Models of Growth Hormone and IGF-1 Deficiency: Applications to Studies of Aging Processes and Life-Span Determination

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The remarkable progress in understanding the genetic basis of life-span determination in invertebrates indicates that impairments in the insulin–insulin-like growth factor 1 (IGF-1) signaling cascade increase longevity. Similarities among insulin and IGF-1-like signaling pathways in invertebrates and mammals raise the possibility that modifications of these pathways may extend life span in mammals. Investigators using Ames, Snell, and growth hormone receptor knockout models have concluded that decreased growth hormone and IGF-1 are responsible for increased life span. In this review, we critique the dwarf models and, based on multiple endocrine deficiencies and developmental anomalies, conclude that these models may not be sufficient to assess the consequences of growth hormone or IGF-1 deficiency on either biological aging or life span. We attempt to resolve some of these issues by presenting an alternative animal model of growth hormone–IGF-1 deficiency. Finally, we propose an integrated explanation of growth hormone and IGF-1’s contribution to the aging phenotype and life-span determination.

OVER the past 60 years, researchers in the field of gerontology have made great strides in the identification of biological changes associated with increasing age. However, only recently has the focus turned to understanding the genetic basis of aging. The remarkable progress in understanding the genetics of life-span determination in invertebrate models, mostly through targeted mutagenesis techniques, has allowed the identification of specific genes and signaling pathways that modulate longevity. For instance, disruption of the daf-2 pathway (including mutations in daf-2 or age-1 genes) in Caenorhabditis elegans (Figure 1) has been shown to extend life span up to 100% (1,2). These findings, and similar results in flies and yeast, support the possibility that modulation of homologous pathways in mammals may influence life span as well.

The signaling pathways that mediate increased longevity in C. elegans are well characterized and homologous to the insulin–insulin-like growth factor 1 (IGF-1) signaling pathways in mammals. Under permissive growth conditions, the receptor encoded by the daf-2 gene binds an insulin-like substrate to initiate a cascade of events, including activation of the age-1 encoded homolog of mammalian phosphoinositide-3-OH kinase (PI3K) (3). This kinase, in turn, is believed to activate the Akt-encoded protein kinase B (PKB). PKB is a serine–threonine kinase believed to antagonize a forkhead-winged helix transcription factor (daf-16), which is required for extended life span of daf-2 and age-1 mutants (3,4).

A related insulin-like signaling pathway is also believed to play a role in longevity of the fruit fly Drosophila melanogaster. The InR gene encodes a receptor with a high degree of molecular similarity to mammalian insulin and IGF-1 receptors, as well as that encoded by daf-2 in the nematode. Recently, a heteroallelic InR mutant has been engineered, yielding a dwarf phenotype whose female cohort lives up to 85% longer than the wild type. Investigators have also mutated another gene, chico, which encodes an insulin receptor substrate, resulting in the extension of female life span by up to 48% in homozygotes and 36% in heterozygotes (5).

Published studies have also shown that mutations in Sch9 (a structural relative of Akt1 and Akt2) and Cyr1 genes of Saccharomyces cerevisiae prolong replicative life span by up to 200%. These genes encode a protein kinase and adenylate cyclase, respectively, and are thought to mediate glucose-dependent signaling that stimulates growth and promotes aging in yeast (6). Researchers have found that another gene, SIR2, which encodes a silencer in the same signaling pathway, is required to produce the life-extending effects of caloric restriction in yeast. SIR2-like genes are believed to encode proteins that suppress gene expression in pathways associated with aging in C. elegans as well (7).

Similarities among insulin–IGF-1-like signaling pathways in invertebrates and mammals reveal evidence for evolutionary conservation, raising the possibility that similar genetic modifications to the insulin–IGF-1 signaling cascade may extend life span in mammals. Within the past 3 years, at least 15 (8–22) original or review papers have addressed the effects of deficiencies in the growth hormone–IGF-1 axis on life span by assessing longevity in dwarf and transgenic models. Al-
though the life-span extension observed in such animals is intriguing and, on the surface, suggests a link between invertebrate and vertebrate models, it should be noted that the consequences of multiple hormone deficiency observed in these animals, particularly the developmental consequences of multiple hormone deficiency, have been largely ignored. Despite the desire of some investigators to link invertebrate and vertebrate studies into a unified hypothesis of aging, at the present time the results of these studies do not support the conclusion that growth hormone deficiency per se increases life span. In this perspectives review, we present the information currently available regarding the regulation of growth hormone and IGF-1 during mammalian aging, as well as the physiological and biochemical changes induced in mammals with reported growth hormone deficiency. We also describe the mammalian models of growth hormone and IGF-1 deficiency currently being used in the aging field, discuss the advantages and disadvantages of these models, and introduce a recently developed model of adult-onset growth hormone deficiency. Finally, we provide a theoretical analysis of the role of the growth hormone–IGF-1 axis in aging.

**Age-Related Changes in the Growth Hormone–IGF-1 Axis in Mammals**

In mammals, growth hormone is secreted in pulsatile bursts from the anterior pituitary gland, a pattern that is necessary to achieve full biological activity. Growth hormone binds with high affinity to its receptor found in tissues throughout the body, and activation of this receptor stimulates the synthesis and secretion of IGF-1 (23). Although 90% of circulating IGF-1 is synthesized and secreted by the liver, many types of cells, including some found in the brain and vasculature, are capable of IGF-1 production (24,25). Binding of the hormone to the IGF-1 receptor causes potent mitogenic effects, including increases in DNA, RNA, and protein synthesis (26). Although heterogeneity exists in the processing of IGF-1 mRNA, these transcripts appear to produce a single peptide that is homologous to the structure of proinsulin. Blood and tissue levels as well as activity of the peptide are regulated by IGF-1 binding proteins (IGFBPs) (26). Although it was initially proposed that all of the actions of growth hormone were mediated through IGF-1, data from several studies (27,28) support direct roles for growth hormone in the regulation of lipolysis and insulin sensitivity that are independent of IGF-1.

Many studies have investigated the changes in the regulation of growth hormone and IGF-1 with age. More than 20 years ago, it was revealed that elderly individuals experience a decline in the ability to secrete growth hormone in response to several stimuli, including insulin-induced hypoglycemia and arginine administration (29). Subsequent studies revealed a loss of the nocturnal surges of growth hormone (30,31) and a decrease in plasma IGF-1 that paralleled the decline in growth hormone pulses (32,33). These early studies in humans have been extended to rodents (34,35), and today, the decline in high-amplitude growth hormone secretion and plasma IGF-1 concentrations are some of the most robust and well-characterized endocrine alterations that occur with age (Figure 2).

The physiological significance of the age-related decline in these hormones has been assessed by the administration of growth hormone to aged animals and humans. Age-related decreases in skeletal muscle mass, bone mass, immune function, and skin thickness are ameliorated by growth hormone administration, and, more recently, age-associated vascular rarefaction and memory deficits are found to be reversed by growth hormone, IGF-1 therapy, or both (36–39). There is now compelling scientific evidence that progressive withdrawal of these hormones contributes to the biochemical and physiological phenotype of aging. Despite the fact that growth hormone and IGF-1 supplementation have been shown to reverse the age-related decline in tissue function, technical caveats and the limited availability of rat growth hormone have hindered progress in this area. In fact, there have been only two studies in which growth hormone was administered for longer than 4 months. Unfortunately, both studies used human growth hormone, which exhibits antigenic and prolactogenic effects, thus confounding the experimental results. Nevertheless, these authors reported either no effect of growth hormone on life span when begun at 18 months of age in rats (40) or an increase in life span when administered to mice beginning at 17 months of age (41).
quences of alterations in these hormones on life span and their influence on the biological processes that may contribute to aging. Several of these models are described as follows (also see Table 1).

**Recessive Mutant Mice**

Snell dwarf mice, first described in 1929 (42), are homozygous for a mutation at the Pituitary-1 or Pit-1 locus (43), which is responsible for differentiation of pituitary cells producing growth hormone (somatotrophs), prolactin (lactotrophs), and thyroid-stimulating hormone (thyrotrophs). As a result, these animals are deficient in all three of these hormones. Phenotypically, they are normal at birth, but they grow slowly and achieve only one third the body weight of normal animals. Snell dwarves demonstrate increased longevity and exhibit a delay in age-related increases in collagen synthesis as well as delayed impairments in immune function (22). The latter results were the first to suggest that decreases in growth hormone and IGF-1 may slow the functional deterioration of tissues often associated with aging.

Another recessive mutant mouse, the Ames dwarf, has a mutation at the prophet of Pit-1 or prop-1 locus, which also results in growth hormone, prolactin, and thyroid-stimulating hormone deficiencies (44). Ames dwarves demonstrate increased longevity (45); however, the wide range of physiological alterations in these animals (similar to the Snell dwarf) suggest that the mechanisms behind the increased longevity may be complex. Phenotypically, they differ from the wild type in a number of ways, including (i) lower body temperature, (ii) increased levels of enzymes responsible for the removal of reactive oxygen species (catalase and Cu/Zn superoxide dimutase), (iii) increased insulin sensitivity, and (iv) smaller body size (20). Although investigators generally attribute the increased life span of Ames and Snell dwarves exclusively to growth hormone deficiency, these results can have multiple determinants. For example, hypothyroidism alone can extend longevity (46), and the reduction in basal glucose levels found in Snell and Ames dwarves leads to a reduction in insulin concentrations that may exert independent effects on longevity. Smaller body size alters cardiovascular parameters and energy requirements, and these factors may influence oxidative stress, independent of growth hormone or IGF-1 deficiency. Finally, increased levels of glucocorticoids found in Ames and Snell dwarves further complicate the task of determining whether or not increases in life span are mediated specifically by changes in the growth hormone–IGF-1 axis.

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**Table 1. Mammalian Models of Growth Hormone Deficiency**

<table>
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<tbody>
<tr>
<td>Snell mouse</td>
<td>Spontaneous pit-1</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑(♂️)</td>
<td>?</td>
<td>↓↓</td>
<td>↓↓</td>
<td>No</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Ames mouse</td>
<td>Spontaneous prop-1</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑(♂️)</td>
<td>?</td>
<td>↓↓</td>
<td>↓↓</td>
<td>No</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>GHR/BP-KO mouse</td>
<td>GH receptor knockout</td>
<td>↓</td>
<td>↑(♂️)</td>
<td>↑(♂️)</td>
<td>↑(♂️)</td>
<td>↑(♂️)</td>
<td>?</td>
<td>↓↓</td>
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</tr>
<tr>
<td>lit mouse</td>
<td>Unknown spontaneous mutation</td>
<td>↓</td>
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*Note:* IGF-1 = insulin-like growth factor 1; TSH = thyroid-stimulating hormone; GH = growth hormone; PRL = prolactin; GHR/BP-KO = GH receptor binding protein knockout; GHRHR = GH-releasing hormone receptor; ↑ = increase; ↓ = decrease; — = no change; ♂️ = males only; ? = unknown at this time.
Ghrhr−/Ghrhr− lit Mouse

The Ghrhr−/Ghrhr− or lit mouse was generated by means of a mutation in the hypothalamic growth hormone-releasing hormone (GHRH) receptor (47). Mice homozygous for this mutation are defective in their response to GHRH, have lower circulating levels of growth hormone and IGF-1, and are approximately two thirds the weight of their wild-type littermates. With age, Ghrhr−/Ghrhr− mice become obese and no differences in life span are evident; however, when placed on a low-fat diet, these animals exhibit a 30% increase in life span compared with that of heterozygous controls (22).

Growth Hormone Receptor Knockout

The growth hormone receptor binding protein knockout (GHR/BP-KO) mouse, first described in 1997, has a disruption in the GHR/BP gene that codes for both the growth hormone receptor and the truncated form of the receptor that acts as a circulating growth hormone binding protein (48). Therefore, the animal is deficient in its response to growth hormone and plasma IGF-1 is reduced by 90% (21). Studies examining longevity in this strain (13) have shown that mice homozygous for the mutated allele have a 50% reduction in body size and demonstrate greater longevity compared with their heterozygous and wild-type counterparts.

A recent study from by Hauck and Bartke (49) argues that the GHR/BP-KO model is similar phenotypically and physiologically to the Ames and Snell dwarves (without the complications of multiple hormone deficiency), thereby supporting the conclusion that the effects seen in the latter models are primarily a consequence of growth hormone–IGF-1 deficiency. Nevertheless, close inspection of the results indicate a reduction in basal levels of glucose compared with wild-type or heterozygous animals. Core body temperature (Tco) is also reduced at several time points over a 24-hour period, as are thyroid hormone levels. Furthermore, insulin levels are severely reduced in females and nondetectable in male GHR/BP-KO mice, whereas basal corticosterone levels are significantly higher in both sexes. Therefore, this model suffers from many of the same complications evident in the Ames and Snell dwarves, although the outcomes are secondary to IGF-1 deficiency.

IGF-1 Knockout Mouse

Multiple primary endocrine deficiencies evident in the Ames and Snell dwarves and secondary endocrine deficiencies present in the GHR/BP-KO mice confound analyses of results related to the specific actions of growth hormone on biological aging and life span. However, one might conclude that more targeted disruption of growth hormone–IGF-1 signaling pathways in mammals may eliminate some of these confounding variables. Transgenic IGF-1 knockout mice resulting from homologous recombination have been generated by Beck and colleagues (50). Unfortunately, this mutation is lethal in a large percentage of animals, and it results in severe growth retardation and deficiencies in development of bone, muscle, and reproductive organ systems in the remainder of the population (51). Although the animals are born live, mortality rates range from 32% to 95% (depending on strain) and death is generally attributed to respiratory difficulties or severe muscle dystrophy. Both males and females are infertile as a result of severe impairments of gonadal steroidogenesis and enlargement of organs such as the kidney, heart, liver, and brain. These effects are possibly due to growth hormone hypersecretion resulting from the absence of negative feedback on the hypothalamic pituitary axis.

In an attempt to improve the postnatal survival of these animals, Liu and colleagues used the Cre/loxP system to create transgenic mice with variable IGF-1 expression (52). Relative to wild-type controls, the resulting homozygous animals are smaller at birth, grow at a slower rate, and have severely reduced levels of IGF-1. This is in contrast to animals with only one recombinant allele, which grow only marginally slower than the wild type, achieve nearly normal size (81–85% weight of wild type), exhibit reduced levels of IGF-1, and are fertile.

In another model of IGF-1 deficiency, a 75% reduction in circulating levels of plasma IGF-1 is achieved by deletion of the gene encoding the IGF-1 peptide in the liver (43). IGF-1 gene expression in other tissues including heart, brain, kidney, and fat is not altered in this model. These animals grow normally and are fertile, and it was suggested that high levels of growth hormone and increased paracrine or tissue IGF-1 levels compensate for the lower plasma levels of IGF-1. Physiological measures and estimates of life span in the aforementioned models of IGF-1 deficiency have not been performed; however, findings from such studies would greatly benefit the field.

Models of Growth Hormone Overexpression

Transgenic mice that overexpress growth hormone were some of the earliest transgenic animals (53) and have been used to indirectly support the argument that growth hormone deficiency increases life span (45). These animals carry either a human or bovine growth hormone gene driven by a metallothionine or phosphoenolpyruvate promoter and, as one might expect, exhibit a drastic reduction in life span. Three groups of transgenic mice were subsequently engineered with varying levels of growth hormone overexpression (MTbGH or high; PCKbGH or medium; MThGH or low), resulting in an inverse relationship between growth hormone expression and life span. The reduction in life span observed with high expression of growth hormone is generally attributed to increased pathologies, primarily renal lesions, hepatic alterations, and a very high frequency of hepatocellular tumors, including adenoma and carcinoma (54). It has been argued that the reduced life span in models of growth hormone overexpression supports the hypothesis that growth hormone reduces life span (45). However, growth hormone levels in the overexpression model are 1,000–10,000 times greater than those found under physiological conditions, a fact that makes the increased pathologies observed of dubious relevance to the investigation of the actions of growth hormone under normal physiological conditions.

In a more directed approach, investigators have created transgenics that overexpress growth hormone or IGF-1 in specific tissues. For example, overexpression of human IGF-1 exclusively in skeletal muscle of transgenic mice...
(55) increases muscle mass and prevents the age-related decline in the number of dihydropyridine receptors that are necessary for excitation–contraction uncoupling (56). Furthermore, (57) selective IGF-1 overexpression in brain increases synaptic density (58). However, these models are directed more toward the investigation of functional parameters than life-span analysis.

**Multiple Endocrine Deficiencies and Developmental Anomalies Confound the Interpretation of Results Related To Growth Hormone Deficiency**

A clearly defined animal model of hormonal aging must allow the dissociation of the effects of a single hormonal deficit from those caused by other factors. Therefore, meticulous investigation into the consequences of manipulating a single variable is required before cause–effect relationships can be established. Unfortunately, many of the spontaneous mutant and transgenic approaches developed to date result in multiple endocrine abnormalities that severely compromise interpretation of the experimental results. Although these models have provided important corollary evidence for a relationship between growth hormone–IGF-1 and longevity, there are several issues that confound the results and thus limit interpretation.

**Multiple Primary Endocrine Deficiencies**

Both the Ames and Snell dwarves possess mutations in transcription factors that result in impaired pituitary cell development and deficiencies in growth hormone, thyroid-stimulating hormone (TSH), and prolactin (43,44). These primary endocrine deficiencies impair tissue growth during the entire developmental period and result in a unique hormonal milieu throughout life (see the section on developmental abnormalities). Individual hormone deficiencies as well as the lack of synergistic interactions among them lead to critical alterations in gene expression and cellular function. Given the fact that growth hormone, IGF-1, thyroid hormone, and prolactin each regulate key aspects of cellular function, it is necessary to separate the intracellular events regulated by these hormones from one another and from those that may contribute to increased life span. Approaches using recently developed techniques including high-throughput DNA arrays may detect the effects of growth hormone and IGF-1 deficiency on gene expression, but they simultaneously show the effects of TSH and prolactin deficiency. Therefore, the changes in genes that are potentially responsible for increased life span in response to growth hormone–IGF-1 deficiency are accompanied by or possibly masked by changes in numerous other genes that are sensitive to TSH and prolactin deficiencies, making analysis and interpretation extremely complex or impossible.

**Multiple Secondary Endocrine Deficiencies**

Although it would be reasonable to suspect that the complexities inherent to multiple endocrine deficiencies could be reduced or eliminated by using a model with a specific mutation or knockout in the growth hormone–IGF-1 axis, studies of GHR/BP-KO mice and IGF receptor knockouts indicate that secondary abnormalities are manifest in these models. For instance, the IGF-1 knockout is nonviable because of severe deficits in muscle development (50). These deficits cause respiratory difficulties, which undoubtedly lead to irregularities in blood chemistry. Although the GHR/BP-KO model does not show these severe problems, this model exhibits decreases in thyroid hormones, increases in glucocorticoid levels, and deficiencies in basal glucose and insulin levels (12). Alterations in insulin and glucocorticoid levels indicate abnormalities in the development of the pancreas and hypothalamic–adrenal axis or physiological compensation for reduced growth hormone–IGF-1 levels. Similar changes in glucose, insulin, and glucocorticoids have also been found in the Ames and Snell dwarves (12), suggesting that these secondary endocrine alterations are not unique to one model but rather represent a fundamental consequence of growth hormone deficiency.

**Developmental Abnormalities**

The organizational role of hormones throughout the development of the organism is a well-recognized phenomenon that has direct bearing on the relevance of transgenic and mutant animals to the study of aging. These animals develop outside the hormonal milieu of wild-type animals, and consequently they show differences in the development of virtually all tissues and organs. These developmental alterations may result in neurological and behavioral deficits, delayed puberty, and impairments of general visceral growth that may not be evident until the animal is fully mature. Impairments in tissue development alter endocrine regulatory mechanisms and raise the concern that dwarf animals are fundamentally different organisms than their wild-type counterparts and thus cannot be compared with wild-type animals.

The profound impact of the role of hormones in the development and organization of tissues has been recognized for decades. Perhaps the most notable example is the ability of androgen to reorganize hypothalamic brain regions during specific developmental “critical periods,” resulting in a permanent male pattern of brain development (59). In contrast, the absence of androgen during these critical periods “feminizes” the brain of male rodents. In reference to the Ames and Snell dwarves, thyroid hormone deficiency results in severe neurological and behavioral deficits that have implications for complex cognitive and sensory development assessed later in life (60–65). Prolactin deficiencies delay the onset of puberty and promote abnormalities in the development of the mammary gland and intestinal mucosa (66,67). Furthermore, growth hormone deficiency impairs somatic growth, delays onset of puberty, and impairs development of muscle and bone (68,69). Unlike the case of androgen, specific critical periods of neonatal development in which growth hormone and IGF-1 exhibit an organizational influence have not, as yet, been identified. However, we have recently found that growth hormone and IGF-1 are necessary for pancreatic maturation in the Lewis rat, leading to impairments in insulin secretion during glucose tolerance tests (see below). Were the glucose-induced insulin secretory response not resolved, a comparison of dwarf to wild-type animals would not only reveal the effects of growth hormone deficiency but also the consequences of increased glucose levels in a borderline diabetic animal. These exam-
ples suggest that hormonally deficient dwarves and their normal size, wild-type siblings are two distinct organisms separated by more than simple growth hormone deficiency. Naively concluding that growth hormone deficiency alone contributes to longevity in these models violates basic principles of endocrinology and decades of groundbreaking research in developmental biology.

Are Dwarf Animals Moderately Calorically Restricted?

The issues related to developmental abnormalities raise the concern that impaired nutrient uptake, transport, or both may lead not only to a model of growth hormone deficiency but to a model of moderate caloric restriction. Clearly, there is substantial evidence that moderate caloric restriction is one of the most potent interventions known to increase life span (70). Studies in the Ames and Snell dwarves have shown a variety of physiological parallels to calorically restricted animals, including decreases in growth rate, overall body size, fertility, and body temperature (12). Glucose, insulin, and thyroid hormone levels are also decreased, whereas corticosterone levels are increased (71). Similar physiological findings are observed in the GHR/BP-KO group (13). Parallels in the physiology of these two groups raise the question of whether dwarf animals are long lived as a result of a caloric restriction type of mechanism rather than growth hormone deficiency. The finding that caloric consumption in dwarf animals, when corrected to body weight, is equivalent to their wild-type counterparts would, on the surface, appear to resolve this issue. Unfortunately, previous studies indicate that, when corrected to body weight, moderately calorically restricted animals have similar food intake compared with ad libitum fed animals. Therefore, food intake alone cannot be an appropriate measure of food utilization. However, a potentially more relevant issue to dwarf models is whether the gastrointestinal tract, including mechanisms responsible for the regulation of enzymes necessary for the absorption of substrates, is sufficiently developed to ensure carbohydrate, protein, and fat uptake and metabolism comparable with that of wild-type animals. It has been well documented that hormones exert an important role in the development of the gastrointestinal tract, and that receptors for growth hormone, prolactin, and thyroid hormones are found in the gastrointestinal tract of a variety of species (67). Deficiency in any one of these hormones during development has been reported to lead to a reduction in mass of the gastric mucosa, a shortening of villi, a reduction in intestinal growth, and a decrease in important gastric hormones such as gastrin, lactase, sucrase, and amino-oligopeptidase (67,72–74). Furthermore, there is some evidence that deficiency in essential amino acids alone increases life span (75). As a result, the long-lived phenotype observed in many of the models detailed here cannot definitively be attributed to growth hormone or IGF-1 deficiency. Future studies using these models must focus on resolving this and other issues associated with multiple hormone deficiencies.

Can Issues Related to Multiple Hormone Deficiency and Developmental Anomalies in Dwarf Animals Be Resolved?

The complex interactions of the growth hormone–IGF-1 axis with other endocrine systems and its influence on tissue development suggest that substantial modifications of the Ames and Snell dwarves, and related animal models (18), are required to test the hypothesis that growth hormone–IGF-1 deficiency per se results in increased life span. We have recently developed a model using a dwarf (dw/dw) rat that is specifically deficient in growth hormone, and we have found that many of the secondary effects of growth hormone deficiency (e.g., alterations in basal glucose, insulin, thyroid hormones, prolactin, and glucocorticoid levels) are not evident (Table 2). Nevertheless, in order to circumvent the confounding variables imposed by the effects of growth hormone on tissue development, we found it necessary to compare dwarf animals treated with growth hormone for several weeks to dwarf animals treated and subsequently removed from growth hormone therapy, rather than comparing dwarf animals to heterozygous or wild-type animals.

The comparison of dwarf animals continuously treated with growth hormone to the adult-onset growth hormone deficient animals ensures that the animals being compared have the identical hormonal milieu during critical stages of tissue development (Figure 3). Analyses of glucose tolerance tests in heterozygous (wild-type) animals, dwarf animals injected with saline, and dwarf animals injected with growth hormone support this conclusion (Figure 4). These analyses reveal an attenuated glucose-induced insulin secretory response in dwarf animals treated with saline alone compared with that in heterozygous animals; the insulin response was restored by the administration of porcine growth hormone for 10 weeks. Were the dwarf animals directly compared with normal heterozygous animals, the effects of growth hormone deficiency would be confounded by impaired insulin secretion in borderline diabetic animals. We suspect that the Ames and Snell dwarves as well as the GHR/BP-KO mice may exhibit similar difficulties with glu-

### Table 2. Food Intake, Cholesterol, and T₄ Levels in dw/dw Dwarf and Heterozygous Animals Treated With GH or Sal

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (mg/dl)</th>
<th>T₄ (pg/ml)</th>
<th>Basal Glucose (mg/dl)</th>
<th>Basal Insulin (µM/ml)</th>
<th>Insulin/Glucose Ratio (×100)</th>
<th>Food Intake (g)</th>
<th>Food Intake/Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>dw/dw + Sal</td>
<td>29 ± 6</td>
<td>6.95 ± 2.75</td>
<td>89.5 ± 4.1</td>
<td>14.3 ± 0.9</td>
<td>16.3 ± 1.4</td>
<td>18.46 ± 0.72**</td>
<td>4.41 ± 0.11</td>
</tr>
<tr>
<td>dw/dw + GH</td>
<td>31 ± 2</td>
<td>6.40 ± 3.83</td>
<td>92.6 ± 4.5</td>
<td>14.3 ± 2.2</td>
<td>15.9 ± 2.7</td>
<td>18.59 ± 0.65**</td>
<td>4.52 ± 0.16</td>
</tr>
<tr>
<td>dw/dw ≥ GH</td>
<td>25 ± 3</td>
<td>58.45 ± 2.42</td>
<td>83.9 ± 1.7</td>
<td>12.7 ± 1.1</td>
<td>15.1 ± 1.2</td>
<td>12.91 ± 0.77</td>
<td>4.17 ± 0.26</td>
</tr>
<tr>
<td>dw/dw + Sal</td>
<td>17 ± 1*</td>
<td>60.75 ± 3.20</td>
<td>82.3 ± 4.3</td>
<td>13.0 ± 0.8</td>
<td>16.0 ± 1.0</td>
<td>11.43 ± 0.91</td>
<td>4.26 ± 0.32</td>
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</table>

Notes: Data represent the mean ± standard error of the mean. GH = growth hormone; Sal = saline.

*p < .05 compared with dw/dw + GH; **p < .05 compared with dw/dw ≥ GH or dw/dw Sal.
cose metabolism. In fact, all three of these groups exhibited decreased basal blood glucose levels, suggesting that additional impairments in glucose regulation are present in these animals. Unfortunately, there have been no thorough analyses of pancreatic function in these models to date, and measures of insulin sensitivity appear to be based solely on the ratio of insulin to glucose levels. The effects of growth hormone deficiency on pancreatic development represent only one example of what we believe are a series of developmental effects that preclude the direct comparison of dwarf to heterozygous animals.

When dwarf animals continuously treated with growth hormone are compared with dwarf animals treated and then removed from growth hormone therapy, several additional advantages of the model become apparent. As previously stated, these animals have identical developmental histories, pancreatic insufficiency is resolved, growth rate and body weight in treated animals are similar to heterozygous animals (until withdrawal of growth hormone in adulthood), and the withdrawal of growth hormone mimics (in magnitude) the decrease in IGF-1 found in aged animals (Figures 5 and 6). In our initial studies, growth hormone was with-
drawn in one step; however, a more gradual decline in growth hormone over a period of weeks or months could easily be implemented. Such a model can also be used to investigate the consequences of growth hormone deficiency apart from other physiological changes known to occur with age, including alterations in a number of other hormonal systems and pathological changes that continuously impair investigations into the role of growth hormone deficiency in aging animals. As a result, this model appears to offer distinct advantages for studies designed to assess the specific role of growth hormone and IGF-1 in determination of lifespan, and the consequences of growth hormone–IGF-1 deficiency.

ROLE OF GROWTH HORMONE AND IGF-1 IN MECHANISMS OF AGING

The above critique of the current dwarf models of aging indicates that the precise role of growth hormone and IGF-1 in regulation of life span remains equivocal. However, it has been suggested that the primary result of growth hormone–IGF-1 deficiency is the slowing of a “biological clock” (19), an effect dissociable from the simple reduction in age-related pathology proposed by others. In support of this perspective, it has been argued that robust changes associated with biological (reduced immune function and increased collagen cross-linking) and behavioral (reduced cognitive ability as measured by passive avoidance tasks) aging are ameliorated in the Ames and Snell dwarves (22). Regardless of the parameters measured, the aforementioned problems with the models persist, making the comparison of dwarf to wild-type animals inappropriate. In addition, the conclusion that growth hormone deficiency delays biological aging is not supported by findings related to the beneficial actions of these hormones in either young and old animals.

Growth Hormone and IGF-1 Reverse the Age-Related Decline in Tissue Function

It has been established that high levels of growth hormone and IGF-1 increase body weight, bolster bone and muscle mass, and enhance immune function. These hormones also modulate the onset of puberty and generally increase reproductive fitness. Furthermore, administration of growth hormone or IGF-1 to aged animals restores many of the deficits associated with age, including declines in immune function, bone mass, and skin thickness (76). In addition to the attenuation of age-related physical deficits, administration of IGF-1 to rodents prevents the development of age-related cognitive impairments (77). These findings, and others not detailed in this review, provide evidence that deficiency in growth hormone and IGF-1 contributes to the aged phenotype.

Growth Hormone and IGF-1 Are Mitogenic and Pathogenic

In contrast to the beneficial actions of growth hormone and IGF-1, it has been recognized for some time that IGF-1...
is an important mitogen that increases the rate of transition from G1 to S phase in the cell cycle. As a result, IGF-1 has the potential to contribute to neoplasia and potentially age-related pathogenesis. This conclusion is supported by the finding that numerous human cancers and transformed cell lines produce IGF-1 or its receptor (78), and that overexpression of the IGF-1 receptor in 3T3 fibroblasts leads to the formation of tumors in nude mice (79). As expected, transgenic rodents expressing high levels of human growth hormone (and subsequently high levels of plasma IGF-1) exhibit an increase in spontaneous mammary tumors (54). In humans, elevated IGF-1 levels have been demonstrated to be a risk factor for numerous cancers, including breast (80–83), lung (84–87), and colorectal cancers (88). Finally, passive immunization with antibodies against the IGF-1 receptor inhibits the proliferation of numerous cell lines and cancers (89–97). A recent study also indicates that lower levels of IGF-1 may be a contributing factor in the protective effect of moderate caloric restriction against age-related pathology. Dunn and colleagues (98) demonstrated that calorically restricted rats that have low levels of IGF-1 are resistant to p-cristine-induced bladder cancer, and that replacement of IGF-1 restored the incidence of bladder cancer to that of ad libitum fed animals. These findings suggest that IGF-1 is required for this type of chemical-induced pathogenesis. Unfortunately, pathological analyses have not yet been performed in any of the dwarf models. Without this information, our ability to reach a conclusion concerning the effects of growth hormone deficiency on age-related pathologies is clearly impaired.

**Integrating the Pleiotropic Actions of Growth Hormone and IGF-1**

The seemingly contradictory actions of growth hormone and IGF-1 on life span and tissue function has been proposed by some investigators to indicate a modulation of two independent pathways: one related to a biological mechanism of aging and the other to age-related pathology (19).

The authors have also concluded that the deleterious effects of growth hormone–IGF-1 deficiency on life span outweigh the beneficial effects of these hormones on tissue function. However, this conclusion fails to integrate the well-characterized actions growth hormone and IGF-1.

As described earlier in this review, growth hormone and IGF-1 are potent anabolic hormones that increase cellular metabolism and as a result enhance the function of numerous tissues. This effect is particularly important during development, when growth hormone and IGF-1 levels are high. We propose that the growth hormone–IGF-1-induced increase in metabolic activity results in increased glucose utilization, increased oxygen consumption, and increased oxidative stress. Over the life span of the animal, these effects lead to deleterious consequences, including pathogenesis and a reduction in tissue function. In support of this hypothesis, we have recently found that growth hormone and IGF-1 increase both glucose metabolism (99) and free radical damage (W. Sonntag, unpublished data, 2001). Therefore, the actions of continuously high levels of growth hormone and IGF-1, either through effects on cell division or metabolism, result in increased pathogenesis. It is not surprising that a reduction of the levels of these hormones throughout the life span retards both collagen cross-linking (through decreased metabolic activity and oxidative stress) and, potentially, the development of age-related pathology. Thus, the pleiotropic consequences of growth hormone and IGF-1 on life span, pathology, and tissue function simply do not necessitate the conclusion that growth hormone and IGF-1 have multiple independent mechanisms of action. Rather, the positive effects of these hormones on tissue function and the negative effects regarding life span and pathogenesis can be accommodated by a single theory of growth hormone–IGF-1 action—all related to the effects of growth hormone and IGF-1 on metabolism.

It is tempting to speculate that growth hormone and IGF-1 fit a model of antagonistic pleiotropy, a recently described theory on the evolution of aging that suggests that the ex-
pression of particular genes are beneficial early in life but become detrimental as the organism ages (100–102). Nevertheless, evolutionary biologists would argue that for antagonistic pleiotropy to be adaptive, growth hormone and IGF-1 would have to increase reproductive efficacy, a criterion clearly supported by a large volume of data. A potential complication in the application of this concept to the growth hormone–IGF-1 axis is that the levels of these hormones decrease rapidly after puberty. In fact, we believe that the age-related decrease in growth hormone/IGF-1 gene expression during this time frame may be an active mechanism designed to balance the beneficial and deleterious consequences of these hormones, possibly resulting in an extension of reproductive life span. These, and other issues related to the actions growth hormone and IGF-1 and their role in aging, await the application of appropriate animal models in which the levels of these hormones can (and must) be carefully and specifically regulated.

Conclusions

The invertebrate studies that have demonstrated a role for insulin–IGF-1 signaling in the regulation of life span are compelling. Our critical analysis of the application of these concepts to mammalian dwarf models of aging (e.g., the Ames and Snell dwarfs) is not meant to detract from the importance of these original findings. In fact, we believe that the potential genetic regulation of life span in mammalian models opens new avenues for research in the field of aging. Nevertheless, we must proceed with caution and avoid frank overinterpretation of results because these models of growth hormone–IGF-1 deficiency or excess are complex and introduce multiple endocrine deficiencies, developmental anomalies, and the possibility of indirect effects related to moderate caloric restriction. No matter how intriguing the findings, theoretical constructs related to the growth hormone–IGF-1 axis cannot be derived from models that are simply not specific to growth hormone and IGF-1. Furthermore, the traditional experimental design of comparing dwarf to wild-type animals introduces confounding variables that compromise an interpretation of results related to the growth hormone–IGF-1 axis.

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