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REVIEW **Open Access**

Experimental models for ageing research

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Summary. Ageing is a biological process caused by the malfunctioning of multiple cellular mechanisms, ascribable to nine hallmarks: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. These ageing pillars have three common traits: (i) they appear during normal ageing; (ii) their experimental intensification accelerates ageing; and (iii) their experimental reduction delays ageing.

The evidence that the elderly are more prone to develop pathologies such as cancer, diabetes and degenerative diseases, together with data showing that the elderly population is steadily increasing, has stimulated an important effort to find specific countermeasures to physiological ageing. Unfortunately, the investigation of ageing processes and the search for countermeasures in humans is very difficult. Therefore, researchers must rely on a wide range of experimental models that span from unicellular to more complex organisms.

Unfortunately, experimental models are not devoid of pitfalls, flaws or obstacles that can have an impact in ageing research. In the present review we describe the most exploited experimental models in the field, such as *in vitro*, animal and human models, highlighting the characteristics that justify their application in the laboratory routine, and translation to human research.

Key words: Ageing, Age-associated diseases, Life-span, Health-span, Experimental models

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Introduction

Ageing is the result of the cumulative alterations of multiple biological processes accompanied by a functional decline of the aged individual, which increases the risk of developing chronic diseases (e.g., diabetes, cancer, cardiovascular and degenerative disorders), and contributes to a negative outcome to health-challenging situations.

Recent research suggests that the biological processes leading to ageing are: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (López-Otín et al., 2013). Each distinct hallmark of ageing has been identified based on three characteristics: (i) it is displayed during normal ageing; (ii) its experimental intensification accelerates ageing; and (iii) its experimental abatement delays ageing (López-Otín et al., 2013).

The multiplicity of the mentioned hallmarks and their possible interaction, make the search for the mechanisms responsible for ageing, their investigation, and, most importantly, their prevention or suppression very difficult. In this context, the rapid growth of the ageing world's population and the increase in life-span, have motivated a large effort in the investigation of the mechanisms underlying ageing, and in the search for possible measures to improve the quality of life for the elderly.

The complexity of the mechanisms that determine ageing is not the only factor that makes research in this field difficult. Actually, in contrast with the need to rapidly find measures for healthy ageing, the study of the ageing process in humans is challenging due to the long duration of the process itself. In humans, both longitudinal and cross-sectional experimental protocols have flaws. Longitudinal protocols deal with the difficulty of maintaining an optimal long-term traceability and continuity. Cross-sectional studies can be influenced by multiple factors that have been shown to change over the years, such as the socio-sanitary conditions, the nutritional regimens and psychophysical activities (Moffitt et al., 2017). To overcome the above-mentioned factors, the heterogeneity of population

characteristics and the presence of ethical issues, researchers resort to a broad range of experimental models, such as *in vitro* systems, non-vertebrate and vertebrate organisms, and laboratory animals.

Nevertheless, working with experimental models is not free of flaws or obstacles. Although theoretical life-span curves are quite homogeneous in shape across several species, they can be enormously different in length (Mitchell et al., 2015). Furthermore, theoretical life-span curves cannot provide information on the real health condition (health-span) of the experimental model (Hamczyk et al., 2020), and if the age-dependent alterations affect specifically distinct tissues or organs. Accordingly, recently, researchers have stressed the complexity in the study of age-related loss of skeletal muscle mass and function (Larsson et al., 2019), or degenerative diseases (Burns et al., 2015) in mouse models due to the evidence that these two ageing processes appear quite different compared to humans.

Based on these observations, what is the best model (or models) to use in the search of ageing mechanisms and their countermeasures? And what could be the criteria that direct the choice of one or more experimental models?

Based on the above-reported indications of Lopez-Otin and collaborators (López-Otín et al., 2013), the exploration of ageing mechanisms should ideally entail organisms that can spontaneously undergo senescence, and can be experimentally modified in order to intensify or abate the investigated hallmark of ageing.

The present review aims at providing an overview of the most utilized experimental models in ageing research, highlighting the characteristics that justify their use in this research branch, and the translation to human application, with a daily-laboratory routine perspective.

In vitro experimental models

Cell lines, induced pluripotent stem cells, and organoids

Cells in culture are an experimental system that allows the study and the manipulation of several aspects of the cellular mechanisms in a Petri dish. Therefore, they are considered an important resource for ageing research. Cells in culture have been shown to both undergo natural senescence (replicative senescence) (Hayflick and Moorhead, 1961), and stress induced senescence (Toussaint et al., 2000). The continuous improvements of the research tools, such as the introduction of genetic modifications, regulation of gene expression, the manipulation of culture conditions (temperature, oxygen and introduction of factors in the medium), makes this model very suitable to study specific cell signalling pathways, protein interaction mechanisms, regulatory actions, etc., therefore providing information on the basic molecular and cellular aspects of ageing pathways (Phipps et al., 2007). The above-mentioned characteristics make cell culture models also very appropriate tools in the screening for anti-ageing compounds (Vatolin et al., 2019).

Interestingly, recent times have seen the employment of induced pluripotent stem cells (iPSC) also in aging research (Liu et al., 2012). This experimental model, despite the fact it requires a more complicated set up of cell culture conditions compared to classical cell cultures, has numerous advantages. iPSCs are obtained by the reprogramming of somatic cells that can be harvested from healthy or diseased adults, such as patients with accelerated ageing or with neurodegenerative disorders (Liu et al., 2012; Machairaki, 2020). Accordingly, although the reprogramming of cells entails the intrinsic potential to reset them to embryonic state, at date there is a wide employment of iPSCs models for Parkinson Disease (Miller et al., 2013), Alzheimer Disease (Machairaki, 2020) and Amyotrophic Lateral Sclerosis (Liu et al., 2012).

Due to their nature, the contribution of cell cultures to the understanding of the mechanism of ageing of the complete organism is limited by the lack of information on defects to tissue and at organ level, such as imbalance of tissue homeostasis, stem cell number, and susceptibility to tissue or organ-specific diseases. This obstacle can be partially overcome by the use of organoids, which can be more proximate to the tissues or organs.

Organoids have gained much interest in the last years due to their potential application in regenerative medicine, and recently their application has also been suggested in ageing research (Hu et al., 2017), based on the conservation of some of the molecular mechanisms involved in ageing. As extensively reviewed by Hu and collaborators (Hu et al., 2017), organoids not only preserve some of the cell-specific aspects of ageing such as DNA hypermethylation (Lewis et al., 2020), but also synthetize the extracellular matrix, which is a structure susceptible to senescence due to glycation and oxidation of its components (Birch, 2018).

At date, researchers can rely on a wide array of human- and mouse-derived organoids to study distinct aspects of basic biology and translational medicine (Kaushik et al., 2018), to investigate the impact of age on the digestive system (Lewis et al., 2020), the progression of neurodegenerative diseases (Machairaki, 2020), and also to explore the mechanisms involved in osteoarthritis (Lozito et al., 2013).

Interestingly, although both IPSCs and organoids obviously fail to fully mirror the organism ageing, they have an important potential in ageing research thanks to the availability of multiple advanced methodological and biological tools, such as genetic manipulation, micromanipulation techniques, and the possibility to apply high-throughput technologies for drug screening (Ebert et al., 2012).

Yeast

The application of yeast in ageing research has been active for 60 years (Mortimer and Johnston, 1959). Although the research involves the use of several distinct

strains, this section will focus on the most exploited yeast, *Saccharomyces cerevisiae*. The budding yeast *Saccharomyces cerevisiae* is a unicellular eukaryotic organism with 6000 completely sequenced genes (Goffeau et al., 1996). Yeast cells have a short, 2-hour life cycle, and proliferate in both haploid or diploid state depending on nutrient abundance. In optimal nutritional conditions, *Saccharomyces cerevisiae* cells proliferate in a diploid state, while subjected to nutrient withdrawal they enter meiosis and spore formation. Spores can survive from hours to months, and in favorable conditions haploid and alpha spores can proliferate and mate to form diploid cells.

In *Saccharomyces cerevisiae*, ageing properties have been associated to the replicative life-span and chronological life-span concepts (Longo, 1999; Longo et al., 2012). The replicative life-span is based on evidence that the budding capacity of a single mother-cell decreases with time (Longo et al., 2012). The chronological life-span consists in the estimation of percentage of viable or metabolic active cells in a liquid culture at the plateau stage (Longo, 1999). The replicative and the chronological life-span are a measure of different biological properties, and therfore, regulated by different molecular pathways (Lin et al., 2000; Longo et al., 2012).

The study of the pathways involved in the regulation of the chronological life-span led to the identification, of the Sir-2 NAD-dependent histone deacetylase pathway (Imai et al., 2000; Lin et al., 2000), responsible for a strict connection between metabolism and ageing, which only later in time was shown to be conserved among species (Smith et al., 2000).

As discussed for cell lines, the use of *Saccharomyces cerevisiae* is considered disputable because it is a unicellular organism that cannot provide information on tissue and organ ageing. However, this experimental model has several advantages, such as the simple laboratory equipment required, short cell cycle, the possibility of tuning the culture medium conditions, and a well-characterized genome with high similarities with mammalian cells, that can be easily modified thanks to the large availability of genetic approaches. Interestingly, these characteristics also make *Saccharomyces cerevisiae* very suitable for high-throughput methodologies (Sarnoski et al., 2017), particularly in the screening of pharmacological anti-ageing compounds (Zimmermann et al., 2018). In this context, the findings showing that the modulation of the Sir-2 NAD-dependent histone deacetylase pathway could extend life-span, led to the designing and screening of numerous caloric restriction mimetics compounds (Howitz et al., 2003).

Non-vertebrate experimental models

Caenorhabditis elegans

Since the 70s the small nematode *Caenorhabditis elegans* has been one of the most used organisms in ageing research and in the study of genetics of ageing (Mack et al., 2018).

In normal temperature and nutritional conditions, *Caenorhabditis elegans* eggs develop passing through four larva stages (L1-L4), to become a reproductive adult hermaphrodite worm in three days. The mean lifespan of the *Caenorhabditis elegans* is around 15 days, and the maximum lifespan around 27 days. Interestingly, in adverse conditions (i.e. temperature or nutrient restriction), worms after the L2 stage can enter into an alternative developmental state called diapause to give the dauer larvae. Dauer Larvae are stress and age-resistant, and in favorable conditions develop into a reproductive adult (Riddle and Albert, 1997).

Although the adult worm is composed of about 1000 cells, these are associated to form distinct tissues and organs with a functional similarity to human organs (Altun and Hall, 2009). During ageing, *Caenorhabditis elegans* worms reduce their activity, become less coordinate and can eventually stop moving (Olsen et al., 2006). Moreover, normal and transgenic *Caenorhabditis elegans* have been shown to display some of the same ageing features as sarcopenia (Herndon et al., 2002), and share some basic mechanisms of neurodegeneration (Oeda et al., 2001; Papaevgeniou and Chondrogianni, 2014).

Caenorhabditis elegans has been adopted as prominent model in ageing mainly because it can be induced to enter, or leave, the dauer stage by altering genes involved in the regulation of life-span. For instance, the study of age-1 and daf-2 natural mutants, which display longer life-spans, allowed to characterize the insulin/insulin-like growth factor signaling pathway, a central mechanism involved in ageing regulation. Only successively, this pathway was found and better characterized in other model animals and humans (Partridge et al., 2011). Consequently, *Caenorhabditis elegans* manipulation has also allowed to show that pathways involved in calorie restriction (Walker et al., 2005) and mitochondrial integrity (Anson and Hansford, 2004) participate in the life-span modulation.

From the laboratory routine perspective, *Caenorhabditis elegans* is an interesting experimental model since it is relatively easy to maintain, its genome is deciphered (Genome sequence of the nematode C. elegans: A platform for investigating biology, 1998), easily modifiable, and has good association to human genes (Wheelan et al., 1999). The presence of a RNAi library that covers about 80% of the genes (Tissenbaum, 2015) allows extensive screens to detect genes involved in the modulation of life-span. Beyond the easiness of its genetic manipulation, *Caenorhabditis elegans* can be exploited in the study of ageing pathways, and relative countermeasures, by various methods, such as control of bacterial food and modulation of specific nutrients, and by pharmacological interventions (i.e. calorie restriction mimetics (Olsen et al., 2006; Calvert et al., 2016)). Moreover, due to its transparency, *Caenorhabditis elegans* is very suitable to the application of live

imaging techniques. Not least, the life-spans of a population have generally no, or little, fluctuation, allowing the identification of factors that increase or reduce the life-span by 10-15% with statical significance (Tissenbaum, 2015).

However, this organism in not free of weaknesses. The simple body organization does not allow a complete comparison with the ageing effects on the human body. Moreover, although the investigation of the mechanisms regulating dauer stage allowed the discovery and elucidated key pathways involved in life-span extension, its correlation with higher organisms, such as vertebrates and mammals, is considered debatable (McElwee et al., 2006).

Drosophila melanogaster

Used for the first time in ageing experiments in 1916 demonstrating that its life-span was food and temperature dependent (Loeb and Northrop, 1916), the fruit fly *Drosophila melanogaster* is still widely used in ageing research. At 25°C, *Drosophila melanogaster* has a life-span of approximately 60 days, which can be reduced or increased by respectively increasing or reducing the growth temperature. Actually, *Drosophila melanogaster* can enter a reproductive diapause upon modulation of the light cycle and temperature. As described for *Caenorhabditis elegans*, diapause is connected to a better stress resistance and increased life-span.

The fruit fly is an obligate aerobe and dioecious organism (Wolf, 2010), and its body presents structures that perform the correspondent functions of the distinct mammalian organs, such as brain, heart, lung, and skeletal muscles (Pandey and Nichols, 2011).

Drosophila melanogaster shows signs of physiological senescence at tissue and organ level (Piper and Partridge, 2018), that result in alterations to behavior, reduction of the reproductive capacity, impairment of stress-resistance, physical activity, skeletal muscle and cardiac function, and modified metabolism (Toivonen and Partridge, 2009). Interestingly, similarly to what is observed in humans, ageing flies display alteration to selected biomarkers, such as advanced glycation end products, or carbonylated proteins (Jacobson et al., 2010).

Drosophila melanogaster is an excellent model to study the genetic complexity of the ageing process. The fly genome is organized in 4 chromosomes with approximately 14000 genes belonging to the same mammalian gene families (Pandey and Nichols, 2011; Taormina et al., 2019), where about the 75% of human genes leading to disease have a correspondent match (Perkins et al., 2015; Taormina et al., 2019). In addition, working with the fruit fly is facilitated by the availability of multiple genetic tools, such as the broad variety of mutants and transgenic strains, the accessibility to temporal, hormone-inducible and tissue specific expression of mutated proteins, the availability of collection of RNAi lines for knockdown screens (Perkins et al., 2015), and the possibility to apply the recent CRISPR-Cas9-dependent gene editing *in vivo* (Bier et al., 2018).

The above-mentioned genetic tools together with the availability of strains with the same genotype, facilitate the feasibility of largescale screens, and provide the advantage of performing demographic studies, making the fruit fly an excellent model for research into ageing, and justifying its exploitation for the validation of findings discovered in humans (Loeb and Northrop, 1916; He and Jasper, 2014). However, needless to say *Drosophila melanogaster* is still far from mirroring the human organism, from the laboratory perspective, it is a model organism with a short life-span, that requires a simple and cost-effective maintenance. Due to its centenary utilization, at date, Researchers have very extensive knowledge of the experimental tools and factors that should be considered when working with *Drosophila melanogaster*.

Vertebrate experimental models

Fish

The fishes include the shortest- and longest-lived vertebrates in nature (Valdesalici and Cellerino, 2003; Trifonova et al., 2018; Maslov et al., 2019). This heterogeneity in longevity provides the possibility to apply comparative analyses to reveal molecular mechanisms involved in the determination of life-span differences (Gerhard, 2003). However, in the view of the laboratory application, work-space and time availability direct the choice towards the small tropical fish species. These species are considered to have the best potential in ageing research because they have short life-spans, they display gradual senescence, and develop degenerative processes and tissue lesions in an age dependent way (Gerhard, 2003). Although the small tropical fish species include known species such as guppy, medaka, and killifish, zebrafish (*Danio Rerio*) is till now the most used model thanks to the broad availability of scientific tools and resources.

Danio Rerio, which has a 2-3 year life-span, has been largely employed in developmental studies. Interestingly, at date, this small tropical species is being increasingly used in other fields and in ageing research for both cross-sectional and longitudinal studies (Gerhard, 2003). This recent application depends on the evidence of signs of senescence, such as spinal curvature, muscle degeneration (Gilbert et al., 2014), and reduced physical ability (Gerhard, 2003).

The zebrafish requires a fairly simple housing, it has a good reproductive capacity, and can provide sufficient amounts of tissues for sampling. The zebrafish presents a conserved genome, which is easily modifiable (Mullins and Nüsslein-Volhard, 1993). Moreover, research with zebrafish is greatly supported by the availability of well-established methodological and biological tools, ranging

from genetic manipulation (Mullins and Nüsslein-Volhard, 1993), live imaging (Keller, 2013), and adaptation to high-throughput screenings (Spaink et al., 2013; Bugel et al., 2014). Interestingly, recent research shows that zebrafish can be exploited for the investigation of neurodegenerative pathologies such as Alzheimer disease, Parkinson disease (Xi et al., 2011), but also osteoporosis (Bergen et al., 2019), sarcopenia (Daya et al., 2020), age-dependent trainability (Gilbert et al., 2014), and as a model for calorie restriction (Giacomello and Toniolo, 2021). Although this model is still far from human ageing, the above-mentioned features together with the fact that this is a more complex organism with defined organs and apparatuses, give zebrafish a prominent role in the search for anti-ageing molecules.

Rodents

Rodents are the most common mammal used in research, and like fishes, they include species with different life-spans, which make them suitable for comparative analyses to elucidate the biological mechanisms involved in ageing (Gorbunova et al., 2008; Vanhooren and Libert, 2013).

Laboratory rodent models include mice, rats, naked rat moles, guinea pigs and others (Gorbunova et al., 2008). Despite the evidence that the mouse model is still the most used, recent times have seen an increase in the use of rats in ageing research thanks to a substantial expansion of the availability of transgenic strains, which allow senescence studies to be performed on neurodegeneration, metabolism and calorie restriction (Yamaza et al., 2004).

Mice and rats have about a 3 year life-span, with slight changes depending on the strain, with inbred strains being more prone to ageing (Quinn, 2005; Flurkey et al., 2007; Ackert-Bicknell et al., 2015). Both mice and rats are similar to humans in their physiology, cellular functions, and, to a lesser extent, in their anatomy. There are several examples of age comparison between mice and humans, and rats and humans age in the recent literature (Quinn, 2005; Flurkey et al., 2007; Ackert-Bicknell et al., 2015), suggesting that there is not a direct correspondence in the ageing patterns in the different species. Actually, factors such as tissue specificity, diet and strain can affect ageing timing and pattern in these models.

Considering their broad use, here we attempt to briefly describe the main advantages and disadvantages of the use of the mouse model in the perspective of planning experiments on ageing.

Mouse ageing has been shown to cause changes in many organ systems, in body composition, in cognition, and to induce a decline in physical function (Flurkey et al., 2007; Ackert-Bicknell et al., 2015). The 2.5 Gbp genome of the mouse, organized in 40 chromosomes, encodes a similar number of genes as the human genome, with 99% of the mouse genes having a human orthologue (Taormina et al., 2019). Ageing research in the mouse model dates back to the characterization of the Ames and the Snell dwarf mice (Snell, 1929; Brown-Borg et al., 1996), which display a longer life-span, ascribable to a naturally occurring point mutation to the Prop1 gene and Pou1f1, which entail an alteration to the insulin/IGF-1 pathway, similarly to what has been demonstrated in *Caenorhabidi elegans* (Hsieh et al., 2002a,b; Wiesenborn et al., 2014).

The use of mice in ageing research relies on several aspects. The large availability of well-characterized inbred and mutant strains together with the rapid advances in gene editing techniques, have made the development and study of mutant strains less complicated and more rapid compared to the past, allowing to delve into basic biological processes *in vivo*. Moreover, the mouse model presents the advantage of working in conditions of genomic homogeneity, which provides good reproducibility in both longitudinal and cross-sectional protocols (Taormina et al., 2019).

The extensive use of mice in ageing research is supported by other factors, such as the large availability of strains that display accelerated or delayed ageing (Kõks et al., 2016), the possibility of easily changing the feeding conditions to simulate obesity or calorie restriction (Giacomello and Toniolo, 2021), to test broad panels of active principles, and, not least, the availability of a wide and well-established spectrum of methodologies (like *in vivo* imaging techniques, analysis of functional and physiological proprieties, behavioral studies), that altogether allow a very long list of varied and in-depth analyses to be performed.

Despite the above-mentioned characteristics, the use of mice in ageing research is debated because they do not fully mirror the human ageing process. This is because the regulation of telomere length, the DNA repair mechanisms, and the immune response differ significantly from humans (Taormina et al., 2019). As a result, some age-related diseases in humans, such as age-dependent neurodegeneration (Burns et al., 2015), or skeletal muscle ageing (Larsson et al., 2019; Giacomello and Toniolo, 2021), do not have an identical correspondence in mice. Particularly in the study of skeletal muscle ageing, the differences in protein expression, the different metabolic properties and the presence of strain-dependent alterations of skeletal muscle fibers, induce caution when translating results from mice to humans (Agbulut et al., 2000; Giacomello et al., 2020; Toniolo et al., 2021).

Human models

The bed rest model

The study of the effects of periods of bed immobilization dates back to the first half of twentieth century when Deitrick and collaborators monitored the loss of bone mass in healthy subjects and patients affected by poliovirus following a bed rest period

(Deitrick, 1948). Subsequently, the use of the bed rest model became very popular after spaceflights, and to date, it is considered the most prominent model of microgravity and accelerated ageing in humans (Pavy-Le Traon et al., 2007). Actually, the application of bed rest studies allowed the collection of an enormous amount of data, which, thanks to comparative studies, have demonstrated the proximity of the bed rest model to the development of frailty in older adults (Kehler et al., 2019). In fact, both microgravity and long periods of immobilization have been shown to cause an alteration of mechano-skeletal and vestibulo-neuromuscular stimuli (Pavy-Le Traon et al., 2007; Kehler et al., 2019), that have detrimental effects on the normal physiology of several organs and apparatuses, such as skeletal muscle, bones, cardiovascular system, and to unbalance several biomarkers, which have been also reported to undergo modification upon ageing (Pavy-Le Traon et al., 2007).

The bed rest protocols can vary in the duration and in the conditions (Pavy-Le Traon et al., 2007). Generally, during a bed rest experiment the subject lies supine or can change position, the bed can have different degrees or no inclination, some protocols envisage urination and defecation while in bed, other allow less restricted parameters.

The application of a bed rest protocol can lead to a redistribution of body fluids and a decrease in plasma volume of 10-15%, with consequent alteration of cardiac function, reduction of baroreflex sensitivity and aerobic capacity, such as in the case of a head down bed rest. In the skeletal muscle tissue, the loss of activity induces a reduction in energy requirements and reduction of muscle mass and function (Pišot et al., 2016; Floreani et al., 2018; Rejc et al., 2018), but also perturbs its metabolic properties (Pišot et al., 2016; Floreani et al., 2018), and impairs the neuromuscular connection (Monti et al., 2021), leading to a condition comparable to sarcopenia. The loss of gravitational force and the concomitant lack of physical activity impacts also the bone structure and density of the lower extremities and the vertebrae (Kim et al., 2003), with the concurrent release of bone-secreted factors that, in turn, can further affect muscle physiology (Bettis et al., 2018).

As a secondary outcome, bed rest studies allow the collection of data on the variables that can influence the effects of long immobilization periods in both young and old patients, to find the most appropriate measures to contrast its detrimental effects. In this context, bed rest studies demonstrated that older adults display a different recovery compared to young subjects (Floreani et al., 2018; Rejc et al., 2018).

Unfortunately, the bed rest experimental model is not free of hurdles. The paucity of patients and the different genetic background (Sirago et al., 2022) introduces a high variability in the outcome. And most importantly, the bed rest introduces several health and ethical concerns, because the tested subjects undergo an important health challenge that can require long recovery times (Kehler et al., 2019). More recently, less invasive models, such as the step reduction protocols (Saoi et al., 2019), and unilateral lower limb suspension (Hackney and Ploutz-Snyder, 2012) have been described to induce metabolic perturbations, which correlate with the risk of sarcopenia.

Conclusions

The increase of the ageing world's population has motivated a large effort in the investigation of the mechanisms underlying ageing, and in the search for possible countermeasures.

To elucidate the hallmarks of ageing, and to translate their knowledge to contrast age-related disfunction, researchers are faced with the evidence that there are conserved mechanisms that regulate life-span, but also important variations in the ageing mechanisms and patterns among different species, and even between related species. Beyond these challenges, researchers must deal with the daily laboratory routine, the availability of appropriate resources, the know-how, the new advances, etc., that influence the selection of one model compared to another, or more models, in a very reasoned way. The biological question or the aim of the study, the time availability, the tissue or organ of interest, the set-up, the availability of dedicated facilities and know-how of the laboratory, highly impact the selection of the most appropriate model.

It is worth mentioning that nowadays researchers can afford numerous important data on ageing pathways, extracted from comparative analyses (Cohen, 2018), and from in silico analysis and simulations (Fortney et al., 2012). These tools could be preparatory to the planning of a research project, since they can help to guide the choice of the most suitable experimental model or models, leading to an optimization of time and resource used in the search of ageing mechanism and possible countermeasures.

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