higher mutation loads in mitochondria from 2 months of age, thereby implying mutation accumulation is occurring during development. Despite bearing a significant mutation load at this age early onset ageing phenotypes are not detectable. Conversely, the mutation rate does not increase significantly by 6 months of age when the accelerated ageing phenotypes are observed. This suggests that the phenotypes arise from an accumulation of dysfunctional mitochondria, possibly by clonal expansion of mutant mitochondria (Trifunovic et al., 2004), resulting in increased levels of ROS/apoptosis and concomitant loss of tissue function.

Among the many benefits endurance exercise brings (reviewed in Mendonca et al. (2016)) is an increased mitochondrial biogenesis (Holloszy and Booth, 1976). An exercise regimen extended the lifespan of PolG mice significantly (Safdar et al., 2011). In addition, exercise had measurable benefits on many of the early onset ageing phenotypes observed in sedentary PolG mice including preventing sarcopoenia, anemia, cardiac hypertrophy and even increased the levels of subcutaneous adipose tissue (Safdar et al., 2011). Not only were there phenotypic benefits, exercise reduced the frequency of point mutations in PolG mice and reduced levels of apoptosis observed in tissues (Safdar et al., 2011). It is thought that ROS induced p53 signaling in PolG mice increases cellular senescence and inhibits mitogenesis and that this is reversed by exercise, thus improving the health outcomes in PolG mice undergoing an exercise regime (Safdar et al., 2016). Indeed it has been hypothesized that exercise also induces the translocation of p53 to the mitochondria where it acts to repair mutations (Safdar et al., 2016) thereby preventing the cascade of events resulting in detrimental phenotypes. Furthermore ROS levels do accumulate with age in PolG mice which may explain the delay in the development of phenotypes in these mice despite the high mutational load in mitochondria in young mice (Safdar et al., 2016).

Homozygous PolG mice have very high mutation rates which may result in the accelerated ageing phenotypes observed in these mice. However, heterozygous KO mice, which exhibit no signs of early ageing, also have a higher mutation rate than aged wild type mice (Vermulst et al., 2007). This suggests that there is a threshold for the accumulation under which there is little observable effect on health during ageing. Indeed, these data also suggest that the rate of accumulation of mitochondrial mutations during normal ageing is not necessarily sufficient to influence lifespan, which has been supported in further studies (Guo et al., 2010). The type of mutations in normal ageing may, however, be different to that observed in PolG mice (Williams et al., 2010). Stem cell function is also affected in PolG mice but may not recapitulate exactly changes seen during ageing, with clear differences in changes in gene expression between HSCs from PolG mice and those from ageing mice (Norddahl et al., 2011). Finally, whilst PolG mice exhibit sarcopoenia they do not recapitulate all of the phenotypes observed in normally aged muscle (Hiona et al., 2010).

In summary, PolG mice develop a greatly increased mutation load in their mtDNA and develop many age-related phenotypes early in life. However the link between mutation load and accelerated ageing, and indeed whether the observed phenotypes are truly accelerated ageing, is not proven conclusively, even if phenotypically the PolG mice appear to age more rapidly. Of note, also another mouse study confirmed a causative link between mtDNA mutations and ageing: homozygous knock-in mice that express a proof-reading-deficient version of PolgA (the nucleus-encoded catalytic subunit of mtDNA polymerase; $PolgA^{mut}/PolgA^{mut}$) displayed reduced lifespan and premature onset of ageing-related phenotypes such as weight loss, reduced subcutaneous fat, alopecia, kyphosis, osteoporosis, anemia, reduced fertility and heart enlargement (Trifunovic et al., 2004).

3.3. Inflammageing

Ageing is characterized by a chronic, low-grade inflammation, and this phenomenon has been termed as 'inflammageing' (Franceschi and Campisi, 2014). Consequently, interfering with inflammatory signaling in mice resulted in several mouse models with accelerated of delayed ageing phenotypes, e.g. brain-specific IKKβ knockout mice, *Nfkb1*$^{-/-}$ mice and IL-10 deficient mice (IL-$10^{tm/tm}$).

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a protein complex that controls transcription of DNA, cytokine production and cell survival and plays a key role in regulating the immune response. In an inactivated state, NF-κB is located in the cytosol complexed with the inhibitory protein IκBα. The enzyme IκB kinase (IKK) is able to phosphorylate IκBα, which eventually results in IκBα degradation, followed by translocation

of activated NF-κB into the nucleus and subsequent transcriptional activation. Interestingly, changes in NF-κB activity severely affect both lifespan and health span of mice (Zhang et al., 2013b; Bernal et al., 2014). Indeed, brain-specific IKKβ knockout mice showed a pronounced phenotype of longevity, with median lifespan 23% longer and maximal lifespan 20% longer than wild type mice. Strikingly, this was also associated with cognitive improvement and protection against ageing-induced muscle and skin atrophy, bone loss, and collagen cross-linking (Zhang et al., 2013b). So while they still age, these brain-specific IKKβ knockout appear to age slower than normal mice (Zhang et al., 2013b). In contrast, loss of *Nfkb1* accelerated observable age-related characteristics leading to a decrease in lifespan: while all *Nfkb1*$^{+/+}$ animals were still alive at 1 year, only 70% of *Nfkb1*$^{-/-}$ mice remained alive at this time (Bernal et al., 2014). Moreover, these mice showed premature age-related skeletal changes, increased tissue inflammation and central nervous system gliosis, reduced apoptosis and increased cellular senescence (Bernal et al., 2014). Interestingly, accumulation of telomere-dysfunctional senescent cells in *Nfkb1*$^{-/-}$ tissues could be blocked by anti-inflammatory or antioxidant treatment of mice, highlighting the potential use of these mice to study age-related interventions (Jurk et al., 2014).

Interleukin-10 (IL-10) is a cytokine with anti-inflammatory activities via amongst others suppression of macrophage activation and inhibition of the production of inflammatory cytokines produced by Th1 cells (Fiorentino et al., 1991a, 1991b). IL-10 deficient mice (IL-10$^{tm/tm}$) were generated to understand the role of the cytokine in immune regulation (Kuhn et al., 1993). These mice are anemic and suffer from extensive enterocolitis, which was limited in proximal colon under the specific pathogen-free (Kuhn et al., 1993). Inflammation in the colon was associated with deficient Th1 cytokine production (Berg et al., 1996). The same study also described that intensity of inflammation was dependent on the mouse strain background. IL-10-deficient mice are growth retarded and expressed increased lethality (Kuhn et al., 1993). Growth retardation becomes evident between ages 3 and 4 weeks, at the age 7–11 weeks around 75% of animals have 30% reduced body weight. The growth retardation follows the course of anemia and development of iron depletion. Lethality increases with age and 30% of animals are dead at the age of 3 months (Kuhn et al., 1993). In T-cell specific IL-10 mutants, the survival is affected only after the immune challenges (Roers et al., 2004). Namely, infection with *Toxoplasma gondii* induced severe hepatitis with focal inflammatory infiltrates and increased lethality (Roers et al., 2004). Interestingly, T-cell specific IL-10 mutants did not have increased mortality after LPS challenge, while full knockouts exhibited significantly reduced survival (Roers et al., 2004). Additional studies with original IL-10$^{tm/tm}$ mice have focused on the similarities with the human frailty syndrome. IL-10$^{tm/tm}$ mice expressed significantly faster muscle strength decline with ageing (Walston et al., 2008). This physical decline was accompanied with the specific gene expression profile and increased levels of IL-6, IL-1β, TNFα, IFN-γ and chemokine ligand 1 levels (Walston et al., 2008; Ko et al., 2012). Mortality and IGF-1 levels of IL-10$^{tm/tm}$ mice were significantly elevated (Ko et al., 2012). IL-10$^{tm/tm}$ mice also have impaired cardiac functions compared to the wild-type littermates (Sikka et al., 2013). One possible cellular mechanism for these pathologies can be altered formation of autophagosomes and increased levels of damaged mitochondria in skeletal muscles of these mice (Ko et al., 2016). In summary, IL-10$^{tm/tm}$ mice are a well-established model for frailty syndrome in humans caused by increased inflammation.

3.4. Other mouse models of accelerated ageing

Other mice with accelerated ageing phenotypes have also been described, such as Klotho-deficient mice and DNA repair mutant mice. These mice have been reviewed extensively (Vanhooren and Libert, 2013; Liao and Kennedy, 2014). Interestingly, the absence of Klotho in mice has strong effects on life expectancy (Kuro-o et al., 1997), whereas overexpression of Klotho extends lifespan (Kurosu et al., 2005) and functional variants of Klotho in humans are associated either with longevity populations or with the onset and severity of human age-related phenotypes (Arking et al., 2002). On the other hand, mice with a mutation in *XPD* (xeroderma pigmentosum, complementation group F), which encodes a DNA helicase involved in both repair and transcription and is mutated in human trichothiodystrophy, exhibit many symptoms of premature ageing, including osteoporosis, kyphosis, osteosclerosis, early graying, cachexia, infertility, and reduced life-span (de Boer et al., 2002). Similarly, genetically engineered mice with disabling mutations in excision repair cross complementing gene (*Ercc1*; a protein that forms a complex with XPF), have defects in DNA repair, accompanied by metabolic stress-induced changes in physiology that result in premature ageing (Weeda et al., 1997).

Patients with autosomal dominant dyskeratosis congenita are now known to carry mutations in either the catalytic component of telomerase (TERT) or in the gene that encodes its RNA template (TERC). These patients have shortened telomeres and reduced lifespan, and they show signs of accelerated ageing and bone marrow failure with increased risk of life-threatening infections (Kirwan and Dokal, 2009). Surprisingly, mice null for either Terc or Tert seemed healthy and phenotypically unaffected, establishing that telomerase activity is dispensable for life (Blasco et al., 1997; Farazi et al., 2006). However, late-generation Terc$^{-/-}$ mice did show a shortened lifespan, overall frailty, decreased fertility and tissue atrophy with impaired organ function (Lee et al., 1998). Additionally, Terc deficiency exacerbated the ageing phenotype of other mutant mice (e.g. Wrn$^{-/-}$ mice as described above), which further shows that multiple factors contribute to ageing and age-related disorders (Sahin and Depinho, 2010).

It is important to note that also environmental factors such as irradiation and stress influence the rate of ageing. Indeed, animal experiments have shown that radiation exposure shortens lifespan. However, this might have been due mainly to the development of cancer (Richardson, 2009). Chronic psychological stress is believed to influence the ageing process: in attempts to restore homeostasis, the body activates several well-known mechanisms, including the autonomic nervous system (ANS), the renin-angiotensin system pathway, and the hypothalamic-pituitary-adrenal (HPA) axis (Groeschel and Braam, 2011; Teixeira et al., 2015; Zhu et al., 2014). As an example, exposing mice daily to rotational stress results in skin ageing manifested in macroscopic changes and increased levels of lipid peroxidation, carbonyl protein content, nitrotyrosine levels, neutrophil infiltration, neutrophil elastase, tissue inhibitor of metalloproteinase-1, and metalloproteinase-8 (Romana-Souza and Santos Lima-Cezar, 2015). Additionally, chronic physiological stress affects other organs, such as brain and muscles, and these effects have been linked to silent mating type information regulation 2 homolog (SIRT1) and to telomere length (Romana-Souza and Santos Lima-Cezar, 2015; Prenderville et al., 2015; Ludlow et al., 2012; McBurney et al., 2013).

3.5. Mouse models of delayed ageing

Mutant mice with extended lifespan are valuable for exploration of the molecular basis of age-related pathophysiological changes of age-related diseases. Several mutations causing dwarfism in mice, such as in Ames (Brown-Borg et al., 1996) and Snell dwarf mice (Flurkey et al., 2001) (Table 2), have been reported to increase lifespan.

Ames dwarf mice contain a recessive point mutation in *Prop1* (homeobox protein prophet of pituitary transcription factor 1 (*Pit1*;

Table 2
Comparison of the two dwarf mouse models.

Name	Gene defect	Affected organ[a]	Impact on lifespan	Impact on physiology[b]	Relevant references[c]
Ames dwarf mice ($Prop1^{df/df}$)	Recessive point mutation in the *Prop1* gene	Defects in pituitary development Low levels of GH, TSH and PRL and suppressive IGF levels.	49–68% increase in mean lifespan 20–50% increase in maximal lifespan	Reduced body size Improved insulin sensitive and glucose tolerance Enhanced memory and learning skills Downregulation of PI3K/Akt/mTOR pathway	(Brown-Borg et al., 1996; Wu et al., 1998; Li et al., 1990; Sharp and Bartke, 2005; Papaconstantinou and Hsieh, 2015; Sanz et al., 2002; Victoria et al., 2015)
Snell dwarf mice ($Pit1^{dw/dw}$)	Spontaneous mutations in the *Pit1* gene	Defects in the pituitary development Lack of GH, TSH and PRL	40–50% increase in mean and maximum lifespan in females	Reduced body size Diminished age-related osteoarthritism and splenanomegaly Decelerated ageing of immune system.	(Flurkey et al., 2001; Silberberg, 1972)

(GH, growth hormone; IGF, insulin growth factor; mTOR, mechanistic target of rapamycin; PI3K, phosphatidylinositol 3-kinase; Pit1, pituitary transcription factor 1; PRL, prolactin; Prop1, homeobox protein prophet of Pit1; TSH, thyroid-stimulating hormone).
[a] Affected organ due to the mutation.
[b] Observed physiological alterations.
[c] Relevant references listed.

Pou1f1 (POU class 1 homeobox 1)). Inactivating mutations in *Prop1* lead to defective differentiation of pituitary somatotrophic, thyrotrophic and lactotrophic cells, which secrete growth hormone (GH), thyroid-stimulating hormone (TSH) and prolactin (PRL), respectively (Wu et al., 1998; Li et al., 1990). The lifespans of male and female Ames dwarf mice are increased by 49% and 68%, respectively, and maximal lifespan is lengthened by 20% and 50% (Brown-Borg et al., 1996). Based on the longevity of Ames dwarf mice, several mechanisms that play a role in ageing have been reported and ageing biomarkers have been described. The longevity of Ames mice has been linked to GH signaling and translation control (Sharp and Bartke, 2005), to the level of phosphorylation of specific insulin receptor substrate 1 (IRS-1) serines together with the insulin/IGF-1 cross-talk (Papaconstantinou and Hsieh, 2015), and to milder oxidative damage to mitochondrial DNA, especially in the brain (Sanz et al., 2002). Additionally, by analyzing circulating miRNAs in Ames dwarf mice and wild-type controls, a 'genotype-by-age' specific circulating miRNA signature was identified (Victoria et al., 2015). This analysis also revealed functional enrichment of miRNA targets related to different processes and pathways important in ageing, including tumor suppression, anti-inflammatory response, modulation of Wnt, insulin signaling, mechanistic target of rapamycin (mTOR) and MAPK signaling pathways (Victoria et al., 2015).

Snell dwarf mice ($Pit1^{dw}/Pit1^{dw}$) have a spontaneous point mutation in the *Pou1f1* gene ($Pit1^{dw}$) that increases mean, median, and maximum lifespans by 40–50% in DW/J females (Flurkey et al., 2002). However, contradictory results have been reported, e.g., shortening of median lifespan in Snell dwarf ($Pit1^{dw}/Pit1^{dw}$) DW/J males by about 25% (Flurkey and Harrison, 1990), which has been explained by the contribution of environmental factors to alteration of lifespan and probably to rates of ageing (Flurkey et al., 2002). Interestingly, Snell dwarf mice show, in addition to increased longevity, retardation of multiple indices of senescence, including diminished osteoarthritis (Silberberg, 1972), prevention of age-dependent splenomegaly, impaired splenic T cell proliferation (Flurkey and Harrison, 1990) and decelerated changes in age-sensitive assays of connective tissue and immune system status (Flurkey et al., 2001). Like Ames mice, Snell mice have been used to identify key factors in the slowing of ageing. In the light of this, proteostatic mechanisms, assessed by the ratio of new protein to new DNA synthesis, were shown to be characteristic of slowed ageing independently of decrease mTORC1 (Drake et al., 2015).

4. Intervention using caloric restriction

It is widely accepted that calorie restriction (CR) is one of the most effective known methods for delaying the ageing process and for supporting healthy ageing. In different mouse models, CR reduces the risk factors for age-related chronic diseases, such as cardiovascular diseases (CVD), diabetes, neuronal, autoimmune, kidney, and respiratory diseases and cancer (Fontana et al., 2010; Anderson and Weindruch, 2016; Trepanowski et al., 2011; Speakman and Mitchell, 2011; Omodei and Fontana, 2011; Taormina and Mirisola, 2014; Weindruch et al., 1986; Fontana and Klein, 2007; Morley et al., 2010; Dogan et al., 2010). Different CR protocols have been applied in mouse model studies on ageing (Van Cauwenberghe et al., 2016). Many researchers restricted caloric consumption by reducing the amount of total food or by reducing the amount of carbohydrates, fat, or protein in the diet. Others used time-restricted feeding (tRF), whereby animals have access to food for only four to eight hours a day but the total number of calories consumed is not limited. In intermittent calorie restriction (ICR) or intermittent fasting (IF), animals have access to food on certain days during the week, as opposed to chronic calorie restriction (CCR), in which food is restricted throughout the study. Most of these studies reported beneficial effects of different types of dietary regimens on ageing or age-related diseases.

Mice are commonly used to study the effects of CR on ageing and age-related diseases. They can be used to study the effect of CR on lifespan or health-span because they are phylogenetically close to humans and are more easily subjected to CR protocols than invertebrates. Studies on mouse models have shown that restricting caloric intake by 40–60% increases lifespan by 30–50% and lowers the incidence of age-related loss of function and diseases, including neurodegeneration (Fontana et al., 2010; Anderson and Weindruch, 2016; Trepanowski et al., 2011; Speakman and Mitchell, 2011; Omodei and Fontana, 2011; Taormina and Mirisola, 2014). Comparison of the disease profiles of Ames dwarf mice to their normal siblings on a CR diet revealed that calorie restriction and Ames dwarfism exert their anti-ageing effects through a common underlying mechanism, namely the change in the endocrine system, especially in GH and IGF-1 levels (Ikeno et al., 2013). However, the pathological profiles of Ames dwarf and CR wild-type mice also showed differences, suggesting that also unidentified independent mechanisms extend lifespan and change age-related pathology in these mice (Ikeno et al., 2013).

4.1. Effect of CR on lifespan is genotype-specific

Although it is widely accepted that CR is one of the most effective methods to attenuate ageing and age-related changes, recent studies on mice have shown genotype-specific effects of CR in mouse models (Forster et al., 2003; Liao et al., 2010b; Rikke et al., 2010; Li et al., 2012; Harper et al., 2006; Turturro et al., 1999). In this context, an early study reported that 40% CR extended the lifespan of both males and females of four different mouse strains or hybrid F1 mice (C57BL/6NNia, DBA/2Jnia, B6D2F1 and B6C3F1) (Turturro et al., 1999). On the other hand, a recent study on 41 mouse strains (both male and female) reported that only 10 of 41 mouse strains had increased lifespan when mice were fed with 40% CR compared to *ad libitum* (AL) feeding (Liao et al., 2010b). Remarkably, CR shortened the lifespan of most of the mouse strains (Liao et al., 2010b). Another study in which male C57BL/6J and DBA/2 mice were fed AL from four months of age revealed no difference in their lifespan, but 40% CR extended the lifespan of C57BL/6J mice by 25% but did not affect the lifespan of DBA/2 mice (Forster et al., 2003). These findings are supported by a study by Dong-Li et al. (Li et al., 2012), who reported that different oxidative stress parameters in skeletal muscle mitochondria increased with ageing in C57BL/6J but not in DBA/2 mice, and that this increase was attenuated by 40% CR (Li et al., 2012). These studies show the genotype-specific effects of CR in mouse models. Therefore, it is important to choose mouse models appropriate for studying ageing and age-related diseases.

4.2. The effects of CR on age-related cardiovascular diseases (CVD)

The prevalence of cardiomyopathies such as hypertension, atherosclerosis, myocardial fibrosis and stroke increases with ageing (Omodei and Fontana, 2011; Lakatta and Levy, 2003; Donato et al., 2013; Guo et al., 2002; Wielinga et al., 2011; McMillen et al., 2013). In recent years, CR varying from 30% to 60% has been proposed as a powerful protective and therapeutic intervention for cardiomyopathies of the ageing heart and vasculature. Long-term CR (up to 30 months) totally prevented the increase in arterial pressure, pulse wave velocity (a measure of arterial stiffness), artery wall hypertrophy, myocardial fibrosis, collagen content, left ventricular stiffness and endothelial dysfunction in large conduit arteries, including the aorta and carotid artery, as well as in cerebral resistance arteries in B6D2F1 mice (Donato et al., 2013; Walker et al., 2014; Weiss and Caloric, 2011; Yan et al., 2013). The authors attributed these improvements to preservation of aortic SIRT-1 protein expression, a mTOR signaling that regulates cellular nutrient sensing in arteries and nitric oxide bioavailability, which suppresses vascular oxidative stress (Donato et al., 2013; Walker et al., 2014; Weiss and Caloric, 2011; Yan et al., 2013). In addition, when mice aged 20 months were subjected to CR for two months, the signs of ageing cardiomyopathy were reversed (Yan et al., 2013; Rippe et al., 2010). This intervention was as effective as long-term CR started from an early age (Yan et al., 2013). Moreover, CR feeding of old mice restored arterial antioxidant and SIRT-1 protein concentrations, indicating the amelioration of cardiomyopathies (Yan et al., 2013, 2012; Rippe et al., 2010).

Many genetically modified mouse models have been used to show the effects of CR on age-related molecules and on CVD. For example, homozygous apolipoprotein E gene (ApoE$^{-/-}$) knockout mice and low-density lipoprotein receptor (LDLR$^{-/-}$) knockout mice were used to show the beneficial effects of CR against atherosclerosis. When these mice were placed on different CR diets, including 60% CR, short term ICR and modification of cholesterol or fat intake, they developed smaller atherosclerotic lesions (Guo et al., 2002; Wielinga et al., 2011; McMillen et al., 2013).

4.3. The effects of CR on age-related metabolic diseases

Dietary effects on age-related metabolic disorders have been shown in different mouse studies (Chaix et al., 2014; Hatori et al., 2012). Mice fed different diets (including high fat diet (HFD), high fructose diet restricted to eight hours a day and without reducing calorie intake) improved hepatic glucose metabolism, rhythms of nutrient utilization and motor function, and increased bile acid production (Chaix et al., 2014; Hatori et al., 2012). In addition, tRF reduced gene expression levels of the inflammatory cytokines IL-6, TNFα and CXCL2 in white adipose tissue, serum cholesterol levels and insulin resistance, and prevented liver damage and hepatosteatosis in C57BL/6J male mice (Chaix et al., 2014; Hatori et al., 2012). Contrary to this, another tRF study (three hours of feeding per day) on C57BL/6J male mice aged 11 weeks increased the levels of inflammatory cytokines such as IL-6, TNFα, NFkB and IL-1 as well as the CRP serum level, which is a potential marker for increased risk of coronary heart disease. In addition, circadian rhythm was robust while mRNA expression of the anti-inflammatory cytokine IL-10 was decreased during tRF (Sherman et al., 2011). On the other hand, tRF did not affect plasminogen activator inhibitor-1 (PAI-1), a possible marker for thrombosis and susceptibility to heart attacks, or arginase (Arg1), a marker for colorectal cancer and liver metastases (Sherman et al., 2011).

4.4. The effects of CR on neurogenesis and age-related brain diseases

CR has also been shown to affect age-related brain diseases such as Alzheimer's, Parkinson's and Huntington's diseases and stroke (Van Cauwenberghe et al., 2016; Bondolfi et al., 2004; Patel et al., 2005; Wang et al., 2005; Park et al., 2013; Wu et al., 2008; Halagappa et al., 2007; Hornsby et al., 2016; Duan et al., 2003; Mattson et al., 2003). Using different mouse models, recent studies have reported beneficial effects of CR (including ICR and small meal size) on neurogenesis, survival of neuronal cells, and restoration of neuronal function following injury (Bondolfi et al., 2004; Patel et al., 2005; Wang et al., 2005; Park et al., 2013; Wu et al., 2008; Halagappa et al., 2007; Hornsby et al., 2016; Duan et al., 2003; Mattson et al., 2003). In addition, it was reported that CR decreases the expression levels of inflammation related genes in the hippocampus, and enhances novel object recognition and contextual fear conditioning memory in different mouse models (Bondolfi et al., 2004; Patel et al., 2005; Park et al., 2013; Wu et al., 2008; Halagappa et al., 2007; Hornsby et al., 2016; Mattson et al., 2003). In this context, Wu et al. (Wu et al., 2008) have shown that CR improved novel object recognition and contextual fear conditioning memory in the conditional double-knockout mouse model for penicillin 1 and 2 (Wu et al., 2008). Using DNA microarray analysis, they found decreased expression of inflammation and increased expression of neurogenesis-related genes in the hippocampus of calorie restricted conditional double-knockout mice (Wu et al., 2008). In another study, Park et al. (Park et al., 2013) reported that CR increases the number of dividing neuronal stem cells and progenitor cells in the dentate gyrus of female Nestin-GFP mice. Based on their observations, they suggested that CR increases the number of divisions that neural stem cells and progenitor cells can undergo in the ageing brain of females (Park et al., 2013). Moreover, Halagappa et al. (Halagappa et al., 2007) reported that different types of CR and IF protect neurons against the negative effects of Aβ and tau pathologies on synaptic function. They concluded that CR and IF dietary regimens can ameliorate age-related deficits in cognitive functions (Halagappa et al., 2007).

5. Summary

Although the mouse might not be an ideal model for studying ageing, its use as a model of mammalian biology will continue to deliver important insights into the pathobiology of disease, fundamental processes involved in ageing, and the relationship between ageing and disease.

Here, we gave some examples of how mouse models of both accelerated and delayed ageing provide insight into the pathogenic mechanisms of the normal physiological ageing process and of premature ageing in progeroid syndromes. The ability of CR to delay ageing has been thoroughly validated in a broad range of mouse models of ageing. Further analysis of the underlying mechanisms might eventually lead to new therapies to postpone age-related diseases. It should be kept in mind that inbred strains are limited in genetic diversity and hence develop phenotypes specific to those strains, but they are easy to manipulate genetically and extensive baseline data are available. On the other hand, outbred mice are more representative of the genetic diversity of humans but they present problems in terms of altering the genome. While many laboratories use similar techniques to study ageing, the development of SOPs and standardized phenotyping, across a range of physiological systems reflecting frailty as seen in patients, is urgently required. This will result in more consistent data on the specific pathways that are critical upon ageing and how pathway modulation affects health.

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