THE GERONTOLOGICAL SOCIETY OF AMERICA®

Journals of Gerontology: Biological Sciences
cite as: *J Gerontol A Biol Sci Med Sci*, 2016, Vol. 71, No. 4, 427–430
doi:10.1093/gerona/glv080
Advance Access publication August 22, 2015

OXFORD

Perspective

Measures of Healthspan as Indices of Aging in Mice—A Recommendation

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Received February 2, 2015; Accepted April 18, 2015

Decision Editor: David Le Couteur, PhD

Abstract

Over the past decade, a large number of discoveries have shown that interventions (genetic, pharmacological, and nutritional) increase the lifespan of invertebrates and laboratory rodents. Therefore, the possibility of developing antiaging interventions for humans has gone from a dream to a reality. However, it has also become apparent that we need more information than just lifespan to evaluate the translational potential of any proposed antiaging intervention to humans. Information is needed on how an intervention alters the "healthspan" of an animal, that is, how the physiological functions that change with age are altered. In this report, we describe the utility and the limitations of assays in mice currently available for measuring a wide range of physiological functions that potentially impact quality of life. We encourage investigators and reviewers alike to expect at minimum an overall assessment of health in several domains across several ages before an intervention is labeled as "increasing healthspan." In addition, it is important that investigators indicate any tests in which the treated group did worse or did not differ statistically from controls because overall health is a complex phenotype, and no intervention discovered to date improves every aspect of health. Finally, we strongly recommend that functional measurements be performed in both males and females so that sex differences in the rate of functional decline in different domains are taken into consideration.

Key Words: Healthspan—Lifespan—Physiological function—Age

Our understanding of the biological underpinnings of the aging process has skyrocketed in the last couple of decades, to the point where research has moved from a primarily descriptive phase through a mechanistic phase and is now poised to enter an intervention phase. Most of the advances have been based on the use of a few animal models—yeast, *Caenorhabditis elegans*, *Drosophila melanogaster*, and rodents (rats and mice). Longevity and/or lifespan—in particular, an increase in both mean and maximum longevity—has traditionally been the method through which an intervention is deemed successful, or not, in altering the aging process. Longevity gives investigators a simple, unambiguous end point (the individual is either alive or dead); however, it does not allow one to evaluate the effect of aging or an antiaging intervention on the physiological functions of the organism. It is generally assumed that if an intervention increases the mean and maximum lifespan of an organism, then the intervention has delayed aging. Implicit in this assumption is that the age-dependent decline in physiological functions has been delayed and/or improved. Although this is a plausible assumption and *may* be true for dietary restriction and possibly for dwarf mice, it is critical that investigators obtain a broad spectrum of health assessment data to evaluate the translation potential of any new interventions to humans.

Over the past decade, the emphasis on healthspan in a human context is often defined as the length of adult life during which a person can perform all activities of daily living (dressing, bathing, eating, toileting, transferring) and instrumental activities of daily living (finances, shopping, transportation, food preparation, managing medications, using the telephone). From this perspective, it is clear that longevity and health are not inevitably linked. For example, women live longer than men in virtually every culture, yet they are more often sick, make more doctor and hospital visits, are more often disabled, and are less likely to live independently later in life than men (1). In fact, since 1990, life expectancy in the United States has increased by 3.0 years, but healthy life expectancy has increased by only 2.3 years (2), showing that lifespan and healthspan are not necessarily as intimately linked as sometimes argued. This is not an easy concept to extend to experimental animals that face relatively few physical or mental challenges. Although invertebrate models have proven particularly useful in genetic studies, they are of limited utility when studying health, physiology, or disease susceptibility. However, such assessments are in principle possible in mice, and assessment of mouse health was the focus of a conference held in Bandera, TX on October 18–21, 2012.

It should be clarified from the onset that while we recognize that the mouse is not the only mammalian model for aging research, mice are the most common mammalian model used in biogerontology. Features that make mice highly attractive for studying aging in a mammalian system are their relative ease of breeding and genetic manipulation, low cost, and short lifespan. Whether such a short-lived mammalian model accurately reflects the biology of aging in long-lived humans is a plausible concern, and some aspects of aging and disease are likely to be difficult to study in rodents and similarly short-lived species. Also the pattern of disease susceptibility with age differs among mice, which are particularly prone to cancer, and humans where heart disease is the primary mortality factor. Nevertheless, as a first-pass mammalian model, however, the mouse remains the animal of choice, and this makes defining parameters of health across the lifespan in mice critical to evaluating the potential human relevance of a putative antiaging intervention. In the same vein, much has been discussed about the fact that so much of biomedical research (in all fields, not just aging) is based on the use of a single sex of one or two inbred model organisms, such as, male C57BL/6 mice. Because both sex and genetic background can have a marked effect on longevity as well as on physiological functions and the types and severity of end-of-life pathology, it is imperative that interventions be studied in both sexes, and in mice with heterogeneous genetic backgrounds, or at least in several inbred mouse strains.

Although the majority of mice appear to die of (or at least with) cancer, they also show a constellation of functional losses as they age, that in some aspects resemble those observed in humans, such as reduction in mobility and physical activity levels. However, each species also has idiosyncratic aspects to its aging [the "private mechanisms" originally described by Martin (3)]. So, while it is important to identify processes that are common to humans and mice, it is equally important to identify those that are not. Studying human-relevant phenotypes in mouse models is valuable even if the process under study does not influence the health or lifespan of the mouse in captivity (eg, studying cardiovascular disease or neurodegeneration). Conversely, there are mouse-specific aging traits, such as olfaction, while not an important role in functional decline in humans, nevertheless it is important within the context of the functional status of a mouse. As long as the mouse is used as a model of aging, it would be a mistake to dismiss these functions as irrelevant because the rate of deterioration as a consequence of a given manipulation should be informative about the processes of aging regardless of whether an equivalence exists between mouse versus human aging morbidity or mortality.

In contrast to humans, where geriatricians generally agree on a definition of healthspan, there is little agreement or a consensus on the definition of healthspan for mice. If we were to use a definition analogous to that used for humans, healthspan would mean the period of life when the mouse is able to move around, feed itself, and care for itself in terms of grooming, etc. This limited range of murine "activities of daily living" is obviously an artifact of captive husbandry. However, in laboratory mice, even these activities are altered primarily in the last few days/weeks of life and are rarely measured. So the measurement of healthspan necessarily has to be different for mice. It is unlikely that any given intervention will positively affect all physiological functions and responses. For example, in dietary restriction, the best studied antiaging paradigm, a significant body of literature indeed supports the notion that the increase in lifespan is largely accompanied by an improvement in health and increased resistance to degenerative disease (4,5). However, even in this well accepted paradigm, there are multiple tests of health in which restricted animals perform less well than controls. Notably, dietary restriction renders mice more susceptible to stresses whose resolution requires a significant output of energy, such as wound healing and influenza or some bacterial infections (6–8). Therefore, it is crucial that future investigators exert utmost care in defining which aspects of health and tissue function are modified and in which direction for any proposed intervention that extends lifespan.

Assays of healthspan in mice

At the Bandera conference, along with follow-up discussions among the conference participants generated a list of suggested

assays for assessing seven physiological domains of function/health that have been shown to decline with age. We consider these to be some of the most important in assessing the health status, or healthspan, of mice. The seven domains of function/health are activity and energetics/metabolism, skeletal muscle function, cardiopulmonary function, inflammation and immune function, sensory function, cognition, and pathology. The specific assays for each domain are described in the Supplementary Material, including the difficulty of performing the assay, whether an antiaging intervention has been shown to delay/reverse the age-related changes, and the corresponding assay used to assess functional status in elderly humans.

As shown in the Supplementary Material, a large number of assays are currently available to study how a genetic, nutritional, or pharmacological manipulation alters a wide spectrum of physiological functions in mice, many of which change with age. The authors encourage investigators and reviewers alike to expect at minimum an overall assessment of health in several domains across several ages before a manipulation is labeled as "increasing healthspan." In the past 2 years, several studies have been published using a combination of assays to describe frailty in mice (9–11). For example, Liu and colleagues (10) used a combination of grip strength, walking speed, rota-rod performance, and voluntary wheel running to develop a score for frailty in C57BL/6 male mice, showing the power of using a combination of assays to assess healthspan. Graber and colleagues (12) also developed a scoring system that would allow investigators to assess neuromuscular healthspan in mice. Recently, it has been demonstrated that 6 months of resveratrol treatment or lifelong 40% calorie restriction reduced the Frailty Index in C57BL/6 male mice (13).

It is important that publications describing healthspan assessment include any tests that were performed in which the treated group did worse than controls, or in which the treated animals did not differ statistically from controls. Overall health is a complex phenotype, and no intervention discovered to date improves every aspect of health. Too often, studies have used one or two measures of physiological function at one age (often a relatively young age) to claim that an intervention alters healthspan, but this interpretation is difficult without knowing the assays that did not show improvement under the treatment. Of course, an all-inclusive characterization of all relevant parameters is not feasible as a first step in determining whether an intervention may have an antiaging phenotype. However, as the aging community identifies more and more manipulations that increase the lifespan of mice, we propose that a comprehensive analysis over a wide variety of different physiological domains and pathology in diverse tissues should be made for the most promising manipulations because it is unlikely that an intervention will affect all or even most tissues/physiological functions similarly and positively. Because aging results in the decline of multiple physiological systems and reduces an organism's ability to respond to stress, assessments are likely to be most informative when the assays integrate the function of multiple systems and when the system(s) under study are stressed in order to reveal functional limits as well as rate of recovery from stress.

Of course, many other considerations need to be taken into account when choosing a panel of tests to assess general health of an aged mouse, such as the age of onset of disability, the difficulty of performing a given assay, the possibility of repeated testing in a longitudinal fashion (particularly problematic in the area of cognitive testing), as well as the technical difficulty of the measurement, its reliability and robustness. These are all addressed, by assay, in the Supplementary Material. One particular matter, sex, deserves special attention given that many interventions have been found to have differential effects depending on sex. For example, sex differences were observed in the effect of rapamycin on many measures of function in C57BL/6 mice, which potentially might be attributed to differences in serum rapamycin concentrations between males and females (14). The authors strongly recommend that measurements of function be made in both males and females so that sex differences in the rate of functional decline in different domains are taken into consideration.

There was universal agreement at the conference that information on physiological function is required before a manipulation be considered for translation to humans. Does the intervention delay or reduce the age-related changes in physiological functions, and just as important, are there negative side effects associated with the intervention? If so, what are they? It is naïve to expect that an intervention that has the power to increase lifespan will have no side effects; in fact, the concept of antagonistic pleiotropy also suggests that such interventions may reduce the fitness of younger individuals (at least in nonprotected environments). Therefore, it is important to know what side effects are observed in mice so that these parameters can be monitored when taken to a nonhuman primate or humans. For example, rapamycin, which increases lifespan in both male and female mice in several genetic backgrounds (15–17) results in an increased incidence of testicular degeneration (15,18), elevated insulin resistance (16), and increased cataract formation in one study (18), but not in another study (15).

While information on how an intervention alters a wide variety of physiological parameters is critical before translating the manipulation to humans, it is not at all clear that healthspan and lifespan data will be concordant or equally relevant to human translation. The difficulty in equating improvements in healthspan to rate of aging is illustrated by recent studies in which the effect of rapamycin on healthspan was measured in more than 200 physiological functions and pathology (14,15,18). While several aging phenotypes were restored by rapamycin (eg, behavior/cognition, several immune parameters, and a number of pathological lesions), many functions were not significantly altered. Interpretations of such "segmental" effects have been controversial (19,20) and demonstrate the difficulties of extrapolating healthspan and longevity data to establish whether an intervention "alters aging."

Must all processes that change with age need to be reversed or improved? Neither dietary restriction nor rapamycin alters all age-sensitive functions/pathologies. Furthermore, if a proposed antiaging drug leads to a rapid improvement in a function before a mouse gets old, then is the improvement in that function really considered as representative as a reduction in the aging process? And, if not all processes have to be altered, what percent of the functional/pathology measures have to be enhanced/improved for an intervention to be considered as antiaging? Or are some functions, such as, cognition, immune function, and cardiac/muscle function, more important than others in assessing the impact of a manipulation of aging? Finally, do any of these matter, so long as an intervention derived from aging studies, can ultimately deliver

a health benefit to humans? To avoid such confusions we propose that the research community abandon the oversimplification of stating that something is "antiaging" or that it "slows aging," but to describe the data as, "treatment X delayed/slowed normal age-related declines in health indicators A, B, and C as well as extended lifespan."

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

References

1. Austad SN. Sex difference in longevity and aging. In: Masoro EJ, Austad SN, eds. *The Handbook of the Biology of Aging*. 7th ed. Amsterdam: Elsevier; 2011:479–495.
2. Christopher JL, Murray AJ, Mohammed K, et al. The state of US health, 1990–2010 burden of diseases, injuries, and risk factors *JAMA*. 2013;310:591–608. doi:10.1001/jama.2013.13805
3. Martin GM. The Werner mutation: does it lead to a "public" or "private" mechanism of aging? *Mol Med*. 1997;3:356–358.
4. Omodei D, Fontana L. Calorie restriction and prevention of age-associated chronic disease. *FEBS Lett*. 2011;585:1537–1542. doi:10.1016/j.febslet.2011.03.015
5. Speakman JR, Mitchell SE. Caloric restriction. *Mol Aspects Med*. 2011;32:159–221. doi:10.1016/j.mam.2011.07.001
6. Clinthorne JF, Beli E, Duriancik DM, Gardner EM. NK cell maturation and function in C57BL/6 mice are altered by caloric restriction. *J Immunol*. 2013;190:712–722. doi:10.4049/jimmunol.1201837
7. Gardner EM. Caloric restriction decreases survival of aged mice in response to primary influenza infection. *J Gerontol A Biol Sci Med Sci*. 2005;60:688–694. doi:10.1093/gerona/60.6.688
8. Reed MJ, Penn PE, Li Y, et al. Enhanced cell proliferation and biosynthesis mediate improved wound repair in refed, caloric-restricted mice. *Mech Ageing Dev*. 1996;89:21–43. doi:10.1016/0047-6374(96)01737-X
9. Parks RJ, Fares E, Macdonald JK, et al. A procedure for creating a frailty index based on deficit accumulation in aging mice. *J Gerontol A Biol Sci Med Sci*. 2012;67:217–227. doi:10.1093/gerona/glr193
10. Liu, H, Graber TG, Ferguson-Stegall, L, Thompson, LV. Clinically relevant frailty index for mice. *J Gerontol A Biol Sci Med Sci*. 2013;68:1326–1336. doi:10.1093/gerona/glt188
11. Whitehead JC, Hildebrand BA, Sun M, et al. A clinical frailty index in aging mice: comparisons with frailty index data in humans. *J Gerontol A Biol Sci Med Sci*. 2014;69:621–632. doi:10.1093/gerona/glt136
12. Graber TG, Ferguson-Stegall L, Kim JH, Thompson LV. C57BL/6 neuromuscular healthspan scoring system. *J Gerontol A Biol Sci Med Sci*. 2013;68:1326–1336. doi:10.1093/gerona/glt032
13. Kane AE, Hilmer SN, Boyer D, et al. A validated mouse frailty index to assess longevity interventions: impact of species, calorie restriction and resveratrol. *J Gerontol A Biol Sci Med Sci*. In press. doi:10.1093/gerona/glu315
14. Zhang Y, Bokov A, Gelfond J, et al. Rapamycin extends life and health in C57BL/6 mice. *J Gerontol A Biol Sci Med Sci*. 2014;69:119–130. doi:10.1093/gerona/glt056
15. Neff F, Flores-Dominguez D, Ryan DP, et al. Rapamycin extends murine lifespan but has limited effects on aging. *J Clin Invest*. 2013;123:3272–3291. doi:10.1172/JCI67674
16. Miller RA, Harrison DE, Astle CM, et al. Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell*. 2014;13:468–477. doi:10.1111/acel.12194
17. Fok WC, Chen Y, Bokov A, et al. Mice fed rapamycin have an increase in lifespan associated with major changes in the liver transcriptome. *PLoS One*. 2014;9:e83988. doi:10.1371/journal.pone.0083988
18. Wilkinson JE, Burmeister L, Brooks SV, et al. Rapamycin slows aging in mice. *Aging Cell*. 2012;11:675–682. doi:10.1111/j.1474-9726.2012.00832.x
19. Richardson A. Rapamycin, anti-aging, and avoiding the fate of Tithonus. *J Clin Invest*. 2013;123:3204–3206. doi:10.1172/JCI70800
20. Johnson SC, Martin GM, Rabinovitch PS, Kaeberlein M. Preserving youth: does rapamycin deliver? *Sci Transl Med*. 2013;5:211fs40. doi:10.1126/scitranslmed.3007316