Pleiotropic effects of growth hormone signaling in aging

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Growth hormone (GH) affects somatic growth, sexual maturation, body composition and metabolism, as well as aging and longevity. Mice lacking GH or GH receptor outlive their normal siblings and exhibit symptoms of delayed aging associated with improved insulin signaling and increased stress resistance. Beneficial effects of eliminating the actions of GH are counterintuitive but conform to the concept of antagonistic pleiotropy. Evolutionary selection for traits promoting early-life fitness and reproductive success could account for post-reproductive deficits. Reciprocal relationships between GH signaling and longevity discovered in mutant mice apply also to normal mice, other mammalian species, and perhaps humans. This review summarizes the present understanding of the multifaceted relationship between somatotropic signaling and mammalian aging.

Physiological actions of GH

GH is a protein hormone secreted by the anterior pituitary in vertebrate animals. Named after its major stimulatory role in the control of somatic growth and adult body size, it exerts a multitude of other effects. GH influences most and probably all tissues and organs either directly or via growth factors. In mammals, insulin-like growth factor-1 (IGF-1) is a major mediator of its actions. Moreover, the effects of GH on carbohydrate, fat and protein metabolism, on nutrient sensing pathways, and on secretory activity of adipose tissue, are important determinants of the internal milieu of an organism and influence virtually every physiological function.

Studies conducted during the past 15 years have firmly established that, in addition to its various growth-promoting and metabolic actions, GH also affects aging and longevity. Surprisingly, the best defined effects of the somatotropic axis [which consists of the hypothalamic GH releasing hormone (GHRH), GH and IGF-1] on longevity are negative. Mutant mice with GH deficiency or resistance outlive their normal siblings by as much as 50% and occasionally even more [1–3]. Genetic disruption of IGF-1 signaling can also extend murine lifespan, whereas transgenic mice with supraphysiological excess of GH and IGF-1 are short-lived (reviewed in [4,5]). One is forced to conclude that the physiological actions of GH involve significant costs in terms of longevity, and this is not entirely surprising in the light of our present understanding of the evolutionary and genetic control of aging. The concept of

antagonistic pleiotropy implies that genes and phenotypes detrimental to post-reproductive survival are not eliminated by natural selection if they confer an evolutionary advantage earlier in life. Thus, in terms of evolutionary fitness the apparent 'pro-aging' effects of GH might be more or less irrelevant, or instead are outweighed by the stimulatory effects of the somatotropic axis on growth, sexual maturation, and fecundity (Figure 1). Importantly, the reciprocal relationship between GH actions and longevity demonstrated in mutant, gene knockout and transgenic mice applies also to genetically normal mice and to other mammalian species including rats, dogs, horses and perhaps also humans [4,6,7].

This article discusses recent progress in identifying mechanisms linking GH actions with aging and longevity in mice and present findings concerning the relationships of GH to aging in other species. This will include information on the phenotypic characterization of humans with GH deficiency or resistance.

Long-lived GH- and IGF-1-related mutants

Studies reported over the past four years have expanded the list of GH-related mutations that extend longevity in mice. Long-lived mice were produced by gene deletions that affect tissue levels of bio-available (free) IGF-1 [8] or levels of insulin receptor substrates (IRS)-1 or -2, mediators of IGF-1 and insulin signals [9,10]. Furthermore, mouse longevity was extended by deleting S6 kinase, which is related to GH actions via mammalian target of rapamycin (mTOR) [10]. The importance of the mTOR pathway in the control of mammalian aging was shown by demonstrating that rapamycin, an inhibitor of mTOR, extends longevity of mice [11]. Our ongoing studies indicate that both sexes of the recently produced mice with isolated GH deficiency due to genetic deletion of hypothalamic GHRH are also long-lived (Spong, A. *et al.*, unpublished).

Comparison of the impact of various somatotropic axis-related mutations on longevity reveals some interesting and not readily anticipated trends. In general, the impact on longevity of mutations affecting IGF-1 levels, IGF-1 receptors (IGF1R) or signaling events downstream from IGF1R is smaller than the effects of disrupting GH biosynthesis or actions [12]. Moreover, GH resistance or deficiency extends longevity in both sexes, whereas several of the mutations directly affecting IGF-1 signaling increase lifespan only in females. Some of these differences could be due to the effects of genetic background, diet or other
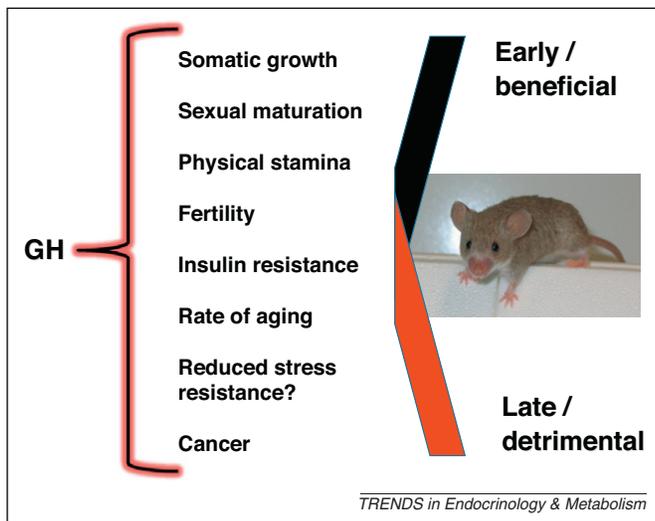
Figure 1. Effects of GH during different life stages. Stimulation of growth, maturation and fertility by GH apparently involves 'costs' of accelerated aging and increased susceptibility to age-related disease. Current understanding of the evolution of aging suggests that the detrimental GH actions late in life have not been eliminated because of beneficial effects of GH on early reproductive fitness.

differences between studies conducted at different laboratories, or to the fact that complete deletion of IGF-1 signaling causes severe developmental problems, including lethality [13]. Consequently, the IGF-1-related mutants available for study have partial suppression of IGF-1 levels or actions, as opposed to the complete absence of GH signals seen in the hypopituitary, GH-resistant or GH-deficient mutants. Nevertheless, the available data suggest that there are some differences between the effects of GH and IGF-1 on aging and longevity. Because IGF-1 expression in many organs is partially or completely GH-independent, eliminating GH action does not interfere with the beneficial actions of locally expressed IGF-1 in the brain, heart or other organs, whereas it reduces circulating (primarily liver-derived) IGF-1 levels, thus protecting the animals from cancer [12,13]. By contrast, genetic suppression of IGF-1, IGF1R or IRS interferes with IGF-1 actions everywhere, thus presumably producing a combination of detrimental and protective effects. Finally, the available data suggest that GH resistance or deficiency can influence aging by mechanisms unrelated to the regulation of IGF-1 expression. Prominent among the likely 'GH-specific' mechanisms affecting longevity is the regulation of insulin sensitivity, an action of GH that is not mediated by IGF-1 and is, in fact, opposite to the IGF-1 effects. Although GH is anti-insulinemic and promotes insulin resistance, IGF-1 is an insulin sensitizer and mimics some of the actions of insulin. Direct actions of GH on adipose tissue are likely to contribute to its impact on insulin signaling via alterations in the levels of circulating adipokines and fatty acids.

The evidence that IGF-1 is importantly involved in the control of longevity was strengthened by a recent demonstration which compared 31 inbred mouse strains: it was found that circulating IGF-1 levels were negatively related to the median lifespan of females [14]. These findings extend previous reports showing that serum IGF-1 levels measured during young adulthood in individual mice were negatively correlated with their survival [15] and that

similar relationships were detected in comparisons of different breeds of domestic dogs [16]. Interestingly, the expected positive correlation between circulating IGF-1 levels and adult body size which was evident in these and many other studies might not apply to comparisons between different species. Stuart and Page [17] recently reported that IGF-1 levels are, surprisingly, lower in larger species. This was based on analysis of published data derived from 36 mammalian species ranging from mice to cattle and polar bears. These data are intriguing and potentially very important for explaining why longevity is generally negatively correlated with body size within a species but, with few exceptions, is positively correlated with body size in comparisons involving different species (e.g. mouse, dog, horse, elephant, whale). The role of IGF-1 in promoting cancer growth together with the apparent 'pro-aging' actions of the somatotropic axis could explain why low IGF-1 levels are associated with increased life expectancy both in large species and in small individuals within a species.

Mechanisms linking reduced GH signaling with extended longevity

Although association of GH deficiency (isolated or combined with deficiency of thyrotropin and prolactin) or GH resistance with extended longevity in laboratory mice is strong, consistent and reproducible, the underlying mechanisms remain to be identified clearly. It was previously suggested that multiple mechanisms are almost certainly involved and that interactions between these mechanisms could amplify their individual effects [4,12] (Figure 2). Based on concomitant reduction of circulating insulin and glucose levels and on the correlation between the effects of different 'longevity genes' and calorie restriction
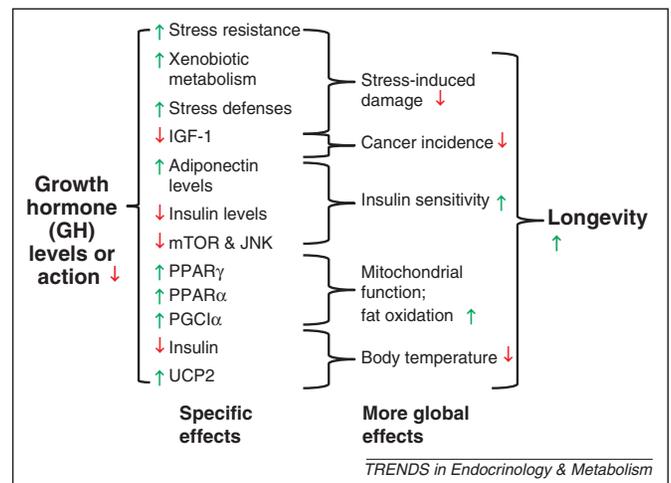
Figure 2. Mechanisms linking reduced GH signaling with extended longevity in GH-resistant or GH-deficient mice. Simplified outline of mechanisms of GH effects on longevity. Interactions and reciprocal relationships omitted for clarity (↑, increase; ↓, decrease). This diagram lists consequences of GH deficiency or resistance that constitute likely mechanisms of extended longevity. The Specific Effects listed in the first column include those detected in long-lived mutants, and most have been linked to GH actions by evidence from other experimental systems. The More Global Effects listed in the second column represent broader functional consequences of these changes known to be involved in the control of aging and longevity. The diagram is not intended to be comprehensive or all-inclusive. For example, plausible mechanisms of extended longevity that have been demonstrated in only one mutant have not been included.

(CR) on longevity and on whole-animal insulin sensitivity, it was further suggested that improved insulin signaling is important in linking reduction in GH actions with increased lifespan [18,19]. Because 30% CR initiated at two months of age had little if any effect on whole-animal insulin sensitivity or longevity in $Ghr^{-/-}$ (GH receptor 'knockout' or GHRKO) mice, whereas both were readily increased in normal animals from the same strain, we examined the interactive effects of CR and Ghr gene deletion on individual steps of insulin signaling in the liver and in the skeletal muscle [20]. In the liver, insulin receptor (IR) levels and its activating phosphorylation in response to acute insulin stimulation were greater in GHRKO than in normal mice, and were increased by CR in normal mice but not in the mutants. Activation of PI3Kinase (PI3K) measured by Tyr99 phosphorylation of its 85 subunit was also increased by CR only in normal animals, but Tyr99 phosphorylation did not differ between the normal and mutant mice – and thus did not reflect the differences in insulin sensitivity detected at the whole-animal level. In hind-limb muscles, IR levels were higher in GHRKO than in normal mice, whereas their acute activation by a large dose of insulin did not differ between genotypes. However, the downstream signaling events – PI3K activation, Akt 1 and 2 levels and activation, and GLUT 4 levels – corresponded to whole-animal insulin sensitivity, in other words they were elevated in GHRKO versus normal mice but were increased by CR only in the latter group. Phosphorylation of IRS-1 at Ser307 is known to be associated with reduced association with PI3K, increased IRS-1 degradation and insulin resistance [22,23]. It was markedly reduced in GHRKO as compared to normal mice [21]. This could be related to reduced JNK1 activation and mTOR signaling and increased adiponectin levels in GHRKO animals [20,23]. Surprisingly, CR did not alter inhibitory (Ser307) IRS-1 phosphorylation in either genotype. Recent studies comparing the effects of surgical removal of intra-abdominal fat in GHRKO and normal mice suggest that the remarkable insulin sensitivity of GH-resistant mutants is importantly related to altered secretory profile of adipose tissue (Masternak, M. *et al.*, unpublished). These studies implicate fat-depot-specific adiponectin secretion as one of the factors responsible for differences in the control of glucose levels [18,24] and lipid oxidation [25] in GH-resistant as compared to normal (wild-type) mice.

Enhanced stress resistance has been identified as another likely mechanism linking reduced GH signals with extended longevity. Several reports have documented increased resistance of dermal fibroblasts derived from hypopituitary (GH-deficient) or GH-resistant mice to a variety of cytotoxic stresses *in vitro* [26,27]. Importantly, in one of these long-lived mutants, increased fibroblast resistance to the toxic effects of Paraquat *in vitro* was associated with increased resistance to the lethal effects of the same toxin *in vivo* [28]. Amador-Noguez and his colleagues reported that long-lived $Ghrhr^{-/-}$ (little) mice with isolated GH deficiency are more resistant than normal animals to hepatotoxins and other xenobiotics *in vivo*. They related these findings to increased activity of hepatic enzymes involved in detoxification [29]. Expression of xenobiotic detoxification genes in the liver is increased

in these mutants, as well as in Ames dwarf mice that lack GH and other pituitary hormones, and this increase can be ascribed to augmented levels of bile acids and their actions via farnesoid X receptors [29,30]. Evidence for a cause–effect relationship between reduced GH signaling and enhanced stress resistance was provided by recent studies of cultured fibroblasts derived from Ames dwarfs that had been injected with GH for several weeks [31]. These cells no longer displayed resistance to multiple cytotoxic stresses and their responses to Paraquat, cadmium, an alkylating agent or a mitochondrial poison resembled those measured in cells derived from genetically normal control mice [31]. These findings suggest that increased resistance to various stressors is an important mechanism linking reduced somatotropic signaling to delayed aging in the mutant mice examined. Moreover, they imply that the association between enhanced stress-resistance and extended longevity, as demonstrated previously in invertebrates, also applies to mammals.

Studies of passive avoidance and spatial memory in hypopituitary and GH-resistant mutants as compared to normal mice provided evidence that the long-lived mutant are remarkably resistant to the age-related loss of cognitive function [32–34]. L. Sun and his colleagues related maintenance of learning and memory into advanced chronological age in Ames dwarfs to normal or elevated expression of IGF-1 in the hippocampus [35,36]. Using organotypic slice preparation, Schrag *et al.* [37] have shown that the hippocampus of Ames dwarf mice is resistant to β-amyloid toxicity as measured by tau protein phosphorylation and levels of apoptosis-related proteins. More recently, S. Sharma *et al.* [38] reported increased resistance of the hippocampus of Ames dwarf mice to oxidative stress induced by kainic acid. Their conclusions were supported by disparate alterations in the glutathione:glutathione disulfide ratio and glutathione peroxidase activity in dwarf as compared to normal mice and by assessment of spatial memory after kainic acid exposure. The remarkable maintenance of cognitive function in the long-lived mouse mutants could therefore be due to neuroprotective effects of local expression of IGF-1 in the brain and enhanced stress-resistance of neuronal circuits involved in learning and memory.

Intriguingly, treatment of senescence-accelerated SAM98 mice with an antagonist of GH-releasing hormone was recently reported to ameliorate various aspects of aging including oxidative stress in the brain and cognition [39]. Moreover, this treatment increased telomerase activity and mean (but not maximal) longevity in the short-lived SAMP8 mice [39].

Several recent studies sought to identify cellular mechanisms that might explain enhanced stress resistance of GH-deficient and GH-resistant mice. The levels of mRNAs for multiple heat-shock proteins were reduced in the liver, kidney and heart of Snell dwarfs (GH-deficient mutants with endocrine phenotype identical to Ames dwarfs) and in GHRKO mice, whereas expression of other heat-shock proteins was elevated in tissues from Snell dwarfs only [40]. In both Snell and Ames dwarf mice the expression of Nrf-2-sensitive genes was elevated in various organs [41]. Moreover, the responses of Ames dwarf and normal mice to treatment with diquat, a known hepatotoxin, differed in

terms of activation of stress-responsive pathways including ERK, JNK, p38MAPK, ERK-dependent and Nrf2-dependent genes, as well as Nrf2 protein [42]. In fibroblasts derived from Snell dwarf mice, exposure to oxidative stress *in vitro* led to reduced activation of ERK but, surprisingly, increased expression of ERK-dependent genes in comparison to the corresponding values measured in fibroblasts derived from normal mice [43]. Ongoing studies aim to identify functional alterations in stress-responsive pathways associated with extension of longevity by various genetic and dietary interventions. Further work should also establish the relationship between these findings and the considerable evidence that Ames dwarf mice have enhanced anti-oxidant enzyme activity and reduced oxidative damage to macromolecules including mitochondrial and nuclear DNA, proteins and lipids [44] – and that the enhanced resistance of these mutants to oxidative stress is related to alterations in methionine and glutathione metabolism [45].

Involvement of other mechanisms in mediating the effects of reduced GH signaling on aging and longevity is suggested by the recent reports that GH-deficient, long-lived Ames dwarf mice differ from normal animals by reduced mutation rate [46], altered expression of multiple microRNA species [47] and genes related to purine metabolism [47], and reductions in the content of extracellular collagen in the heart [48] and in the size of cardiomyocytes [48]. Reduced cell size probably reflects reduced levels of translation due to diminished levels and activation of mTOR in these animals [20]. Moreover, improved maintenance of bone-marrow stem cells was recently reported by Ratajczak *et al.* in GH-resistant mice [49].

Increased levels of adiponectin [23], diminished expression of inflammatory markers [50], and delayed aging of the immune system [3] in various GH-deficient and GH-resistant mutants suggest that thorough exploration of immune function and inflammatory processes in these animals, and of how these findings relate to the established anti-inflammatory and anti-atherogenic effects of adiponectin, represents another potentially exciting area for future studies.

Role of GH in mediating the effects of calorie restriction
Reduction in food intake generally reduces GH release, and the impact of reduced GH levels on circulating IGF-1 under conditions of diminished energy intake is amplified by reduced responsiveness of the liver to GH signals. Reduced levels of GH and IGF-1 are believed to be one of the mechanisms by which CR delays aging and extends longevity. The impact of CR on the somatotropic axis is complex and time-dependent. In rats, short-term CR reduces GH levels whereas long-term CR maintains pulsatile GH release [51], presumably by postponing age-related alterations in the hypothalamic control of GH secretion. In humans, short-term CR has little or no effect on circulating GH and IGF-1 levels unless protein intake is substantially reduced [52]. In hypopituitary Ames dwarf mice, 30% CR initiated at approximately two months of age produces an additional increase in longevity, whereas the identical CR regimen has no effect on the longevity of GH-resistant GHRKO males and a very small effect on the longevity of GHRKO females [18,53]. These findings, together with the analysis of gene

expression in Ames dwarf, GHRKO and CR-exposed mice, indicate that GH and CR influence longevity by different but overlapping pathways.

Recent findings reopened the issue of the role of GH in mediating the effects of CR [54]. Based on studies in mice with brain-specific deletion of the *Sirt1* gene encoding one of mammalian sirtuins, this group suggested that CR-induced changes in *Sirt1* expression lead to suppression of the somatotropic axis, including reduced GH release, and that these hormonal changes play a key role in the shift from growth and reproduction to maintenance and repair. This shift is generally believed to represent activation of the evolutionarily conserved survival program and provides an attractive explanation for extended longevity under nutritionally challenging or adverse conditions.

Interaction of GH and nutrient availability was addressed in a recent study of domestic cattle. Comparison of the gene encoding GH in seven breeds of *Bos indicus* and *Bos taurus* revealed relatively low frequencies of the wild-type (*G1*) allele and a preponderance of a 'domestic' (*G2*) allele [55]. The frequency of the *G2* allele increased further under selection for post-weaning weight gain under *ad libitum* feeding conditions. Interestingly, under CR conditions, *G1* promoted better growth whereas *G2* was detrimental. These findings support a suggestion that domestication and selection of domestic, companion and laboratory animals lead to enrichment of genetic variants (primarily single nucleotide polymorphisms) that favor growth and reproduction under conditions of unrestricted or optimized feeding but could reduce the likelihood of thriving under more challenging conditions.

GH and human aging
In contrast to the major and well documented impact of GH resistance and GH deficiency on aging and longevity in mice, the impact of the corresponding endocrine syndromes on human longevity is unclear and certainly not major. Association of genetic defects in GH signaling with both a reduction and an increase in life expectancy have been reported [56,57], and there is evidence that some hypopituitary and GH-resistant individuals can reach very advanced, although not extraordinarily old age [57,58]. Within the past few years, multiple aging-related traits were examined in large cohorts of individuals with isolated GH deficiency or GH resistance who had not been given hormone replacement therapy. M.H. Aguiar-Oliveira, R. Salvatori and their colleagues reported that subjects with isolated GH deficiency due to *GHRHR* gene mutations were characterized by proportional dwarfism, delayed menarche, reduced parity, indications that menopause could be advanced, and obesity – which, unexpectedly, was associated with increased levels of adiponectin but no changes in the levels of leptin. These individuals were partially protected from atherosclerosis and exhibited no differences in various parameters used to assess the quality of life with the exception of reduced physical endurance. Although mortality of young women was increased, there were no differences from matched normal controls in the overall risk of death or lifespan of those that reached the age of 20 years [59,60].

The Laron dwarfism syndrome of GH resistance is associated with protection from cancer and preservation

of normal endothelial function [61,62]. Long-term follow-up of an unrelated cohort of 99 Ecuadorian individuals with mutations leading to GH receptor deficiency revealed the expected reduction in circulating IGF-1 levels, dwarfism and reduced insulin levels. Insulin sensitivity was enhanced despite increased incidence of obesity. Diabetes was absent and there was only one case of malignancy. Analysis of age distribution compared to the general population suggested increased early mortality and comparable rates, but not causes, of mortality afterwards. In particular, GH receptor-deficient individuals appeared to be less likely to die of stroke but more likely to die of accidents, alcohol-related events and perhaps also cardiac disease [63]. Drastic reduction or absence of GH signaling in humans therefore appears to have no major or consistent effect on life expectancy but is associated with protection from some age-related diseases.

Concluding remarks

Recent studies of the role of the somatotropic axis, and specifically GH, in the control of aging and longevity have added new evidence for the anti-aging and life-extending effects of GH resistance and GH deficiency in laboratory mice. These studies have also begun to identify the endocrine and molecular mechanisms involved in these effects and have provided new evidence linking extended longevity of GH-related mutants to enhancement of their stress resistance. Further studies of stress resistance and stress-induced damage in mice with global or organ-specific *Ghr* gene deletion should identify pathways related to the enhanced stress-resistance of cells that have not been exposed to GH signals. The recent report of apparently IGF-1-independent effects of muscle-specific GH resistance on body composition and metabolic characteristics probably related to aging and longevity [64] indicates that this approach should be very fruitful. Studies of GH-deficient and GH-resistant humans have provided evidence that some, although definitely not all, of the findings in mutant mice with similar endocrine syndromes apply to our own species.

In contrast to the effects of reduced GH signaling, expression of transgenes that increase GH levels markedly above the physiological range causes many changes resembling aging in mice and also in fish [65,66], and hypersecretion of GH by pituitary tumors in patients with acromegaly increases their risk of various age-related diseases and reduces life expectancy [67,68]. Perhaps the pathological features of acromegaly and gigantism could be interpreted as accelerated aging.

There is accumulating evidence for the anti-aging and protective effects of GH deficiency and resistance and the apparent 'pro-aging' effects of pathological GH excess. This raises an important question: how are variations in GH signaling within the normal (physiological) range related to aging and longevity? The likelihood that there is such a relationship in the human advises extreme caution in the widely promoted use of GH as an anti-aging agent.

Acknowledgments

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