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Adult-Onset Growth Hormone and Insulin-Like Growth Factor I Deficiency Reduces Neoplastic Disease, Modifies Age-Related Pathology, and Increases Life Span

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Disruption of the insulin/IGF-I pathway increases life span in invertebrates. However, effects of decreased IGF-I signaling in mammalian models remain controversial. Using a rodent model with a specific and limited deficiency of GH and IGF-I, we report that GH and IGF-I deficiency throughout life [GH deficiency (GHD)] has no effect on life span compared with normal, heterozygous animals. However, treatment of GHD animals with GH from 4–14 wk of age [adult-onset (AO) GHD] increased median and maximal life span by 14% and 12%, respectively. Analysis of end-of-life pathology indicated that deficiency of these hormones decreased tumor incidence in GHD and AO-GHD animals (18 and 30%, respectively) compared with heterozygous animals and decreased the severity of, and eliminated deaths from, chronic nephropathy. Total disease burden was reduced by 24% in GHD and 16% in AO-GHD animals. Interestingly, the incidence of intracranial hemorrhage increased by 154 and 198% in GHD and AO-GHD animals, respectively, compared with heterozygous animals. Deaths from intracranial hemorrhage in AO-GHD animals were delayed by 14 wk accounting for the increased life span compared with GHD animals. The presence of GH and IGF-I was necessary to maximize reproductive fitness and growth of offspring early in life and to maintain cognitive function and prevent cartilage degeneration later in life. The diverse effects of GH and IGF-I are consistent with a model of antagonistic pleiotropy and suggest that, in response to a deficiency of these hormones, increased life span is derived at the risk of functional impairments and tissue degeneration. (*Endocrinology* 146: 2920–2932, 2005)

THE REMARKABLE progress in understanding the genetics of life span in invertebrates, primarily through mutagenesis techniques, has permitted the identification of specific genes and signaling pathways that modulate longevity. For example, disruption of signaling in the daf-2 pathway (including mutations in the daf-2 or age-1 genes) in *Caenorhabditis elegans* extends life span up to 100% (1, 2). Heteroallelic mutation of *InR* extends female life span in *Drosophila melanogaster* by 85% (3). Similarly, mutation of *Saccharomyces cerevisiae* Sch9, which encodes a protein kinase involved in glucose-dependent signaling that is homologous to that encoded by mammalian *Akt*, can double replicative life span (4). Because these invertebrate genes exhibit substantial homology to the insulin/IGF-I receptor and signaling pathways in other species, the possibility exists that genetic modification to the insulin/IGF-I signaling cascade may represent a conserved pathway for regulating life span (5).

In mammals, IGF-I is regulated by the secretion of GH from the pituitary gland, and several reports demonstrate that mutations at the *pit-1* and *prop-1* locus, resulting in the complete absence of GH, TSH, and prolactin, extend life span in mice (6, 7). The extended life span in these and related models, including the GH receptor (GHR) knockout (GHRKO), $IGFR^{+/-}$ (IGF receptor) transgenic and $GHRHR^{-/-}$ mutations in mice, have been widely interpreted as a specific consequence of GH and IGF-I deficiency (6, 8, 9). Nevertheless, the effects of these mutations on tissue and organ development and the general modification of the hormonal milieu that have been reported in the majority of these mutations (9, 10) raise the issue of whether these effects are a primary result of GH deficiency (GHD) and IGF-I deficiency or are secondary to other endocrine and/or nonendocrine alterations that are present in these models. Other models of GHD and IGF-I deficiency including the GH antisense mutation and mice expressing a GHR antagonist demonstrate a modest or no increase in life span (11). Nevertheless, the concept has evolved that, as in invertebrate species, the presence of GH and IGF-I accelerates biological aging and inhibition of this axis extends life span.

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Abbreviations: AO, Adult onset; DMBA, dimethylbenzanthracine; GHD, GH deficient/GH deficiency; GHR, GH receptor; GHRHR, GHRH receptor; GHRKO, GHR knockout; OA, osteoarthritis.

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In contrast to the aforementioned studies, experiments in both humans and animals clearly indicate a progressive decrease in GH and IGF-I with age, and replacement of GH has been shown to reverse the age-related decline in IGF-I and the decline in lean body mass, bone density, skin thickness, immune function, learning and memory, myocardial function, and the increase in adiposity that is part of aging (12–16). The results of these numerous studies have been interpreted to suggest that the aged phenotype results from a deficiency in anabolic hormones, of which, a deficiency of GH and subsequently IGF-I has a particularly important role. These two disparate concepts—that GH and IGF-I ameliorate functional impairments of biological aging and that the presence of GH and IGF-I accelerate biological aging (and limit life span) are at the center of the current controversy.

To develop models of specific GH and IGF-I deficiency that are devoid of other endocrine and nonendocrine changes that may impact aging and life span, two issues need to be resolved. First, deficiencies in GH alone, or in combination with other hormones, before or immediately after birth may impair the development of tissues/organs resulting in functional changes throughout life and thus have the potential to influence pathology and life span through secondary mechanisms (10, 17). Additionally, the role of GH in the regulation of nutrient homeostasis suggests that a complete deficiency of the hormone at any age results in compensatory responses in other endocrine systems (*e.g.* insulin, glucocorticoids). Either of these two issues have the potential to compromise interpretation of the effects of a primary deficiency of GH and the assessment of its impact on aging and life span. To address these issues, we developed an animal model that exhibits a specific and limited deficiency in GH and IGF-I in adulthood and assessed effects on pathology, life span, and several functional markers of aging (10, 18). Our results demonstrate that a specific and limited deficiency of GH and IGF-I alone does not increase life span but regulates age-related pathology. In addition, our results emphasize the importance of the peripubertal rise in these hormones for regulation of life span and the importance of GH and IGF-I during adulthood for maintenance of tissue function.

Materials and Methods

Animal model

Homozygous *dw/dw* rats originally derived from the Lewis strain were purchased from Harlan Industries (Indianapolis, IN). Previous studies indicate that these animals have a recessive mutation in the transcription factor necessary for development of the somatotroph and hence pituitary GH synthesis and plasma GH levels are reduced without changes in other anterior pituitary hormones (19–21). Although prolactin secreting cells are increased with this mutation, we found that dwarf females did not exhibit increased basal plasma prolactin levels (4.78 ± 2.4 and 8.42 ± 1.6 ng/ml, in dwarf and heterozygous females, respectively). Homozygous dwarf male rats were bred with females of the Lewis strain to produce heterozygous offspring of normal size. Thereafter, normal-size females, heterozygous for the dwarf trait (*dw/+*), were mated with dwarf males to produce both homozygous (dwarf) and heterozygous (normal size) littermates for use in these studies. Preliminary studies validated that plasma IGF-I and body weights in adult heterozygous animals were equivalent to wild-type animals.

As expected, no differences in body weights between homozygous and heterozygous littermates were observed throughout the early developmental period (d 1–24). Around d 25 (the age at which GH pulse amplitude increases in rodents), a bimodal distribution of body weight was evident. Differences between the two groups reached statistical significance on d 28 ($P < 0.01$), and this information was used to classify animals as dwarf (*dw/dw*) or heterozygous controls (*dw/+*). Offspring were subsequently divided into one of four treatment groups (Fig. 1) and injected twice daily with vehicle or 200 μg highly purified porcine GH kindly provided by Dr. A. F. Parlow, Director, National Hormone and Peptide Program (Torrance, CA). This preparation was highly purified by solvent extraction, ammonium sulfate fractionation, and size exclusion chromatography. *In vivo* biologic potency is 1.8 IU/mg in terms of the International Standard for Bovine GH. The porcine GH is highly homogeneous according to criteria of physical-chemical purity including sodium dodecyl sulfate-gel electrophoresis and analytical gel chromatography. Contamination with other pituitary hormones is low (<0.01%). As expected, analysis of plasma samples after injection with porcine GH for up to 17 months revealed no antibodies against porcine GH. This finding is consistent with the parallel weight gains in dwarf animals treated with porcine GH and heterozygous animals injected with saline and the homology between rat and porcine GH (91.66% amino acid similarity based on information from the Swiss Protein Bank). For these studies, a constant dose of GH (200 μg) was used (despite a decreasing concentration on a per body weight basis as the animal matures) because preliminary data demonstrated that this regimen is sufficient to normalize the increase in body weight and plasma IGF-I levels compared with heterozygous animals.

After d 28, heterozygous animals and GH-injected dwarf animals exhibit substantial increases in body weight (Fig. 2). Body weight gains in dwarf saline-treated animals were consistently less than their normal size siblings (66% that of heterozygous littermates). Experimental groups for life span studies were as follows: 1) heterozygous animals injected with saline from 4–14 wk of age (normal size = normal GH/IGF-I levels); 2) GHD dwarf animals administered porcine GH from 4–14 wk of age [adult-onset (AO) GHD]; and 3) GHD dwarf animals administered saline from 4–14 wk of age (dwarf, GHD). For specific studies described in these series of experiments, an additional group of dwarf animals were raised that received GH injections from 4 wk until the animals were killed (GH replete). In this case, all other treatment groups were injected with vehicle until the animals were killed. At death, pituitaries and plasma were collected and analyzed for GH and IGF-I concentrations, respectively, to independently validate group assignments.

Animals were maintained on a 12-h light, 12-h dark cycle (lights off at 1800 h) in a climate-controlled room with food (Purina Mills, Rich-

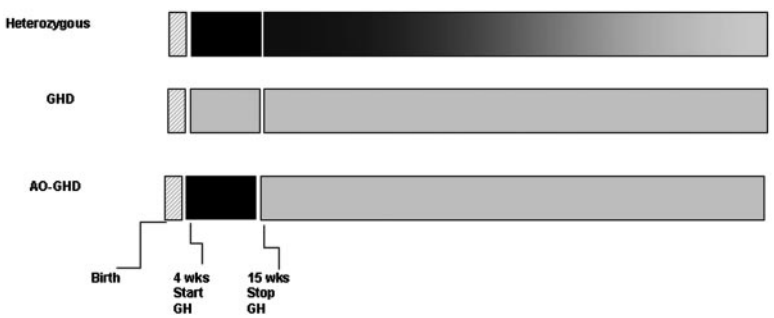

FIG. 1. Experimental design. Females, heterozygous for the dwarf trait (*dw/+*), were mated with homozygous dwarf males to produce both homozygous and heterozygous littermates for use in these studies. Offspring were weaned on d 21 and body weights followed at 3-d intervals. On d 28, dwarf animals were identified by decreased body weight compared with heterozygous siblings. Animals were subsequently divided into one of three treatment groups: heterozygous animals (normal size and GH/IGF-I levels) treated with vehicle; AO-GHD, dwarf animals administered GH (200 μg twice daily) for 10 wk; and GHD, GHD dwarf animals administered vehicle alone. *Shaded regions* represent elevated plasma GH and IGF-I levels.

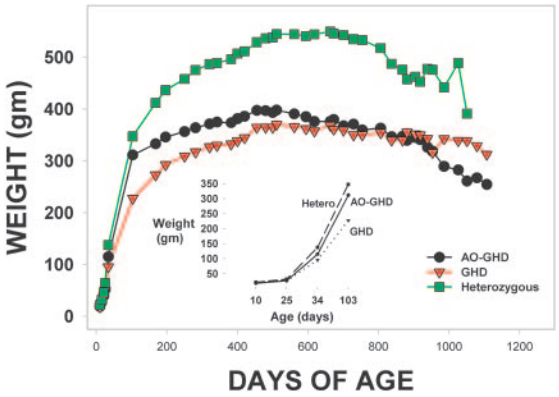

FIG. 2. Body weights in male subjects. As expected, no differences in body weights between experimental groups were observed throughout the early developmental period (until d 28). After d 28 (initiation of treatment), heterozygous vehicle-treated and GH-replete dwarf animals exhibited parallel increases in body weight until 14 wk of age when GH injections were terminated to create AO-GHD animals. No significant differences in basal plasma T_4, corticosterone, glucose, insulin, or insulin/glucose ratios were observed between the experimental groups. Similarly, no differences in weight of heart, liver, spleen, or kidney were evident when corrected to body weight (data not shown).

mond, IN) and water available *ad libitum*. Routine microbial analyses of sentinel animals within our animal colony (at 4- to 6-month intervals throughout the studies) did not reveal the presence of typical rodent bacterial and viral pathogens. Body weights and health were monitored biweekly and daily, respectively. The animal facilities at Wake Forest University School of Medicine are fully accredited by the American Association for Accreditation of Laboratory Animal Care and comply with all Public Health Service-National Institutes of Health Institutional Policies and Standards for Laboratory Animal Care. All protocols were approved by the Institutional Animal Care and Use Committee.

Life span and end-of-life pathology

General. Animals in this experiment included a total of 58 male (25 heterozygous, 17 AO-GHD, and 16 GHD animals) and 61 female rats (22 heterozygous, 20 AO-GHD, and 19 GHD animals). These animals were allowed to live out their life span and survival (in days) was recorded as the outcome measure. A complete pathological analysis was performed on all spontaneously dead animals in the life span study. Necropsies were performed generally within 6 h to minimize autolysis and deterioration of the tissues. A profile of pathological lesions was constructed for each animal that included the prevalence and severity of both neoplastic and nonneoplastic diseases, the probable cause of death, and the effect of the pathology on longevity. Potential interrelationships between hormonal interventions, disease status, and aging were examined.

The complete pathological analysis involved the following: body weight at death, gross and microscopic analysis of all visible tumors as well as brain, pituitary gland, heart, lung, trachea, esophagus, stomach, small intestine, colon, liver, pancreas, spleen, urinary bladder, thyroid/parathyroid gland, adrenal gland, sternum, spinal cord, vertebra, nasal passage, ventral abdominal skin, eyes, and gonadal tissue including the testes, preputial gland, epididymis, prostate and seminal vesicle in the male or the ovary, oviduct, uterus and vagina in the female. The complete necropsy also included gross visual examination of the animal carcass, the weight of major organs, and the dimensions, the weight, and mass of visible tumors. After gross inspection, all of the tissues were fixed immediately in 10% neutral, buffered formalin. The small tissues (adrenal gland, pancreas, preputial gland, and aorta) were kept in a specimen bag to avoid possible loss of the samples. The fixed tissues, which were processed conventionally, were embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin-eosin.

Classification of lesions. The severity of the lesions was assessed based on histological grading systems (22) similar to the system Dr. Ikeno has used for neoplastic and nonneoplastic lesions in Fischer 344 rats (23). Chronic nephropathy was graded in the order of increasing severity based on the grading system described by Yu *et al.* (24): Grade 0 (no lesions), Grade 1, Grade 2, Grade 3, Grade 4, and Grade E (end-stage kidney disease). Photomicrographs of each grade of lesion have been published. In previous studies, elevated levels of blood urea nitrogen and serum creatinine were observed only in rats with Grade 4 or Grade E lesions.

The severity of pituitary tumors was determined by the criteria cited by Shimokawa *et al.* (25), which was further redefined by Dr. Ikeno (23) based on the size of the tumor: hyperplasia (less than 1 mm in diameter); adenoma (lesions larger than 1 mm in diameter); Grade 1 (1–2 mm); Grade 2 (2–4 mm in diameter); Grade 3 (4–5 mm in diameter), and Grade 4 (> 5 mm and indentation on hypothalamus of brain). Grades 3 and 4 were considered to contribute to the death of the animal.

The severity of other neoplastic lesions were based on criteria reported previously (25), which was further redefined (23) based on histopathological findings of the involvement of tumor cells as follows: Grade 1 (primary site only), Grade 2 (two to three organs), Grade 3 (three to four organs), and Grade 4 (more than five organs or Grade 3+ additional pathology, *e.g.* pleural effusion, ascites *etc.*). Hydrothorax, ascites, hemorrhage, and severe congestion and edema in lung were common complications associated with fatal neoplastic lesions.

Body composition

Whole body differences in fat and lean mass were determined by dual x-ray absorptiometry in a subset of animals that completed the life span study (GHD, n = 10; AO-GHD, n = 7; heterozygous, n = 11) at 12 and 18 months of age. Before the scan, animals were anesthetized with a combination of ketamine/xylazine. Body composition measurements (lean and fat mass) were obtained with small animal software on a human dual x-ray absorptiometry scanner (Delphi ATM, Hologic, Inc., Bedford, MA). The percent coefficients of variation are 0.40% for lean mass and 1.66% for fat mass.

Telemetry

In a separate study, AO-GHD and heterozygous males (190 d of age, n = 4 each) were anesthetized with a mixture of halothane and nitrous oxide. An incision was made into the abdominal muscle and a telemetry probe (TL11M2-C50-PXT; Data Sciences International, St. Paul, MN) implanted. This probe is designed to assess both core body temperature and several cardiovascular parameters. The catheter tip was inserted into the descending aorta, secured in place with absorbable suture and the transmitter body anchored to the abdominal musculature. The muscle and sc tissues were sutured using absorbable 5-0 suture and the skin closed with 3-0 Vetafil. Seven days after surgery, the telemetry receiver was placed under the animal's home cage and data acquired for 1-min periods every 5 min for 48 h using a Dataquest Advanced Research Technology software system. Temperature, heart rate, and systolic and diastolic pressure were recorded and averaged for each animal.

Behavioral/functional testing

Inclined plane. For the inclined plane (26) (a measure of muscle tone and stamina), the animal was placed on a 60-degree tilted mesh screen 1.6 meters above a 7.6-cm foam pad. Latency to fall was recorded with a maximum latency of 30 min. Animals were tested at 12, 19, and 25 months of age.

Grip strength. Forelimb grip strength was determined using a computerized electronic pull strain gauge (Grip Strength Meter-Columbus Instruments, Columbus, OH). The mean force (grams) was calculated over three trials each at 12, 19, and 25 months of age.

Morris Maze. Learning was assessed using a variation of the Morris Maze (27). Animals were given one trial each day for 9 d with the platform located in the same position across all days of testing. At the beginning of the trial, the animal was placed in the water maze, from one of three start locations, facing the inside of the pool. A hidden platform was submerged underneath the water (1–2 cm) made opaque with nontoxic paint. To escape, the animal must find the platform within 60 sec. A curtain surrounded the pool and visual objects were positioned at var-

ious locations to serve as cues for the location of the platform. Latency to find the platform (in seconds) was the dependent measure with shorter latencies indicating better performance. For analysis, data were collapsed into three three-trial blocks. Each three-trial block represented all three start locations, randomized across the 3 d in the block of testing. Animals were tested at 8, 16, and 27 months of age.

Object recognition

Apparatus. For this task (28), test objects were presented in a square arena (1×1 m) with walls 0.46 m high. The arena was opaque with a floor covered in standard bedding. For the object recognition task, one arena was employed for all phases of testing. The objects to be discriminated were made of wood, plastic, metal or glass, but pairs of objects to be discriminated were made of the same material. The objects varied in size, the largest being approximately $15 \times 15 \times 14$ cm and the smallest being approximately $7 \times 7 \times 12$ cm. The behavior of the animal was monitored by an overhead video recorder and later scored by two observers blinded to the experimental treatments and tested for interrater reliability.

Handling and habituation. Rats were handled daily for 1 wk before cognitive assessment. Animals were given five habituation sessions. For object recognition, animals were randomly exposed to the testing environment. Testing began the day after the last habituation session.

Procedure. Two test sessions were spaced approximately 48 h apart. Each session was divided into a sample phase and a test phase. The environment remained constant through these two phases for a given rat. In the sample phase, two identical objects (A1 and A2) were placed in two adjacent corners of the arena approximately 10 cm from the edges. The rat was then placed in the arena facing away from the objects and allowed to explore the arena and objects for 3 min. The animal was then returned to its cage for a 5-min interval. In the test phase, two objects (A3 and B1) were placed in the corners of the arena and the rat allowed to explore the arena for 3 min. Object A3 was identical with the sample objects (A1 and A2) and therefore appeared familiar, whereas object B1 was a novel object. The position (left or right) of the novel object in the test phase was balanced between sessions.

Measurements. The basic measurement of memory was the time the rat spends exploring a novel object. Exploration of an object was defined as directing the nose at the object (<2 cm from the object) and actively exploring it. Turning around or sitting on or next to the object was not considered exploration. Several measurements were determined to examine exploration and discrimination as follows:

E1: Total time spent exploring objects in sample phase.

E2: Total time spent exploring objects in test phase.

D1: Index of discrimination—the difference in time spent exploring objects (A3 and B3) in test phase (B-A3).

D2: Discrimination ratio—the difference in exploration time (D1) expressed as a proportion of the total time spent exploring in test phase (D1/B+A3). For this measure, a value of zero indicated no difference in exploration of the two objects, values greater than zero indicated greater exploration of the novel object, whereas values less than zero indicated a greater exploration of the familiar object.

Reproduction studies

The effects of chronic GH and IGF-I deficiency on reproduction were compared in two groups: heterozygous and GHD females. The decision not to use AO-GHD or GH-replete animals for these studies was based on preliminary data indicating that the pattern of GH replacement (200 μg twice daily) in females had an independent effect on estrous cycles that may bias experimental results. Successful pregnancies, live births, and neonatal weight of offspring were assessed at 4 and 11 months of age by housing females with a proven male breeder for 10 d.

Estrous cycles in heterozygous and GHD animals were assessed by evaluating vaginal smears over 9 d in 3-month-old animals. For analysis of ovarian follicles, ovaries from these animals were collected, fixed overnight in Bouin's solution, dehydrated in graded ethanol, and embedded in paraffin. Mid-saggital sections (5 μm) were cut and stained with hematoxylin and eosin. Follicles of various maturational stages (primordial to large antral follicles) were evaluated in each group.

To investigate whether the presence of elevated GH and IGF-I in the maternal circulation is important for postnatal growth of offspring, we compared body weight gain from d 3–60 of age in offspring from breeding homozygous dwarf males and females. The homozygous dwarf offspring were cross-fostered to a separate cohort of GHD or GH-replete mothers and body weights of the offspring followed at 5-d intervals through d 60.

RIAs

IGF-I (Bachem, Torrance, CA) was radiolabeled using the lactoperoxidase-glucose oxidase method and purified on a Sep-Pak silica cartridge (Waters, Milford, MA). Plasma was extracted in acid-ethanol and IGF-I measured by RIA as previously described (29). The intra- and interassay coefficients of variance were 8% and 13%, respectively. Materials for analysis of pituitary GH and prolactin and plasma IGF-I were the generous gift of Dr. A. F. Parlow and the National Hormone and Peptide Program. T_4 and corticosterone were measured using materials purchased from Diagnostic Products Corp. (Los Angeles, CA).

Statistics

Survival data were analyzed using the Kaplan-Meier procedure. Data for both grip strength and latency to fall from the inclined plane were calculated as a percentage of change from baseline (12 months) at 19 and 25 months of age and analyzed using a 2×3 (age, 19, 25 months $\times$ condition: heterozygous, GHD, and AO-GHD) repeated-measures ANOVA. For body composition analysis, data were analyzed using a three group (heterozygous, GHD, and AO-GHD) $\times$ time (12 and 18 months) repeated-measures ANOVA. *Post hoc* analyses were assessed by Student-Newman-Keul's test as required. Data on body temperature, heart rate, systolic, and diastolic pressure for heterozygous and AO-GHD animals were analyzed by repeated measures ANOVA. Data for the Morris Maze were calculated as change from baseline performance (block 1 *vs.* block 3) at each time point and analyzed using a repeated measures 3×3 [age: 8, 16, and 27 months $\times$ condition: GHD (dwarf saline), AO-GHD, and heterozygous)] ANOVA. Data for body weight were analyzed using repeated ANOVA and all other comparisons were made using one-way ANOVA followed by Newman-Keul's tests or a modified Bonferroni analysis as required.

Results

Animal model

No significant differences in basal plasma T_4, corticosterone, glucose, insulin, or insulin/glucose ratios were observed between the treatment groups (males) under basal conditions (Table 1). Similarly, no differences in weight of heart, liver, spleen, or kidney were evident when corrected to body weight (data not shown). Analysis of food intake at wk 26 indicated that GHD and AO-GHD animals consume less food than heterozygous or GH-replete dwarf animals; however, these differences were eliminated when corrected to body weight.

Dwarf animals injected with saline only (GHD) and those animals removed from GH treatment (AO-GHD) exhibit a 40–53% reduction in IGF-I levels compared with heterozygous controls or dwarf animals treated with GH (GH replete; $P < 0.001$ each). No differences in plasma IGF-I levels were evident between GH-injected dwarf animals and heterozygous littermates (Fig. 3).

Life span

We found no differences in mean or maximal life span between GHD animals and their heterozygous siblings (Fig. 4). Heterozygous and GHD males exhibited a median life span of 907 and 899 d and a maximal life span of 1071 and 1117 d, respectively. Surprisingly, treatment of GH/IGF-I-

TABLE 1. Plasma hormone levels and food intake

Group	T_4 (pg/ml)	Corticosterone (ng/ml)[a]	Basal glucose (mg/dl)	Basal insulin (μM/ml)	Insulin/glucose ratio ($\times$100)	Food intake/ body weight[b]
Heterozygous	65.9 ± 2.8	51.2 ± 7.6	89.5 ± 4.1	14.3 ± 0.9	16.3 ± 1.4	4.4 ± 0.1
GHD	60.8 ± 3.2	43.6 ± 12.5	82.3 ± 4.3	13.0 ± 0.8	16.0 ± 1.0	4.3 ± 0.3
AO-GHD	58.5 ± 2.4	ND	83.9 ± 1.7	12.7 ± 1.1	15.1 ± 1.2	4.2 ± 0.3
GH replete	69.4 ± 3.8	39.1 ± 10.6	92.6 ± 4.5	14.3 ± 2.2	15.9 ± 2.7	4.5 ± 0.2

Plasma samples for insulin and glucose were taken from animals fasted for 12 h; other samples were taken from trunk blood at the time animals were killed. ND, Not determined.

[a] Corticosterone levels were not assessed ion AO-GHD animals. [b] Data expressed as food intake (g)/100 g body weight and represent mean ± SEM. [Some data derived from Ref. 10.]

deficient animals with porcine GH for 10 wk (AO-GHD animals) resulted in a 13.6 and 14.6% increase in median life span and a corresponding increase in maximal life span (12.4% and 7.8%) compared with heterozygous or GHD animals, respectively ($P = 0.004$). This increase in life span in male AO-GHD animals was not observed in females with the same treatment. Females, regardless of treatment group, exhibited a mean life span of approximately 720 d. Although females with the dwarf phenotype appeared to show increased mortality early in life compared with heterozygous animals, this effect was not significant but may be the consequence of early onset on pituitary tumors (see below).

Pathology

Analysis of end-of-life pathology indicated that 88% of heterozygous males exhibited tumors at necropsy and incidence of this pathology was partially reduced in GHD animals ($P = 0.34$) and reached significance in AO-GHD animals ($P = 0.04$). Fatal neoplastic disease was found in 57% of male heterozygous animals and was commonly associated with other pathological lesions including pleural effusion, ascites, hemorrhage, or severe congestion and edema in the lung (Table 2). Although not significant, dwarf rats (both GHD and AO-GHD) exhibited a numerically lower incidence of both fatal pituitary adenomas and total tumors compared with heterozygous animals. The major fatal nonneoplastic disease in heterozygous rats was chronic nephropathy that was found in 74% of animals at death. In GHD and AO-GHD animals, nephropathy was not fatal and severity was significantly reduced ($P < 0.001$ each). Interestingly, the reduced incidence of fatal neoplastic lesions and fatal chronic nephropathy in GHD animals was not associated with increased life span. On the contrary, we found that both GHD and AO-GHD animals exhibited increased incidence of intracranial hemorrhage and thrombus in the heart. The average age of occurrence of these fatal nonneoplastic diseases (thrombus and intracranial hemorrhage) was 31.9 months in heterozygous, 30.7 months in GHD, and 34.1 months in AO-GHD animals. Finally, total disease burden was reduced in GHD and AO-GHD animals compared with heterozygous controls ($P = 0.009$, $P = 0.067$, respectively). Therefore, the delayed occurrence of nonneoplastic disease, together with reduced fatal neoplastic lesions in AO-GHD rats, has an important role in their extended life span compared with either heterozygous or GHD animals. Although dwarf females (GHD and AO-GHD) exhibited a similar reduction in nonpituitary neoplastic disease compared with heterozygous animals, the high incidence of pituitary adenomas in all groups was the primary cause of mortality and may have contributed to the gender differences observed between treatment groups.

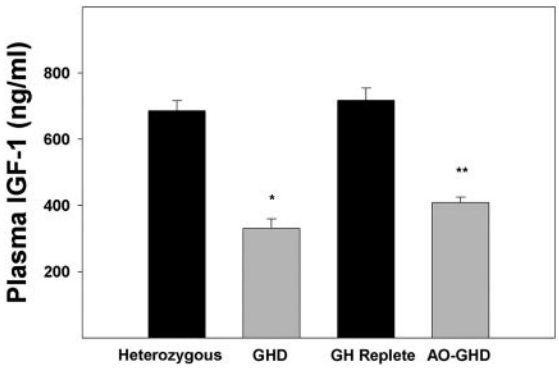

FIG. 3. Plasma levels of IGF-I in experimental groups (males). Blood samples for analysis of IGF-I levels were taken at wk 17 (2 wk after termination of hormone replacement in AO-GHD animals). GHD and AO-GHD animals exhibit a 40–53% reduction in IGF-I levels compared with heterozygous controls or GH-replete (continuous GH replacement) animals ($P < 0.001$, each). No differences in plasma IGF-I levels were evident between GH-replete dwarfs and heterozygous littermates. Data were analyzed by one-way ANOVA and expressed as mean ± SEM. *, $P < 0.01$; **, $P < 0.001$ compared with heterozygous or GH-replete animals.

Body composition analysis

Fat mass. An increase in fat mass occurred between 12 and 18 months of age (Table 3) in heterozygous animals ($P < 0.05$) with slight but nonsignificant changes in GHD and AO-GHD animals ($P > 0.05$). When expressed as a percentage of body weight, both GHD and AO-GHD groups exhibited a marginally larger percentage body fat relative to heterozygous controls ($P = 0.07$).

Lean mass. Heterozygous animals had a larger lean body mass at 12 and 18 months of age relative to either the GHD or AO-GHD ($P < 0.001$, each; Table 3). A significant increase in lean body mass was evident in all groups between 12 and 18 months ($P = 0.01$); however, when expressed as a percent of total body mass, only a marginal difference between groups was found with heterozygotes exhibiting a larger percent of body mass as lean body weight relative to either the GHD or AO-GHD groups ($P = 0.07$).

Telemetry

Average temperature readings in Celsius were recorded at hourly intervals over a 24-h period and are shown in Fig. 5.

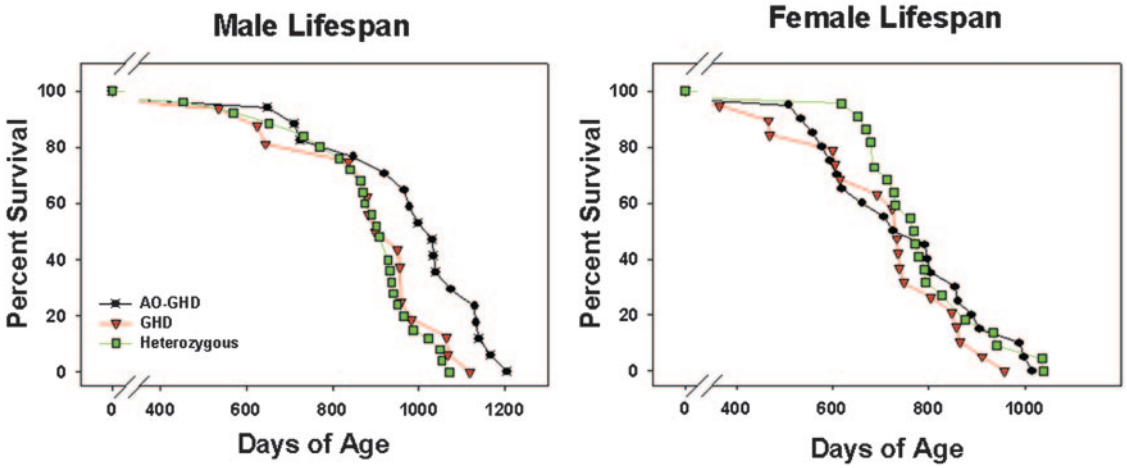

FIG. 4. Life span was determined on a total of 58 male (25 heterozygous, 17 AO-GHD, and 16 GHD animals) and 61 female rats (22 heterozygous, 20 AO-GHD and 19 GHD animals). Information on dates of birth and death were recorded and necropsies performed generally within 6 h of death (see Table 1). Survival analysis using the Kaplan-Meier procedure indicated that both mean and maximal life span were increased in male AO-GHD animals compared with either heterozygous or GHD animals ($P < 0.004$, each). No differences in life span of females were evident between the treatment groups.

Diurnal variations in body temperature were evident in both AO-GHD and heterozygous animals with lower temperatures occurring during the light (nonactive) phase of the light-dark cycle. No differences were evident between groups. The mean 24-h body temperature was 37.3 ± 0.04 and 37.4 ± 0.05 C in AO-GHD and heterozygous animals, respectively. No differences in systolic, diastolic pressure, or heart rate were evident between AO-GHD and heterozygous animals (Table 4).

Functional tests

Both GHD and AO-GHD animals exhibited superior performance on the inclined plane compared with heterozygous animals that we attribute to reduced body mass in these groups (Fig. 6). However, when these data were expressed as a change from baseline performance, performance in all groups decreased with age ($P < 0.001$, each) and no differences were evident between groups ($P = 0.28$).

Grip strength was similar in all groups at 12 months of age, reached maximum levels at 19 months and declined substantially thereafter (Fig. 6). Heterozygous animals exhibited superior performance at 19 months of age compared with the other treatment groups; however, these differences were not statistically significant. Importantly, no differences in the rate of decline in grip strength were evident in the treatment groups between 19 and 25 months of age.

At 12, 18, and 24 months of age, learning was assessed by comparing the decrease in latency to find a submerged, hidden platform in the Morris Maze (Fig. 7). In this study, we found that heterozygous animals improved their learning over trials (37.7% decrease in latency), whereas GHD and AO-GHD animals demonstrated impaired learning ability at all ages (2.2% increase and a 5.4% decrease in latency to locate the hidden platform). Similar findings were evident in an independent experiment that assessed working memory at 17 months of age using an object recognition task. In addition to GHD, AO-GHD, and heterozygous animals, a cohort of dwarf animals were raised and treated with GH continuously from 4 wk to 17 months of age (GH replete); the time of the behavioral tests. Heterozygous and GH-replete animals exhibited superior memory compared with GHD animals ($P < 0.05$). These results are consistent with numerous studies in both rodent and human models indicating that the presence of GH and IGF-I are required for optimal performance on tests of learning and memory throughout life and that GH/IGF-I replacement to older animals reverses the age-related decline in cognitive function (16, 30, 31). Using measures of cognitive function and physical performance,

TABLE 2. End-of-life pathology

Pathology	Heterozygous	GHD	AO-GHD
% Tumor bearing	88	73 ($P = 0.34$)	63 ($P = 0.04$)
Tumor burden (mean)	1.3 ± 0.2	0.92 ± 0.2 (P = ns)	1.0 ± 0.2 (P = ns)
% Fatal tumor	57	36 ($P = 0.21$)	31 ($P = 0.11$)
% Fatal pituitary adenoma	39	21 ($P = 0.30$)	13 ($P = 0.08$)
Grade of pituitary adenoma	1.57 ± 0.3	1.69 ± 0.4 (P = ns)	1.19 ± 0.3 (P = ns)
% Fatal chronic nephropathy	74	0 ($P < 0.0001$)	0 ($P < 0.0001$)
Severity of nephropathy	4.0 ± 0.3	0.8 ± 0.2 ($P = 0.01$)	1.0 ± 0.2 ($P = 0.001$)
% Intracranial hemorrhage/thrombus	17	50 ($P = 0.06$)	44 ($P = 0.14$)
Age of occurrence of hemorrhage/thrombus (months)	31.9	30.7	34.1
Disease burden (total)	6.6 ± 0.5	5.0 ± 0.5 ($P = 0.009$)	5.5 ± 0.4 ($P = 0.067$)

ns, Not significant.

TABLE 3. DXA analysis of body composition at 12 and 18 months of age

Group	Fat (g)		Fat (%)		Lean (g)		Lean (%)	
	12	18	12	18	12	18	12	18
Heterozygous	110.4 ± 6.7	135.4 ± 11.1[a]	22[b]	24[b]	368.3 ± 1.0[b,c]	396.6 ± 1.7[b,c]	75	73
GHD	95.4 ± 6.2	99.8 ± 11.4	27	27	241.4 ± 1.4	254.2 ± 2.4	70	71
AO-GHD	110.1 ± 6.7	106.1 ± 8.6	29	27	260.2 ± 1.3	267.0 ± 1.6	69	70

[a] $P < 0.05$ compared with 12-month values in the same treatment group; [b] $P < 0.05$ compared with GHD or AO-GHD at the same age; [c] $P < 0.001$ compared with GHD or AO-GHD at the same age.

we conclude that a deficiency in GH and IGF-I in adulthood does not increase life span by modifying the age-related decline in tissue function.

Reproduction

Because the effects of elevated GH and IGF-I on neoplastic disease and nephropathy are not manifest until late in life, we reasoned that there could be no evolutionary advantage to limit the rise in these hormones in early adulthood. On the contrary, the increase in these hormones around puberty must impart a selective advantage either by increasing reproductive fecundity and/or the growth/survival of offspring. To address this question, reproductive fecundity was assessed in GHD and heterozygous females by breeding animals at several points during the life span (Fig. 8). The normal age-related decline in parity was evident, but no differences were found between the treatment groups. Nevertheless, litter size was reduced by 29.2% in GHD compared with heterozygous animals at 4 months of age ($P < 0.02$). By 11 months, litter size in GHD animals was reduced by 68% compared with heterozygotes ($P < 0.001$). Analysis of primordial follicles and number of corpora lutea in an independent cohort of animals supported this finding. At 3 months of age, the number of primordial follicles was reduced by 35% ($P < 0.05$), ovaries and ovarian follicles were smaller and estrous cycles were irregular in GHD compared with heterozygous animals. The reduction in primordial and primary follicles in GHD animals was also evident at 9 and 12 months of age. These results demonstrate that optimal reproductive fecundity requires adequate concentrations of GH and IGF-I and suggest that reduced concentrations of these hormones accelerate specific aspects of reproductive aging.

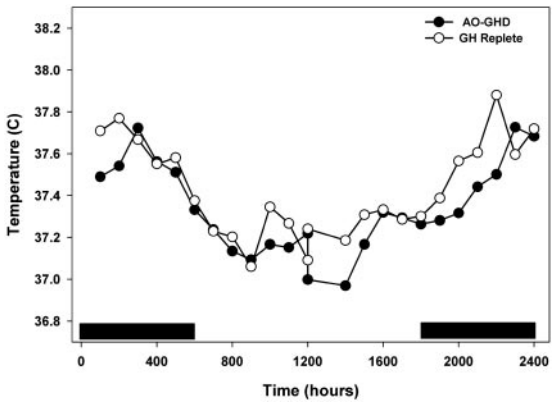

FIG. 5. Temperature variations over a 24-h period in AO-GHD and GH-replete animals at 160 d of age. Temperature was assessed remotely using an implanted telemetry probe and receiver placed under the animal's home cage. Data were acquired for 1-min periods at 5-min intervals for 48 h and mean hourly temperature values calculated using a Dataquest Advanced Research Technology software system. Mean (hourly) body temperature is expressed as degrees Celsius for four animals per treatment group. *Dark bars* represent dark phase of the light-dark cycle

The effects of maternal GH and IGF-I on postnatal growth of offspring were also evaluated (Fig. 9). For this study, homozygous dwarf male pups were cross-fostered to GHD or GH-replete mothers. No differences were observed in neonatal weight between the treatment groups; however, postnatal growth was consistently greater in male offspring raised by GH-replete compared with GHD mothers. By d 21 (day of weaning), body weights of pups from GH-replete mothers were approximately 42% greater than those from GHD mothers ($P = 0.012$). Although differences in body weights between the treatment groups were partially ameliorated after weaning, the effects of circulating maternal GH and IGF-I were still evident on d 60 (18.8% increase in body weight of pups raised by GH-replete compared with GHD mothers; $P = 0.02$). These findings support the conclusion that the rise in GH and IGF-I in adults maximizes reproductive success, increases postnatal growth of offspring, and delays reproductive aging. We suspect that, in the wild, in the presence of disease, limited resource availability, and predation, levels of maternal GH and IGF-I exert an even more profound impact on reproductive success and survival of offspring than under the current laboratory setting.

Discussion

Together, these studies indicate that a limited and specific reduction in GH and IGF-I (40%), initiated in adulthood and continued throughout the life span, reduce neoplastic disease, nephropathy, and total disease burden. The critical nature of the rise in the levels of these hormones immediately before and after puberty is supported by the absence of increased life span in GHD animals despite the substantial reduction in specific age-related pathologies (chronic nephropathy and disease burden) compared with heterozygous animals. Our results support the conclusion that the absence of increased life span in GHD animals is the result of a delay/perturbation in tissue and organ maturation that result from GH and IGF-I deficiency during critical developmental periods. For example, GH has important effects on pancreatic development (17) and we have previously reported that GHD animals exhibit impairments in tests of glucose tolerance that are resolved by short-term GH treatment (10). Similar findings have been reported in the GH-RKO transgenic animal, in that, the development of the pancreatic islet is impaired in the absence of IGF-I as early as 10 d after birth (16). In addition to pancreatic development, the present study suggests that vascular development and/or maintenance is also impaired by GHD because the presence

TABLE 4. Cardiovascular parameters and body temperature (24 h mean values)

Group	Heart rate	Pulse pressure	Systolic pressure	Diastolic pressure	Body temperature
Heterozygous	316 ± 3	43.5 ± 0.2	152 ± 1.2	107 ± 0.7	ND
GHD	322 ± 5	36.8 ± 0.6	143 ± 1.3	106 ± 0.9	37.24 ± 0.09
AO-GHD	332 ± 5	30.1 ± 0.2	145 ± 1.1	120 ± 0.7	37.3 ± 0.044
GH Replete	334 ± 4	33.7 ± 0.1	154 ± 0.8	115 ± 0.6	37.4 ± 0.046

Heart rate is in beats/minute, pressures in mm Hg, and temperature in degrees Celsius. Data represent mean ± SEM for three to five animals/group. ND, Not determined.

of GH for 10 wk early in life delayed the appearance of cerebral hemorrhage in AO-GHD compared with GHD animals by approximately 12 wk. These results demonstrate that early perturbations in GH and/or IGF-I levels can have profound effects on tissue development that are manifest later in life as alterations in age-related pathology and life span.

Importantly, the modest reduction in GH and IGF-I in either GHD or AO-GHD animals was not sufficient to induce alterations in basal levels of T_4, insulin, glucocorticoids, or glucose (or prolactin in females) as has been reported for the Snell and Ames dwarf mice that are homozygous for mutations at the *pit-1* and the *prop-1* locus, respectively (6, 33). Mutations in these transcription factors inhibit the development of pituitary cells responsible for production of GH (somatotrophs), prolactin (lactotrophs), and TSH (thyrotrophs) (34, 35). Similar endocrine anomalies (with the exception that prolactin increases) have been reported in transgenic GHRKOs (36) that most likely result from the absence of IGF-I during early development. Consequently, the increased life span reported in the Ames and Snell dwarf mice or related models that produce secondary alterations in other endocrine and nonendocrine systems cannot be attributed to GH or IGF-I deficiency alone but rather suggest that impairments in other systems, induced by GH and/or IGF-I deficiency, may be the specific modulator of increased life span in these models.

The increase in life span in response to a specific and limited reduction in GH and IGF-I reported in the present study is similar to that found with suppression of GH using an antisense transgene (37) but relatively modest compared with the 50–60% increase in life span reported for either Ames or Snell mice. Although the specific mechanisms for the increased life span in these models has not been established, innate differences in susceptibility to age-related pathology between animal strains/species may be a contributing factor in life span extension induced by GH and IGF-I deficiency. Studies of end-of-life pathology in the Ames dwarf revealed that greater than 95% of wild-type mice exhibit neoplastic disease and the *prop-1* mutation results in an 88% decrease in adenocarcinoma of the lung (the primary neoplastic lesion in the wild-type animal) and fatal neoplastic disease occurs at an older age (38). In contrast, only 57% of heterozygous (control) animals in the present study exhibit fatal neoplastic disease and, although GH and IGF-I deficiency substantially reduce tumor incidence, the reduction in number of deaths from neoplastic disease in heterozygous animals may limit the impact of GH and IGF-I deficiency on life span. Thus, the possibility exists that the overriding variable in the magnitude of life span extension induced by GH and IGF-I deficiency is directly related to the incidence of neoplastic disease of the parent strain and possibly the degree of suppression of IGF-I.

Interestingly, animals expressing a GHR antagonist exhibit no increase in life span and the increase in life span in GHRKO mice ($GHR^{-/-}$) appears to be both strain and gender dependent. For example in the C57BL/6J background, no differences in life span were evident between $GHR^{-/-}$ animals compared with controls at 50% mortality (although modest effects were detectable at different ages), whereas the

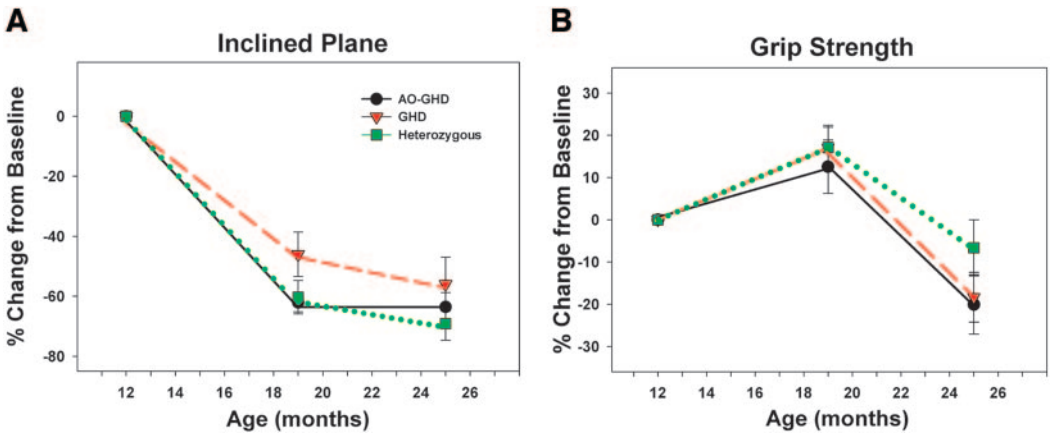

FIG. 6. Analyses of the effects of GH and IGF-I on physical performance were assessed using the inclined plane and grip strength procedures. Data for both measures were calculated as a percentage of change from baseline (12 months) at 19 and 25 months of age. A, For the inclined plane (a measure of muscle tone and stamina), results indicate that performance in all groups declined with age and that there were no differences in the rate of decline among the groups. B, Forelimb grip strength was determined using a computerized electronic pull strain gauge and mean force (grams) calculated over three trials at each age. All groups improved between baseline and 19 months and then declined between 19 and 25 months. No differences between the treatment groups were evident ($P = 0.42$).

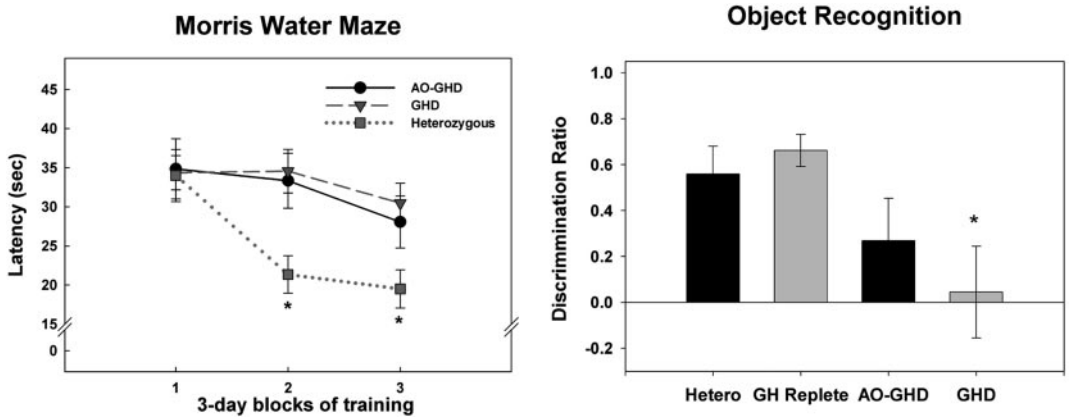

FIG. 7. Analysis of cognitive changes between treatment groups revealed that deficiency of GH and IGF-I results in impaired learning and memory. A, In the Morris Maze, latency to find the platform (in seconds) was the dependent measure with shorter latencies indicating better performance. Results indicated a shorter latency in heterozygous relative to either the AO-GHD or GHD animals ($P = 0.031$ and $P = 0.02$, respectively); *, $P < 0.05$. B, Effects of GH and IGF-I on memory were assessed by performance in the object recognition task at 17 months of age. For this study, an additional cohort of dwarf animals was raised that were treated continuously with GH from 28 d of age until testing at 17 months of age (GH replete). Heterozygous and GH-replete animals demonstrated superior performance on this task compared with GHD animals (*, $P < 0.05$). Performance of AO-GHD animals was reduced compared with either heterozygous or GH-replete animals, but these effects were not significant ($P = 0.177$ and $P = 0.194$, respectively). Data are expressed as mean ± SEM.

effects of the mutation on life span appeared to be more robust in C57BL/6J females and in both males and females of the Ola-Balb/cJ background (11). The importance of the underlying pathology of the parent strain is further supported by our analysis of life span in females using the model of AO-GHD described here. In contrast to males, no increase in life span of either GHD or AO-GHD animals was observed compared with heterozygous females. Analysis of end-of-life pathology revealed the presence of large, prolactin-secreting pituitary tumors in all groups regardless of treatment that undoubtedly was the primary factor in death in all groups. In fact, there appeared to be early deaths from pituitary tumors in GHD and AO-GHD females (beginning around 400–500 d of age in GHD and AO-GHD animals and 600 d in heterozygous females), but this effect did not reach statistical significance. Despite this potential acceleration of pituitary pathology, GH and IGF-I deficiency appeared to reduce the number of palpable mammary tumors in both AO-GHD and GHD animals by approximately 50% (data not shown). These latter results are consistent with the absence of dimethylbenzanthracine (DMBA)-induced mammary cancer in GHD animals and the dose-related rise in DMBA-induced mammary cancer in response to GH administration previously reported by our laboratory (39). Thus, the presence of plasma IGF-I-dependent and plasma IGF-I-independent pathologies that we have identified in this model are certain to have an impact on life span and consequently the assessment of whether GHD influences aging and/or life span. We suspect that back-crossing this mutation into females of other strains not susceptible to such frank pituitary tumors may reveal effects similar to that observed in males. These studies are currently in progress. Nevertheless, without specific knowledge of end-of-life pathology of the parent

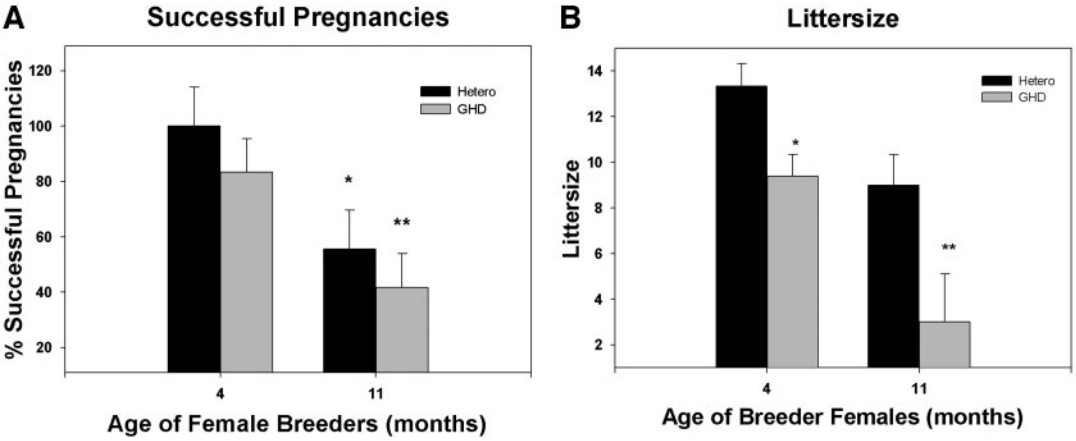

FIG. 8. In heterozygous animals (A), successful pregnancies decreased 44% from 4–11 months of age. Although the percent successful pregnancies were reduced in GHD animals at 4 months and 11 months of age compared with heterozygotes (17% and 21% decrease, respectively), these differences were not significant. B, As expected, litter size decreased significantly between 4 and 11 months of age in heterozygous females ($P = 0.012$). GHD animals exhibited a reduced littersize at 4 months (27% decrease compared with heterozygous animals) and, by 11 months, litter size was reduced 57% compared with heterozygous animals of the same age ($P = 0.016$ and 0.005, respectively).

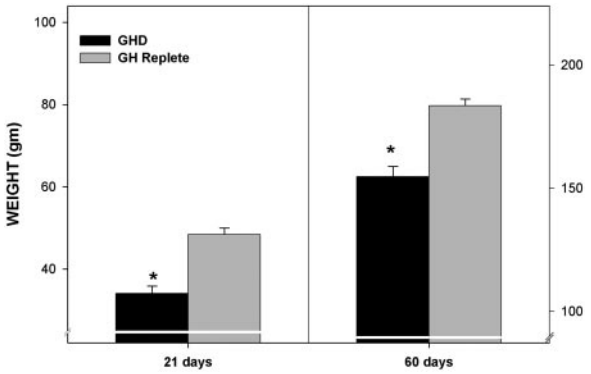

FIG. 9. Body weights of male homozygous dwarf (*dw/dw* offspring) raised by GH-deficient dwarf mothers treated with either GH (GH replete) or vehicle (GHD). Data indicate that maternal GH influences growth of offspring before weaning (d 21) and this effect is maintained for a minimum of 60 d. Data represent mean ± SEM. *, $P < 0.01$ compared with animals raised by GH-replete mothers).

strain and the mutant, the interpretation of interventions designed to assess aging and life span is compromised.

In addition to effects on age-specific pathology, others have proposed that GH and IGF-I deficiency modifies the rate of biological aging. Data from the long-lived *GHRHR*$^{-/-}$ (GHRH receptor) transgenic mouse (maintained on a low-fat diet), for example, indicate a delay in the age-related rise in collagen cross-linking and in markers of immune function that are associated with immune senescence (6). Similarly, *Igf1r*$^{+/-}$ heterozygous female mice exhibit increased life span after paraquot-induced oxidative stress compared with *Igf1r*$^{+/+}$ animals (9). Delays in biological aging also have been reported in the Ames and GHRKO dwarfs (40) and in cell lines from Ames and GHRKO mice (41). These studies, and others, have been used to support the concept that specific genes (*e.g.* IGF-I) regulate biological aging in mammals. Unfortunately, there are no accepted markers of biological aging and thus no specific measure can be used to conclude that a mutation either accelerates or slows the rate of biological aging. Rather, one can conclude that correlates of aging are modified that may, or may not, be associated with life span.

The conclusion that the regulation of aging and life span are separable events is further supported by recent studies by de Magalhaes *et al.* (42). In their analyses, they found no evidence that either *GHRHR*$^{-/-}$, *IGF1R*$^{+/-}$, or *prop1* mice exhibited alterations in biological aging. Rather, the results of their analyses pointed to age-independent factors that contributed to increased life span in these animals. The investigators did find evidence that GH transgenic mice accelerate and that *GHR*$^{-/-}$ and *pit1* mutants delay biological aging. However, the authors urged caution in interpretation. In this regard, the GH transgenic animals are reported to exhibit increased pathology compared with wild-type animals (43) and independent studies demonstrate that large increases in GH and IGF-I induce hepatomegaly and increase spontaneous mortality (44). Such an increase in pathology would certainly have an effect on measures of cognitive function that were reported to be impaired in these animals and is part of the justification for the conclusion that aging is accelerated in this model (45). Thus, the issues related to the regulation of biological aging and determination of life span are complex and confounded by age-related pathology.

In our own studies, we find that several biological correlates of aging including cognitive and reproductive decline as well as osteoarthritis (OA) appear to be either increased or accelerated in GHD animals. For example, the effects of chronic deficiency of GH and IGF-I on the development of OA in joint tissues were investigated using the model of GHD presented in this manuscript (46). Analysis of medial and lateral femoral condyles and tibial plateaus, articular cartilage, and subchondral bone thicknesses and areas were evaluated by histomorphometry and scored using a comprehensive OA histologic grading scheme (47). Loss of matrix staining for cartilage proteoglycan, a marked loss of chondrocytes in the superficial third of the articular cartilage and superficial fibrillation were present in sections from GHD rats but not in GH-replete animals. Assessment of articular cartilage lesions of osteoarthritis revealed less severe lesions in GH-replete animals when compared with GHD animals. In addition, severity of cartilage lesions characteristic of osteoarthritis was reduced in those animals with higher GH/IGF-I levels or those treated with GH for 10 wk (GH replete<heterozygous<AO-GHD<GHD). These analyses provide additional support for the conclusion that GHD negatively impacts articular cartilage and that the cartilage degeneration that normally accompanies aging is accelerated by the absence of GH and IGF-I.

It is of interest that our results in the *dw/dw* rat are in contrast to studies demonstrating a slowing of articular and vertebral cartilage aging in the Ames dwarf (48, 49). Similar differences between our results and those of the Ames dwarf have been noted for cognitive function (40). Although the specific reasons for these differences are unclear, increased paracrine IGF-I expression has been reported in brains of Ames dwarf mice (50), whereas we have not found such increases in either GHD or AO-GHD rats compared with heterozygous controls (Sonntag, W. E., D. R. Riddle, and J. K. Brunso-Bechtold, manuscript in preparation). Such increases in paracrine IGF-I activity in dwarf mice, if confirmed in other tissues, would challenge the concept that the mechanism for the increased life span in Ames dwarf mice is related to reduced IGF-I signaling. Additionally, the consequences of hormonal deficiency during early adulthood and/or thyroid hormone deficiency present in the Ames dwarf also may be another important component of these differences. These issues merit further investigation.

Even though there are a large number of studies demonstrating that GH replacement to older animals and humans improves immune function (51), cognitive function (31, 52–54), muscle mass (55), as well as the function of other tissues (see reviews in Refs. 10, 14, and 18), such data do not allow us to conclude that GH and IGF-I deficiency accelerate biological aging. Rather, the only conclusion that can be made is that GHD after adolescence accelerates the functional decline in specific tissues and that GH or IGF-I replacement ameliorates this decline. Such an interpretation would be consistent with a vast experimental literature.

The diverse or pleiotropic actions of GH, either directly or through its anabolic mediator, IGF-I, are consistent with its effect as a modulator of tissue growth and nutrient ho-

meostasis. It is well known that GH regulates cellular DNA and protein synthesis, fat metabolism, insulin sensitivity, vascular growth, and the coordinated development of body growth. In fact, the early effects of GH and IGF-I on pancreatic development previously discussed and its well-known effects on reproductive function (56) suggest that these hormones have a critical role in survival by optimizing reproductive function and the ability of the organism to compete for limited resources. Although the complete absence of GH has a profound effect on reproduction and many of the animals are sterile, we find that a 40% decrease in IGF-I has only a modest effect on successful pregnancies and litter size. Nevertheless, the presence of GH and IGF-I delayed the age-related decline in reproductive function (*e.g.* litter size) in GHD animals and increased the growth of offspring. These results are consistent with the conclusion that higher levels of GH and IGF-I during the period of reproductive competence exert a significant evolutionary advantage to the species.

It has been proposed that the role of GH and IGF-I on tissue growth make these hormones an important factor in the genesis of neoplastic disease (57). In this regard, decreases in plasma IGF-I appear to be one of the mediating factors in the decrease in pathology in response to moderate caloric restriction (58). Although the specific mechanisms remain unclear, moderate caloric restriction is the most robust intervention capable of increasing life span in rodents and decreases both age-specific pathology and modifies the rate of biological aging (59–64). In response to caloric restriction, plasma levels of IGF-I decrease approximately 40%, which compares favorably with the reductions in IGF-I found in the GHD and AO-GHD models reported here. The decline in plasma IGF-I appears to be necessary for some of the effects of caloric restriction because resistance to chemical-induced pathogenesis induced by caloric restriction is reversed by administration of IGF-I (65). Similar findings are evident in GHD animals with low levels of IGF-I. As previously reported, GHD animals are resistant to DMBA-induced mammary cancer and increasing IGF-I levels produces a dose-related rise in both tumor incidence and numbers of tumors/tumor bearing animal (39). A decline in metastasis of transplanted GI cancer cells also has been demonstrated in transgenic models with a reduction in plasma IGF-I (66). Furthermore, the association between plasma IGF-I and age-specific pathology is supported by epidemiological studies demonstrating a modest correlation between high levels of IGF-I (compared with age-matched controls) and the incidence of lung, breast, and GI cancer in humans (67). However, these associations are generally assessed by correlating plasma IGF-I levels in older individuals with subsequent neoplastic disease. The results presented here suggest that plasma IGF-I assessed throughout the life span may be a better correlate of cancer risk than those levels obtained later in life.

The close relationship between caloric restriction and the reduction in age-related pathology raises the question of whether the increased life span in AO-GHD animals found in this study may be an indirect effect of caloric restriction. However, four findings argue against this possibility. First, no differences in measures of diurnal body temperature (a well-documented consequence of caloric restriction) or food intake are evident between GHD, AO-GHD, and heterozygous animals. Second, GHD animals fail to demonstrate increased life span despite similar food intake, body weights, and levels of IGF-I (after 15 wk of age) compared with AO-GHD animals. Third, percent body fat is modestly increased in AO-GHD and GHD animals. Fourth, no age-related differences in cognitive function or physical performance are evident between GHD or AO-GHD animals despite the differences in their life spans. Therefore, we conclude that, in contrast to moderate caloric restriction, AO-GHD animals demonstrate an increase in life span primarily via a reduction or delay in specific pathologies of aging.

There has been substantial controversy surrounding the question of whether GH and/or IGF-I contribute to the development of the aged phenotype, whether administration of these hormones to the elderly is warranted and whether the actions of these hormones on the aging phenotype have evolutionary significance. Our findings demonstrate that GH and IGF-I are important for reproductive success early in life, for developing an adequate tissue architecture to delay degenerative changes that contribute to disability (*e.g.* loss of cartilage, vascular degeneration) and for providing optimal tissue function throughout life (*e.g.* maintenance of cognitive function). However, we also found that levels of GH and IGF-I found in normal animals throughout the life span contribute to the development of both neoplastic and nonneoplastic disease. Although the optimal period of GH and/or IGF-I treatment in our model remains to be determined, the paradoxical actions of these hormones are consistent with a model in which expression of specific genes influence biological aging through both beneficial and deleterious effects on the organism at different stages of the life span—antagonistic pleiotropy (32, 68). In reference to GH and IGF-I, effects on reproductive success result in selection for high levels of these hormones in early adulthood; however, there is little or no evolutionary pressure to suppress expression of these hormones because the pathological actions are not manifest until after reproductive senescence. Our findings demonstrate that the reduction in age-associated neoplastic disease, nephropathy, pathological burden and increased life span possible through a modest reduction in GH/IGF-I levels after puberty are obtained at the risk of increased functional impairments and degenerative disease with age. Finally, the diverse tissue alterations induced in models of accelerated/delayed aging demonstrate that multiple species as well as pathology and functional endpoints rather than life span analysis alone are required to interpret the effects of interventions on aging.

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