

Growth hormone signaling and longevity. Why dwarf mice are long-lived and what does this tell us?



SUBMIT A COMMENT ON THIS TOPIC/PAPER AND ENTER TO WIN A FREE MEETING REGISTRATION AT THE 34TH ANNUAL MEETING OF THE AMERICAN AGING ASSOCIATION (hotel and airfare not included).

Winner will be announced in our April edition of the Newsletter.

Comments will be published in subsequent editions of the AGE Newsletter.

**SEND COMMENT via
FEEDBACK FORM on
the website
or
EMAIL TO:
AmerAging@aol.com
or
FAX at:
+1.610.565.9747**

Andrzej Bartke, Geriatrics Research, Department of Medicine, Southern Illinois University School of Medicine, P.O. Box 19628, Springfield, IL 62794-9628, USA,
Email: abartke@siu.edu, Telephone 217/545-7962, Fax 217/545-8006.

Hypopituitary dwarf mice, lacking growth hormone (GH), prolactin and thyrotropin, and GH resistant "Laron dwarf" mice live much longer than their normal siblings (Brown-Borg et al., *Nature* 384:33, 1996; Flurkey et al., *PNAS* 8:6736, 2001.; Coschigano et al. *Endocr* 144:3799, 2003). Although these observations were initially received with considerable skepticism, evidence for extended longevity of these animals is now undisputable and evidence that aging is retarded in these animals is very strong (Flurkey et al. *PNAS* 98:6736, 2001; Kinney et al. *Horm. Behav.* 39:277, 2001; *Physiol. Behav.* 72:653, 2001; Ikeno et al., *J. Gerontol. Biol. Sci.* 58A:291, 2003, & unpublished). In Ames dwarf (Prop1df), Snell dwarf (Pit1dw) and Laron dwarf (GHR/GHBP-KO) mice, both the average and the maximal life span is significantly increased with an occasional animal reaching an age of over four years. This is a truly remarkable age for a laboratory mouse living under standard laboratory conditions with constant access to high energy food. Association of delayed aging with absence of GH signaling raises a number of important questions which are being addressed in current studies and are likely to suggest directions for future research.

First of all, it is not understood how absence of GH action leads to delayed aging and long life. However, data available to date suggest a number of possible mechanisms which singly or, more likely, combined might account for the "longevous phenotype" of Ames dwarf, Snell dwarf and GHR-KO mice.

These include (i) reduced circulating levels of IGF1, and reduced somatic growth, (ii) reduced secretion of insulin combined with enhanced sensitivity to its actions, (iii) reduced body temperature and generation of reactive oxygen species (ROS) together with improved antioxidant defenses, and (iv) increased cellular resistance to multiple forms of stress. The involvement and the suggested importance of these mechanisms is supported by data obtained in these and in other long-lived mutant mice (reviewed in Bartke et al., *J. Gerontol. Biol. Sci.* 56A, B340, 2001; *Exper. Gerontol.* 36:21, 2001), as well as by extrapolation of findings obtained in genetically normal animals differing in body size (Rollo, *Evol. Dev.* 55:55, 2002; Miller et al. *Aging Cell* 1:22,2002), in normal animals subjected to caloric restriction (Weindruch & Sohal, *N. Engl. J. Med.* 337; 986:1997; Masoro, *Handbook Biol. Aging, Acad. Press* 2001), and in transgenic animals overexpressing GH (Bartke, *Neuroendocrinology* 78:210, 2003). However, it should be noted that evidence supporting involvement of mechanisms listed above, although substantial, is indirect being derived from the studies of the association of various physiological characteristics with aging and life span.

In addition to suggesting likely mechanisms linking reduced GH and insulin signaling with longevity, comparisons of long lived mutants to calorically restricted (CR) animals reveal some interesting and informative differences. For example, adiposity is reduced in CR animals but increased in GHR-KO mice (Bartke & Heiman, in press) while Ames dwarfs

Growth hormone signaling and longevity. Why dwarf mice are long-lived and what does this tell us? (cont.)

exhibit relatively minor age-related changes in adiposity (Heiman et al. *Endocrine* 20:149, 2003). This contrasts with the situation in fat-specific insulin receptor knock out (FIRKO) mice in which extension of life span is associated with extreme leanness (Blüher et al., *Science* 299:572, 2003). We suspect that alterations in the secretory profile rather than the mass of adipose tissue will prove important in the control of aging, acting, most likely, via alterations in insulin sensitivity.

Another important question raised by the findings in dwarf mice is whether and if so, to what extent the conclusions from studies in these animals may apply to the human. Delayed aging and long life of mice lacking GH signaling is at odds with the ability of injected GH to ameliorate some of the symptoms of human aging and with the enthusiastic promotion of GH, GH releasers, and various GH-related products as “scientifically proven” means to feel younger, look younger, and combat a host of age-related problems. Moreover, GH deficiency in the human is considered a risk factor for cardiovascular disease, and reduced life span was recently reported in a cohort of genetically GH deficient individuals (Bessen et al., *JCEM* 88:3664, 2003). However, hypopituitary patients with a mutation homologous to one of the life extending mutations in the mouse are not short-lived and, in fact, can reach a very advanced age (Krzisnik et al., *J. Endocr. Genetics* 1:9, 1999). Furthermore, ablation of the pituitary was reported to reduce mortality of diabetic patients, at least during the first 5-10 years following irradiation (Klein et al., *J. Diab. Complic.* 12:246, 1998). While more work is clearly needed to resolve these controversies, I believe that it is exceedingly unlikely that a mechanism involved in the control of aging in organisms ranging from worms to mice (and probably operating also in unicellular yeast) does not play a similar role in the human. Extension of life by reducing IGF-1/insulin or homologous signaling appears to represent an ancient mechanism facilitating survival under adverse conditions and promoting enhanced stress resistance and repair capacity at the expense of growth and reproduction when energy resources are scarce (Tatar et al., *Science* 299:1346, 2003). In support of this reasoning, enhanced sensitivity to insulin which characterizes long lived dwarf and Laron dwarf mice (Dominici et al., *J. Endocr.* 166:579, 2000; 173:81, 2002) was reported also in exceptionally long-lived people (Paolisso et al., *Am. J. Physiol.* 270:E890,1996).

However, the relative impact of reduced actions of IGF-1 and/or insulin on life span will likely prove to differ between species. For example, reduced activity of the somatotrophic axis may be universally related to reduced risk of neoplasia, but tumors are a much less common cause of death in humans than in mice. Conversely, IGF-1 may be protecting against cardiovascular disease (Shut et al., *Stroke* 34:1623, 2003) which is a leading cause of death in humans but not in mice. Moreover, in comparison to other mammals, and particularly to mammals of comparable body size, humans are rather inordinately long-lived and therefore there may be less “room for improvement” in the human than in mice, flies or worms.

Ames, Snell and Laron dwarf mice are clearly outside the range of normal variation in body size, longevity, and other characteristics of the laboratory stocks of house mice. However, the extreme features of these diminutive animals offer exciting opportunities to discover and elucidate physiological mechanisms that control aging and longevity in genetically normal individuals and likely apply broadly, including our own species.

COMMENTARIES on “Growth hormone signaling and longevity. Why dwarf mice are long-lived and what does this tell us?”

The following commentaries were submitted by Drs. Norm Wolf and Steve Austad on our January discussion led by Dr. Andrzej Bartke and titled "Why dwarf mice are long-lived and what does this tell us?" (Review the discussion text on page 4.)

Comment submitted by Dr. Norm Wolf, Univ. of Washington:

Dr. Bartke has briefly and effectively summarized the status and importance of the GH-defective models, a subject to which he has made many important contributions. The consensus seems to be that the removal of GH or GHR results in consequent disappearance of circulating IGF-1 and, this (along with possible reductions in TH in most models) produces the phenotype of the models. One aspect that was not discussed and that I think is of great importance is the quite recent article by Muraki, Salmon, Miller and others, including Dr. Barke, himself, reporting that adult fourth passage fibroblasts from Snell and GH deficient mice were extremely resistant to H₂O₂, paraquat and other pro-oxidants in vitro, as well as to a non-oxidant alkylating agent, MMS. These findings suggest that the oxidation-resistance characteristics of the tissues of living dwarf mice are not only the result of contemporary circulating GH and IGF-1 deletion, but are somehow intrinsic and lasting in the cells (fibroblasts) after 4 passages in vitro, due to an induced cellular status. Apparently, it takes some period of life-span before this resistance is induced (it is not present in mice only a few days old). Also, given that 4th passage cells retain this resistance, would this advantage extend the length of continuing replications for murine cells in vitro without crisis and transformation? It is notable that de Cabo and co-workers have shown that blood serum from CR rats extends protection from stress to cells in culture. Is this the same phenomenon and can serum from the dwarfs do the same?

Comment submitted by Dr. Steven Austad, Univ. of Texas Health Sci. Ctr.:

Three thoughts spring immediately to mind on reading Dr. Bartke's lucid discussion piece.

First, I am not quite yet prepared to agree with Dr. Bartke that aging is retarded in the dwarf mouse models. Clearly, they are long-lived and **some** markers of aging seem retarded. However, if aging is a progressive and generalized decline in function, then relatively few functions have been investigated so far. In particular, I'm curious as to whether sensory function, muscle strength and endurance, and bone mineral density decline more slowly in dwarfs than in controls. These are of particular interest because key features of human Laron Syndrome (LS), which is genetically identical to the GHR/GHBP-KO mice, are muscle weakness, osteopenia, and aberrant retinal blood vessel morphology.

Second, with respect to whether findings from the mouse studies extend to humans, we already have hints at a few answers because humans with LS have been extensively characterized (Laron, JCEM 84:4397, 1999; Laron, JCEM 89:1031, 2004). Ultimately, there are likely to be both similarities and differences in the syndrome between mice and humans. Data so far are too sparse to determine whether LS humans live exceptionally long, but it is quite clear that they are not exceptionally short-lived. On the other hand, they do not appear to age more slowly by external signs. Although obese, they have thin skin and early wrinkling. In addition to the muscle weakness and osteopenia mentioned above, adults frequently develop hyperinsulinemia, hypercholesterolemia, and glucose intolerance or diabetes – all associated with aging in the general population. Also intellectual performance is lower on average in LS humans. Not all of these clinical signs may be related to growth hormone resistance. People with LS are invariably the product of consanguineous marriages, so some effects may be due to homozygosity for deleterious alleles at other genetic loci. However this constellation of clinical signs does not seem to be replicated in mice.



SUBMIT A COMMENT ON THIS TOPIC/PAPER AND ENTER TO WIN A FREE MEETING REGISTRATION AT THE 34TH ANNUAL MEETING OF THE AMERICAN AGING ASSOCIATION (hotel and airfare not included).

Winner will be announced in our April edition of the Newsletter.

Comments will be published in subsequent editions of the AGE Newsletter.

SEND COMMENT via FEEDBACK FORM on the website or EMAIL TO: AmerAging@aol.com or FAX at: +1.610.565.9747

COMMENTARIES on “Growth hormone signaling and longevity. Why dwarf mice are long-lived and what does this tell us?” (cont.)

Third, if aging can reasonably be thought of as unrepaired damage to cells, tissues, and organs, we still have only the faintest clue as to the origin or nature of that damage. In enumerating four possible mechanisms by which absence of GH delays aging, only two (reduced ROS production, enhanced stress resistance) are clearly associated with cellular damage. That is, exactly how something like increased IGF-I or insulin signaling might lead to the sort of cellular damage we associate with aging is a mystery.

The discovery and creation of these long-lived dwarf mice have given us exciting new opportunities to address fundamental questions about the modulation of aging in mammals. It might be worth remembering, however, that no animal model of extended longevity has yet to be characterized that does not also have some less-than-desirable side-effects. In our focus on the length of life, let us not forget about its quality.

Dr. Andrzej Bartke's Response:

Drs Wolf and Austad brought up many important issues in their Commentaries. The results of recent studies of the resistance of fibroblasts isolated from long-lived mouse mutants to various forms of cytotoxic stress indicate that stress resistance is probably a very important part of the "longevity phenotype" of these animals. These findings also suggest that the association of longevity with stress resistance which is very well documented in invertebrates applies also to mammals. To answer the question posed at the end of Dr. Wolf's comments, preliminary data obtained by Dr. de Cabo indicate that serum from Ames dwarf mice resembles serum obtained from calorically restricted animals by exerting "protective" effects on cultured cells.

I certainly agree with Dr. Austad that retardation of aging is difficult to document. However, there is a rather long list of physiological characteristics of hypopituitary or GH resistant long lived mutant mice that indicate delayed aging of these animals. These include data reported in the 70s on cartilage and joint disease and more recent data obtained by Flurkey and Miller concerning immune function, collagen characteristics, renal pathology and cataracts in Snell dwarf mice, studies of tumor incidence in Ames and Snell dwarfs by Ikeno and Miller and a series of studies by Kinney who examined various measures of cognitive function in Ames dwarfs and in GHRKO mice. I am tempted to add a personal footnote here: one of the reasons many years ago we decided with Holly Brown-Borg and Kurt Borg to examine longevity of Ames dwarf mice was that they appeared to us to look younger than their chronological age.

In the human, hyperinsulinemia and insulin resistance in some GH deficient or GH resistant individuals is likely related to their obesity. Growth hormone exerts anti-insulinemic actions and promotes insulin resistance in both mice and men but it appears that in the human, lack of lipolytic effects of GH may override the effects of reduced GH signal on sensitivity to insulin action.

As pointed out by Dr. Austad, the mechanisms linking reduced somatotrophic signaling with extended longevity remain to be identified. With available data we can only speculate about the importance of reduced oxidative metabolism and ROS generation, improved anti-oxidant defenses, reduced non-enzymatic glycation etc.

Concerning the "costs" of prolonged longevity in animals with reduced GH or IGF-1 signaling; IGF1R +/- mice studied by Holzenberger et al were reported to be only slightly smaller than normal and fully fertile. Perhaps more importantly, studies of genetically normal (i.e. not mutant, gene knock-out or transgenic) mice demonstrated consistent and significant negative correlation of adult body size (presumably a measure of somatotrophic signaling) and life span. Of course, one could bring up an issue of smaller animals having smaller litters or likely being at some disadvantage in a competitive situation. Perhaps as one of our former presidents have said, there are no free lunches.....

COMMENTARIES on “Growth hormone signaling and longevity. Why dwarf mice are long-lived and what does this tell us?”



SUBMIT A COMMENT ON THIS TOPIC/PAPER AND ENTER TO WIN A FREE MEETING REGISTRATION AT THE 34TH ANNUAL MEETING OF THE AMERICAN AGING ASSOCIATION (hotel and airfare not included).

Winner will be announced in our April edition of the Newsletter.

Comments will be published in subsequent editions of the AGE Newsletter.

**SEND COMMENT via
FEEDBACK FORM on
the website
or
EMAIL TO:
AmerAging@aol.com
or
FAX at:
+1.610.565.9747**

The following commentaries were submitted by Drs. Norm Wolf and Steve Austad on our January discussion led by Dr. Andrzej Bartke and titled "Why dwarf mice are long-lived and what does this tell us?" (Review the discussion text on page 4.)

Comment submitted by Dr. Richard Miller, Univ. of Michigan:

I've read Austad's commentary on the Bartke paper in the American Aging Association Newsletter, and I wish to take up his challenge to find a situation in which a dwarfing mutation has slowed aging without leading to some ill effects (such as, for example, the tendency of Snell dwarf mice to be slow, cold, infertile, and poor fighters, despite their excellent vision, kidneys, joints, intelligence, oxygen resistance, and youthful-looking skin and tendons).

This is, I think, an easy challenge to meet: viz Chihuahuas, toy poodles, and other miniature breeds. Not all dwarfing mutations produce healthy and vigorous sports, but some do, and dog breeders have kept these for a variety of purposes, such as sitting on laps, living in cramped apartments, and darting down rat-holes. I defy anyone who has owned a West Highland White Terrier, like our late lamented "Tiger," to call these dogs effete or wimpish. Nature, apparently, can figure out ways to construct long-lived dwarfs that are healthy and active at ages at which their wolfhound and Newfoundland cohort-mates are long gone to their heavenly reward, though mouse breeders have not yet caught on to the secret method. Yet.

Comment submitted by Dr. Steven N. Austad:

I believe the challenge is a little more formidable than suggested. As I recall, I was commenting on dwarfs due to single gene mutants. I heartily admit that nature produces fit and feisty dwarves again and again. However humans crippling a single gene in an otherwise well-integrated genome is unlikely to produce such a fit and feisty critter as a result. In fact, I know of no such example, although I am waiting to be proven wrong.

Dr. Andrzej Bartke's Response:

With regard to the recent exchange of views about ill effects of dwarfing mutations, I would like to say how I view the "relevance" of these animals to the issue of aging. I would obviously not argue that Snell dwarf, Ames dwarf or "Laron dwarf" (GHRKO) mice are fully fit or likely to be successful in competition with normal animals under natural conditions. I have spent many years of my professional life on analysis of reproductive deficits in these animals and the underlying hormonal mechanisms. My own fascination with these mice is related to the fact that they demonstrate the dramatic impact of specific endocrine defects on aging and longevity and thus facilitate identification of the pathways and the mechanisms involved

The obvious question is to what extent findings in these mutant animals may apply to genetically normal individuals. Providing an answer that would satisfy everyone is not easy. However, analysis of the relationships between adult body size (that can reasonably be assumed to represent a marker of GH and IGF-1 actions) and longevity in mice strongly suggests that the major extension of longevity in animals lacking GH or its action represents an extreme case of a physiological relationship that exists in normal animals. I am referring to numerous studies in different stocks of normal mice as summarized in a recent meta-analysis by David Rollo and to analysis of individual differences in longevity in a stock of genetically heterogeneous normal mice by Richard Miller. Studies of the same signaling pathways in various organisms, the relationships of body size to

COMMENTARIES on “Growth hormone signaling and longevity. Why dwarf mice are long-lived and what does this tell us?” (cont.)

longevity in other species, analysis of polymorphism of human genes related to IGF-1, and studies of glucose metabolism in exceptionally long-lived people provide further (although admittedly indirect) indications that findings in the various types of dwarf mice can help us understand normal biological control of the aging process.