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Role of the GH/IGF-1 axis in lifespan and healthspan: lessons from animal models

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Abstract

Animal models are fundamentally important in our quest to understand the genetic, epigenetic, and environmental factors that contribute to human aging. In comparison to humans, relatively short-lived mammals are useful models as they allow for rapid assessment of both genetic manipulation and environmental intervention as related to longevity. These models also allow for the study of clinically relevant pathologies as a function of aging. Data associated with more distant species offers additional insight and critical consideration of the basic physiological processes and molecular mechanisms that influence lifespan. Consistently, two interventions, caloric restriction and repression of the growth hormone (GH)/insulin like growth factor-1/insulin axis, have been shown to increase lifespan in both invertebrates and vertebrate animal model systems. Caloric restriction (CR) is a nutrition intervention that robustly extends lifespan whether it is started early or later in life. Likewise, genes involved in the GH/IGF-1 signaling pathways can lengthen lifespan in vertebrates and invertebrates, implying evolutionary conservation of the molecular mechanisms. Specifically, insulin and insulin-like growth factor 1 (IGF-1)-like signaling and its downstream intracellular signaling molecules have been shown to be associated with lifespan in fruit flies and nematodes. More recently, mammalian models with reduced growth hormone (GH) and/or IGF-1 signaling have also been shown to have extended lifespans as compared to control siblings. Importantly, this research has also shown that these genetic alterations can keep the animals healthy and disease-free for longer periods and can alleviate specific age-related pathologies similar to what is observed for CR individuals. Thus, these mutations may not only extend lifespan but may also improve healthspan, the general health and quality of life of an organism as it ages. In this review, we will provide an overview of how the manipulation of the GH/IGF-axis influences lifespan,

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highlight the invertebrate and vertebrate animal models with altered lifespan due to modifications to the GH/IGF-1 signaling cascade or homologous pathways, and discuss the basic phenotypic characteristics and healthspan of these models.

GH/IGF-1 axis and aging: the controversy continues

GH synthesis and secretion are well documented to decline with normal aging in all mammalian species studied to date [1–3]. Accordingly, as GH secretion declines there is a concomitant decrease in IGF-1 levels. This well established phenomenon is sometimes referred to as somatopause [4]. In humans as well as other species, a reduction in the GH/IGF-1 axis is correlated with increased percentage of total body and visceral fat, decreased muscle mass, decreased physical fitness, decreased immune function, and physiological declines in estrogen and androgen concentrations [5–7]. Thus, the natural declines in GH and IGF-1 that accompanies age-related degenerative processes implies that the GH/IGF-1 axis may be a causative determinant. To further support this notion, various GH dosing regimens in GH deficient adult populations have been documented to benefit body composition, circulating lipids, fitness and bone density [8–12]. Further, dwarfism that accompanies untreated GH deficiency was reported to significantly shorten median lifespan [13]. These data have led many to embrace the use of GH therapy as an anti-aging regimen.

There is no question that GH treatment is important for the health and well being of GH deficient individuals. However, there is not general agreement (nor sufficient data) regarding the physiological benefits of reestablishing ‘youthful’ GH levels to normal, aging individuals. Thus, a controversy arises over whether this decline could be considered overall beneficial or detrimental to aging and aging-related problems. After all, abnormally high levels of GH, as occurs with acromegaly, increase the incidence of morbidity and mortality in both rodent models and humans [14–17]. However, this level of GH excess does not divulge the impact of restoring more moderate levels of GH on disease progression or aging. While the benefits of GH administration are diverse and well documented, it is also clear that not all clinical outcomes of restoring GH levels are favorable, with even low doses reported to increase diabetes and glucose intolerance in healthy adults [11,18] and to increase mortality in patients that are critically ill [19]. To resolve the debate, one might assume that lifespan data would be the best gauge to appraise the role of this axis on aging. Overwhelmingly, the evidence suggests that a reduction in GH/IGF-1 signaling in vertebrates or its homologous pathways in invertebrates extends lifespan as compared to control or normal siblings. Some data in humans also supports increased longevity with a reduction in this axis [20,21]. These two disparate concepts, namely GH and IGF-I ameliorate functional declines of aging while limiting lifespan, are at the center of the current controversy. Thus, GH, despite having some positive effects, is unlikely to be the answer to our society’s aging woes.

Invertebrate models relevant to GH/IGF-1 axis

The short lifespans of invertebrate models make them powerful systems to establish molecular pathways that may influence longevity. Mutants in both *Caenorhabditis elegans* and *Drosophila melanogastor* have revealed repeatedly that a reduction in intracellular signaling pathways homologous or similar to that induced by insulin or IGF-1 signaling (IIS) increases longevity [22–25]. A comparison of IIS pathways in these species as compared to mammals is offered in Figure 1.

A thorough discussion of the IIS genes that are implicated in longevity were comprehensively and recently reviewed [26–28]. Briefly, in *C. elegans*, genes (and corresponding protein/function in parentheses) involved in IIS include *daf-2* (DAF-2 or insulin/IGF-1-like receptor), *age-1* (AGE-1 encoding the catalytic subunit of phosphatidylinositol-3 kinase PI3K), *pdk-1*,

akt-1, akt-2, sgk-1 (serine-threonine kinases PDK-1, SGK-1, Akt1 and Akt-2), as well as *daf-16* (forkhead transcription factor and the major target of insulin-like signaling in *C. elegans*) and *daf-18* (PTEN, a phosphatase to dephosphorylate phosphatidylinositol (3,4,5)-trisphosphate resulting in a dephosphorylation activity important in inhibition of the AKT signaling pathway). Recently, SKN-1, having distinct but some overlapping functions as the FOXO-like protein DAF-16, was shown to be a second transcription factor inhibited directly by IIS and to influence longevity [29]. Loss of function mutations in *daf-2, age-1, pdk-1, akt 1,2* and *sgk-1* result in increased lifespan while those in *daf-16*, *daf-18* and *skn-1* reduce lifespan. Likewise, in Drosophila, InR (insulin-like receptor), insulin receptor substrate (Chico), PI3K (Dp110 is the catalytic subunit and Dp60 is the regulatory subunit of PI 3-kinase), PTEN, Akt/PKB, and FOXO are components of IIS and particular mutations in many of these genes alter lifespan, albeit sometimes in a tissue specific manner.

Since both invertebrate pathways point to role of IIS in longevity and because of the marked evolutionary conservation of this pathway, it is intriguing to speculate that the pathways have similar influence on longevity in vertebrate species. However, it is important to appreciate that the connection between IIS and longevity in vertebrates is much more complex, having functionally distinct insulin and IGF molecules, multiple receptors and downstream signaling molecules with distinct tissue specific expression profiles, as well as the added dimension of GH expression. Thus, elucidating the similar yet distinct biological activities of GH versus IGF-1 and insulin is of paramount importance. Also, establishing the precise roles of particular intracellular signaling proteins, protein modifications, and tissue and cell specificity of the response to GH, IGF-1, and insulin remains a challenge and a source of intense study.

Mouse models with altered activity in the GH/IGF-1 axis and with increased longevity

Several mouse models with reduced GH and/or IGF-1 signaling have been shown to have extended lifespan as compared to control siblings. Evaluation of these mouse models and new models relevant to this axis, as well as mice with altered GH signaling that do not show increased lifespan, has offered some clues as to factors that contribute to aging. For example, it is clear that the role of the GH/IGF-1 axis overlaps partially, but is distinct from, the effect of CR, a well-recognized means to delay aging [30]. Although the mechanisms linking the GH/IGF-1 axis with delayed aging remain to be determined, there are some commonalities among the long-lived mice, such as reduced IGF-1 signaling, enhanced insulin sensitivity, improved stress resistance, and protection from carcinogenesis, which likely contribute to the improvement in longevity. A comparison of several common traits of long lived mouse models are provided in Table 1. Future studies comparing these mouse models will undoubtedly provide valuable insight into additional factors that contribute to the extension of lifespan.

Mice with defects in anterior pituitary function - Snell and Ames Dwarf Mice

Snell mice, as well as dwarf mice from a spontaneous mutation in the same gene generated at Jackson labs, are homozygous for a defect in the Pit-1 (pituitary specific transcription factor 1) gene [31]. The Ames dwarf mouse, so named because it was first reported by researchers at Iowa State University [32], has a recessive point mutation in the PROP-1 (prophet of Pit1 or paired-like homeodomain transcription factor in the Prop-1) gene that encodes a transcription factor that is required for Pit-1 activation [33]. As Pit 1 and Prop-1 proteins are required for differentiation of hormone-specific cell types in the anterior pituitary, both types of homozygous mutant mice lack GH, prolactin, and thyroid stimulating hormone producing cells resulting in severe endocrine deficiency of these three hormones. Likewise, the Ames and Snell mice have similar phenotypes (Table 1). Snell and Ames mice are dwarf and characterized by female sterility and severely reduced circulating levels of insulin, IGF-1, glucose, and

thyroid hormones (as reviewed, [34]). Both of these strains of mice are also remarkably long lived and exhibit a greater than 40% increase in longevity. For Ames dwarf, females were reported to have an impressive 68% increase in lifespan (1206 ± 32 days vs. 718 ± 45 days) while males showed a 49% increase in mean lifespan (1076 ± 56 days vs. 723 ± 54 days) [35]. This extension in lifespan for Ames dwarf mice has been consistently reported, varying from 35–69% depending on diet [36–38]. For Snell dwarf mice, the extension in lifespan is less consistent and seems to vary with genetic background, housing and breeding practices of the mice. For Snell dwarfs, one report showed an increased lifespan of 42% with no significant gender difference (1,178 ± 235 days vs. 832 ± 158 days for controls) [39] and another showed a 50% increase in only female mice [40].

Mice with defects in specifically GH function - Lit/Lit and GH Receptor gene disrupted mice (GHR −/−)

Lit/Lit mice have a missense mutation in the extracellular domain of the GH releasing hormone (RH) receptor [41]. As GHRH receptor is specifically targeted, the *lit/lit* mice are suppressed in GH production but have no apparent defect in prolactin or thyroid stimulating hormone as with the Snell and Ames dwarf mice. Lit/Lit mice share some features of Snell and Ames dwarf mice as they are also dwarf (50–75% smaller than wild-type mice), have reduced serum IGF-1 levels and marked increase in adiposity [42,43]. In a C57BL/6J background, lit/lit mice are longer lived than heterozygous (+/−) controls with a 23% increase in males (1093 ± 186 days versus 886 ± 148 days) and a 25% increase in females (1070 ± 127 days versus 857 ±169 days) [39]. Mice with complete removal of GH action by disrupting or knocking out the GHR/binding protein (BP) gene (termed GHR−/−) were generated in our laboratory nearly a decade ago [44]. These GHR−/− are dwarf, GH resistant or insensitive and are characterized by elevated levels of GH yet markedly reduced levels of IGF-1 [44,45]. Other notable features of these mice include delayed puberty [46,47], decreased fasting insulin and glucose levels [45,48], and diminished pancreatic islet size [48]. The GHR−/− mice also are protected from both diabetes-induced nephropathy [49] and prostate and mammary carcinogenesis [50,51]. While longevity is increased in GHR−/− mice, the increase again varies according to background strain. In a mixed genetic background, GHR−/− mice had an increase in mean lifespan of 55% in males to 38% in females [52]. When GHR−/− mice were backcrossed into the C57BL/6J strain, lifespan was still significantly increased but to a lesser degree with an average increase of 26% and 16% in males and females, respectively [45]. As with the lit/lit mouse, the longevity data in GHR−/− mice implicate the GH/IGF axis and not other pituitary hormones with improving lifespan. Notably, one GHR−/− mouse from a colony managed by Dr. Andrzej Bartke lived 1,819 days and holds the ‘longevity record for laboratory mice’[53].

Mice with defects in IGF-1 or IGF-1 receptor

One common feature of the mouse models with increased longevity mentioned thus far is a very low level of circulating insulin-like growth factor-1 (IGF-1). Likewise, studies conducted on heterogeneous wild-caught mice, wild-caught mice crossed with common inbred strains of mice, and mice selectively bred for small body size all point to a role of reduced IGF-1 levels on extending longevity [54,55]. So what are the aging profiles in genetically homogeneous mice that lack either IGF-1 or its receptor? Regarding targeted genetic modification, mice with a gene disruption in either IGF-1 or the IGF-1 receptor unfortunately die at birth or shortly after, hampering the ability to study their role on longevity [56]. However, mice heterozygous for the IGF-1 receptor gene disruption (IGF1R +/−), which have a 50% reduction in receptor levels, do show a 33% increase in lifespan for females, while male mice show no statistically significant increase in longevity [57]. Interestingly, the female mice have an increased lifespan and are not dwarf, unlike the previous models discussed, suggesting that dwarfism is not a requirement for the extended lifespan. Two other mouse models to be discussed ($p66^{shc}$ –/–

mice and KLOTHO transgenic) also shed doubt on the requirement of smaller body size for increased longevity.

Pregnancy-associated plasma protein-A (PAPP-A) is a secreted metalloproteinase that has been reported to degrade inhibitory insulin-like growth factor binding proteins (IGFBPs). Thus, PAPP-A increases IGF-I availability in vascular tissues without altering IGF-1 expression (as reviewed in [58]). PAPP-A KO mice, which presumably have reduced localized IGF-1 bioavailability yet normal circulating levels of IGF-1, were recently shown to have a striking 33% and 41% increase in lifespan for males and females, respectively (Conover, Aging Cell 2007). Interestingly, this extension was not associated with any significant alteration in circulating levels of insulin, glucose, GH, IGF-1 or cholesterol or in level of dietary intake. Thus, these mice are living longer without altered insulin sensitivity and without voluntary calorie reduction. Similar to other mouse models with increased longevity and reduction in GH/IGF axis, these mice displayed a marked reduction in the incidence of spontaneous tumors [59] and vascular disease [60], hinting to the importance of this protein and the GH/IGF-1 system in development of disease pathologies. One potential advantage of PAPP-A KO mice is that they may offer a means to separate the effects of GH directly from those specifically of IGF-I.

Mice with defects in downstream IGF-1 Signaling Molecules

p66^{shc}—Shc proteins mediate growth factor mitogenic actions by activation of the MAPK pathway. Ligand binding and subsequent activation of IGF1R, as well as other growth factor receptors, results in recruitment of p66^{shc}, a specific protein isoform of the proto-oncogene *shc* locus that functions in the intracellular pathways converting oxidative signals into apoptosis [61], to the receptors where it is phosphorylated and activated. The p66 isoform has recently been suggested to regulate mitochondrial oxidative capacity with its absence increasing the reliance on glycolysis as opposed to oxidative metabolism [62]. Mice lacking this IGF-1 receptor substrate, p66^{shc} −/−, live 30% longer than their normal littermates and have enhanced resistance to oxidative stress [63]. The p66^{shc} −/− mice also are protected from disease progression as they have been shown to have reduced incidences of age-related endothelial dysfunction [64], angiotensin II-induced myocardial damage [65], some behavioral aspects of aging [66], and diabetic glomerulopathy [67], all presumably by reducing oxidative stress. Of note, p66^{shc} −/− mice also confirm that smaller body size is not required for increased longevity..

KLOTHO—The Klotho protein is expressed principally in the distal tubules of the kidney and the choroid plexus in the brain [68,69]. Klotho is a transmembrane protein with homology to B-glycosidases, but evidence also suggests that a fragment of Klotho is found in circulation, implying it may also act as a hormone. In relationship to the GH/IGF-1 axis, the Klotho protein is hypothesized to bind and repress intracellular signals of insulin and IGF-1 [70]. Mice homozygous for a mutation in *Klotho* exhibited growth retardation, osteoporosis, arteriosclerosis, CNS neuronal alterations and atrophy of the skin, implying accelerated aging [70–72]. Not surprisingly, this gene disruption also results in premature death [69]. In contrast, over expression of Klotho in mice extends lifespan with mice carrying the transgene outliving wild-type controls by 20–31% in males and by 19% in females [70]. In contrast to previously discussed insulin sensitive mouse models with increased lifespan, the Klotho transgenic mice are hyperinsulinemic and insulin resistant. Further, there is evidence that a homologue of Klotho declines in association with human aging [73]. It is important to point out that Klotho has other proposed functions, such as altering Wnt induced intracellular signaling and altering the activity of multiple ion channels. These activities also need to be considered when evaluating the role of Klotho on aging.

IRS-1 and IRS-2—Insulin receptor substrate (IRS) proteins are major substrates of both insulin receptor and IGF receptor kinases and serve as docking proteins between these receptors and a complex network of intracellular signaling pathways. In mammals, there are at least 4 distinct proteins, IRS-1 through IRS-4 with distinct tissue distributions and differences in the signaling capacity [74]. IRS-1 and IRS-2 display differential sensitivities and are more widely expressed than the other IRS proteins. Selman et al. [75] performed longevity studies in homozygous (IRS-1 −/−) and heterozygous (IRS1 +/−) mice as compared to wild-type controls. Female IRS-1 −/− mice displayed a statistically significant increase in longevity (32%) relative to controls. No significant increase in lifespan was noted for males or for heterozygotes of either gender. Features of the longer lived IRS-1 −/− female mice included reduced body mass, normal circulating GH and IGF-1 levels, and some resistance to age-sensitive markers such as motor/neurological function, immune function, bone density and idiopathic dermatitis [75]. Also, these gene disrupted mice had higher circulating insulin and mildly impaired glucose tolerance starting in early adulthood when compared to controls, suggesting that improved insulin sensitivity was not responsible for the enhanced longevity in these mice.

Two separate laboratories have studied the impact of IRS-2 on mouse lifespan, with some inconsistencies in their findings. IRS-2 gene disrupted (IRS-2 −/−) mice have dramatically shortened lifespan, with the reduction more striking in male versus female mice [75]. These mice develop a severe diabetic phenotype due to pancreatic β cell failure [76]. The lifespan of heterozygotes is at the center of the discrepant findings. Selman et al. [75] reported no change in lifespan for heterozygotes (IRS-2+/−) of either gender as compared to control mice. In contrast, Taguchi et al [77] reported that IRS-2+/− mice (presenting data combined for both sexes) live 17% longer than littermate controls. Although IRS 2+/− mice were previously reported to display a relatively normal metabolic phenotype when young [76], Taguchi et al. [77]reported that the mice have slightly increased insulin sensitivity, increased body weight, and no change in food consumption in older IRS-2 +/− mice as compared to wild-type controls. Taguchi et al. [77] also produced mice with conditional disruption of the IRS-2 gene in the brain (bIRS-2 −/−), that resulted in an 18% extension in lifespan, implicating that reduced IRS-2 signaling specifically in the brain was likely to be responsible for the observed extension in lifespan. However, older bIRS-2−/− mice were overweight, hyperinsulinemic, and glucose intolerant when compared with control mice. Also, bIRS-2 −/− mice were more active and displayed greater glucose oxidation during meals, and they displayed stable superoxide dismutase-2 concentrations in the hypothalamus.

Mice with a repression in GH/IGF-1 axis but with no change in longevity

Not all mouse models with reduced GH or IGF-1 levels exhibit improvements in longevity. Mice that express a transgene for a growth hormone antagonist (GHA) represent one such example [78,79]. GHA mice express a molecule that competes with endogenous GH for GHR binding and results in marked reduction of GH-induced intracellular signaling [80] and, as a result, these mice are dwarf and have reduced levels of IGF-1 [78,79]. Unlike the models previously discussed with a reduction in GH/IGF-1 signaling, the GHA transgenic mice are reported to have no extension in lifespan as compared to littermate controls [45]. This model offers an interesting exception to the models previously mentioned. That is, GHA mice are dwarf, have a partial inhibition of GH action and concomitant decrease in IGF-1, but do not exhibit increased longevity. However, there are several key phenotypic differences besides lifespan between GHA and GHR−/− mice. While they have circulating levels of IGF-1 that are significantly lower than control littermates, the reduction is much less pronounced in GHA mice than other models such as the GHR−/− mice (80% decrease in GHR−/− while GHA have only a 20–25% decrease) [45]. Other striking differences between GHR−/− and GHA mice are the weight gain profile and degree of insulin sensitivity. GHA transgenic mice, although dwarf in early months, eventually gain weight that approaches that of control littermates and do not

exhibit extreme insulin sensitivity, whereas GHR−/− remain substantially dwarf and notably insulin sensitive even in older mice [45].

Mice with excess in GH/IGF-1 signaling and longevity

Further confirmation that the GH/IGF-1 axis is an essential contributor to lifespan is offered by animal models with an excess in GH and IGF-1 signaling. Mice that express a GH transgene have been generated by multiple independent laboratories. These mice have fairly uniform phenotype regardless of genetic background or species of GH used. That is, GH transgenic mice are larger in size, have reduced adiposity, advanced puberty, elevated IGF-1 and insulin levels and are insulin resistant (as reviewed, [14]). Importantly, lifespan of these GH transgenic mice is drastically reduced (~50%) compared to non-transgenic controls [34,81–83]. In mice, an interesting exception to this phenomenon comes from a recent study that reported overexpression of IGF-I specifically in the heart increased longevity by about 20% and improved several measures of cardiomyocyte health [84]. It is worth noting that cancer tends to be the most common cause of death (70–85% of all deaths) in this and many strains of commonly used laboratory mice, which may have contributed to this discordant finding [85].

Other mammalian models

While the majority of lifespan data in vertebrates has been generated from mice, they are not the only animal models available to assess the impact of the GH/IGF-1 axis on aging. For example, livestock are commonly treated with exogenous growth hormone and other animals, such as rabbits [86] and pigs [87], have been genetically manipulated to express a GH transgene, resulting in acromegalic-like animals. Although these other models share many metabolic features with GH transgenic mice and GH treated or acromegalic humans, information about aging in these animals is not available and more difficult to attain because of their longer lifespans. For this review, we will limit the discussion of lifespan data available for other animal models rodents and dogs.

Other Rodents

Mice have been the model of choice for the study of longevity in part because of their smaller size, shorter lifespans, and ease of genetic manipulation. However, several studies have focused on the use of other rodent models. Data from rats have not been as clear cut or as systemic in the evaluation of the GH/IGF-1 axis on aging although some data does support that repression of this axis may be beneficial for aging. For example, a heterozygous rat model expressing an antisense GH gene shows a modest improvement in lifespan (7–10%). Interestingly, this improvement was not found for rats homozygous for the transgene, as these rats were reported to have lifespans 5 to 10% shorter than normal controls. The homozygous mice were reported to die prematurely from neoplastic diseases and to have immune system alterations as compared to control rats [88]. The *dw/dw* rats (rats with profound GH deficiency due to an unknown genetic defect that leads to a 95% reduction in pituitary GH [89]) do not have altered lifespan [90]. However, Sonntag et al. [90], using these rats, developed a model with a specific and limited deficiency of GH in adulthood. This was done by treating *dw/dw* rats with recombinant GH from 4–14 weeks of age and then stopping treatment. These so called adult-onset GH deficient rats did show an increased maximal lifespan by 12%. The adult-onset GH deficient rats also showed improvement in cognitive and cardiac function [91,92]. These results suggest that a specific and limited deficiency of GH and IGF-I may be sufficient to increase lifespan. Rodent models with natural exceptional longevity may also prove useful to provide unique insights not readily available from short-lived models. One such long-living small mammal that has been suggested as useful is the naked mole-rat, which has the longest reported lifespan of any rodent (~28.7 years) [93]. Research tools to study the hormonal milieu of these

exceptionally long-lived rodents are not currently available hampering their current use but providing a potential avenue of future exploration.

Dogs

While the domestic dog descended from the gray wolf over 15,000 years ago, it is only within the last 300 or 400 years that the enormous diversification of breeds has occurred. This diversification is largely due to human pressures as opposed to natural selection, leading to unparalleled variations in body mass, metabolism and lifespan within a single species. Mass, for example, can vary from 1.4 kg in the Chihuahua to over 100 kg in the St Bernard. Lifespan also varies tremendously with some studies reporting 8 to >15 years for pet dogs. This genetic diversity poses a unique opportunity to evaluate genes that contribute to lifespan. Repeatedly, lifespan in dogs has been shown to be inversely related to body size [94–96]. Although a cause and effect relationship has not been established, several lines of evidence support a role for the GH/IGF-1 axis. For example, Favier et al. [97] demonstrated that differences in adult body size of medium-sized and giant dog breeds are preceded by differences in GH release although they did not find differences in circulating IGF-I or IGF-II concentrations. Other studies have reported strong positive correlations between body weight of dogs and plasma IGF-1 concentrations [98,99]. More recently, Sutter et al. [100] reported that a single IGF-1 haplotype is a major contributor to size variation in dogs. Although not specifically addressed and more difficult to assess in these longer lived mammals, it is tempting to speculate that this pathway may likewise contribute to aging in dogs.

Healthspan of these animal models

Thus far in this review, lifespan data have been used as the main criterion to evaluate factors that influence the aging process. However, longevity, or the average length of time an organism survives, is not giving adequate attention to the quality of that organism's existence. The concept of healthspan implies 'successful aging' and although no one single definition exists, the term usually refers to life without disability and free of disease. Most long-lived animals would be expected to have some improvement in healthspan, which ultimately enabled them to achieve the longer lifespan. So how do the aforementioned models measure up in terms of healthspan? As the concept of healthspan is quite broad, we will provide a focus on areas that have been most heavily studied. As the mouse models mentioned previously have been subjected to thorough characterization, several common traits emerge as potential mechanisms, including improved insulin sensitivity, improved oxidative stress resistance, reduced incidence of cancer and renal disease, and improved cognitive functioning. On the other hand, musculoskeletal changes, at least in some respects, may be less favorable for healthspan.

Diabetes and insulin sensitivity

Insulin resistance is well documented to be a risk factor for a number of major diseases such as cardiovascular disease and type 2 diabetes mellitus. Likewise, plasma levels of insulin are inversely correlated with survival in humans [101] and insulin sensitivity [20]. Therefore, it is reasonable to hypothesize that a reduction in insulin release and improved insulin sensitivity could be, in part, responsible for increased longevity and improve healthspan by preventing type 2 diabetes and its associated maladies. In 1936, Houssay showed that an "excess of anterior pituitary lobe aggravates the diabetes or produces it in normal animals" and found that the anterior pituitary lobe extract increases the resistance to insulin in normal and hypophysectomized dogs [102]. Thus, the first correlation between a pituitary 'factor' inducing insulin resistance was presented. Since then, numerous studies have shown that GH possesses anti-insulin action and is a diabetogenic molecule [103–110]. Therefore, GH would be suspected to generate insulin resistance in tissues, such as adipose, muscle and liver, which respond to both GH and insulin. Indeed, liver [111,112], adipose tissue [113–117] and muscle

[115,117–120] have been shown to be impacted by GH to induce insulin resistance. The molecular mechanisms responsible for GH induced insulin resistance is not completely known; however, recent data suggest that GH up regulates the regulatory subunit of PI3 kinase which then down regulates insulin signaling [118,121]. Indeed, several of the long-lived mice, such as GHR−/− mice, have reduced plasma insulin levels and improved insulin sensitivity [48, 122]. Since CR GHR−/− mice do not show a further enhancement in longevity, it may suggest that the extreme insulin sensitivity found in these mice may be at an ultimate level for improved lifespan. However other long-lived models do not exhibit improvements in insulin sensitivity. For example, both Klotho transgenic and IRS-1−/− mice exhibit increased insulin resistance despite an improvement in longevity [70,75]. These 'outliers' still share a reduction in the signaling downstream of IGF-1 and insulin receptors, albeit by different mechanisms. Given the established cross-talk between the GH/IGF-1 axis and insulin signaling as well as the diabetogenic nature of GH, reduced insulin signaling remains a viable mechanism for the increase in longevity observed with the animals described here.

Tissue-specific gene disruption in mice has also implicated several tissues as key mediators of the extended longevity or altered insulin sensitivity. For example, the fat-specific insulin receptor gene-disrupted (FIRKO) mouse initially displays a normal growth curve, but at 3 months shows a 15–25% decrease in body weight and 50–75% lower fat mass. These lean mice are resistant to the age-related decline in glucose tolerance seen in their non-transgenic littermates and have increased median and maximal lifespan [123]. Finally, the IGF-I gene has been conditionally disrupted in the liver of mice [124]. These mice (termed LID) have ~75% reduction in circulating IGF-I, elevated GH, and a fourfold increase in serum insulin with normal glucose levels. The LID mice do not show increased longevity (Ikeno, personal communication). Interestingly, the insulin insensitivity observed in these mice appears to be mainly at the level of muscle, and treatment with either recombinant human IGF-I or GH-releasing hormone antagonist, which reduces circulating GH levels, increases insulin sensitivity [119]. Also, when LID mice are crossed with GHA mice, insulin resistance disappears [112]. Thus, the insulin resistance seen in the LID mice is due the increased levels of GH.

Resistance to stress

Stress resistance would presumably improve healthspan and contribute to longevity. In invertebrates, reduction in IIS results in resistance to multiple forms of stressors, including oxidants, heat and heavy metals (for recent reviews, see [125–128]. Can stress resistance also be observed in vertebrate models of improved longevity? In addition to other stressors, reactive oxygen species (ROS) and free radicals are involved in a variety of physiological and pathological processes including degenerative diseases. Accumulation of oxidative damage has been implicated in aging and occurs due, in part, to an imbalance between generation rates of ROS and the activity and amount of antioxidant defense systems. There is evidence that several of the mouse models with reduced activity in the GH/IGF-1 axis have improved resistance to oxidative stressors either by improved antioxidant defense or through reduced production of ROS. The $p66^{shc}$ −/− mice display increased resistance to the oxidative stress induced by paraquat, which generates superoxide anions upon cellular intake, and are characterized by a decreased incidence of aging-associated diseases by reportedly reducing oxidative stress [64–67]. Other models also suggest increased tolerance towards oxidative stress in mouse models with reduced activity of the GH/IGF-1 axis. For example, Ames dwarf mice have reduced lipid, protein, and DNA oxidation in brain in comparison to normal siblings [129,130] and increased antioxidant defense in skeletal muscle [131]. Likewise, fibroblasts derived from Snell dwarfs are resistant to a variety of cellular stressors, including UV light, heat, heavy metal exposure and paraquat [132]. Finally, IGF1R+/− mice challenged with paraquat treatment live longer than IGF1R+/+ mice [133]. Data for resistance to oxidative

stress is less consistent for GHR−/− mice with these mice showing increased, decreased or no change in oxidative measures [134–136]. Some of the differences observed among the different models not only relate to the tissue/cell type assayed and the type of stressor, but also to the age of the animal. For example, the stress resistance of fibroblasts derived from Snell dwarf mice is dependent on age of the mouse from which the tissue was harvested [137]. Stress resistance has also been observed in long-lived mice with klotho overexpression [138]. However, it should also be noted that the stress response itself has been shown to trigger a suppression of the GH/IGF-1 axis; this suppression has been suggested to be a potential adaptation to shift energy use from growth/proliferation to self- protection from further damage by stressors [139]. Regardless, decreased vulnerability to a variety of stressors is a common theme among tissues and cell types of the long-lived mouse strains and still remains a likely mechanism by which these animals display extended longevity.

Cancer incidence

Available data support that GH/IGF-1 status may influence neoplastic tissue growth; however, this is still a very controversial area. For example, IGF-1 exerts potent effects on key stages of cancer development, having been implicated in many types of cancer (as reviewed, [140–143]); thus, inhibiting IGF-1 signaling has been suggested as an anticancer therapy [144]. On the other hand, the apoptotic influence of IGF-1 on cancer cells has others suggesting IGF-1 a potential target for anticancer therapy [145]. A debate also looms regarding GH therapy and its potential to increase cancer risk as a cancer initiation factor [146]. Animal models with a reduction in the GH/IGF-1 axis and with altered longevity would provide valuable insight into this debate, especially since mice have high mortality rate due to cancers [85]. Indeed, GHR −/− mice have protection from prostate carcinogenesis [51] and estrogen-independent mammary carcinogenesis [50]. Likewise, Ames dwarf mice are reported to have delayed occurrence of total neoplastic lesions and reduced incidence of adenocarcinoma in lung [37]. PAPP-A −/− mice also exhibit a pronounced reduction in cancer incidence. This reduction in cancer incidence is also shared with CR rodents [147] and rat models with reduced GH/IGF signaling[148,149]. Notably, GH treatment in spontaneous dwarf rats, which are protected from mammary carcinogenesis, can be made vulnerable by restoring GH levels, strongly implicating GH and/or IGF-1 in the development of neoplastic tissue and its growth[149]. GHA mice also share some reduction in mammary cancer incidence [150] yet do not have an increase in longevity, indicating that the reduction in cancer incidence may not be the sole factor important in influencing longevity. Recently, humans with GHD or Laron Syndrome have been reported to have lower incidences of cancer [151]. Finally, GH has been reported to be an autocrine cancer causing molecule [152]. Thus, the GH/IGF-1 axis may contribute to cancer propagation or progression which would clearly and negatively impact healthy aging.

Renal Health

Kidney function alters with advancing age due to, or even in the absence of, overt pathology. Many of the mouse models with enhanced longevity also appear to have protection against natural or experimentally-induced forms of kidney damage, which likely improves healthspan. For example, $p66^{shc}$−/− [67] and GHR−/− [49] mice are protected from diabetes-induced kidney damage. Likewise, mice transgenic for bovine growth hormone develop glomerular lesions characterized by a disproportionate increase in glomerular volume, mesangial sclerosis as well as an increase in glomerular lipid accumulation [153,154]. However, some protection to kidney damage is also offered to GHA mice, which do not reap the benefits in lifespan, suggesting that protection from kidney damage is not sufficient to improve lifespan in this species [155]. Klotho is expressed predominantly in the distal renal tubules of the kidney and, thus, one might expect an important function of this protein in renal health. While little data has been published related to kidney function in the long-lived Klotho transgenic mice, kidney pathologies have been noted for the kidneys of Klotho −/−, which exhibit accelerated aging.

These mice have displacement of cotransporters, which function at the proximal renal tubules in reabsorption of phosphate ions, implicating Klotho in calcium homeostasis [138]. Moreover, klotho −/− mice have elevated expression of plasminogen-activator 1 and an increase in renal glomerular fibrin deposition [156]. Accordingly, Klotho expression in humans is greatly suppressed in the kidneys of patients with chronic renal failure [157]. While the molecular mechanisms are not fully resolved, the recent appreciation that Klotho protein binds to fibroblast growth factor receptors and functions as a regulator of fibroblast growth factor 23 (FGF23) signaling will undoubtedly reveal its critical role in the kidney for calcium/phosphate homeostasis (as reviewed, [158]).

Cognitive Function

In humans, aging is associated with a wide spectrum of cognitive ability, ranging from intact cognitive function to clinically manifested dementia that impacts quality of life. Brain aging is typically characterized by changes in cognition function, brain volume, and neuronal function with a loss of synaptic contacts and neuronal apoptosis being common. Neural redundancy and plasticity of brain networks, promoted by mental and physical challenges, promote maintenance of brain activity. GH and IGF-1 are critical players in many aspects of central nervous system development, maintenance and plasticity. How then do the long-lived mouse models compare on basic assessments of cognitive function? Ames dwarf and GHR−/− mice appear to have an apparent delay in mental aging and improvement in some measures of cognitive function [159,160]. This apparent discrepancy may be explained by local production of IGF-1 and subsequent PI3/AKT activation in the hippocampus, reported for Ames dwarf mice [161], that may compensate for low circulating levels of these hormones. Further, hippocampal production of IGF-1 activates an anti-apoptosis signal transduction pathway through PI3/AKT, which contributes to the survival of new neurons [162]. In contrast, excess GH signaling, as with mice expression a GH transgene, exhibit enhanced cognitive function in early life, but earlier-onset of age related declines in cognitive function [163]. The demonstration that brain specific deletion of IRS2−/− influences longevity, also points to the central role of both the brain and insulin-like signaling in lifespan [77]. Much more information will be forthcoming in this area which is a fertile area of research.

Muscloskeletal Health

While there appear to be many benefits to healthspan in decreasing GH/IGF-1 axis, one might expect to find musculoskeletal health to be compromised. For muscle, aging is associated with a decline in skeletal muscle mass, sometimes referred to as “sarcopenia of old age.” There are several underlying mechanisms that have been implicated in contributing to this age-related muscle wasting. In particular, decreased protein synthesis, reduced enzymatic activity (especially in glycolytic and glycogenolytic pathways), a reduction in energy reserves, increased oxidative damage, and changes in ion content likely contribute (reviewed in [164]). GH and IGF-1 have a significant anabolic effect on skeletal muscle and so their decline with aging likely contributes to the decline in muscle mass. For example, GH, in tandem with IGF-I, promotes mitosis, protein synthesis, satellite cell proliferation and nerve sprouting, while preventing apoptosis [165,166]. GHR−/− mice have been reported to have reduced muscle mass [167] and while this decline may influence the lifespan of free-living animals, there is no apparent issue with animals maintained in controlled conditions. There is also evidence that GH influences muscle fiber type [168–170]. Using GHR−/− mice, the Pende laboratory was able to demonstrate that GH action is needed to increase skeletal muscle size by increasing myofiber size but not myofiber number [171]. Further, GH promotes fusion of myoblasts with nascent myotubes leading to increased myonuclear number, and cell fusion independent of IGF-1 [171]. On the other hand, the GH regulates substrate metabolism in muscle by antagonizing insulin-stimulated glucose disposal and by increasing lipolysis. Insulin also has a profound impact on muscle, as skeletal muscle is the principle tissue responsible for insulin-

stimulated glucose disposal. Thus, muscle represents a major site responsible for insulin resistance. Recently, GH has been found to affect intracellular insulin signal transduction in muscle [117] and has been reported to induce an increase in the regulatory subunit of PI3 kinase [172]. PI3K has been found to be a potent negative regulator of skeletal muscle insulin signaling that ultimately results in insulin resistance [118,173]. This, coupled with the data of Rabinowitz [115] in which GH induced insulin resistance in the human forearm, reveals the importance of GH action in muscle.

Skeletal health may also be compromised in vertebrates with reduced GH/IGF-1 signaling. Like muscle, normal aging is associated with both quantitative and qualitative changes in bone including alterations in bone architecture, in mineralization, protein content and the accumulations of microfractures. GH and IGF-1 have major effects on growth plate chondrocytes and all bone cells (as reviewed, [174]) The separate effects of GH and IGF-1 on bone growth were established years ago [175,176]. GHR−/− mice and Ames dwarfs were reported to have reduced bone mineral density, bone mineral content, and bone area, but these factors did improve with advancing age, suggesting these dwarf mice have an attenuation of the age-related increase in bone mineral density [177,178]. GHR−/− mice were also shown to have no alteration on trabecular bone modeling [167]. Longer-lived female IRS-1−/− have increased bone volume, trabecular numbers and reduced trabecular segregation despite reports of having osteopenia and low bone turnover [75,179]. PAPP-A−/− mice also exhibit skeletal insufficiency in mass, density, architecture and strength [180]. Klotho, with its role in phosphate calcium homoestasis is clearly important in skeletal health, as its absence results in severe osteoporosis [69], yet it is unclear if the enhancement of lifespan in Klotho transgenic mice comes along with major improvements in bone health. That is, in an inducible Klotho expression system using Klotho −/− mice, researchers were able to alleviate many of the aging-related phenotypes except no improvement in osteopenic changes were observed [181]. Comparison of bone health in these long-lived models, both in early and later life, is warranted to better understand the long-term impact of repressed GH/IGF-1 signaling on bone health.

Reduction in GH/IGF-1 axis is distinct from caloric restriction—CR is recognized as one of the most successful means to delay aging. Interestingly, many phenotypic characteristics of animals subjected to CR are shared by many, but not all, of the animals with repression in the activity of the GH/IGF-1 axis. That is, CR results in reduced body weight, reduced GH and IGF-1 levels, decreased plasma levels of insulin and glucose, reduced fertility, and delayed puberty. Based on these similarities, it is feasible that CR and reductions in the GH/IGF-1 axis may increase lifespan through similar processes. It is important to note that voluntary food restriction in vertebrate models is unlikely to contribute to their increased lifespan. That is, several long-lived mouse models (GHR−/−, Ames dwarf and IRS-1−/− mice) eat more calories than littermate controls when food consumption is normalized to body weight [45,75,182] or eat the same level of calories (PAPP-A−/−, IGF1R+/−) as littermate controls [57,59].

Several studies have attempted to distinguish whether the mechanisms by which CR and GH/IGF-1 lead to increased longevity are distinct or overlapping by using CR on dwarf mouse models. When long lived Ames dwarf mice were subjected to CR, both mean and maximal lifespan were increased as compared to CR in non-dwarf controls or Ames dwarf mice fed ad libidum [36]. Since CR further increased lifespan in this dwarf model, it was suggested that distinct mechanisms were involved. Moreover, specific genes associated with insulin signaling were reported to be altered differently when Ames dwarf mice were subjected to CR [183], further pointing to separate mechanisms. In contrast, similar studies performed using our long lived, dwarf GHR−/− mouse, showed quite different results from those obtained using Ames dwarfs. When GHR−/− mice were subjected to CR, mean lifespan was unchanged in both males and females, and only a slight impact was observed in females when maximal lifespan

was reported [30]. Results from this study suggest that more of an overlap exists than previously thought, especially when compared to results for CR in Ames dwarfss. While this may suggest that GH resistance and CR work via similar mechanisms, gene expression profiling still suggests some distinctions. For example, hepatic mRNA levels of IGF-1 are severely reduced while levels of several other genes, including AKT1 and IRS-1, are significantly elevated compared to control mice. CR was not shown to alter expression of these hepatic genes in comparison to mice without CR [184–186]. Recently, a comprehensive study that simultaneously compared hepatic gene expression in Ames, Snell, GHR−/− and lit/lit mice to various CR regimens was reported [187]. Using microarray technology, 43 genes were identified as being related to longevity with 13 genes having similar differential expression patterns in all four dwarf, long-lived strains and another 30 genes that were similarly expressed in both the calorie restricted and the dwarf strains. Numerous other examples of differences in gene expression in other tissues have been reported [184,188–190] suggesting that GHR−/− mice are not mere mimetics of CR animals.

Summary

The similarities in the insulin/IGF-1 and homologous regulatory systems between invertebrates and mammal models with increased longevity suggest that a fundamental mechanism of aging is likely evolutionarily conserved. A common trait shared among the long-lived models described in this review is decreased IGF-I activity. The mechanism for a reduction in IGF-1 differ in these animal models with some resulting from decreased GH action (GHR−/− mice, Lit/Lit mice, rat GH antisense transgenics), in increasing IGF-1 resistance (IGF1R+/− mice, PAPP-A) or from inhibiting IGF-1 signaling (Klotho transgenics, IRS-1 −/− mice). Further, there are no consistent phenotypic outcomes shared by all models with increased lifespan and even disparate results with the same mutant (IRS-2 +/− mice) point to the need for systemic and careful scrutiny of single studies that demonstrate extension of lifespan. While mice are routinely the animal model of choice due to genetic and physiological similiarities to humans as well as the ease of genetic manipulation, the relevance of mouse lifespan and healthspan data to the human condition are still controversial. Regardless, IGF-1 is a potent anabolic hormone that increases cellular metabolism and proliferation, enhances the function of numerous tissues, and participates in glucose homeostasis; therefore, one might expect that various animal models and humans with altered IGF-1 signaling are likely to experience alterations in longevity. A challenge in the future will be to unravel the molecular link or links and the phenotypes that tie these models together and to discern between those pertinent to humans versus those specific to the animal model of choice.

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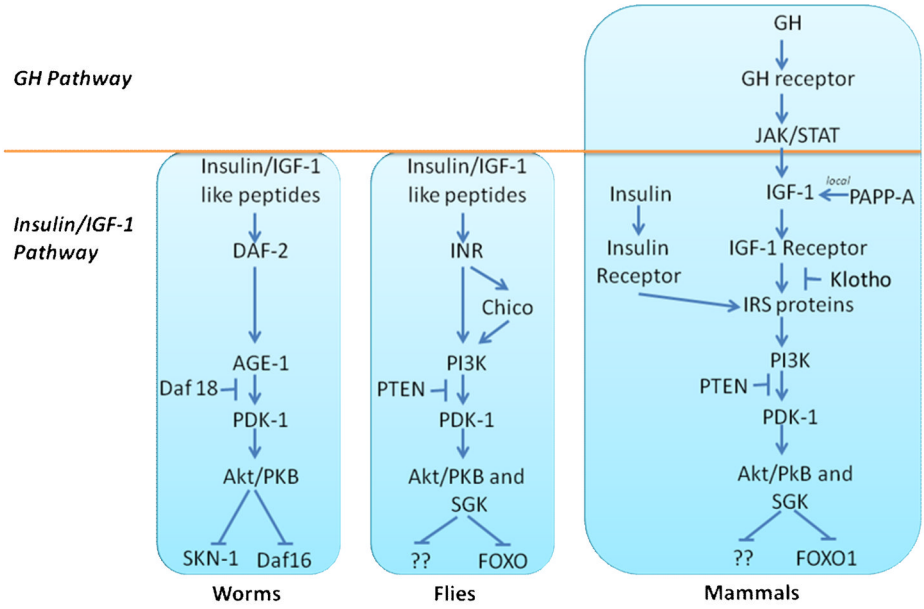

Figure 1. Comparison of GH/IGF-1/insulin signaling pathway in *C. elegans, Drosophila* and mammals

Schematic adapted from two recent reviews[191,192]

Table 1

Phenotypic comparison of mutant mice with reduced activity of GH/IGF-1 axis with calorie restricted mice[a]

Mouse Model	Ames dwarf[a] (Prop1[df])	Snell dwarf[a] (Pit1[dw])	Ghrh[b] (lit/lit)	GHR−/−[c]	IGF1R+/−[d]	p66[shc] −/−[e]	Klotho transgenic[f]	PAPP-A−/−[g]	IRS-1−/−[h]	IRS-2+/−[i]	CR[a]	GHA transgenic[j]
Primary Effect	GH, PRL, TSH deficiency	GH, PRL TSH deficiency	GH deficiency	GHR deficiency/ GH resistance	Partial IGF-1 resistance	Stress resistance	Inhibit IGF-1 and insulin signaling	Reduction in IGF-1 bio-availability	Post-IIS receptor signaling	Post-IIS receptor signaling		Reduced GH signaling
% increase in mean lifespan	49% ♂	26% ♂	23% ♂	26–55% ♂	33% ♀	30%	20–31% ♂	41% ♂	32% ♂	↔ or 17%	Varies	Normal
	68% ♀	42% ♀	25% ♀	16–38% ♀			19% ♀	33% ♀				
Plasma levels												
GH	↓	↓	↓↓	↑	ND	ND	ND	↔	↔	↔	↓	↑
IGF-1	↓↓	↓↓	↓↓	↓↓	↑	ND	ND	↔	↔	↔	↓	↓
Glucose	↓	↓	↓	↓	↔	↔	↔	↔	↔	↓	↓	↔
Insulin	↓	↓	↓	↓↓	↔	↔	↑	↔	↑	↓	↓	↔
Growth												
Body size	↓↓	↓↓	↓↓	↓↓	↔	↔	↔	↓↓	↓↓	↔ to ↑	↓	↓
% body fat	↑	↑	↑↑	↑	ND	ND	↓↓	ND	↓	ND	↓	↑
Energy Balance												
Food Intake	ND	ND	↔	↑	↔	↔	↔	↔	↔	↔	↓	↑
Metabolic Rate	↓	↓	↔	↑	↔	↔	↔	ND	ND	ND	↓	ND

ND, no available data

↑ refers to elevated; ↔ indicates normal levels and ↓ refers to reduced levels, with large differences indicated by double arrows.

[a] As reviewed [193,194];

[b] [43,195–197].

[c] [44,52,83,198]

[d] [133]

[e] [63]

[f] [70]

[g] [59]

[h] [75]

[i] [75–77]

[j] [78,79,83,198,199]