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Alive and Well? Exploring Disease by Studying Lifespan

Jamie O. Brett^{1,2,3} and Thomas A. Rando^{1,2,3,4}

¹Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine; Stanford, CA USA

²Paul F. Glenn Laboratories for the Biology of Aging; Stanford University School of Medicine; Stanford, CA USA

³Department of Neurology and Neurological Sciences; Stanford University School of Medicine; Stanford, CA USA

⁴Neurology Service and Rehabilitation Research and Development Center of Excellence; Veterans Affairs Palo Alto Health Care System; Palo Alto, CA USA

Abstract

A common concept in aging research is that chronological age is the most important risk factor for the development of diverse diseases, including degenerative diseases and cancers. The mechanistic link between the aging process and disease pathogenesis, however, is still enigmatic. Nevertheless, measurement of lifespan, as a surrogate for biological aging, remains among the most frequently used assays in aging research. In this review, we examine the connection between “normal aging” and age-related disease from the point of view that they form a continuum of aging phenotypes. This notion of common mechanisms gives rise to the converse postulate that diseases may be risk factors for accelerated aging. We explore the advantages and caveats associated with using lifespan as a metric to understand cell and tissue aging, focusing on the elucidation of molecular mechanisms and potential therapies for age-related diseases.

Aging, aging phenotypes, and age-associated diseases

Aging is undeniably linked to declining health, bringing increased disease susceptibility and “aging phenotypes,” the diminished functions of tissues and organs that afflict the older population universally and the younger population rarely [1]. The promise of research in the biology of aging is to reduce the disability that comes with age-associated disease and dysfunction. Success in the prevention or treatment of age-associated diseases not only improves healthspan, but also extends the average population lifespan. While this directional relationship between healthspan and lifespan is intuitive, it does not predict the trajectory of decline at the end of life – is morbidity simply delayed, or is it also compressed, or even prolonged (Figure 1)? Conversely, with increased lifespan as a goal and metric of many

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studies of the biology of aging, is there an equally tight directional relationship implying that longer lifespan means improved healthspan? This complex association of lifespan and healthspan, centered on age-related disease and tissue dysfunction, is being brought into sharp relief as studies of the biology of aging provide empirical evidence.

The overwhelming conclusions from studies that use increased lifespan as a primary endpoint indicate that lifespan and healthspan tend to lengthen together. For instance, the drug metformin, used in the treatment of diabetes, not only prolongs mouse lifespan, but also delays aging phenotypes and tumorigenesis [2•,3,4]. As another example, the pleiotropic protein Klotho, which modulates FGF signaling, insulin/IGF-1 signaling, ion homeostasis, and vitamin D metabolism, extends life when overexpressed in mice, and it also protects against aging phenotypes, stress-induced cardiac hypertrophy, and kidney failure [5–7]. Caloric restriction (CR) extends lifespan in rodents, along with preventing cognitive decline, neoplasia, cataracts, and sarcopenia [8–11]. Inhibition of mTORC1 activity in mice extends lifespan and counters age-related neoplasia, vascular dysfunction, neurodegeneration, and cardiac dysfunction [12,13,14••,15•,16•,17]. Most other studies that show lifespan enhancement also demonstrate improved aging phenotypes and reduced disease risks. Because the upstream and even immediate causes of death are difficult to determine, however, these studies raise the question of the extent to which extended lifespan is due to slowing of the aging process as opposed to the prevention of fatal disease, or indeed the extent to which that distinction is meaningful.

Shedding light on this issue is the molecular similarity of cells and tissues from healthy aged individuals, adult individuals with chronic diseases, and adult or even young individuals with segmental progerias. For example, skin cells from elderly individuals, from animals bearing the genetic defect of Hutchinson-Gilford progeria syndrome (HGPS), or from individuals with radiation dermatitis, squamous cell carcinoma, or psoriatic skin disease all manifest pro-inflammatory transcription, including NF- κ B activity and the senescence-associated secretory phenotype [18,19•,20–22,23•,24,25]. Inhibition of NF- κ B is able to reverse the skin aging phenotype, radiation dermatitis, and psoriasis [19,23•,25]. Hepatocyte dysfunction and impaired liver regeneration in aged animals, animals with the genetic defect of Werner's syndrome, and young animals with experimentally-induced non-alcoholic steatohepatitis are associated with oxidative damage and inflammation [26–29]. Individuals with sarcopenia, cancer-induced cachexia, or ovariectomy-induced muscle atrophy all exhibit increased circulating IL-6 and TNF α , muscle insulin resistance, and NF- κ B activation in muscle [30–36]. Studies of corneal endothelial cells in old individuals, mice with the genetic defect of XFE progeroid syndrome, and individuals with Fuchs' corneal endothelial dystrophy all implicate unrepaired DNA damage [37–41]. These examples suggest that the molecular vulnerabilities of each cell type are exposed during the passage of time, in premature aging phenotypes, and by disease etiologies.

These associations support more than one candidate explanation. In a conventional model, old age is a risk factor for disease. Indeed, as aged and diseased cells possess similar molecular profiles, older cells may have a head start on the track to cell failure. In both mice and humans, aged individuals present with smaller deviations from homeostasis when they develop disease [42••]. Cardiac arrest occurs with smaller vital signs deviations and delirium

is more easily triggered in aged individuals [43–45]. Aged tissues have more baseline mitochondrial dysfunction, DNA damage, inflammation, abnormal intercellular communication, and depletion of their reserve capacity used to return to homeostasis after further disturbance [46••].

As intuitive as this simple model may be, it is interesting to consider the converse: many diseases can be risk factors for aging itself if we characterize aging by the decline in tissue function noted above. For example, metabolic syndrome is associated with an earlier onset of aging phenotypes like declines in renal and cognitive functions [47,48]. Chronic kidney disease and kidney failure are associated with earlier onset of aging phenotypes like periodontal decay and skin inflammation [49–51]. Chronic obstructive pulmonary disease is associated with renal insufficiency, muscle wasting, and cognitive decline [52–54]. Individuals with HIV infection also suffer from premature frailty, cognitive decline, vascular dysfunction, and reduced bone density [55]. The treatment for HIV infection likely contributes to this deterioration [55], but another possibility is that the infection itself also accelerates the appearance of aging phenotypes [56–59]. Perhaps the increased incidence of many diseases with age is not caused by aging *per se*, but rather is more akin to a positive feedback loop: age is a risk factor for the development of many diseases, and various disease states pose a risk factor for accelerated aging.

Lifespan as a metric – of what?

Lifespan is a common metric for studies of organism aging, so it is important to consider the advantages of lifespan assays and the caveats of equating lifespan with healthspan. In short-lived organisms, the lifespan assay provides an efficient screen for interventions that may be beneficial for many aspects of health. Interest in Sirtuin activity as affecting animal health was raised by the discovery of a lifespan-extending mutation in *sir4* in yeast and the later identification of resveratrol as a Sirtuin-activating compound in yeast, nematode, and fly lifespan assays [60–62]. In many mouse studies resveratrol or SRT1720 (a Sirt1 activator) have effectively prevented high-fat diets from inducing metabolic derangements like insulin resistance, obesity, and dyslipidemia [63–66]. Although later studies found that resveratrol does not consistently increase lifespan in laboratory yeast and animals – perhaps because it acts in a strain- and diet-dependent manner [67,68•,69–71] – the initial findings in yeast lifespan assays and later observations in mice fed high-fat diets have stimulated a number of studies on the benefits of resveratrol for humans [72,73]. Even for compounds already with specific medical purposes, lifespan studies can suggest more general uses. For example, the mTORC1 inhibitor rapamycin has long been used clinically as an immunosuppressant. Lifespan studies in nematodes and yeast showed that inhibition of mTOR signaling dramatically extends lifespan, suggesting that the mTOR pathway affects organism health more generally [74,75]. Since then, inhibition of mTOR signaling in mouse models has been shown to delay a plethora of aging phenotypes and prevent many diseases, including neurodegeneration, heart failure, obesity, and even psychiatric diseases [12,13,14••,76–79]. Metformin has been used for over half a century as a treatment for diabetes. The discovery that it activates AMP-activated protein kinase and may serve as a CR mimetic [80,81] led to studies of its use in preventing and treating cancer and cardiac disease, even in individuals without diabetes [4,82–84]. Following these studies demonstrating alternative uses of

metformin were results showing that metformin extends healthy lifespan and reduces aging phenotypes in nematodes and mice [2•,85], although not in flies or rats [86,87]. These examples demonstrate how studying longevity may accelerate our discovery and promote research of compounds and pathways broadly beneficial for health.

Pursuing mortality as the sole endpoint, however, can give misleading results about aging. It is possible to increase life expectancy without affecting aging phenotypes or disease susceptibility. Entering the dauer larval state in nematodes increases lifespan, but the dauer worm is in stasis. Upon exit from this arrested growth stage, expected remaining lifespan is normal, as if the time spent in this dauer state were subtracted from nematode age [88]. It is critical to test whether new lifespan-extending interventions simply “pause” life or whether they indeed allow organisms to live longer and productively [89•]. At the other extreme of the course of life are death-targeted interventions. For instance, artificial life support can delay death as an organism continues to age. Much of the historical increase in human life expectancy is attributable to the prevention of early death from infectious disease [90,91]. For some female semelparous animals, death related to resource allocation to offspring can be delayed, such as by removing the optic glands of octopuses after egg-laying, later breeding of female Pacific salmon, or producing slow-growing or no offspring for antechinus shrews [92–94]. As aging research continues, it will be important to consider whether lifespan-extending interventions pause life or postpone death, or whether they truly affect aging phenotypes and diseases.

One of the challenges of lifespan screens is to distinguish those hits that reveal novel and distinct mechanisms from those that simply provide another way to induce an established mechanism for lifespan extension. For instance, *eat-2* or *eat-18* mutation in nematodes extends lifespan by reducing pharyngeal pumping, thereby restricting food intake [95]. These mutations serve as an alternative method of CR, and pharyngeal function itself is biologically interesting, but studying the molecular functions of *eat-2* or *eat-18* would be unlikely to yield insight into nematode aging and disease. Similarly, some interventions may extend lifespan by reducing appetite [96], or if administered in food, by making food unpalatable and inducing CR. Upstream mechanistic research would end up focusing on appetite and food intake control, rather than on pathways of cell dysfunction and health. Well-planned studies address this caveat by measuring food consumption and body weight [97,98]. Thus, seemingly novel processes or mechanisms identified in lifespan studies may simply point to known interventions.

Importantly, it is now clear that lifespan studies, especially in mammals, can lead to essentially false negative results in the search for interventions to improve healthspan. For example, Sirtuin activation is broadly beneficial for health and delays aging phenotypes in mice, but it does not alter normal mouse lifespan [68•,99]. One possible explanation is that resveratrol does not prevent lymphomas, a major cause of death in mice [68•]. Indeed, resveratrol even increased lymphoma incidence in a mouse model of Werner’s syndrome [100]. It does improve cardiovascular disease, however, which is more important for human mortality [68•]. As another example, elimination of senescent cells does not affect lifespan, at least in a progeria model, but it improves many aging phenotypes [101•]. In monkeys already fed a healthy diet, CR does not extend lifespan, but it reduces age-related diseases

and multiple aging phenotypes [102••]. Long-term aerobic exercise often does not alter lifespan in rodents, although in these studies it prevents frailty, metabolic decline, and cognitive decline [103–105]. It would seem that these interventions do benefit many aspects of aging, but perhaps not the few most lifespan-limiting factors in these models. Lifespan is thus a poor measure of age-related health in these situations, and lifespan studies alone would overlook these interventions as potentially valuable for the prevention of age-related disease and aging phenotypes.

Another caveat to lifespan studies is that environmental and genetic contexts determine intervention outcome (Figure 2). For example, overexpression of telomerase extends lifespan in mice genetically resistant to cancer [106] but causes cancer and early death in wild-type mice [107,108]. Resveratrol extends lifespan in a mouse model of HGPS [109], wherein progerin inhibits SIRT1 activity, but not in normal mice or in a mouse model of Werner's syndrome [69,100,110]. Lifespan extension occurs by treating a mouse model of XFE syndrome with intraperitoneal administration of wild-type muscle progenitors or by treating HGPS mice with recombinant IGF-1 or depletion of the lamin-interacting protein SUV39H1 [111–113], but whether these interventions would extend normal mouse lifespan is undetermined. Such interventions may address the specific mechanisms of a particular progeria which are lifespan-limiting in those conditions but might not alter the fundamental mechanisms of aging in non-progeroid organisms. This caveat of context dependence applies to all laboratory lifespan studies and will affect the translation of basic aging research to our understanding of human aging and disease.

Lifespan-extending interventions in the controlled laboratory setting may in fact not be predictive of lifespan changes by those same interventions in the wild. For example, mice deficient for the oxidative stress regulator p66Shc live longer and healthier in a protected laboratory setting [114,115], but in a controlled natural setting, their survival and fitness are reduced [116••]. In the wild, under food and temperature stress, alterations in the energy metabolism of these mice become a handicap rather than an advantage [116••]. Nematodes deficient for the PI3 kinase component of insulin/IGF-1 signaling live longer and have normal fitness in standard laboratory conditions, but they have reduced fitness in cyclic starvation conditions that mimic a natural setting [117]. Although CR does not extend the lifespan of diet-controlled monkeys [102••], it may extend the lifespan of monkeys fed *ad libitum* [118]. In addition, CR actually shortens the lifespan of many mouse strains, in association with their capacity for fat reduction [119••,120,121]. Superoxide dismutase overexpression dramatically increases female and male laboratory fly lifespan, but in wild-caught, naturally longer-lived flies, it increases lifespan in only one-half of strains for females and one-tenth for males [122]. If lifespan-extending interventions in laboratory animals actually lead to unchanged or shortened lifespan in the wild, the effects of parallel interventions in healthy humans are uncertain.

Conclusions

The strong association between lifespan and healthspan has made lifespan a useful metric for studies aiming to identify and elucidate pathways relevant for aging and diseases. A number of limitations of the lifespan metric, however, arise when lifespan and healthspan

are uncoupled. Lifespan can be extended by pausing life or delaying death rather than by altering aging. Conversely, some pathways relevant for aging and diseases should not be ignored even if they fail to extend organismal lifespan. The identification of healthspan biomarkers will be key to moving beyond lifespan assays to more directly measure health during aging.

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Figure 1. Longer lifespan and healthspan may come with unchanged, compressed, or even prolonged end-of-life morbidity

The graphs represent the decline in overall health with age for an individual under four theoretical conditions. For normal aging, there is an extended period of essentially normal functional capacity, followed by an inflection when functional capacity begins to decline, and a trajectory of declining functionality at some rate until death. Most reported interventions that extend lifespan also extend healthspan, but their effects on the trajectory of decline around and after the inflection point may differ and have not been well-studied. Theoretically, there are different patterns of decline that could follow the period of extended health. On the one hand (illustrated by the red line), the trajectory of decline would not differ from the control; it would just begin later. At another extreme (illustrated by the green line), the same extension of healthspan would precede a much longer and slower trajectory of decline. This would result in an even greater extension of lifespan, but the increased portion of life would be spent during a period of disability and functional limitation. Finally, there is the trajectory (illustrated by the blue line) that is consistent with the hypothetical “compression of morbidity” in which an extended period of good health is followed by a much shorter and more rapid period of decline before death [123]. Thus, it is important to assess not just how interventions affect lifespan and healthspan, but also how they affect the time of onset and pace of progression of age-related morbidity.

Figure 2. Intervention effects on lifespan and healthspan depend on genetic and environmental context

In assays of lifespan, each genotype for a given range of organisms (e.g., strains of mice or strains of yeast), is likely to respond to any lifespan-extending intervention in a unique way in terms of optimal “dose” (e.g., extent of caloric restriction or amount of metformin). Therefore, increasing the dose of the intervention could lead to increased lifespan of one strain but decreased lifespan of another strain (A). This explains the commonly observed phenomenon that a particular intervention has negligible effects on lifespan for many strains, increases lifespan for a subset, and decreases lifespan for a subset (B).