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HEAD-TO-HEAD DEBATE ON THE UTILITY OF SHORT LIVED ANIMAL MODELS FOR AGING: PROS AND CONS

'Accelerated aging': a primrose path to insight?

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Summary

Organism envy afflicts most researchers who work on aging in mice; how frustrating it is to see the worm and fly biologists nail down milestone after milestone, citation after citation! Surely genetic trickery can produce mice that age in a comparable jiffy? Alas, our near-total ignorance of what times the aging process makes it hard to guess what genes to tweak, if indeed aging can be mimicked *a presto*. Building a case that a given short-lived mutant ages quickly is a steep and thorny path, requiring more than just plucking a symptom here and there from a list of things that sometimes go wrong in old people or old mice. The hallmark of aging is that a lot goes wrong more or less at the same time, in 2-year-old mice, 10-year-old dogs and 70-year-old people. Finding ways to damage one or two systems in a 6-week or 6-month-old mouse is not too hard to do, but the implications of such studies for improved understanding of aging *per se* are at best indirect and at worst imaginary and distracting.

Key words: longevity; mouse models; mutants; pathology; segmental aging; skepticism.

Snippity snippity
Dr. Jan Hoeijmakers
Tired of working with
Slow-aging beasts
Lops out one deoxy-
Ribonucleotide;
Now his mice age just as
Quickly as yeasts.
(Chaffey, 2003; Hasty *et al*., 2003)

There is a long and distinguished tradition of mistaking mortality risk for aging. On balance, people would rather not die; if they must, they would prefer to do so later rather than sooner. The obituary pages are devoted mostly to people who die at ages most readers have not yet reached, because the risk of mortality goes up almost exponentially with age in a modern society. The close association between death and aging is so obvious that children incorporate it into their fundamental models of life and its trajectories, and when these children grow up into scientists, it remains all too easy to assume that analyses of lifespan are, for all practical purposes, analyses of aging.

Modern biological gerontology has, through its historical development, made the conceptual disentanglement of aging and mortality more, rather than less, difficult. Textbooks and handbooks of biological aging research always include a chapter, very early on, that presents a demographer's view of aging, complete with Gompertz curves and discussions of the pros and cons of various ways to summarize life history tables, as though the only really useful measure of aging were a count of dead bodies at specific ages. Students are taught early that claims that a specific mutation or diet slows aging must be tested by looking for increased mean or maximal longevity; such evidence is by cliché and common agreement the 'gold standard' against which other potential measures of diminished aging can be evaluated. The rationale for this prejudice is a fairly good one: dramatic postponement of mortality risk is indeed hard to achieve without modulation of aging, because alterations of risk of any one disease have minimal impact on the overall life table (Olshansky *et al*., 1990). ['Hard to achieve', but not impossible: administration of clotting factor to a population all of whose members die of haemophilia would provide a counterexample; see Smith & Walford (1977) for a real-life example of MHC-mediated retardation of death in a lymphoma-prone stock of mice.] Use of age at death as a key outcome measure for comparisons of aging rate is also sanctioned by the collective failure of the gerontological community to develop and validate a good series of surrogate measures ('biomarkers') as an alternate index of aging rate.

If drugs, diets and genes that produce increases in lifespan act through a delay or deceleration of aging, then it seems plausible that interventions that diminish lifespan act in the opposite way, i.e. by acceleration of aging. A moment's thought shows this idea to be incorrect, of course: there are uncountable ways to shorten lifespan, including thousands of mutants that cause lethal developmental abnormalities, millions of poisons that kill quickly or slowly, and lots of diets that are just not very good for you. No one (I hope) would accept a lead-poisoned mouse or a mouse born without lungs or an immune system as a model for accelerated aging. Just what should it take to convince a sophisticated scientist that his/her short-lived mutant will teach

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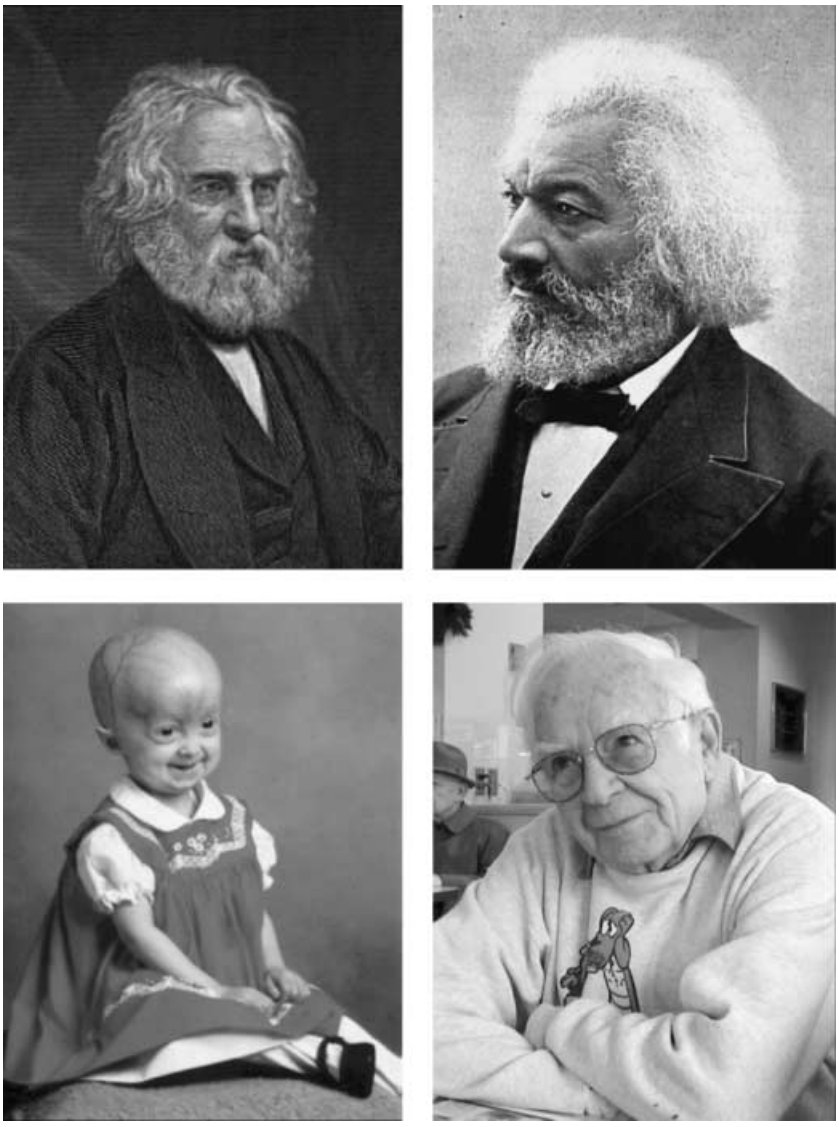

Fig. 1 Portraits of three old people and a person with a disease thought to represent accelerated aging. Can you spot the ringer? The pictures of Frederick Douglass (top right) and Henry Longfellow (top left) are reproduced courtesy of The General Libraries, The University of Texas at Austin. The picture of the child with Hutchinson-Gilford progeria is reproduced courtesy of Dr. W. Ted Brown.

Fig. 2 The left panel shows a 24-month-old mouse hemizygous for a mutation encoding a C-terminal fragment of p53. Compared with normal aged mice (see right panel) the mutant shows dramatic loss of adiposity and muscle mass and pronounced kyphosis of the spine (Tyner *et al*., 2002). The middle panel shows two 8-week-old mice homozygous for the klotho mutation (Kuro-o *et al*., 1997). These mice show growth retardation, kyphosis, dysgenesis of external genitals in both sexes, an abnormal walking pattern, destruction of the alveoli and arteriosclerosis, none of which is characteristic of normal aged mice. The right panel shows, for comparison, an old mouse, age 1420 days, the oldest survivor of a study of wild-derived mice (Miller *et al*., 2002).

us something important about aging, the process that converts teenagers into seniors, and thence unto dust? How does one decide that a specific fast-dying mouse merits commitment of thousands of days and millions of dollars which might instead be devoted to the study of aging *per se*?

When in a cynical mood, I sometimes feel that what it takes is an ability to overlook the obvious. Consider Fig. 1, which contains portraits of three old people and someone with a disease too often represented, to both lay and scientific audiences, as a form of 'accelerated' aging. Portraits emphasize surface

features and differences in bone modelling, but more detailed biochemical data would confirm the initial impression: patients with Hutchinson–Gilford's disease (so-called 'progeria') differ from normal old people in many ways, lacking many characteristics of normal aging and presenting many characteristics not seen among the elderly (Brown, 1991). Figure 2 presents a similar rogue's gallery of portraits, this time with a single, easy-to-identify authentic old mouse easy to spot among poseurs. The position that these developmental abnormalities are in fact the same as aging, though conveniently faster, does not withstand very much scrutiny, and advocates of accelerated aging models typically now abandon this line of argument if you look them straight in the eye.

The fallback position, typically, is that the model in question shows 'segmental' aging, i.e. it is like aging in some cells or organs or systems, though not in all. This is a tougher position to rebut, in large part because we do not yet know enough about aging to tell when some abnormality is 'like aging' to an important degree. Old people often have thin skin and fragile bones: so should a mutation that causes skin atrophy and osteoporosis be accepted as a model for 'segmental' aging? In the absence of any real knowledge of why old people and old mice develop skin atrophy and brittle bones, it is possible that an analysis of mutants that exhibit such signs might shed light on the way in which aging leads to the same changes. It is at least equally possible, though, that skin atrophy and brittle bones might be the end result of many dozens or hundreds of alterations in genes or metabolic processes that modify turnover and differentiation of connective tissue cells, and that many of these work in ways quite distinct from the mechanism by which aging produces the same result. If the mutation in question also leads to outcomes (examples: a 10-fold increase in mesenchymal tumours with no corresponding increase in epithelial tumours, a dramatic increase in UV sensitivity, or exaggerated kyphosis) not seen in aging bodies, the bet that its detailed analysis will help us understand the mechanisms of aging becomes progressively riskier. There are many ways to speed up the 'aging' of an automobile, ranging from sugar in the petrol tank to dilution of motor oil by marinara sauce; analysis of these 'segmental automobile aging' models is not an efficient way to investigate why Hondas might last longer than Yugos.

A second habit of thought, based on theoretical ideas about how aging might work, also feeds the passion for models of accelerated aging. If a scientist has a hunch (often an educated and justifiable hunch) that aging involves lots of damage to DNA, then a mutant with poor DNA repair that dies young fairly calls out 'study me' to the pretuned ear. If one then discovers a few other abnormalities that look like aging, at least in some respects – premature deafness, or some overlap in liver gene expression patterns or diminished gonadal function – suspicion hardens into conviction, the enemy of scientific inquiry. A proper Popperian would take the opposite tack, looking for examples in which poor DNA repair, or low levels of Mn-SOD or poor control of mitochondrial mutation rate do not interfere with robust health or prevent survival to a ripe old age. Such hypothesis-testing approaches have done a fine job of disposing of clever and interesting theories that turned out not to be correct, such as the proposed association between metabolic rate and longevity across species (Austad & Fischer, 1991; Miller and Austad, 1999), and the idea that positive feedback loops might cause aging by modification of amino acid sequences (Gershon, 1979).

How would one go about proving that a particular mutant, or a particular drug-treated animal, actually was worth studying as a model of accelerated aging? Because we do not yet have any defensible idea of how aging works to produce the synchronized signs and symptoms of aging, such arguments are necessarily indirect. A good approach might be to evaluate, in mutants and controls, a dozen or so well-validated age-dependent traits in multiple tissues and organs. Do the mutants show the typical pattern of immune system changes – but earlier? Do they also show age-dependent changes in muscle fibre type and motor unit distribution – but earlier? Do they show collagen cross-linking, altered wound healing, cognitive decline and changes in two dozen preselected mRNA levels and in IGF-I levels – but a good deal earlier than controls? Or, alas, do they simply die at an early age, because of the effects of cardiac and liver lesions not seen in normal aging (Li *et al*., 1995), or intestinal atrophy (Herrera *et al*., 1999), or autoimmuity and amyloidosis (Takeshita *et al*., 1982; Umezawa *et al*., 1993)?

The caloric restriction (CR) community, trying to make a case that the CR rodent really does age slowly, has set a superb example, with now hundreds of tests of the basic claim that CR rodents show most of the typical signs of aging – but do so later. Those of us who work with mouse mutants that exhibit unusually long lifespans are just now beginning to build a parallel case, with evidence that IGF-I-deficient mice do indeed show delays not merely in mortality risk, but also in joint changes (Silberberg, 1972), collagen cross-linking and T-cell subset pattern changes (Flurkey *et al*., 2001), cognitive performance (Kinney *et al*., 2001a,b), and age-adjusted tumour incidence rate (Ikeno *et al*., 2003). Very few mutations (Miller, 2001; Holzenberger *et al*., 2002; Bluher *et al*., 2003), and only one or perhaps two (Zimmerman *et al*., 2003) dietary interventions, are known to increase lifespan, and the case that these models actually do reflect a fundamental retardation (or perhaps delay; see Bartke *et al*., 2001) in aging rate is already strong and getting stronger. There are by contrast a vast number of ways to shorten lifespan, and the claim that a specific member of this bulging set is an authentic form of speedy aging should require correspondingly strong support before it receives comparable commitment of funding, effort and acclaim.

Although the gladiatorial format of the 'Head-to-Head' series encourages advocacy and polemic, scientific decency requires that one seek a point of balance, rather than mere devastation of an entire field of endeavour populated largely by one's friends, present and former. There is a baby, perhaps several babies, in the turbid bathwater of the 2026 PubMed references on 'accelerated aging', a literature which grew, in 2002, at the rate of one published paper every 37 h. For one thing, there

is much to be learned regarding the effects of DNA damage, telomere length, oxidation damage, abnormal p53 levels, etc., on cell and developmental biology and pathobiology; high-quality work on these systems is of high value, whether it is marketed as 'aging research' or not. Aging could well be due (who knows, really?) to DNA damage, or mitochondrial mutations, or alterations in apoptotic responses or modulations of stem cell differentiative pathways, and therefore new discoveries in these areas, however motivated, may well come back someday to facilitate studies of aging. Second, the synchronicity of age-dependent changes is a critical puzzle for biogerontology. It is possible to develop cataracts by age 2 (mice do it), and postpone sarcopenia until age 60 (we do it); why, then, do horses get both at about 15–20 years of age, an age at which they also get serious joint and immune disorders? Some of the more attractive models advertised as accelerated aging do show synchronous changes in multiple age-sensitive tissues, and insights into the mechanism of synchronization might give clues into parallel mechanisms that work in real aging.

Much of the effort devoted to studies of short-lived mutant animals (and their human counterparts) is therefore productive and informative. In my view, however, portraying work in this area as analysis of 'accelerated aging' has two serious consequences. First, it tends to confuse still further the terminological morass that afflicts discussions of aging, just as the use of the term 'cellular aging' to describe replicative failure of fibroblast cultures has for years made it more difficult to think about and discuss the kind of aging that turns young adults into old people. Second, resources for the study of aging are far lower than the field deserves, and commitment of funds (and, just as importantly, the seduction of talented and committed researchers) to analysis of these malleable and highly marketable model systems makes it even tougher to make progress in other areas more likely to produce insights into aging.

As it happens, we already have a fine model for accelerated aging. It is called the mouse. Like people, mice develop cancer, cataracts, muscle weakness, immune abnormalities, cognitive impairment, impaired fertility, joint problems, central obesity, skin atrophy and myriad other aspects of normal mammalian aging; but they do so in 2 years rather than 60 years, a full 30-fold acceleration with none of this 'segmental' stuff to worry about. There are certainly differences between old mice and old humans, but also unmistakable similarities at the level of cell biology, tissue organization and systems biology. At the biochemical and histological level, lens, liver, thymus, skin and muscle biopsies from young adult mice are almost indistinguishable from biopsies taken from young adult humans, yet one set of tissues is built to last a few years, and the other many decades. Elucidation of the factors (telomeric safety valves? altered DNA repair mechanisms? antioxidant defences? lots of heat-shock proteins?) that underlie the 30-fold difference in the rate of progression of a highly overlapping suite of age-dependent changes should be the number one priority for experimental gerontologists. This is a lamp-post that not only has the bulb on, but has actual keys lying nearby.

Acknowledgments

The impolitic opinions expressed here are my own, but I find it comforting to remember that at least one other person seems to agree with many of them: Harrison (1994). I am grateful to Dr. W. Ted Brown for the charming photograph of the child with Hutchinson-Gilford syndrome shown in Fig. 1. The long-lived mouse shown in Fig. 2 enjoyed free room and board for 1449 days, all courtesy of NIH grant AG13711.

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