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Genes That Prolong Life: Relationships of Growth Hormone and Growth to Aging and Life Span

Andrzej Bartke,[1] Karen Coschigano,[2] John Kopchick,[2] Varadaraj Chandrashekar,[1] Julie Mattison,[3] Beth Kinney,[1] and Steven Hauck[1]

[1]Department of Physiology, Southern Illinois University School of Medicine, Carbondale.
[2]Edison Biotechnology Institute and Department of Clinical Research, Ohio University College of Osteopathic Medicine, Athens.
[3]National Institute on Aging, National Institutes of Health Animal Center, Poolesville, Maryland.

Mutant mice with a combined deficiency of growth hormone (GH), prolactin, and thyrotropin, and knockout mice with GH resistance, live longer than their normal siblings. The extension of life span in these animals is very large (up to 65%), reproducible, and not limited to any particular genetic background or husbandry conditions. In addition to demonstrating that genes control aging in mammals, these findings suggest that GH actions, growth, and body size may have important roles in the determination of life span. We describe the key phenotypic characteristics of long-living mutant and knockout mice, with an emphasis on those characteristics that may be related to delayed aging in these animals. We also address the broader topic of the relationship between GH, growth, maturation, body size, and aging, and we attempt to reconcile the well-publicized antiaging action of GH with the evidence that suppression of GH release or action can prolong life.

STUDIES in yeast, worms, and flies have provided considerable evidence for the existence of genes that control aging and life span (1–9). Reports that silencing or overexpressing of some of these genes can greatly prolong life (1,4,7–9) attracted wide interest and media attention. These exciting developments raised an obvious question as to what extent findings obtained in these organisms may be relevant to vertebrates and especially to mammals and to the human. Here we review recent studies of gene mutations that extend life span in the mouse and discuss alterations in endocrine function that appear to be responsible for the prolonged longevity of these animals. We focus on the search for mechanisms that may be involved, and on the possible relevance of these studies to the human. This focus includes a discussion of the highly controversial issue of the role of growth hormone in aging.

Long-Living Mutant and Knockout Mice

Ames Dwarf Mice

In 1996, we reported that Ames dwarf mice live much longer than their normal siblings (10). The extension of life span in these animals is very large, approximately 50% in males and over 60% in females (10), and it is reproducible (11; also unpublished data, 2000). Ames dwarf mice (12) are homozygous for a recessive mutation at the *Prop-1* locus, which interferes with development of a specific cell lineage in their anterior pituitary during fetal development (13). This leads to the absence of three adenohypophyseal cell types, which are somatotrophs, lactotrophs, and thyrotrophs, and to a deficiency of the three hormones these cells normally produce, namely growth hormone (GH), prolactin (PRL), and thyroid-stimulating hormone (TSH) (14; review in 15,16). The affected animals are approximately normal size at birth but their growth lags behind that of their normal siblings, and the body weight of adult Ames dwarf mice is approximately one third that of normal mice. Plasma insulinlike growth factor-I (IGF-I) and thyroxine levels are extremely low, and sexual maturation is delayed (16–18). Females can produce fertilizable ova but are infertile, because in rodents PRL is absolutely required for support of corpora lutea and production of progesterone, which is necessary for maintenance of pregnancy (16,19). Only some of the males can sire litters in spite of complete and apparently normal spermatogenesis. The effects of this mutation on the life span are almost certainly due to deficiencies in GH, PRL, and TSH, rather than to some unknown effects of this mutation or interference with expression of the neighboring genes. In support of this conclusion, a comparable extension of life span was detected in Snell dwarf mice (20; Flurkey and Harrison, personal communication, June, 1996), animals with an identical endocrine phenotype caused by mutation of a different gene located on a different chromosome (details below).

Snell Dwarf Mice

Snell dwarf mice (21,22) are homozygous for a recessive mutation at the *Pit-1* locus (23), which is located on chromosome 16 (22,23). The *Prop-1* locus, which is mutated in the Ames dwarf mouse, was mapped to chromosome 11

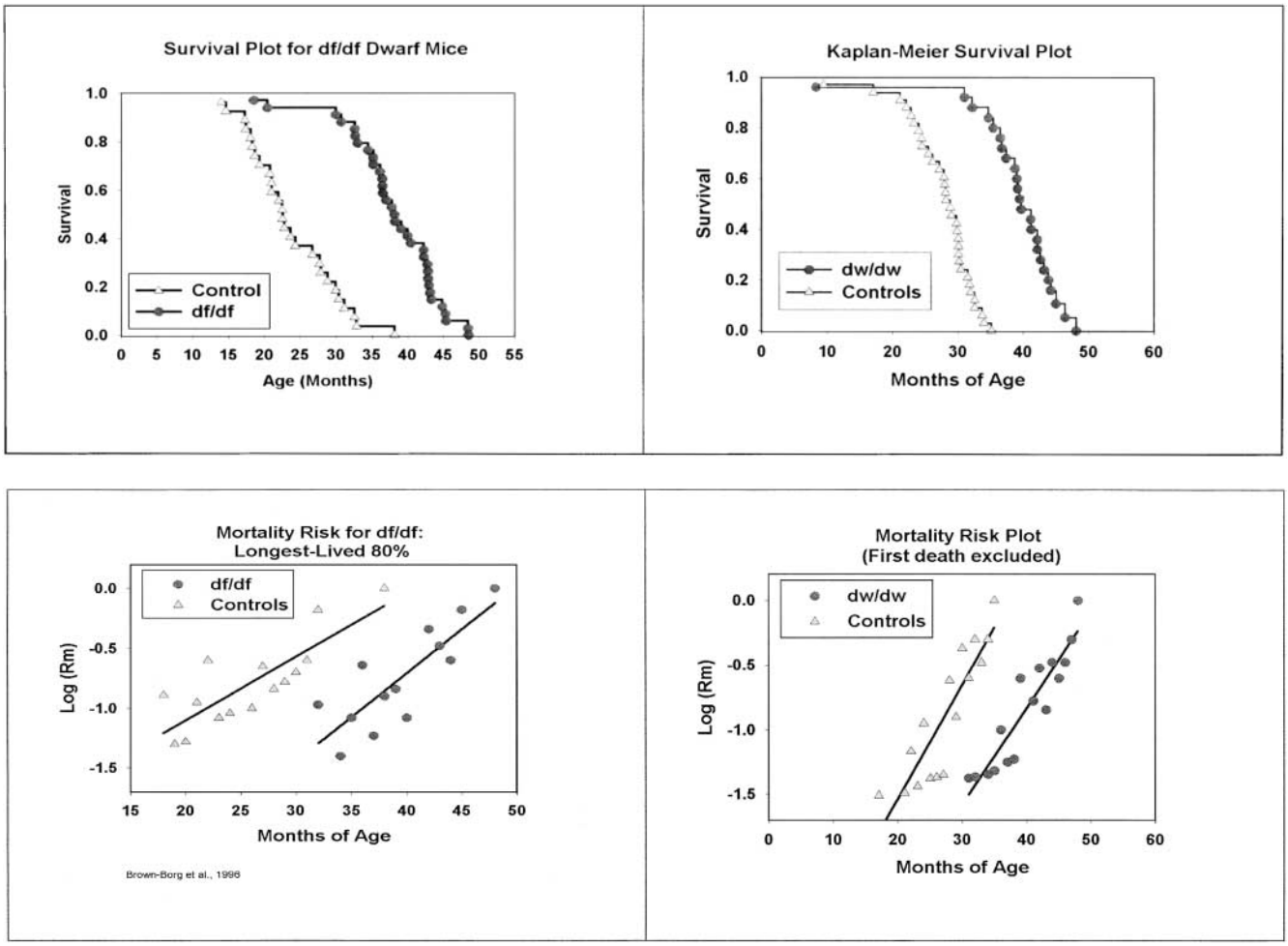

Figure 1. Comparison of survival plots (top) and mortality risk plots (bottom) in Ames dwarf mice (left) and Snell dwarf mice (right). Ames dwarf data are replotted from those reported by Brown-Borg and colleagues (10); Snell dwarf data are reproduced from Miller (20). Mortality risk plots were constructed by using the longest-lived 80% of animals (Ames dwarfs) or by excluding the first death (Snell dwarfs).

(24). The *Pit-1* gene controls differentiation of somatotrophs, lactotrophs, and thyrotrophs at a step distal to the action of the *Prop-1* gene, and homozygosity for a loss-of-function mutation at this locus leads to deficiency of GH, PRL, and TSH; that is, there is a phenotype identical to that of Ames dwarf mice (15,23,25). The longevity of Snell dwarf mice was a matter of some controversy in early literature, with one laboratory reporting premature deaths caused by a susceptibility to infectious diseases (26), and several investigators reporting normal or "at least normal" life spans (review in 27). In 1972, Silberberg (28) referred to the "unusually long life span" of these mutants but provided no data or citations to support this statement. Conclusive evidence that Snell dwarf mice live longer than their normal siblings was recently provided by Miller (20).

Data on the longevity of Ames and Snell dwarf mice were recently analyzed by Miller (20 and personal communication, April 2000). Results of this analysis revealed that slopes of the graphs describing relationships between survival and age and between mortality risk and age are parallel in dwarfs and in matched normal mice (Figure 1). This implies that, although aging in dwarf mice is significantly delayed, the rate of aging is not altered. This is a very important point because it implies that the prolonged longevity of these animals is not due to a longer period of senescence but rather to a delayed onset of aging, that is, a prolonged "health span." Results of a quantitative assessment of age-related changes in learning and memory in Ames dwarf versus normal mice (29) support this conclusion. Using the so-called passive avoidance test, we have shown that whereas normal mice exhibited an expected significant decline in cognitive function between the ages of 2–4 and 20–23 months, dwarf mice did not.

Identification of hormonal alterations responsible for the effects of genes at the *Prop-1* and *Pit-1* loci on aging and longevity is difficult because of the primary deficiency of three hormones (GH, PRL, and TSH) in the affected mutants. However, recent results obtained in other models (details follow) are very helpful in this regard and suggest that a deficiency of GH signaling may be an important and perhaps the key factor in mediating the effects of these genes on longevity.

Table 1. Origin and Key Characteristics of Long-Living Mutants of the House Mouse (*Mus musculus*)

Parameter	Snell Dwarfs	Ames Dwarfs	GH Receptor Knockouts
Original description	Snell, 1929 (21)	Schaible & Gowen, 1961 (12)	Zhou et al., 1997 (32)
Origin	spontaneous mutation at the *Pit-1* locus	presumably spontaneous mutation at the *Prop-1* locus (found in stock used previously in radiation experiments.)	targeted disruption of GH-R/GHBP gene
Chromosomal location	chromosome 16	chromosome 11	chromosome 15
Nature of mutation	loss of function	loss of function	loss of function
Mode of inheritance	recessive	recessive	recessive (with mild effects on growth in heterozygotes)
Propagation	mating of heterozygous carriers or hormone-treated homozygates	mating of heterozygous carriers or hormone-treated homozygotes	mating of heterozygous carriers or homozygous males with heterozygous females
Fertility	all females & most males are sterile	all females & most males are sterile	both sexes are fertile but fertility is quantitatively reduced, part. in females
Primary effect	absence of anterior pituitary cells producing GH, PRL, and TSH	absence of anterior pituitary cells producing GH, PRL, & TSH	absence of GH receptor & GH binding protein
Gross phenotypic characteristics	reduced postnatal growth & adult body size; infantile body proportions	reduced postnatal growth & adult body size; infantile body proportions	reduced postnatal growth & adult body size
Extension of life span	~42%	50–65%	38–55%

Notes: Details and references are in the text; reference numbers are given parenthetically in the table. GH = growth hormone; PRL = prolactin; TSH = thyroid-stimulating hormone; GH-R/GHBP = GH receptor/GH binding protein.

Little Mice

Mice homozygous for the mutation "little" (lit/lit) have an isolated GH deficiency that is due to a loss-of-function mutation of the gene encoding the receptor for the GH-releasing hormone (30). These animals exhibit reductions in growth rate and adult body size. For reasons that are not clear at present, they continue to grow slowly throughout much of their adult life, and thus the difference in body weight between the little mice and their normal siblings gradually decreases with age (31). Recent findings of K. Flurkey (personal communication, February 2000) indicate that lit/lit mice live significantly longer than normal animals from the same line, providing that they are fed a low-fat diet to prevent development of obesity.

GH-Resistant Knockout Mice

A group headed by Dr. Kopchick succeeded in producing animals with targeted disruption ("knockout"; KO) of the GH receptor/GH binding protein (GH-R/GHBP) gene (32). These GH-R-KO mice produce GH but cannot respond to it because of absence of GH-R. Consequently, they exhibit a deficiency of IGF-I in peripheral circulation and retardation of postnatal growth. The body weight of adult GH-R-KO mice is slightly less than half of that of their normal siblings (30). A disruption of the GH-R gene results in GH resistance, which is the single primary endocrine defect in the GH-R-KO mice. Plasma PRL levels are not suppressed and, in fact, are significantly elevated (33). This may represent a compensatory response to the absence of GH signaling. Plasma levels of thyroid hormones, thyroxine (T_4), and triiodo-thyronine (T_3), are slightly but significantly suppressed (34), presumably as a secondary consequence of reduced IGF-I levels (25,35). Both females and males are fertile, although puberty is delayed and fertility is reduced (32,33,36). In males, the effects of exogenous luteinizing hormone–releasing hormone (LHRH) and luteinizing hormone (LH) and the release of LH and testosterone are reduced (33). Recently, Coschigano and colleagues (37) reported a significant extension of life span in GH-R-KO as compared with normal mice. The differences in the average life span between GH-R-KO and normal mice ranged from 38% to 55%, depending on gender and using only +/+ or both +/+ and +/− animals as normal controls. Results of our ongoing study, now entering its fourth year, are in complete agreement with these findings. At this time, 7 of 68 of normal animals (10.3%) and 25 of 43 of age- and sex-matched GH-R-KO animals (58.1%) are still alive; $p < .001$. These results demonstrate that an isolated primary defect in the ability to respond to GH is sufficient to prolong life and that PRL deficiency, infertility, and/or primary defect in the function of the pituitary-thyroid axis are not necessary for this effect.

The origin and key phenotypic characteristics of Ames dwarf, Snell dwarf, and GH-R-KO mice are summarized and compared in Tables 1 and 2. Results of behavioral studies in GH-R-KO mice (38) indicate that, similar to the situation that occurs in Ames dwarf mice, prolonged longevity of those animals is associated with a delay of cognitive aging.

Other "Longevity Genes" in Mammals

In 1999, Migliaccio and his colleagues (39) reported a 30% increase in life span in mice homozygous for targeted disruption of $p66^{shc}$; $p66^{shc}$ is encoded by the proto-oncogene *SHC* locus and is a part of a signal transduction pathway that regulates apoptotic responses of cell lines to stress. Homozygous $p66^{shc}$ −/− mice had apparently normal phenotype with no significant alterations in food intake or body weight, increased resistance to paraquat, and prolonged life span. It is presently unknown whether mechanisms responsible for prolonged longevity of $p66^{shc}$ KO mice overlap

Table 2. Endocrine, Metabolic, and Reproductive Characteristics of Long-Living Mutants of the House Mouse

Parameter	Snell Dwarfs	Ames Dwarfs	GH Receptor Knockouts
Plasma GH	absent	absent	elevated
Plasma IGF-I	greatly reduced	greatly reduced	greatly reduced
Plasma thyroid hormones	severely reduced	severely reduced	reduced
Body core temperature	reduced	reduced	slightly reduced
Plasma corticosterone	normal (?)	elevated in males	elevated in males
Plasma insulin	reduced	reduced	greatly reduced
Plasma glucose	reduced	reduced	reduced or normal
Glucose response to insulin	?	enhanced	enhanced
Plasma PRL	absent	absent	elevated in males
Plasma TSH	reduced*	reduced*	reduced in males
Plasma LH	reduced or normal*	reduced or normal*	normal
Plasma testosterone in males	reduced*	reduced*	reduced or normal
Sexual maturation	delayed*	delayed*	delayed
Spermatogenesis	complete, quantitatively reduced*	complete, quantitatively reduced*	complete, quantitatively reduced
Estrous cycle and ovulation	sporadic*	sporadic*	irregular; quantitative deficits
Pregnancy	absent because of luteal failure	absent because of luteal failure	prolonged; quantitative deficits in fetal development

Notes: Details and references are in the text. GH = growth hormone; PRL = prolactin; TSH = thyroid-stimulating hormone; IGF-I = insulinlike growth factor-I; LH = luteinizing hormone.

*With respect to these characteristics, Snell and Ames dwarf mice exhibit a range of phenotypes, depending on their genetic background.

those involved in the delayed aging of dwarf and GH-R-KO mice.

Why Do Dwarf and GH-R-KO Mice Live Longer?

Mechanisms responsible for delayed aging and prolonged longevity of Ames dwarf, Snell dwarf, and GH-R-KO mice have not been elucidated and are the subject of intense studies in several laboratories. However, some of the physiological characteristics of these animals are likely to contribute to their longevity. These characteristics are summarized as follows.

Improved Antioxidant Defenses

The activity of two important antioxidant enzymes, catalase (CAT) and Cu–Zn superoxide dismutase (SOD) in the liver, kidney, and hypothalamus is higher in Ames dwarf mice than in sex- and age-matched normal controls (40–42). Significant increases in the activity of these enzymes were detected in each of these organs, with the differences being generally greater and more consistent in young animals. Inasmuch as generation of free oxygen radicals and oxidative damage of DNA, lipids, and proteins are established as key mechanisms of aging at the cellular level (43,44), an increased activity of antioxidant enzymes in Ames dwarf mice may provide them with increased protection against damage by reactive oxygen species (ROS) and thus contribute to their increased longevity. In support of this possibility, Brown-Borg recently demonstrated that Ames dwarf mice have significantly reduced oxidative DNA damage and protein carbonyl content (45). Moreover, the levels of inorganic peroxide were shown to be reduced in the livers of Ames dwarf mice compared with those of normal mice (46). Increased CAT and SOD activity in young (3–5 months old) dwarf mice suggests that protection from oxidative damage early in life may contribute to delayed aging of these animals.

In further support of the conclusion that Ames dwarf mice are "protected" from detrimental effects of ROS, they were recently shown to have an increased resistance to a high toxic dose of paraquat (Hauck, Wright, and Bartke, unpublished data, 2000), a compound that induces oxidative stress (47). Further studies will be needed to determine whether the metabolism of paraquat to toxic products may be altered in dwarf mice and thus contribute to these findings. It should also be emphasized that without extensive studies of mitochondrial function, ROS generation, ROS levels, and oxidative damage in various tissues of dwarf mice, it is not possible to verify or refute the proposed role of improved antioxidant defenses in delayed aging of these animals.

Reduced Body Temperature and Metabolic Rate

Snell dwarf mice were reported to have a reduced body temperature (48) and oxygen consumption (49; review in 21). Using miniature transmitters implanted into the abdominal cavity and 24-hour telemetric recording, we have compared body core temperature (Tco) in Ames dwarf and normal mice under basal conditions and after exposure to food deprivation or mild stress, stimuli known to, respectively, reduce or increase Tco in wild-type mice. Tco was found to be reduced by an average of 1.5°C in Ames dwarfs as compared with normal mice, and this difference persisted during fasting and after mild stress (50). We have recently shown that GH-R-KO also have a reduced Tco, but the difference from normal animals is relatively small (~0.4°C) and reaches statistical significance only at certain times of the diurnal cycle, including several hours immediately preceding "lights on" (34).

Reduction in Tco in Ames dwarf and GH-R-KO mice and comparison with the results obtained in Snell dwarfs suggest that metabolic rate is reduced in these animals. Reduction in both Tco and metabolic rate would certainly be consistent with severe hypothyroidism of Snell and Ames dwarfs and a small but significant reduction in plasma T_4 and T_3 levels in GH-R-KO mice. Experimentally induced hypothyroidism was shown previously to prolong life in rats (51). However, the extension of life in hypothyroid rats was

very small in comparison with that recorded in dwarf and GH-R-KO mice, and therefore the delayed aging of these animals can be assumed to be primarily due to other factors. Growth hormone deficiency or resistance in these animals may also contribute to the observed reduction in Tco and the suspected suppression of metabolic rate, because GH exerts both anabolic and calorigenic actions (52). Unexpectedly, in Ames dwarf mice the amount of food consumed per gram of body weight is increased rather than reduced (11), suggesting that their metabolic rate may be higher than that of their normal siblings. Further studies will be necessary to fully characterize energy balance and metabolic status of these animals. For example, increased food consumption in the dwarf mice may be associated with reduced, rather than increased, metabolic rate if food absorption and/or utilization is less efficient in dwarfs than in normal mice. This possibility is supported by the stimulatory effects of GH and IGF-I on the gastrointestinal tract (53).

In poikilothermic (cold-blooded) animals, body temperature is an important determinant of metabolic rate, and life can be significantly extended by lowering the environmental and thus the body temperature (54). In homeothermic (warm-blooded) animals, the relationship of body temperature and metabolic rate to aging and longevity is complex. For example, caloric restriction, which very effectively prolongs life in rats and mice, reduces body temperature, but the metabolic rate adjusted for body weight (or lean body mass) is reduced only transiently (55). Although the "rate of living" theory of aging is thought to apply in a fairly limited number of cases, we suspect that reduction in Tco and the probable reduction in metabolic rate contribute to delayed aging in Ames and Snell dwarfs and perhaps also in GH-R-KO mice. It seems reasonable to assume that reduced thermogenesis and metabolism may lead to a reduced production of ROS, and this, combined with the increased activity of antioxidant enzymes in these animals, reduces oxidative stress and thus delays aging.

Hypoglycemia and Hypoinsulinemia

In both Ames dwarf and GH-R-KO mice, plasma glucose levels are reduced (34,56), although in GH-R-KO mice this reduction is small and was not detected in every study (57). However, significant reductions in plasma glucose were detected in GH-R-KO animals that had unrestricted access to food (34), as well as in animals subjected to an overnight fast (58). Reduced plasma glucose levels were also detected in young Snell dwarf mice (Flurkey and Harrison, personal communication, June 2000). Plasma insulin levels are reduced in Snell dwarfs (Flurkey and Harrison, personal communication, June 2000), in fasted Ames dwarfs of both sexes (Turyn and Bartke, unpublished data, 1999), and in ad-lib fed Ames dwarf males (56). Insulin levels are severely reduced in GH-R-KO mice (34,57,58). Concomitant reduction in glucose and insulin levels implies an increased sensitivity to insulin. This is not unexpected in GH-deficient and GH-resistant animals, because GH is well known to induce insulin resistance (59,60). The administration of a single dose of insulin produced greater suppression of blood glucose levels in GH-R-KO and in Ames dwarf mice than in the corresponding normal controls (57; Pazo, Mattison, and Bartke, unpublished data, 1999). Unexpectedly, the ability to dispose of a glucose load was reduced rather than enhanced in both GH-R-KO and Ames dwarf mice (57; Pazo, Mattison and Bartke, unpublished data, 1999). This might reflect a reduced capacity to increase insulin secretion in response to an acute demand. Recent studies of insulin signaling in the liver of Ames dwarf and GH-R-KO mice indicate that an increased sensitivity of these animals to the action of insulin is due to different mechanisms: increase in the level of insulin receptor substrates 1 and 2 in the dwarfs, and increase in the level of insulin receptors in GH-R-KOs (58; Dominici, Turyn, and Bartke, unpublished data, 2000).

Because glycation is believed to represent an important mechanism of aging (61), reduced plasma glucose is very likely to contribute to delayed aging in both dwarf and GH-R-KO mice. Hyperglycemia and hyperinsulinemia are associated with a reduced life expectancy in diabetic patients and in various animal "models," including transgenic mice that overexpress GH (60,62).

It is most intriguing that *daf-2*, one of the longevity genes in a worm, *Caenorhabditis elegans*, exhibits some homology to the mammalian insulin receptor (4). This raises a possibility that involvement of insulin and IGF-I in the control of aging and longevity may be evolutionarily conserved. Comparative studies of genes involved in insulin signaling pathway in *C. elegans* and in the mouse are of intense current interest (J. Papaconstantinou, personal communication, November 2000).

Small Stature

In contrast to the generally positive correlation between the size of animals from different species and their longevity, body size within a species is usually negatively correlated to life span. Lines of mice with significantly different adult body size were developed by selection in several laboratories. A consistent finding in these studies was that mice from lines with small body size live longer than mice from lines with large body size (63–65). Caloric restriction, which reduces growth rate and adult body size, delays aging and prolongs life in rats, mice, and almost certainly monkeys. Small breeds of dogs appear to have reduced responsiveness to GH as evidenced by low plasma levels of IGF-I (66,67) and live significantly longer than larger breeds (68). Giant transgenic mice overexpressing GH genes have drastically reduced life spans (62,69–71).

The evidence for small individuals, strains, or breeds outliving the large ones is particularly strong for mice (63–65,71) and domestic dogs (68), but it also applies to other species. Samaras and his colleagues assembled considerable evidence that this relationship applies also to the human (72,73). Although the mechanisms responsible for the negative correlation of body size and longevity within a species are unknown, the possible roles of improved rheological efficiency of a smaller cardiovascular system (within a species-specific body plan) and reduced energy intake and utilization have been suggested (72,73). Regardless of the mechanisms involved, Ames dwarfs, Snell dwarfs, and GH-R-KO mice are all at or near the minimum of body size of house mice, and thus it could be argued that the major ex-

tension of their life span is not unexpected on that basis alone.

Reduced Number of Cell Divisions

Early studies in Snell dwarf mice provide evidence that the number of cells in different organs of these animals is significantly reduced (74,75). This is consistent with reduced IGF-I levels in dwarf mice (17) because IGF-I is mitogenic, and it suggests that the number of cell divisions is lower in dwarf than in normal mice. It is reasonable to assume that a reduced number of cell divisions may be associated with fewer opportunities for somatic mutations and for the development of neoplasia. Preliminary data on the incidence of neoplastic lesions in Ames dwarf mice dying from natural causes suggest that, in comparison with normal animals, Ames dwarf mice develop tumors with a comparable frequency but at a later age (11).

Hypogonadism

Data derived from different control populations of animals of the same species, as well as comparisons of different strains or lines produced in selection experiments, suggest that delayed puberty and reduced fertility (particularly early in life) tend to correlate with increased longevity (63–65,76). There are also reports of increased life span of gonadectomized, as compared with intact, individuals (77). In dwarf and GH-R-KO mice, puberty is delayed and fertility reduced (15,16,32,33,36). This is particularly pronounced in Snell and Ames dwarf mice, in which all females are infertile as a result of PRL deficiency (16,19), and, depending on the genetic background, puberty may be indefinitely delayed (16,19,22,27). Thus, hypogonadism may contribute to delayed aging and prolonged longevity of these animals. In support of this possibility, the relative extension of life span, the "longevity advantage" of Ames dwarf females that are hypogonadal and invariably infertile, is greater than that of Ames dwarf males, which have considerable testicular development and occasionally are capable of siring litters (10,27). However, we believe that the role of hypogonadism in delayed aging of dwarf and GH-R-KO animals is minor, at best, because the reported effects of gonadectomy on life span are very small (77), and there is only a small difference between the relative extension of life span in Ames and Snell dwarf mice, which are almost invariably sterile, and in GH-R-KO mice, in which both sexes can reproduce.

Altered Gene Expression

Data summarized in the preceding sections of this paper suggest that delayed aging of Ames and Snell dwarf mice (and most likely also GH-R-KO mice) is due to multiple mechanisms and functional changes in multiple organ systems. Therefore, it is reasonable to postulate that the expression of numerous genes is altered in these animals. This possibility is consistent with recent reports that aging is accompanied by altered expression of a large number of genes in mouse skeletal muscle (78), liver (79), and brain (80), and in human fibroblasts (81). In a collaborative study with Drs. Miller and Dozmorov, we are currently comparing the levels of hepatic expression of approximately 600 genes in Ames dwarf versus normal mice at different ages. Results obtained to date (82) indicate differences in the level of expression of individual genes and in the temporal patterns of gene expression between normal and Ames dwarf mice. A list of genes that are differentially expressed in the liver of Ames dwarf as compared with normal mice includes several genes related to IGF-I production and binding (82), and at least one gene that is known to be under GH control, namely *p38* MAP kinase (83). Further work will be necessary to verify these preliminary observations, to begin identifying the functional implications of altered expression of individual genes, and to determine which of the observed changes are candidate mechanisms, as opposed to markers, of delayed aging in these animals.

Interpretation of Findings in Long-Living Mice

Association of Delayed Aging With Reductions in Growth and Reproductive Fitness

At first glance, it is surprising and difficult to understand why animals that are small, appear to be frail, and have reduced reproductive competence outlive their normal and outwardly much more robust and "fit" siblings. However, these findings are not at all surprising when they are viewed from the evolutionary perspective. Consideration of the evolution of aging leads to the conclusion that the process of aging does not appear to serve any identifiable biological function. Under natural conditions, most animals succumb to predation, environmental stress, or disease early in life, and the timing of the functional decline or the limits of the life span of the remaining few are probably of very little, if any, consequence for the survival and success of the species. It was, therefore, suggested (84) that the genes that accelerate aging, induce functional deficits late in life, or reduce life expectancy persist in the genetic pool of the populations only because they have beneficial effects early in life. Genes that promote growth, early sexual maturation, and high fertility would be highly beneficial; that is, they would increase the probability of passing genes of an individual to the next generation and thus would fit well in this category. This relationship would imply that mutations that prolong life are likely to involve loss or reduced function of these particular genes and thus can be expected to have detrimental effects on growth, maturation, and reproductive competence. In support of these arguments, mutations that extend life in worms and insects often result also in reduced body size and in suppressed fertility (Austad, personal communication, November 2000). Delayed aging of dwarf, GH-R-KO, and lit/lit mice can thus be interpreted as indication that genes involved in promotion of growth, maturation, and fertility, including *Prop-1*, *Pit-1*, GHRH-R and GH-R/GHBP have a role in determining the onset of aging; when these genes are silenced, aging is delayed.

The cause–effect connection between genetic disruption of GH production or action and delayed aging can also be viewed in terms of the role of reductions in oxidative metabolism, Tco, mitotic activity, nutrient processing, plasma glucose, and so on in the control of aging and longevity. These relationships were brought up earlier in this review in the context of discussing putative mechanisms of delayed aging in Ames dwarf, Snell dwarf, and GH-R-KO mice. Ev-

idence from studies in calorically restricted animals and from mutant, transgenic, or knockout invertebrates that supports the role of these mechanisms in the determination of aging and life span cannot be reviewed here because of the limitations of space.

Whether relationships of growth and fertility to aging are viewed from the point of view of the evolutionary emergence of wild-type genotypes or from the point of view of physiological alterations associated with prolonged life, one is left with the conclusion that hormonal signals that control growth and maturation are also involved in determination of aging and life span.

This line of reasoning and the negative association of life span with growth, maturation, and fertility are certainly consistent with the well-documented effects of caloric restriction. Caloric restriction (CR) is the only treatment that reliably produces a major delay of aging and a major extension of life span in mice and other mammalian and nonmammalian species (85,86). It is particularly effective when started early in life. In addition to beneficial effects on aging and longevity, CR reduces growth and adult body size, delays puberty, and reduces some indices of fertility (e.g., litter size) (85,86). In other words, rapid growth, early maturation, and attainment of large body size and great reproductive potential may be linked to significant "costs" in terms of earlier onset of aging and reduced longevity. We are presently studying the interactions between the effects of CR and Ames dwarfism (11). Unexpectedly, the results obtained to date indicate that CR significantly extends life of these long-lived mice. Apparently, Ames dwarf mice are not CR mimetics.

Are Findings in Long-Living Mice Relevant to the Human?

Increased longevity of hypopituitary dwarf mice and GH-resistant knockouts appears to be at odds with some observations in the human and with clinical practice. Children with GH deficiency or hypothyroidism are routinely given replacement therapy aimed at producing normal growth and development. Hypopituitarism and GH deficiency are believed to constitute risk factors for cardiovascular disease (87). Finally, patients who became hypopituitary after treatment for adenohypophyseal tumors and were not given GH replacement were reported to live shorter lives than normal healthy controls (88). Although the clinical findings mentioned above provide a solid basis for providing hypopituitary children with appropriate replacement therapy, they do not constitute evidence that the relationship between GH signaling, growth, maturation, and aging is fundamentally different in humans than in mice. In fact, there is increasing evidence that the negative correlation of adult body size and longevity, which is well documented in mice, dogs, and other animal species, applies also to the human (72,73). Moreover, tall stature was shown to be a risk factor for several age-related diseases (89–92). Most intriguing in the context of this discussion are results of studies in individuals with hypopituitarism caused by a mutation at the *Prop-1* locus, the same locus that is mutated in Ames dwarf mice (93). It was recently reported that individuals with such mutations can survive to a very advanced age, apparently longer than normal individuals in the same population (93). It should be emphasized that these "little people of Krk" who lived to be over 60, and in one case 91 years old, were not given hormonal replacement.

It is also of interest that chronic elevation of plasma GH levels outside the physiological range is associated with reduced life expectancy in both mice (62,69–71) and humans (94,95). However, information on the effects of elevated GH levels on longevity in humans and mice is derived from studying individuals with hypersomatotropism of different etiology (adenohypophyseal tumors in humans, transgenes in mice) and different age of onset (adult onset in most humans; prenatal or perinatal onset in mice). Furthermore, the mechanisms responsible for reduced life expectancy in acromegalic patients and GH transgenic mice differ in important ways (69,94–96), and it can be argued that they represent GH-induced pathology rather than premature onset of normal aging. A detailed discussion of indirect evidence that transgenic mice overexpressing GH exhibit various symptoms of premature aging (62,69,97) is outside the scope of this article.

Can the Proposed Role of GH Signaling in the Genetic Control of Aging Be Reconciled With the Antiaging Effects of GH?

The antiaging actions of GH have been widely publicized, and GH as well as GH-releasing agents are actively promoted for human use. This appears totally incompatible with the delayed aging of GH-deficient and GH-resistant animals and the proposed negative relationship between GH signaling and longevity. However, we would like to suggest that these two concepts are not necessarily conflicting because they refer to different actions of GH and to different stages of life. More specifically, the antiaging actions of GH refer to its effects on body composition and function in elderly individuals rather than to its role in determining life expectancy. Release of GH from the pituitary declines with age (98,99), and reduced levels of GH almost certainly contribute to age-related loss of muscle mass, increase in adiposity, loss of bone mineral (100), and perhaps also to impairments of cognitive function (101). Indeed, these changes resemble those observed in adult GH deficiency (102,103) and can be reduced or reversed by GH therapy (100,102). The use of GH in antiaging medicine is further supported by the observation that in GH-deficient individuals, treatment with GH has a positive impact on several measures of psychological well-being and has been reported to result in general improvement of the quality of life (102,104). Although reduction in obesity and particularly in trunkal obesity is believed to reduce the risk of diabetes and cardiovascular disease, there is no data on the effects of GH therapy in the elderly on life expectancy. Detailed discussion of risks and benefits of GH therapy is outside of the scope of this article, but many reports raise significant concerns as to the safety and undesirable side effects of GH administration (105,106).

Currently available data from the studies in animals with targeted disruption of the GH-R gene or with hereditary dwarfism do not allow for the separation of effects of the somatotropic axis on development from their effects on

adult functions. However, extrapolation from the results obtained in calorically restricted animals and in animals differing in adult body size suggests that the effects of this axis early in life may be particularly important. Consequently, we would like to suggest that the normal stimulatory effects of GH on linear growth, metabolism, sexual maturation, and adult body size are also involved in timing the normal rate of aging and life span. Supraphysiological stimulation of these processes by excessive GH can shorten life, whereas their inhibition as a result of reduced GH signaling can delay aging and prolong life. We suspect that in dwarf and GH-R-KO mice, the positive impact of GH deficiency or resistance on events and processes that "program" the organism for long life must be masking and, indeed, far outweighing the possible negative effects of GH deficiency on physiological functions related to longevity. Thus, findings in the animals with extreme alterations in the somatotropic axis, growth, and adult body size help us uncover the normal role of GH and IGF-I in the control of aging and life span.

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Address correspondence to A. Bartke, Ph.D., Professor and Chairman, Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL 62901-6512. E-mail: abartke@siumed.edu

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