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Animal Models of Aging Research: Implications for Human Aging and Age-Related Diseases*

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Abstract

Aging is characterized by an increasing morbidity and functional decline that eventually results in the death of an organism. Aging is the largest risk factor for numerous human diseases, and understanding the aging process may thereby facilitate the development of new treatments for age-associated diseases. The use of humans in aging research is complicated by many factors, including ethical issues; environmental and social factors; and perhaps most importantly, their long natural life span. Although cellular models of human disease provide valuable mechanistic information, they are limited in that they may not replicate the in vivo biology. Almost all organisms age, and thus animal models can be useful for studying aging. Herein, we review some of the major models currently used in aging research and discuss their benefits and pitfalls, including interventions known to extend life span and health span. Finally, we conclude by discussing the future of animal models in aging research.

INTRODUCTION

The aging process is associated with a time-dependent progressive increase in disease susceptibility. Almost all known organisms age, and although the maximum life span differs between organisms, the shape of the curve, often considered representative of the health of the organism, is remarkably consistent across species (**Figure 1**). In the human context, aging is becoming an increasing socioeconomic problem for countries around the world. By the end of the twenty-first century, the percentage of the population aged above 65 is projected to increase from approximately 7% to more than 20% worldwide (**http://esa.un.org/wpp/**). Further adding to this aging epidemic, the older population, and indeed the population in general, is becoming increasingly unhealthy independent of a slight increase in life span over the past decades (1). Further, at least 80% of health care costs are accrued after a person turns 45 years of age (2). It is thus clear that society is facing an enormous economic challenge in the decades to come, and investigating interventions that ensure healthy aging is becoming increasingly important.

In the past decades, research into the underlying causes of aging has led to remarkable breakthroughs, not only in the understanding of mechanisms of aging but also in interventions that may increase life span and, more importantly, health span. Model organisms have been at the forefront of this research and have yielded a wealth of information, allowing us to find conserved pathways that may also regulate human aging.

One of the most successful examples was the initial discovery that inhibition of the target of rapamycin (mTOR) pathway increases life span in yeast, nematodes, and flies, with later work demonstrating these life-extending properties appear to be conserved in vertebrates (3–7). This led to the discovery that rapamycin (named for its discovery on Easter Island, Rapa Nui), an inhibitor of mTOR, may be able to ameliorate aspects of the accelerated aging diseases Hutchinson-Gilford progeria and Cockayne syndrome (8, 9), as well as extending life span in mice (3, 10). Another

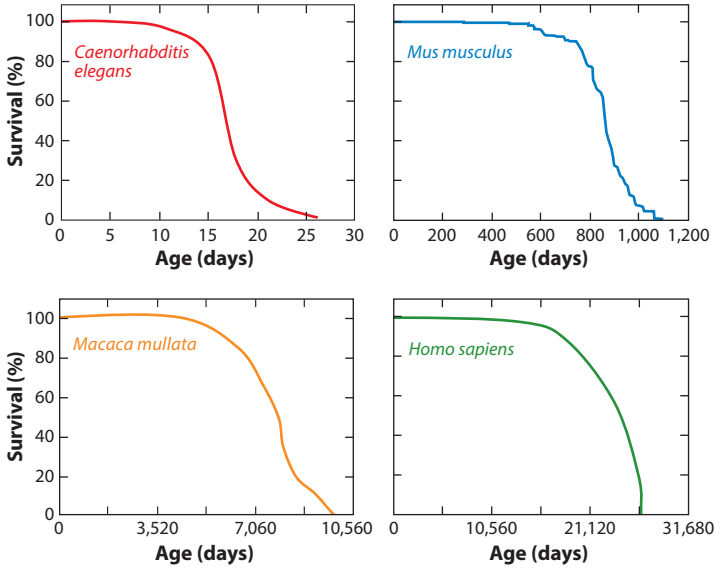

Figure 1

The universality of aging. Theoretical life-span curves depicting the similarity in the aging process across model organisms relative to humans. Despite the differences in life span, the shape of the curve, often considered a measure of the health (or health span) of the organism, is similar.

famous, but controversial, discovery underscoring the use of model organisms was the finding that overexpression of the sirtuin Sir2 in yeast, nematodes, and flies leads to life-span extension (11–13). The implication of Sir2 in aging across several species led to the identification of the small molecule resveratrol, which was able to activate Sir2 as well as the mammalian homolog SIRT1 (14). Later, resveratrol was found to extend the life span of mice fed a high-fat diet, as well as having beneficial effects in nonhuman primates (NHPs) fed a high-sugar, high-fat diet (15–18). Compounds with higher specificity and potency as SIRT1 activators were later synthesized, and two of these, SRT1720 and SRT2104, have been shown to extend the life span of mice fed a standard diet (19, 20). These animal studies have led to the initiation of several clinical trials using SIRT1 activators in humans (**http://www.clinicaltrials.gov/ct2/results?term=resveratrol&Search=Search**).

Model organisms continue to form the basis of aging research, as ethical issues, long natural life span, environmental influences, genetic heterogeneity, and various other limiting factors complicate use of human subjects in aging research (**http://www.afar.org**). But how do we assess the ability of an intervention to improve both the health and longevity of an organism? Great strides have been made since the pivotal reports of McCay describing the life-span extension of rats on caloric restriction (CR) in the early twentieth century (21). A host of more sophisticated assessments of health span and life span are now available (**Figure 2**). Nevertheless, we must still consider the limitations of these models to accurately reflect human aging. In this review, we attempt to describe vertebrate animal models that have been used to study aging and age-related diseases, as well as suggest future directions for this research.

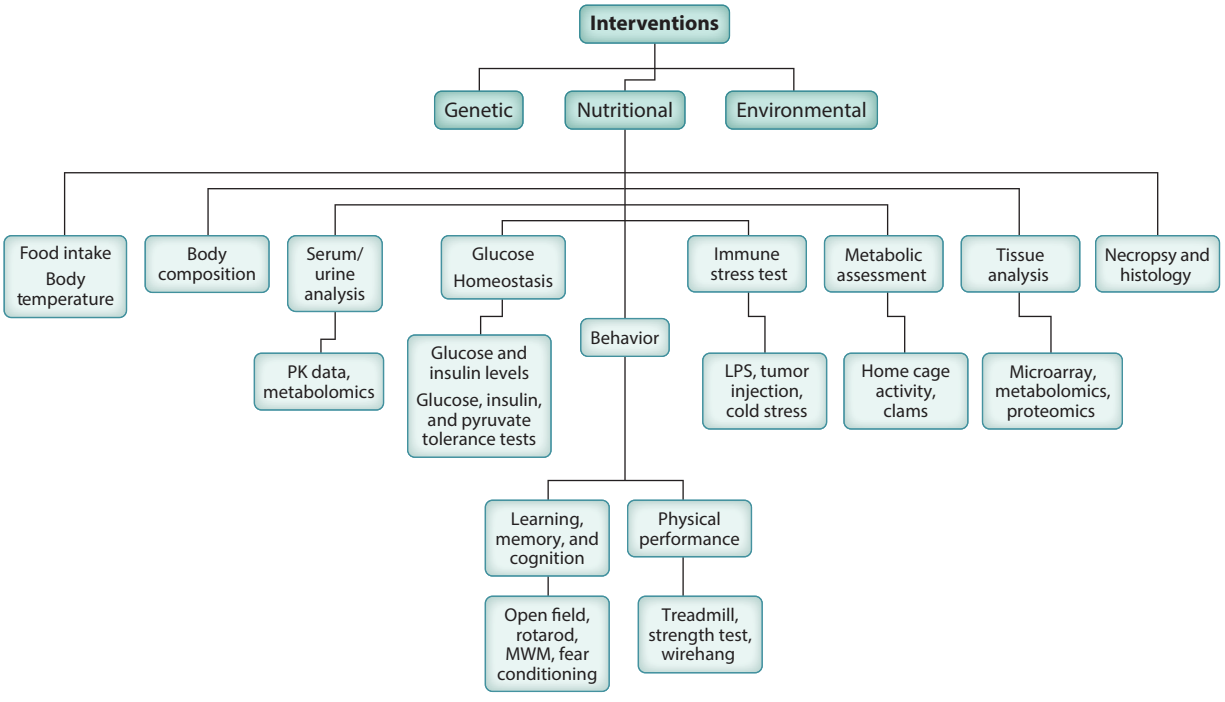

Figure 2

Testing interventions for longitudinal studies. Longitudinal assessments every three to six months on at least $n = 10$ animals per intervention are performed on animals across their life span to assess health span. Abbreviations: LPS, lipopolysaccharide; MWM, Morris water maze; PK, pharmacokinetic.

RODENT MODELS OF AGING

The laboratory mouse and rat are common models for the study of aging and age-related diseases. The wealth of background knowledge, convenience of use, capacity to regulate environmental factors, genetic manipulability, and expense have led to an explosion of aging-related research focused on these models. Furthermore, their short life span relative to humans makes them easier to study than long-lived animals. Indeed, rodents paved the way for both dietary and genetic interventions in aging, as best illustrated by the discovery that CR extends rodent life span, as well as the finding that mutations in certain genes are associated with longevity. In the following sections, we discuss these common aging models, including their possible limitations.

Mouse Models

Inbred mice. Inbred mice have been the most extensively used strains for the study of aging and age-related diseases to date. This method of breeding between relatives (usually a brother and sister) increases the genetic similarity between the offspring; thus, differences between animals of the same genetic strain can be attributed to environmental or treatment effects. The idea is to minimize other factors that may affect an outcome or complicate interpretation of a study. Although inbred strains have had considerable use in the study of aging, the major concern surrounding their use is that some commonly used strains show only a limited range of pathology. For example, C57BL/6 mice, upon which 70% of published animal studies have relied, show high prevalence of lymphoma and increased susceptibility to metabolic dysregulation (22). But whether one strain is more appropriate than another remains contentious. In particular, the assessment of health span in inbred mice can be confounded owing to premature vision or hearing loss compared with other inbred strains (23). Furthermore, differences in reported mean life span can vary up to 20% depending on the strain and sex of the mouse, despite the same genetic background and environment (24, 25). The power of inbreeding is remarkable given its capacity to minimize genetic variability; however, conclusions must be interpreted with caution, as data from a single inbred strain may not be representative of the species as a whole. Further, the resulting genetic uniformity of inbred strains is not representative of the human population.

With this in mind, the body of information surrounding the development, reproduction, physiology, behavior, and genetics of these mice is vast. The Mouse Phenome Project conducted by the Jackson Laboratories is an in-depth study of the physiology and life span of 31 genetically diverse inbred mouse strains (**http://phenome.jax.org/**). Launched in 2001, the Mouse Phenome Database (MPD) is the data coordination center for the international Mouse Phenome Project. The MPD integrates quantitative phenotype, gene expression, and genotype data into a common annotated framework to facilitate query and analysis (26). With more than 3,500 phenotype measurements or traits relevant to human health, including cancer, aging, cardiovascular disorders, obesity, infectious disease susceptibility, blood disorders, neurosensory disorders, drug addiction, and toxicity, the MPD represents an important resource for the study of aging biology and its relevance to human disease.

Outbred and F1 mice. Outbred and F1 mice are generally used for the same reason: hybrid vigor, with long life spans, high disease resistance, early fertility, large and frequent litters, rapid growth, and large size. However, unlike F1 hybrids, outbred mice are genetically undefined. This brings an advantage, as they can be considered to be more representative of the human population; however, it represents an obstacle when assessing the benefits of an intervention (**Figure 2**). These outbred stocks should not be used in situations where smaller numbers of mice from a range of inbred

strains would give optimal results, such as determining sensitivities to substances or examining physiological parameters (27). Caloric and methionine restriction are two of the most frequently used interventions to extend life span in mice. In (BALB/cJ x C57BL/6 J)F1 mice, methionine restriction has been shown to increase maximal life span as well as lower levels of serum insulin-like growth factor 1 (IGF-1), insulin, glucose, and thyroid hormone (28). Forty percent CR extends maximal life span in male B6D2F1 mice by 20% relative to ad libitum–fed controls (29); however, whether this effect also extends to females and to other F1 and outbred strains remains to be seen.

Wild-derived mice. But what about the wild-derived mice? It has been suggested that laboratory mice eat roughly 20% more than wild mice under ad libitum laboratory conditions on a weight-adjusted basis, indicating that they are metabolically obese (30). Thus, the life-span extension in these fat CR mice may simply be due to the reduction of food intake to what they should normally be eating if they were in the wild (31). Nevertheless, CR does extend life span in wild mice, but whether there is a beneficial effect on health span remains to be determined (31). These results do agree with some aspects of the CR literature in that the incidence of tumors was remarkably reduced in wild mice on CR (31). Few studies since Harper et al. (31) in 2006 have used wild-derived mice, most likely owing to the tedious nature of catching wild mice. However, one should consider genetically heterogeneous models of mice and their utility in aging research, in particular the four- or eight-way cross (see next section).

Genetically heterogeneous mouse models. Genetically heterogeneous mouse models provide many advantages for research on aging but have been used infrequently. These mice are the offspring of four or eight different grandparent lines. In this cross, each mouse is genetically unique, but replicate populations of essentially similar genetic structure can be generated quickly, at low cost, and of arbitrary size from commercially available, genetically stable hybrid parents (32). A recent study of genetically heterogeneous mice created from four inbred strains (BALB/c, C57BL/6, C3H, and DBA2), referred to as HET3 mice, found that more than 90% died of cancer (33). Although this homogeneity in the cause of death could be considered beneficial under some circumstances, it highlights the importance of natural variation in causes of death for mouse models to parallel the human situation. We do know that CR extends life span in HET3 mice (34).

Accelerated Aging

An important step in our understanding of aging was the description of several inherited human diseases that show accelerated aging (35). Notably, all of these diseases appear to be caused by mutations in genes that are involved in maintaining genome integrity, supporting the idea that the accumulation of DNA damage may be involved in aging. Each of the diseases displays only a few features of normal aging phenotype, and the disorders are therefore also called segmental progerias. The diseases include Werner syndrome (36), Hutchinson-Gilford progeria (37), Rothmund-Thomson syndrome (38), Bloom syndrome (39), Nestor-Guillermo progeria (40), dyskeratosis congenita (41), ataxia telangiectasia (42), xeroderma pigmentosum (43), and Cockayne syndrome (44). The genetic mutations underlying all of these disorders have been characterized, and knockout mouse models have been created for most of them (45). Interestingly, many of these knockout mice show much milder phenotypes, and premature aging often occurs only when multiple genes are knocked out. Thus, it appears that backup systems exist for some of these key pathways in mice that likely involve genes not yet defined. We describe some of the mouse models in greater detail below.

Human Werner syndrome is the accelerated aging disorder that most closely reflects human aging and is caused by mutations in the RecQ-like DNA helicase *Wrn*. Patients develop normally until they reach early adulthood. At this time, premature aging, such as cerebral atrophy, hair loss, cataracts, osteoporosis, diabetes, and cardiovascular disease, becomes apparent, and the patients generally die of heart disease at approximately 40–50 years of age (46, 47). In the late nineties, a mouse model was generated that showed little phenotype (48). However, crossing the $Wrn^{-/-}$ mice with mice lacking the tumor suppressor P53 led to a modest decrease in life span, whereas knockout of the telomerase complex in a $Wrn^{-/-}$ background replicated many features of human aging, such as osteoporosis and diabetes, possibly owing to defects in cellular replicative potential (49, 50).

Hutchinson-Gilford progeria is caused by mutations in the *LMNA* gene, which encodes the nuclear filament proteins lamin A and C. The *LMNA* mutation creates an alternative splice site leading to the formation of a shortened mRNA transcript encoding a protein dubbed progerin that accumulates in cells from Hutchinson-Gilford progeria patients. Accumulation of progerin disrupts normal nuclear architecture, leading to DNA damage and replication problems (51). Patients suffering from Hutchinson-Gilford progeria display aging-associated pathology, such as cardiovascular disease, osteoporosis, hair loss, and loss of adipose tissue, and have a mean life span of approximately 12 years (52). Hutchinson-Gilford patients in general do not display neurological aging to any significant extent. Several mouse models have been created that recapitulate aspects of the disorder, such as cardiovascular disease and osteoporosis (53–55). More recently, an inducible transgenic mouse that overexpresses progerin was created that showed premature skin aging and hair loss (56). Notably, the skin aging was reversed when progerin expression was inhibited. Possible cardiovascular changes in this mouse model were, however, not reported.

Dyskeratosis congenita is caused by mutations in genes believed to be involved in maintenance of telomeres, the specialized structures that form the ends of the chromosomes. These include the RNA component of telomerase, TERC, and protein components of telomerase, such as TERT and DKC1 (57). Dyskeratosis congenita is characterized by the triad of nail dystrophy, reticular hypopigmentation, and leukoplakia. In addition, bone marrow failure, idiopathic lung fibrosis, graying of hair, and hair loss occur with varying penetrance. The disease thus shows only relatively minor features of normal aging. Telomeres have, however, been implicated in aging, particularly at the cellular level. This correlation originally stemmed from the observation that primary cells in culture divide only a limited number of times. This phenomenon, termed the Hayflick limit, is believed to be caused by telomere shortening that occurs with each division owing to problems in replicating the very ends of the telomeres (58). Telomerase helps to maintain the telomere length, and deficiencies in this enzyme lead to defects in proliferating cells, such as stem cells in the skin. Interestingly, mice have very long telomeres, and knockout of TERC or TERT does not lead to any immediate phenotype. However, inbreeding of telomerase-deficient mice leads to progressive loss of telomere length with each generation. Thus, third-generation telomerase null mice show accelerated aging (58–60). As in humans, proliferative tissues are particularly prone to telomere shortening, and TERC or TERT knockout mice may therefore represent good models for interventions that aim at maintaining stem cell pools. It remains largely unexplained why telomerase deficiency affects the largely nonreplicating lung and has no phenotype in the vigorously replicating intestinal mucosa. Furthermore, it is not well explained why mice that have long telomeres have short life spans, while humans have short telomeres and live substantially longer. In addition, although telomere shortening has been shown in circulating leukocytes with age in several studies, it is unknown whether this correlates with increased mortality (61–63). It is most certain that telomeres play a role in cellular senescence in vitro; however, the effect on aging in vivo is still being questioned.

Xeroderma pigmentosum, particularly complementation group A, as well as ataxia telangiectasia and Cockayne syndrome are the only accelerated aging disorders in which severe neurodegeneration is highly prevalent (64–66). Xeroderma pigmentosum is caused by mutations in several genes (*XPA, XPB, XPC, XPD, XPE, XPF, XPG,* and *XPV*) involved in a DNA repair pathway called nucleotide excision repair (43). Cockayne syndrome is most commonly caused by mutations in *CSA* or *CSB*, two proteins involved in transcription-coupled nucleotide excision repair. Ataxia telangiectasia is caused by mutations in the ATM kinase, an enzyme primarily involved in the signaling cascade after double-stranded DNA breaks (64). Although they work in different DNA repair pathways, the neurodegenerative phenotypes are relatively similar, with cerebellar degeneration, ataxia, and neuropathy. All of these diseases manifest in early childhood, and patients have an average life span of 12 years (Cockayne syndrome) to 30–40 years (ataxia telangiectasia and xeroderma pigmentosum group A). Several mouse models have been created to describe these diseases. None of them capture the severity of the disease in humans, and only very minor degeneration of the cerebellum has been reported in one model of ataxia telangiectasia (38).

The Cockayne syndrome mice mirror some aspects of human diseases, such as wasting and loss of cells in the inner ear, and show a 10% reduction in brain size (9). *XPA* mice, however, appear completely normal, although they show higher propensity for UV-induced skin cancer (65). Crossing the *XPA* mice with the Cockayne syndrome mice produces a profound neurodegenerative phenotype with greatly shortened life span and global neurological deterioration (67, 68). *ATM* null mice display only minor neurodegenerative phenotypes, although they do recapitulate aspects of ataxia telangiectasia, such as immunodeficiency (69, 70). $ATM^{-/-}$ mice may thereby represent an interesting model for the study of immune senescence. More recently, mice harboring catalytically dead ATM show early embryonic lethality, perhaps indicating that nonfunctional ATM may interfere with a general DNA damage response and that other kinases may compensate if the ATM protein is completely absent (71).

Because of the idea that deficient DNA repair may contribute to aging, several mouse models have been created with disruption of various enzymes in this pathway. One interesting model is the ERCC1 and XPF knockout mice. ERCC1, in complex with the endonuclease XPF, participates in nucleotide excision repair as well as interstrand crosslink DNA repair. Interestingly, *ERCC1* and *XPF* knockout mice show a strong multisystemic degeneration and die of liver failure upon weaning (72, 73). The hepatic phenotype and early death of *ERCC1* mice can be rescued by overexpression of liver-specific *ERCC1*, which leads to survival after weaning and death from kidney failure at two to three months of age (74). Notably, transcriptional profiling in the liver of *ERCC1*-deficient mice at postnatal day 15 shows attenuation of the IGF-1 axis (75). As we touch on below, loss of IGF-1 signaling is known to extend life span in mice and nematodes, indicating that loss of this pathway in the *ERCC1*-deficient mice may be a compensatory response to DNA damage accumulation. Indeed, the same transcriptional changes are observed in $Xpa^{-/-}/Csa^{-/-}$ double-knockout mice (76).

Accumulation of mitochondrial damage has been proposed to be the underlying cause of aging (77). Considerable research has supported a role for mitochondria in the aging process, and a large number of animal models have been generated that support the mitochondrial theory of aging. The most famous example of this may be the mutator mouse. This mouse model harbors a mutation in the proofreading domain of the murine mitochondrial DNA polymerase gamma (*POLG*) (78). This leads to the accumulation of mutations in mitochondrial DNA but interestingly does not lead to increased reactive oxygen species (ROS) production. The phenotype of the mice is characterized by weight loss, alopecia, osteoporosis, cardiomyopathy, and hypogonadism and thereby shows significant overlap with many features of human aging.

Notably, the mice show stem cell renewal defects but no overt neurodegenerative phenotype (79). This is in contrast to humans with mitochondrial diseases, in whom neurodegeneration is prominent but osteoporosis, hair loss, and anemia are rare (80). Even though no increase in ROS production is observed in the mutator mouse, other mouse models have supported the role of free radicals in aging. Particularly strong support came from the observation that overexpression of catalase, a ROS scavenging enzyme, targeted to mitochondria leads to life-span extension in mice (81). However, other models with decreased capacity to scavenge ROS have not demonstrated shortened longevity (82).

Delayed Aging

Caloric (83) and methionine (84) restriction remain the only non-genetic, non-pharmacological interventions to increase life span in mice. In fact, it has been nearly a century since the potential life-extending effects of CR were first reported, and we are still searching for the elusive mechanism. CR extends life span in most species tested (as reviewed in 85). But recent evidence suggests that the subtleties of CR may be more complex than initially thought. Indeed, the effect of 40% CR on 41 recombinant inbred strains (ILSXISS) of mice, both males and females, found a huge variation in the response to CR, with CR being detrimental to some strains (86). There are clear examples of the differential response to CR in the literature. For example, reports of CR on the DBA2 strain show anywhere from a detrimental effect on life span of approximately 6% to a beneficial effect on life span of 20–50% depending on the sex of the animals (87–89). Furthermore, diet composition plays a major role. Most recently, it has been shown that longevity can be manipulated through altering macronutrient content, with mice fed a low-protein, high-carbohydrate diet having maximal life span (90). And this is before we even consider the effect (if any) of CR. However, the translational potential for humans is low given the proven difficulty of altering diet to manage diseases in people and the aversion to consuming 40% less calories for years. Thus, alternative strategies are in demand. Perhaps if one understood how CR works, an alternative approach could be developed.

In 2000, the Interventions Testing Program was developed to systematically study the effects of diets, drugs, or other interventions on life span in mice. Unfortunately, this program is reserved specifically for mice, as the number of rats required to obtain statistical significance for a particular intervention far outweighs the space and financial availability to conduct these studies. One of the first compounds tested, rapamycin, was found to extend median and maximal life span of both male and female genetically heterogeneous mice when fed beginning at 600 days of age. Based on age at 90% mortality, rapamycin led to an increase of 14% for females and 9% for males (3). Rapamycin administered in the food from 9 months of age to genetically heterogeneous mice resulted in significant increases in life span, including maximum life span, with an associated increase in median survival of approximately 10% in males and 18% in females (33). Other pharmacological interventions, such as resveratrol, metformin, and sirtuin activators, have been demonstrated to increase life span in mice (15, 19, 91), through modulation of the nutrient sensing pathways controlled by AMP-activated protein kinase and sirtuin 1 (92, 93). However, the efficacy of these interventions might be sex and strain specific, and this warrants further investigation. It is important to consider both males and females when determining the success of an intervention, genetic, pharmacological, or otherwise (**Figure 1**). Indeed, male, but not female, transgenic mice overexpressing Sirt6 (94) exhibit increased life span. Similarly, nordihydroguaiaretic acid and aspirin significantly increased life span in heterogeneous male, but not female, mice (95). And more recently, it has been shown that life-span extension of HET3 mice on rapamycin is independent of insulin sensitivity (96).

Genetic Models of Delayed Aging

In looking for the fountain of youth, several models have been identified through which genes are shown to play a major role in the extension of life span. The Ames dwarf, Snell dwarf, and growth hormone (GH) receptor knockout (GHRKO) mice are the classical mouse models of delayed aging. These strains display exceptional longevity through alteration in the GH pathway resulting in low-circulating IGF-1 (97, 98).

The Ames and Snell dwarf mice have loss-of-function mutations in their *Prop-1* and *Pit-1* genes, respectively, resulting in deficiencies in circulating levels of thyrotropin, prolactin, and GH, which lead to life-span extension (99). Interestingly, there is a sex-specific difference in maximal life span of Ames dwarf mice, with an observed increase of 20% in males and 50% in females. Snell mice, however, live up to 50% longer than their wild-type littermates (97, 99). These mice show some of the characteristics of CR, including lower core body temperature (100, 101), improved insulin sensitivity (98), enhanced antioxidant defenses (102), and delayed onset of neoplasia (103, 104), which may play roles in their increased longevity. A defect in the *Klotho* gene leads to a premature aging phenotype characterized by arteriosclerosis, osteoporosis, age-related skin changes, and ectopic calcifications, together with short life span and infertility (105). Conversely, the transgenic mice that overexpress *Klotho* exhibit significant resistance to oxidative stress associated with moderate resistance to insulin/IGF-1, which may partly explain why these mice live longer than wild-type mice (106). The GHRKO mouse was generated through the targeted disruption of the GH receptor and GH-binding protein (97). These mice are long-lived and have a reduction in glucose, insulin, thyroid hormones, and core body temperature that is in agreement with observations reported for the Ames dwarf mouse (100). The GHRKO mice show a similar increase in life span between males and females of 23% and 25%, respectively (97). Reductions in these parameters may be important to the underlying mechanisms of delayed aging in these animals. Interestingly, the GHRKO mice are obese but insulin sensitive (97), which is paradoxically opposite to what is observed in CR. A recent study that examined the role of the visceral fat in adiposity and insulin sensitivity found that removal of visceral fat resulted in an improvement in insulin sensitivity in wild-type mice but made the GHRKO mice more insulin resistant (107). When GHRKO mice are put on CR, there is no life-span extension (108, 109), perhaps because CR reduces adiposity, which may not be beneficial to these animals (107). Consistent with this idea of altered fat signaling, removal of visceral fat at five months of age (110) leads to increased medial and maximal life span in rats. The GHRKO mice achieve life-span extension by a mechanism that appears to overlap the effects of CR given that CR cannot augment the effect. Thus, the available tools to examine the mechanisms behind aging and potential interventions are vast.

Rats

Rats have been extensively used in the laboratory for research into many areas, including cardiovascular disease, neurological disorders, neurobehavioral studies, cancer susceptibility, and renal disease, as well as for behavioral studies of cognition. Such research has relied on the widespread use of inbred Fischer 344 (F344) rats as well as other genetically defined (F1 hybrids) and outbred rat populations. The National Institute on Aging (NIA) aging animal colony has provided F344 rats since inception, possibly accounting for the relatively widespread use of this model in aging research even today. Three options for aging rats, all genetically defined, are now available under the NIA program: the F344, Brown-Norway (BN), and F1 hybrid of F344 × BN strains. Interestingly, F344 × BN rats are used as models for progressive aortic vasculopathy, as changes in the thoracic aorta have been shown to display age-related pathology similar to what

occurs in humans (111). In cognitive studies, it is important to understand the phenotype of the model that you are using to identify any pathologies or disabilities, which may affect the outcome. For example, age-associated blindness can negatively impact and confound cognitive assessments. Another issue is the occurrence of a single severe disease in inbred animals that can confound the interpretation of an aging study; for instance, nephropathy in F344 rats is the major cause of mortality (112).

Transgenic models. Although the use of transgenic mice in research has steadily increased over the past years, this has not been the case for transgenic rats. There have been hurdles to the development of transgenic rats, such as sensitivity of rats' fertilized eggs under in vitro conditions. Nevertheless, recent advances in the development of transgenic rats have meant they are gaining importance in cognitive research. In Alzheimer's disease, it has been suggested that rats are a more appropriate model for the human disease given that rats are closer to humans and have a predictable and multifaceted behavioral display (113). However, rat Alzheimer's disease models do not display the human-like neurofibrillary tangles that some mouse models do (113). Transgenic rat models have been used for the study of retinal degeneration, including the P23H transgenic albino rat for the study of the retinitis pigmentosa mutation (114) and the Royal College of Surgeons transgenic rat used for the study of human retinitis pigmentosa (115).

Interventions for life span extension. McCay et al. (21) presented the very first report of extended life span in his white rats upon dietary restriction. Since this pivotal report, many labs have confirmed this finding in rats (116–118). Notably, removal of the pituitary gland in male Wistar rats at 70 days of age produced similar life span–extension effects as CR begun at the same time point (119). Further supporting the role of GH-IGF-1 in longevity, heterogeneous GH knockout rats had life-span extension of approximately 10% relative to control rats, although the homozygous GH knockout rats are actually shorter lived (120). However, not all interventions are successful; take, for instance, metformin, which is successful in mice (91) but not in F344 rats (121), and 2-deoxyglucose, which does not extend life span in F344 rats (122) but does in *Caenorhabditis elegans* (123). Moving forward, integrated approaches of both mouse and rat models will together advance our understanding of aging and age-related diseases.

NAKED MOLE RATS

The naked mole rat (NMR; *Heterocephalus glaber*), also known as the sand puppy or desert mole rat, is the longest-living rodent known to man, with a maximum life span of approximately 30 years (124). These mouse-sized rodents live up to five times longer than expected based on their small body size, but they are highly socialized rodents that are commonly used in behavioral, neurological, and physiological research (124, 125). NMRs are common to the subterranean burrows in the arid and semiarid regions of the horn of Africa. They are the first mammals discovered to exhibit eusociality, with the presence of a female queen and one to three reproducing males, with the rest of the members of the colony functioning as workers for gathering food and protection (124). But it is their biology that makes them so attractive to gerontologists. Indeed, NMRs aged >24 years do exhibit signs of aging consistent with humans, such as retinal degeneration and osteoarthritis (125), but display negligible senescence, no age-related increase in mortality, and high fecundity until death. The possibilities for translation to human health are undoubtedly significant if we discover the mechanism behind their well-preserved health.

Potential Mechanisms for Longevity in NMRs

Initially, enhanced antioxidant defense was thought to be one of the major mechanisms through which NMRs had enhanced longevity (126) and extreme resistance to experimentally induced tumorigenesis (127). The activity levels of the antioxidants, such as superoxide dismutases (SOD1–2), do not change with age in NMRs, although they do decline with age in mice (128). Thus, maintenance of the activity of SOD1 and -2 rather than an enhanced activity may contribute to the extended life span of NMRs. Indeed, CR maintains the levels of these enzymes into old age in mice (129).

The insulin/IGF signaling pathway is another important modulator of life span. In CR, maintenance of this pathway is proposed to be one of the major factors influencing longevity (130). Interestingly, NMRs display an abnormal response to a bolus of glucose as measured using the glucose tolerance test with prolonged hyperglycemia (131). Their pancreata show an unusual distribution of endocrine cells relative to most other rodents, which may explain their unusual hyperglycemic condition. These animals show lower insulin levels (126), which further highlights the complexity of the IGF pathway in longevity. These lower insulin levels and reduced levels of IGF-1 are consistent with changes reported in CR (130, 132).

NMR cells produce fewer aberrant proteins, supporting the hypothesis that the more stable proteome of the NMR contributes to its longevity (133, 134). Recently, it was shown that NMRs have high levels of basal autophagy (135). Increased translational fidelity may play a role in the NMR's longevity, and differences in translational fidelity may be important in determining life span (133). A whole genome sequencing analysis of the NMR genome found that genes related to the degradation of macromolecules, mitochondrial encoded genes, were not altered with age in NMRs (136). Furthermore, telomerase reverse transcriptase showed stable expression regardless of age (136). Taken together, these results highlight differentially expressed patterns of expression of NMR genes, which may underlie longevity mechanisms in this animal. Furthermore, it would be of significant interest to compare the gene expression profile of mice or rats on CR and on ad libitum feeding to that of NMRs. Given the tenfold difference in life expectancy of mice and NMRs and the likely high degree of genetic homology between the species, any differences detected are likely to be important in explaining the differences in longevity. Would CR further extend the life span of NMRs, or would it be detrimental?

PRIMATES

NHPs are perhaps the most appropriate model for the study of aging and age-related diseases. Traditionally, rhesus macques (*Macaca mulatta*) have been the prime focus of aging research. Two programs, one at the NIA of the National Institutes of Health and the other by the University of Wisconsin–Madison, have studied this species in ongoing longevity studies for more than 30 years. Rhesus monkeys are commonly used in biomedical research owing to their similarity to humans across a wide range of variables, including genetics, endocrinology, physiology, neuroanatomy, and cognitive function. However, there are drawbacks to the use of these monkeys in research. Their weight and strength pose difficulties in husbandry, and sophisticated equipment is needed to navigate daily life in these facilities. Furthermore, the strict social hierarchies and potential for aggressive behavior mean that these incredibly intelligent animals need special consideration and substantial environmental enrichment to keep them appropriately cared for. Monkeys can carry and transmit many dangerous pathogens, making it expensive to study them in the context of aging. Furthermore, the costs and ethical concerns of supply alone limit the contribution of NHPs to research.

Interventions for Longevity and Health

In recent years, two studies have highlighted the importance of the study environment for calorie restriction and its application to humans. Most notably, the University of Wisconsin–Madison, and the NIA NHP CR studies have highlighted the subtle differences in response to CR (137, 138). Although we can all agree that CR delays the onset of age-associated diseases, the data on whether this is also associated with life-span extension are conflicting. Indeed, further studies and analysis are needed to definitively address this question. Interestingly, we have recently shown that two years of resveratrol treatment improved the metabolic syndrome associated with a high-fat, high-sugar diet in rhesus monkeys (16–18). Clearly, the translation potential for compounds like resveratrol is great, as resveratrol now is in clinical trials for use in humans, with at least 80 different trials ongoing or completed as of the publication of this article (**http://www.clinicaltrials.gov**).

ALTERNATE AGING MODELS

Although primates and rodents have supplied a wealth of information regarding the aging process, alternative models are useful to test ongoing hypotheses of aging. This is particularly pertinent because species-specific changes may influence results and data interpretation. The rate-of-living hypothesis of aging is an example of a theory that initially explained many observations in aging but was later questioned based on data from other species. This theory was based on observations more than a century ago by the physiologist Max Rubner, who found that longer-lived species generally have a lower resting metabolism per gram body weight than shorter-lived species do (139). Although this relationship has been found across several species, there are several exceptions. Birds, for example, appear to defy this relationship by living considerably longer than expected for their metabolic rate (140, 141). To understand aging, it is therefore clear that information from multiple species across the phylogeny of life is of value. We now discuss a few of the alternative vertebrate aging models that have been reported in the literature. Although these models may appear rather extraordinary, each has its own strengths and weaknesses.

Fish

Fish have been surprisingly robustly present in the aging field throughout the years. This may partially stem from some rather controversial claims in the early twentieth century that fish do not age (142), a statement that was later repudiated (143). Nevertheless, fish have emerged as an interesting model system in general biology and aging research. The zebrafish (*Danio rerio*) remains the most common fish in the lab setting. It has a life span of approximately two to three years and may therefore not be particularly advantageous for life-span studies as compared with rodents. However, zebrafish have remarkable regenerative capabilities that could be of interest for tissue repair and thus for longevity (144).

Another species that shows promise as a model for longevity is the turquoise killifish (*Nothobranchius furzeri*). *N. furzeri* have several advantages compared with other vertebrate aging models. First, the fish has one of the shortest life spans (~13 weeks) of any vertebrate species (145). Second, the fish can be kept at relatively high population densities, allowing for larger and cheaper population studies than usual for rodent life-span studies. Third, the eggs are resistant to desiccation and can be kept at room temperature for months. Storage of strains of fish is therefore much easier than for rodents. Fourth, each female produces several hundred eggs, allowing for rapid expansion of a colony (146). In addition, these fish respond with an increase in life span in response to CR and show life-span extension after resveratrol treatment under standard diet

conditions (147). Based on these observations, *N. furzeri* represents an interesting and inexpensive model system for interventions in aging and could thus represent an ideal model system for higher throughput screening of putative life span–extending compounds. Indeed, several labs are currently pursuing research with this model (see **http://www.nothobranchius.info/**).

Dogs and Cats

Domesticated species, such as dogs and cats, represent interesting model systems for aging. Even though the average canine life span of 10–12 years discourages longevity studies, dogs spontaneously develop many age-related phenotypes, such as muscular and neurological decline, as well as cardiovascular disease (148–151). Rodents, however, do not develop significant neurodegeneration with age unless severely genetically manipulated (152). Dogs may therefore be particularly interesting in the study of cognitive deterioration and age-associated neurodegenerative disorders (153). In addition, the physiology and pathology of dogs have been extremely well characterized. Similarly, cats represent another physiologically well-characterized domesticated animal that has been used in aging studies (149, 154–156). As in dogs, several pathological age-associated processes occur in felines, including kidney disease, arthritis, sarcopenia, and neurological decline (149, 154–156). Cats live an average of 12–14 years, and life-span studies in this species are therefore also problematic (157); however, their aging phenotype may make them attractive models.

Birds

When looking across the life span of multiple species, longevity tends to scale according to the size of the animal, in agreement with the rate-of-living hypothesis of aging. Birds, however, live a remarkably long time when considering their relatively small body size (158). Interestingly, birds maintain blood glucose levels one- to threefold higher than most mammals but with low insulin and high glucagon levels (159). This could indicate that the insulin/IGF-1 pathway might be involved in the longevity of birds. Indeed, although birds retain very high GH and IGF-1 levels during development, the levels of these hormones decrease in adulthood (158). Other possible explanations for the apparent longevity of birds have been related to decreased susceptibility to oxidative stress and increased telomere length (158). In addition, fertility appears to be well preserved with age in birds (160). Several bird species can be kept in a lab setting, and their high reproductive capacity makes them easy models to work with. Life-span studies are, however, difficult owing to their inherent longevity. The Japanese quail is a common lab bird that lives for a maximum of six years and interestingly responds similarly to CR as mammals (161). Birds represent an interesting animal for comparative cross-species studies of the interplay of metabolism and aging.

FUTURE DIRECTIONS

Animal models form the basis for preclinical biomedical research and will undoubtedly continue to do so, as their life span, although shorter, essentially mimics that of humans, highlighting the universality of the aging process (**Figure 1**). Transgenic mice have contributed greatly to our knowledge of a multitude of different biological processes; however, this animal model also has its drawbacks. In particular, inbred mouse strains are prone to numerous diseases, perhaps masking true physiological responses to various interventions. This is widely acknowledged, and many large-scale investigations, such as the Aging Interventions Testing Program, now use the four-way

cross. Nevertheless, even outbred strains of mice are still significantly limited in the aging phenotype. For example, normally aged mice do not develop neurodegeneration and have very low prevalence of cardiovascular disease (162, 163). It is perhaps not surprising that some organisms, such as mice, age differently than humans; however, this is important to remember when attempting to extrapolate from murine data to human physiology. It is thus possible that with expanding physiological knowledge of species not conventionally used in aging research, many nonmurine animal models may contribute to our understanding of aging. In particular, transgenic primate models have now been generated, and useful primate models for studying genetic pathways involved in aging could therefore be created (164). However, rodent models still represent one of the best tools in our toolbox, and much translational knowledge can still be gathered from these models. In conclusion, a multifaceted approach using different model organisms is the key to further understanding human aging and age-related diseases.

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