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Aged-Rodent Models of Long-Term Growth Hormone Therapy: Lack of Deleterious Effect on Longevity

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Studies were carried out to examine the effects of long-term recombinant human growth hormone (GH) therapy on longevity in rodents. In the first study, 150 18-month-old female F344 rats were divided into three groups of 50 rats per group: Group 1, solvent vehicle; Group 2, 10 μg GH/kg body weight three times per week; Group 3, 50 μg GH/kg body weight three times per week. GH and solvent vehicle therapies were started at 18 months of age and continued until all the animals died spontaneously. Serum insulin-like growth factor (IGF)-I was measured at 18 and 29 months of age and on 3-month-old rats. Serum IGF-I level decreased between 3 and 29 months of age. GH therapy reversed the decrease in a dose-dependent manner, with the 50 μg GH dose returning the serum IGF-I level to that of 3-month-old animals. However, statistical analysis revealed no significant effect of GH therapy on median life span, 10th percentile life span, or maximum life span. Similar observations on longevity were made on aged F344 male rats and on aged Balb/c mice, even when the dose of GH was increased to 1.0 mg/kg body weight two times per week. The main pathologic lesions in control animals were nephropathy, cardiomyopathy, leukemia, and testicular interstitial cell tumor; the prevalence of these lesions was not significantly altered by GH therapy. We conclude that long-term low-dose GH therapy that includes doses in the range that is given to humans in clinical trials in GH deficiency and to revert age-related physiologic declines has no overt deleterious effects on longevity and pathology in aged rodents.

ALTHOUGH growth hormone (GH) is best known for stimulating growth, it is secreted even after linear growth has ceased. However, its serum levels are high in the young and decrease with aging in both humans and rodents (1,2). Knowledge about the exact cause of age-related decreases in GH secretion is incomplete, but studies in rodents indicate that it may involve low growth hormone–releasing hormone (GHRH) secretion, diminished responsiveness of somatotropes to GHRH, increased secretion of somatostatin, and a reduced density of pituitary somatotropes (3,4). The age-related decrease in GH levels has been implicated in the alterations that occur with aging in body composition (5), and there has been a long-standing interest in the therapeutic use of GH, in part, to reverse the age-related alterations. However, species specificity and limited availability of human GH from cadaveric sources have limited its use.

In the last decade, recombinant human GH has become available from genetic biotechnology and this has ushered in a new era of clinical and scientific investigation of problems related to GH. Initially, GH therapy was administered mainly to severely short-statured children to enhance their linear growth (6). More recently, it is given increasingly to GH-deficient adults and to older individuals as a result of the recognition that GH deficiency is prevalent in the elderly and may be responsible, in part, for some of the adverse metabolic deteriorations associated with human aging, such as decreased protein synthesis, decreased percent lean body mass, decreased bone mass, and increased percent body fat (1,5). Clinical studies with GH continue to expand. It has been administered to young athletes to examine its effects on body composition and endogenous GH secretion (7), to obese adults to facilitate nitrogen conservation and to accelerate loss of body fat during restriction of caloric intake (8,9), to malnourished older individuals to enhance and maintain their weight (10), to burn patients to increase protein synthesis and tissue repair (11), to postsurgical patients to attenuate postoperative catabolic response and facilitate convalescence (12), and to osteopenic elderly individuals to assess its effect on bone loss due to aging (13). Some of the potential clinical applications of GH would require that the hormone be given over a prolonged period. The deleterious consequences of persistent high circulating GH due to oversecretion of the hormone, such as in acromegaly, are not in question (14,15), and excessively high GH levels in transgenic animals are known to shorten life span (16–20). However, the aim in GH therapy in humans is to give the hormone intermittently at doses that are relatively low (1). Although low-dose GH therapy slows the progression of some age-associated physiologic declines, there is no proven association of GH therapy with life span (21). In one study in mice, Khansari and Gustad (22) concluded that long-term GH therapy prolongs life span, and although this important conclusion has not been confirmed, the claim has entered the gerontologic literature (16,17,21). The purpose of this study is to develop rodent models of long-term, intermittent, low-dose growth hormone therapy, and to begin to bridge the information deficit on the effects of such therapy. Because the ultimate toxicologic test is effects on

longevity, we have carried out pilot studies on the effects of long-term, intermittent, low-dose GH therapy on longevity in aged rodents.

Materials and Methods

Animals

Male and female F344 rats and male and female Balb/c mice, 16–17 months of age, were purchased from NIA rodent colonies. The mice came from Charles River Laboratories, Inc. (Kingston, N.Y.), and the rats came from Harlan Sprague Dawley, Inc. (Indianapolis, IN). On arrival at our institution, the animals were housed in a specific pathogen free (SPF) facility in rooms maintained at 26°C and 14-hour light and 10-hour dark cycles. Male rats were housed two per cage, female rats three per cage, and mice five per cage, and different species and sexes were kept in different rooms. Sentinel animals were housed in these rooms and euthanized periodically to monitor their SPF status. During the experimental period, all animals were fed a Teklad diet (Madison, WI) that contained 0.93% calcium, 0.65% phosphorus, and 3.0 units of vitamin D per gram and allowed free access to drinking water. The animals were used to carry out the following longevity studies.

Experiment 1, Female F344 Rats

One hundred and fifty 18-month-old female F344 rats were divided into three weight-matched groups of 50 rats per group. Group 1 served as controls and received solvent vehicle; Groups 2 and 3 received injections of 2 and 8 mg GH per kg body weight, respectively, three times per week. The doses were based on reports that doses in this range are required for studying the in vivo effects of GH therapy on bone in rats (23). The doses were later changed to 10 µg and 50 µg GH per kg body weight for reasons described in Results. The latter doses are in the range that is given to elderly humans on GH therapy (1). Hormone and solvent vehicle injections were given subcutaneously starting from 18 months of age. For the determination of serum IGF-I, baseline control rats were bled and sacrificed at 18 months of age. Three-month-old female F344 rats from the same supplier were also bled, and at 29 months of age, animals were picked at random from the three treatment groups and bled from the tail for the determination of serum IGF-I. Serum was stored at –20°C until required for analysis.

Experiment 2, Male F344 Rats

Seventy-five 18-month-old male F344 rats were divided into three weight-matched groups of 25 rats per group. Group 1 served as controls and received solvent vehicle; Groups 2 and 3 received injections of 50 µg and 1.0 mg GH per kg body weight, respectively, two times per week. The 1.0 mg dose was used because of a report that it increased longevity in mice (22). Hormone and solvent vehicle injections were given subcutaneously starting from 18 months of age. As in Experiment 1, animals were bled at 3 months, 18 months, and 28 months of age for the determination of serum IGF-I, and serum was separated and stored at –20°C until required for analysis.

Experiment 3, Male Balb/c Mice

Forty 17-month-old male Balb/c mice were divided into two weight-matched groups of 20 mice per group. Group 1 served as controls and received solvent vehicle; Group 2 received injections of 1.0 mg GH per kg body weight two times per week. Hormone and solvent vehicle injections were given subcutaneously starting from 17 months of age.

Experiment 4, Female Balb/c Mice

Forty 17-month-old female Balb/c mice were divided into two weight-matched groups of 20 mice per group. Group 1 served as controls and received solvent vehicle; Group 2 received injection of 1.0 mg GH per kg body weight two times per week. Hormone and solvent vehicle injections were given subcutaneously starting from 17 months of age.

Experiment 5, Female Balb/c Mice

Forty 22-month-old female Balb/c mice were divided into two weight-matched groups of 20 mice per group. Group 1 served as controls and received solvent vehicle; Group 2 received subcutaneous injections of 1.0 mg GH per kg body weight two times per week. Hormone and solvent vehicle injections were given subcutaneously starting from 22 months of age.

In all studies, all animals were weighed and their food intakes monitored at monthly intervals. GH and solvent vehicle injections were continued from the beginning of the study until all the animals died spontaneously. The recombinant human GH used in these studies was kindly provided by Genentech, Inc. (South San Francisco, CA).

Serum IGF-I Assay

Serum IGF-I was determined as in our previous studies (24). Briefly, IGF-I was extracted from serum with the acid-ethanol extraction procedure, and immunoreactive IGF-I was measured on diluted serum extracts with an radioimmunoassay (RIA) kit obtained from Nichols Institute (San Juan Capistrano, CA).

Physical Appearance of Animals

At 26 months of age animals from control and GH-treated mice were photographed to record their physical appearance.

Procedure for Histopathologic Examination

Histopathologic analysis was carried out on organs and tissues from control and GH-treated male F344 rats. The following organs and tissues were removed for histopathologic examination following spontaneous death: brain, pituitary gland, heart, lungs, trachea, esophagus, kidneys, adrenal glands, salivary glands, pancreas, testes, urinary bladder, thymus, spleen, liver, and thyroid gland. The heart, brain, liver, lung, spleen, kidneys, thymus, adrenal glands, pituitary gland, and testes were weighed and fixed immediately in 10% neutral buffered formalin. Other organs were not weighed but were fixed immediately. The fixed organs were embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin-eosin. The microscopic slides were evaluated by two pathologists without knowledge of the groups.

The frequency of lesions and grade of lesions were analyzed with Fisher's exact test (25).

Grading of Lesions

Chronic nephropathy.—The grading of kidney lesions was performed according to Yu and colleagues (26) and is briefly described here: Grade 0, no lesions; Grade 1, lesions of minimal severity primarily involving glomerular capillary basement membrane and mesangial matrix including an occasional hyaline cast; Grade 2, lesions of mild severity involving glomerular capillary basement membrane, mesangial matrix, and the invariable presence of tubular proteinaceous casts; Grade 3, lesions of moderate severity involving the same structures as Grade 2, but more extensively, plus thickening of Bowman's capsule, lymphocyte infiltration, and mild interstitial fibrosis; Grade 4, very severe lesions involving all the structures described for Grade 3, but more marked, plus segmental or diffuse glomerular sclerosis and frequent adhesion of glomerular tuft to Bowman's capsule; Grade E, end-stage lesions involving widespread glomerular sclerosis, obsolescence of glomeruli, diffuse interstitial fibrosis, frequent calcifications, and marked tubular dilatation with numerous proteinaceous casts.

Cardiomyopathy.—Grade 0, no lesions; Grade 1, occasional focal myocardial degeneration with atrophy and vacuolation of muscle fibers plus minimal fibrosis; Grade 2, frequent focal myocardial degeneration with extensive fibrosis; Grade 3, widespread and confluent myocardial degeneration involving massive fibrosis with occasional calcification.

Hepatic bile duct hyperplasia.—Grade 0, no lesions; Grade 1, number of bile ducts is increased in only a few of the portal areas (less than 10%) with slight thickening of duct basement membrane; Grade 2, the number of bile ducts is increased in 10% to 30% of the portal areas with occasional involvement of fibrosis and lymphocytic infiltration; Grade 3, the number of bile ducts is increased in more than 30% of the portal areas with extensive fibrosis.

Hepatic fatty change.—Grade 0, no lesions; Grade 1, a few small fat droplets in hepatocytes near portal area; Grade 2, many moderate sized fat droplets in hepatocytes near portal area as well as the midzonal region; Grade 3, many large fat droplets in hepatocytes diffusely distributed throughout the liver.

Leukemia.—Grade 0, no lesions; Grade 1, lesion involved only in spleen; Grade 2, lesion involved two organs including spleen; Grade 3, lesion involved more than 3 organs.

Statistics

In all longevity studies, the Kaplan-Meier (27) statistical method was used to estimate and compare survival curves among groups; the log-rank test (27) was used to assess the equality of survival functions across strata, and the quantile test (28) was used to examine whether the strata has the same quantile survival. Other data analysis involved estimation of means and standard errors and analysis of variance (ANOVA) (29). One-way ANOVA was performed using the SAS statistical package (Sas Institute, Inc., Cary, NC). When the ANOVA indicated significant mean differences among doses of GH or times, the differences between the doses or times were evaluated by Tukey-Kramer multiple comparison test (30). A value of $p \leq .05$ was considered statistically significant.

Results

Experiment 1, Female F344 Rats

Body weight and food intake.—Experiment 1 consisted of three groups of female F344 rats with a beginning age of 18 months. Group 1 served as control and received solvent vehicle; Groups 2 and 3 initially received 2 mg and 8 mg GH per kg body weight 3 times per week, respectively. Both doses increased the body weights of Groups 2 and 3 rats within 1 week (Figure 1A). However, from 1 to 3 weeks the increase in body weight was not dose dependent, and this

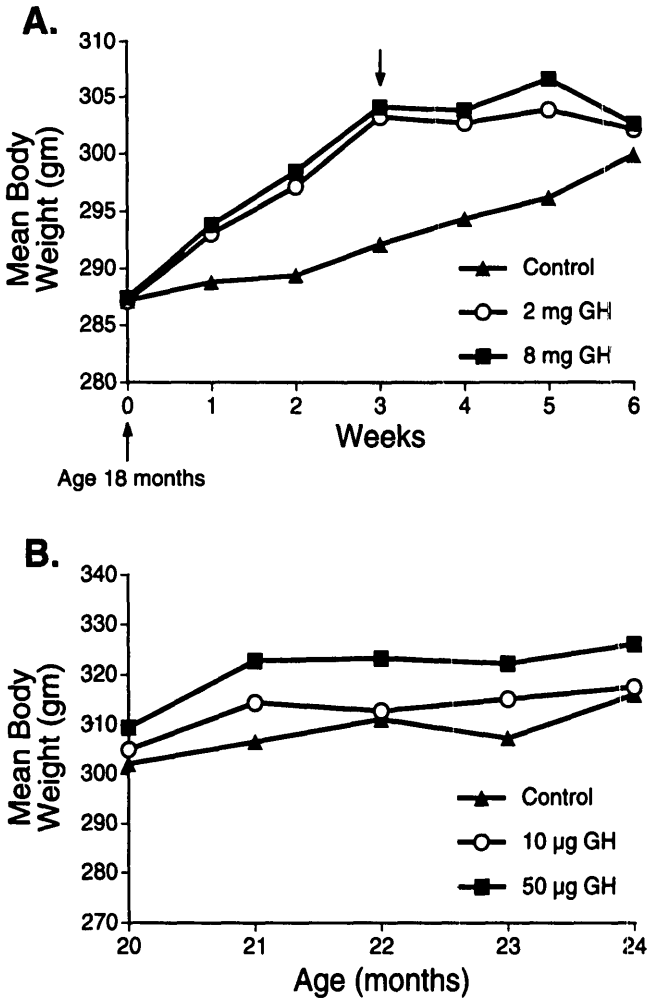

Figure 1. Determination of the appropriate dose of GH for long-term GH therapy in rodents. The arrow pointing downwards indicates when GH administration was suspended (Figure 1A). GH administration was resumed with lower doses at 20 months of age (Figure 1B).

suggested that the doses might be too high. As a result GH therapy was suspended and no further injections were given until the body weights of the GH-treated animals returned to the level of the control animals 3 weeks later. GH therapy was then resumed at the lower doses of 10 μg and 50 μg GH per kg body weight 3 times per week for animals in Groups 2 and 3, respectively, starting at 20 months of age (Figure 1B). These doses are in the range of doses that are given to humans in clinical studies (1). At these lower doses the increase in the body weights of rats due to GH resumed, and in a dose-dependent manner, with the 50-μg dose causing a greater increase in body weight than the 10-μg dose (Figure 1B). These lower doses and solvent vehicle injection to control animals were then maintained throughout the study.

The body weights of control animals continued to increase from 18 months of age until about 26 months of age (Figure 2A); subsequently, the control animals still surviving experienced a progressive loss of body weight with advancing age. In contrast, the GH-treated animals did not experience age-related loss of body weight, and the 50 μg GH group maintained higher body weights than the 10 μg GH group for a substantial part of the experimental period (Figure 2A). The apparent increase in the mean body weight of the 10 μg GH group from 27 months of age was due to selective deaths of animals with low body weights. There was no statistical difference between the amounts of food ingested per day by control and GH-treated rats (Figure 2B).

Longevity.—The survival curves for the rats in Experiment 1 are shown in Figure 2C. The mean life spans of the control group, the 10 μg GH group, and the 50 μg GH group are 29.1, 28.6, and 28.7 months, respectively; their median life spans are 29.9, 28.4, and 28.1 months, with a 95% confidence interval of 28.4–30.8, 27.7–29.2, and 27.2–29.6 months, respectively; their 10th percentile survival times are 32.6, 33.4, and 32.5 months, with a 95% confidence interval of 32.5–36.1, 31.3–35.6, and 31.7–37.6 months, respectively; and their maximum life spans are 36.1, 35.6 and 37.6 months, respectively. A quantile test (28) indicates that there is no significant difference in either median life span or the 10th percentile survival time among the three groups; a log-rank test of equality of survival functions across strata (27) indicates that there is no significant difference in survival among the three groups (p = .6666), and a Kruskal-Wallis test (28) indicates that there is no significant difference in their mean life spans.

Serum IGF-I, female F344 rats.—Serum IGF-I levels decreased between 3 and 29 months of age, and GH therapy reversed the decrease in a dose dependent manner, with the 50 μg dose returning the serum IGF-I level of 29-month-old female F344 rats to that of 3-month-old animals (Figure 3A).

Experiment 2, Male F344 Rats

Body weight and food intake.—Experiment 2 consisted of three groups of male F344 rats with a beginning age of 18 months. Group 1 served as controls and received solvent vehicle, and Groups 2 and 3 were treated with 50 μg and 1.0 mg GH, respectively, two times per week. After an initial increase in body weight in the first 2 months, all animals experienced a slow but progressive loss of body weight with no significant difference between the groups (Figure 4A). There was no significant difference between the amounts of food ingested by control and GH-treated rats (Figure 4B).

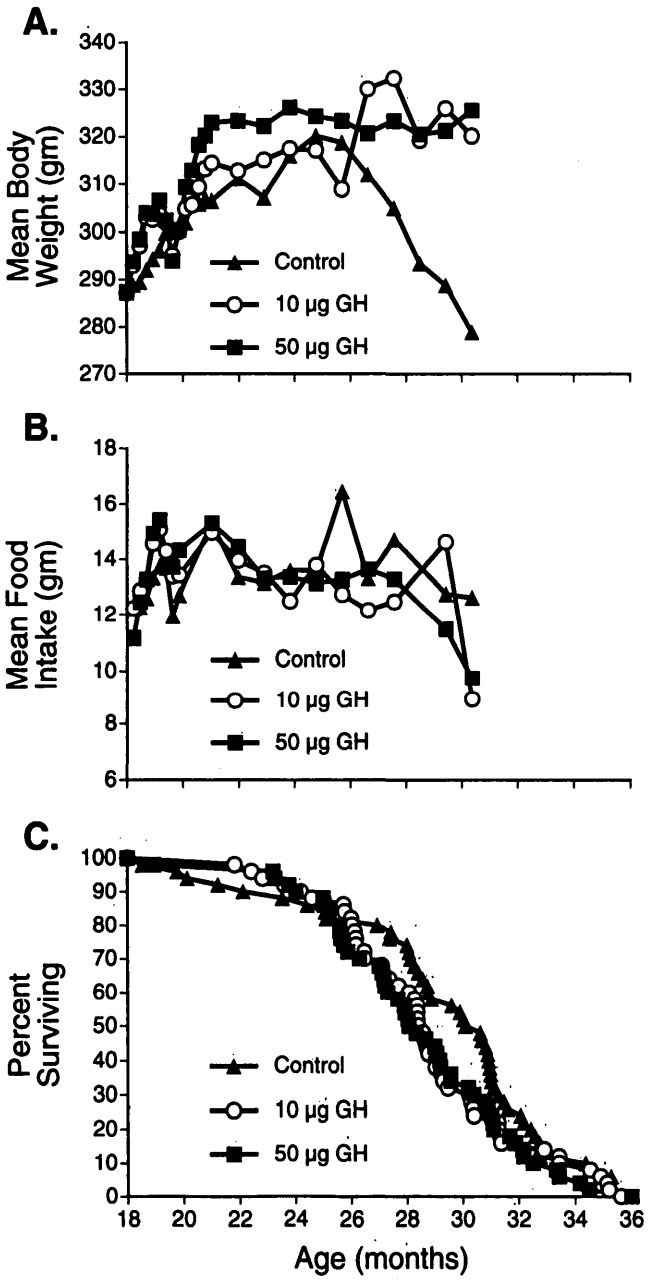

Figure 2. Effects of long-term low-dose GH therapy on body weight, food intake, and longevity in female F344 rats. In panels A and B each point is the mean of data from animals in the different experimental groups. In panel C each point represents one animal death. In this and subsequent figures, mean body weight and mean food intake data were no longer plotted when there were too few animals remaining to provide meaningful data, and standard error bars were excluded for clarity. A log-rank test of equality of survival functions across strata (27) indicates that there is no significant difference in survival among the three groups (p = .6666), and a Kruskal-Wallis test (28) indicates that there is no significant difference in their mean life spans.

Longevity.—The survival curves for the rats in Experiment 2 are shown in Figure 4C. The mean life spans for the control group, the 50 μg GH group, and the 1.0 mg GH group are 27.6, 26.7, and 27.3 months, respectively; their median life spans are 27.8, 27.1, and 28.2 months, with a 95% confidence interval of 25.2–29.5, 24.7–28.8, and 25.1–29.8 months, respectively; their 10th percentile survival times are 30.4, 31.1, and 31.7 months, with a 95% confidence interval of 30.0–34.0, 30.6–33.2, and 31.7–34.2 months, respectively; and their maximum life spans are 34.0, 33.2, and 34.2 months, respectively. A quantile test (28) indicates that there is no significant difference in median life span or the 10th percentile survival time among the three groups; a log-rank test of equality of survival functions across strata (27) indicates that there is no significant difference in survival among the three groups (p = .7374), and a Kruskal-Wallis test (28) indicates that there is no significant difference in their mean life spans.

Serum IGF-I, male F344 rats.—Serum IGF-I level was highest in the youngest animals (3-month-old) and decreased progressively with aging (Figure 3B). GH therapy reversed the age-related decrease with the 1.0-mg dose returning the serum concentration to the levels observed in 3-month-old animals.

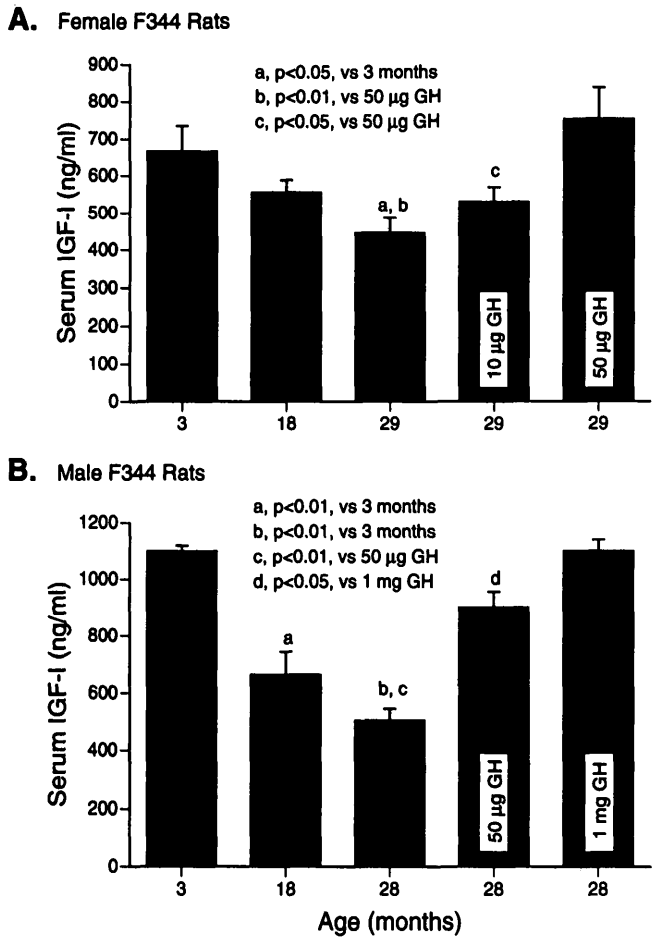

Figure 3. Effects of aging and long-term low-dose GH therapy on serum IGF-I levels in F344 female (A) and male (B) rats. Each bar is mean ± standard error. Male and female GH-treated rats and their controls were bled for serum IGF-I determination at times that were close to the median life span of the respective controls. One-way ANOVA was employed in the data analysis. In one ANOVA, time (3, 18, and 29 months for females; 3, 18, and 28 months for males) was the main effect. In another ANOVA, dose of GH (0 μg GH, 10 μg GH, and 50 μg GH for females; 0 μg GH, 50 μg GH, and 1 mg GH for males) was the main effect. The number of rats examined was 5 to 6 (A) and 7 to 10 (B). Rats were picked at random, except at 28 months (B), when all the rats still living were studied.

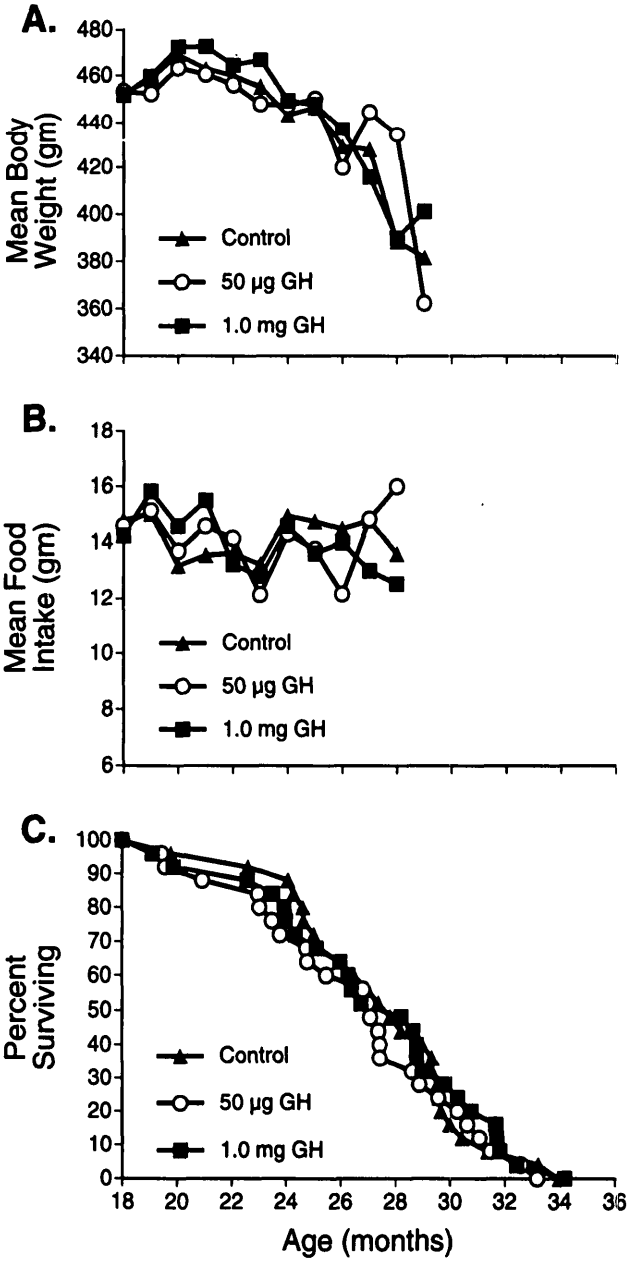

Figure 4. Effects of long-term low-dose GH therapy on body weight, food intake, and longevity in male F344 rats. In panels A and B each point is mean of data from animals in the different experimental groups. In panel C each point represents one animal death. A log-rank test of equality of survival functions across strata (27) indicates that there is no significant difference in survival among the three groups (p = .7374), and a Kruskal-Wallis test (28) indicates that there is no significant difference in their mean life spans.

Experiment 3, Male Balb/c Mice

Body weight and food intake.—Experiment 3 consisted of two groups of male Balb/c mice with a beginning age of 17 months. Group 1 served as controls and received solvent vehicle, and Group 2 received 1.0 mg GH per kg body weight two times per week. The solvent vehicle–treated control animals experienced a progressive decrease in body weight with aging, and the decrease was retarded by GH therapy (Figure 5A). The level of mean food intake among the groups exhibited a periodic variation with time, and there was no significant difference between the intakes of the control and the GH-treated mice (Figure 5B).

Longevity.—The survival curves for the rats in Experiment 3 are shown in Figure 5C. The mean life spans for the control group and the 1.0 mg GH group are 24.7 and 26.4 months, respectively; their median life spans are 22.6 and 26.2 months, with a 95% confidence interval of 19.8–29.3 and 23.5–28.5 months, respectively; their 10th percentile survival times are 32.6 and 32.8 months, with a 95% confidence interval of 30.2–34.3 and 29.1–35.2 months, respectively; and their maximum life spans are 34.3 and 35.2 months, respectively. Although the median life span of GH-treated mice was 3.6 months more than that of control animals, a quantile test (28) indicates that there is no significant difference in either median life span or the 10th percentile survival time among the two groups; a log-rank test of equality of survival functions across strata (27) indicates that there is no significant difference in survival among the two groups (p = .4275), and a Kruskal-Wallis test (28) indicates that there is no significant difference in their mean life spans.

Experiment 4, Female Balb/c Mice

Body weight and food intake.—Experiment 4 consisted of two groups of female Balb/c mice with a beginning age of 17 months. Group 1 served as controls and received solvent vehicle and Group 2 received 1.0 mg GH per kg body weight two times per week. In the solvent vehicle–treated control animals, an initial drop in body weight commonly associated with solvent vehicle injection (Orhii and Kalu, personal observation) was followed by an increase in body weight up to 20 months of age (Figure 6A). Subsequently, body weights declined and the decline was prevented by GH administration. The apparent increase in body weight in control animals from 25 months of age was due to selective deaths of animals with low body weights. GH-treated animals maintained higher body weights for a substantial part of the experimental period. The level of mean food intake exhibited periodic variations with time, and there was no significant difference between the intakes for the control and the GH-treated mice (Figure 6B).

Longevity.—The survival curves for the rats in Experiment 4 are shown in Figure 6C. The mean life spans of the control group and the 1.0 mg GH group are 25.6 and 25.2 months, respectively; their median life spans are 24.4 and 24.1 months, with a 95% confidence interval of 21.4–28.6 and 21.9–28.7 months, respectively; their 10th percentile survival times are 31.7 and 30.2 months, with a 95% confidence interval of 28.6–38.5 and 28.7–36.6 months, respectively; and their maximum life spans are 38.5 and 36.6 months, respectively. A quantile test (28) indicates that there is no significant difference in either median life span or the 10th percentile survival time among the two groups; a log-rank test of equality of survival functions across strata (27) indicates that there is no significant difference in survival among the two groups

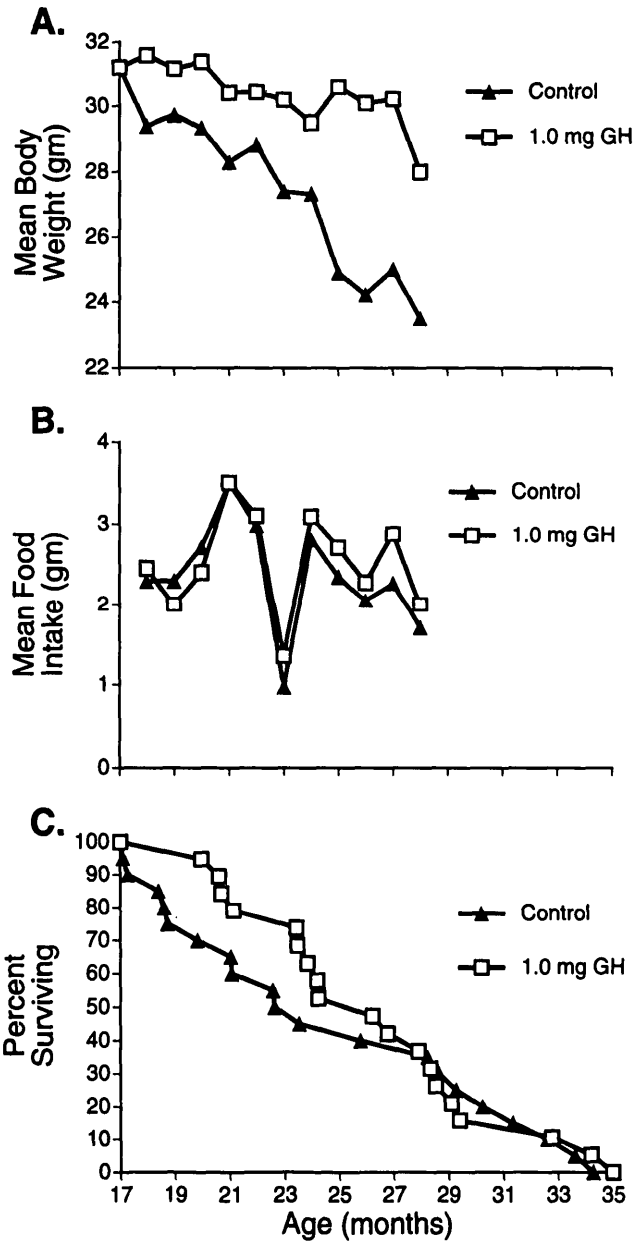

Figure 5. Effects of long-term low-dose GH therapy on body weight, food intake and longevity in male Balb/c mice. In panels A and B each point is the mean of data from animals in the different experimental groups. In panel C each point represents one animal death. One mouse died from the GH treated group just before the initiation of injections. A log-rank test of equality of survival functions across strata (27) indicates that there is no significant difference in survival among the two groups (p = .4275), and a Kruskal-Wallis test (28) indicates that there is no significant difference in their mean life spans.

(p = .8334), and a Kruskal-Wallis test (28) indicates that there is no significant difference in their mean life spans.

Experiment 5, Female Balb/c mice

Body weight and food intake.—In Experiment 4, GH administration was started at 17 months of age and the female control mice continued to increase in weight with aging. For this reason, Experiment 5 was carried out with older animals whose body weights had stabilized and were no longer increasing with age. Therefore, Experiment 5 consisted of two groups of female Balb/c mice with a beginning age of 22 months. Group 1 served as controls and received solvent vehicle injections, and Group 2 received 1.0 mg GH per kg body weight two times per week. The body weights of the animals were stable up to 25 months of age following which there was a progressive age-related decrease in body weight that was not modulated by GH therapy (Figure 7A). As in all

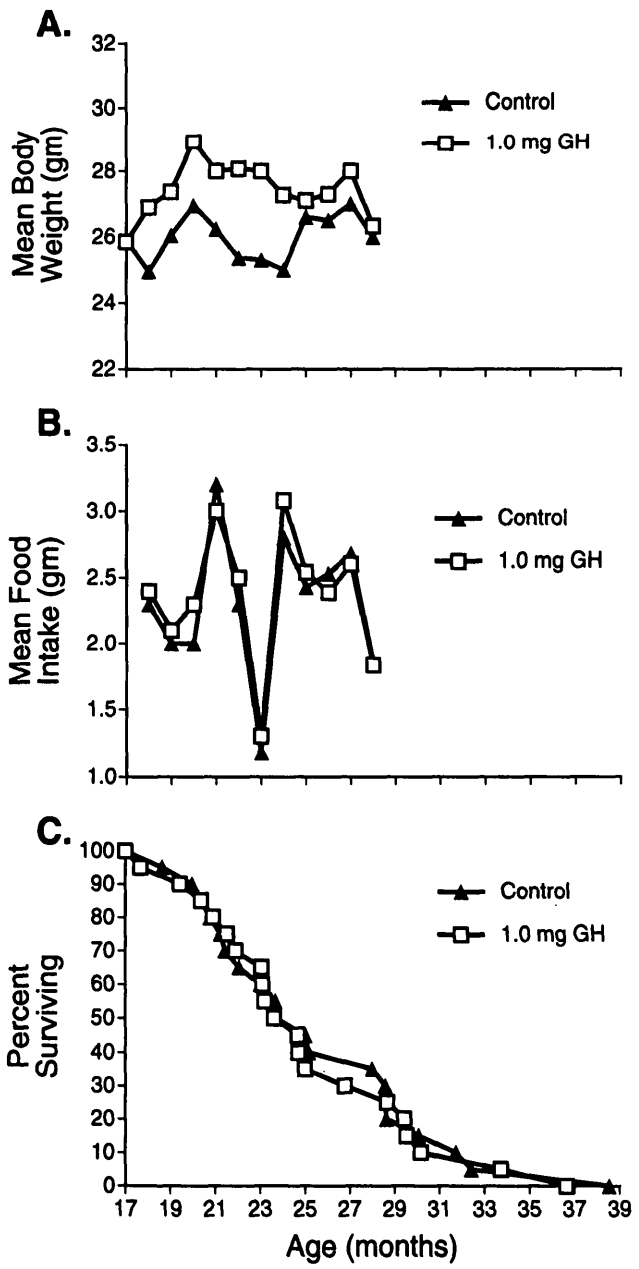

Figure 6. Effects of long-term low-dose GH therapy on body weight, food intake, and longevity in female Balb/c mice with a beginning age of 17 months. In panels A and B each point is the mean of data from animals in the different experimental groups. In panel C each point represents one animal death. A log-rank test of equality of survival functions across strata (27) indicates that there is no significant difference in survival among the two groups (p = .8334), and a Kruskal-Wallis test (28) indicates that there is no significant difference in their mean life spans.

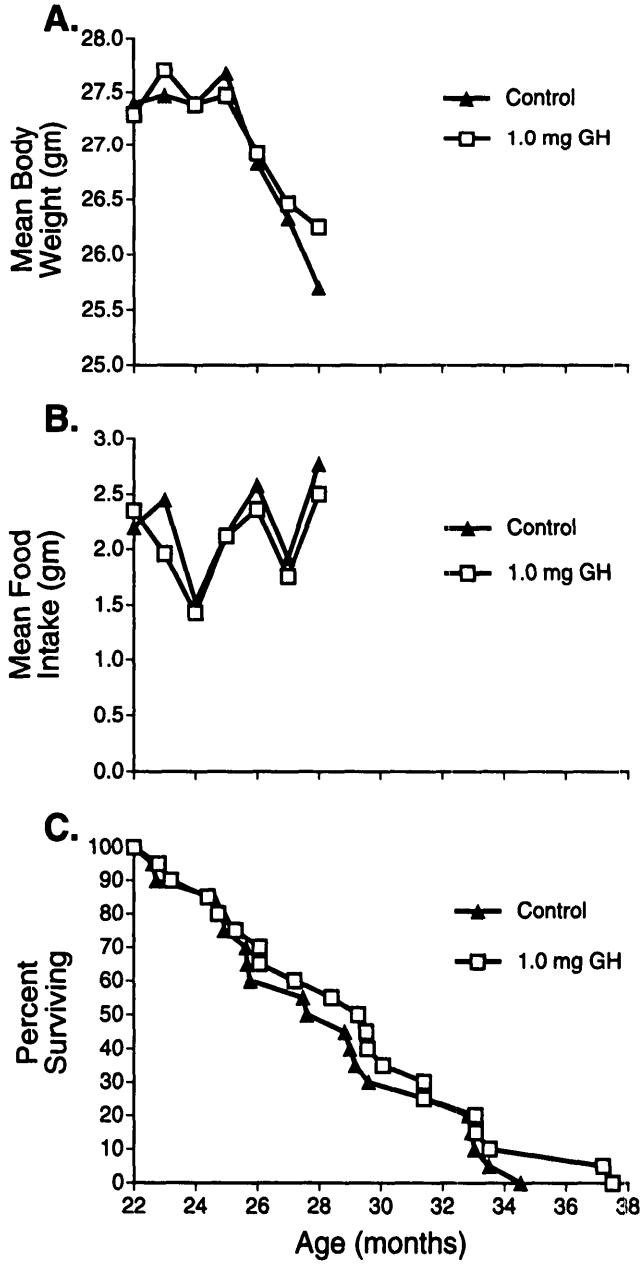

Figure 7. Effects of long-term low-dose GH therapy on body weight, food intake, and longevity in female Balb/c mice with a beginning age of 22 months. In panels A and B each point is the mean of data from animals in the different experimental groups. In panel C each point represents one animal death. A log-rank test of equality of survival functions across strata (27) indicates that there is no significant difference in survival among the two groups (p = .3097), and a Kruskal-Wallis test (28) indicates that there is no significant difference in their mean life spans.

our studies with mice, the level of mean food intake exhibited periodic variations with time and there was no significant difference between the intakes for the controls and the GH-treated mice (Figure 7B).

Longevity.—The survival curves for the mice in Experiment 5 are shown in Figure 7C. The mean life spans of the control group and the 1.0 mg GH group are 28.3 and 29.2 months, respectively; their median life spans are 28.2 months and 29.4 months, with a 95% confidence interval of 25.6–31.4 and 26.1–31.4 months, respectively; their 10th percentile survival times are 33.0 and 33.5 months, with a 95% confidence interval of 31.4–34.5 and 31.4–37.5 months, respectively; and their maximum life spans are 34.5 and 37.5 months, respectively. A quantile test (28) indicates that there is no significant difference in either median life span or the 10th percentile survival time among the two groups; a log-rank test of equality of survival functions across strata (27) indicates that there is no significant difference in survival among the two groups (p = .3097), and a Kruskal-Wallis test (28) indicates that there is no significant difference in their mean life spans.

Histopathologic Examination

Histopathologic observations on organs and tissues obtained from control and GH-treated F344 male rats are summarized in Table 1 and briefly described as follows.

Chronic nephropathy.—The severity of nephropathy increased with age (Table 1). In the control group, rats dying before 26 months of age did not have lesions more severe than Grade 2. In the low-dose GH group, one of nine rats dying before 26 months of age had Grade 3 lesion and the rest did not have lesions more severe than Grade 2. The percentage of rats with lesions more severe than Grade 3 in the control, low-dose GH, and high-dose GH groups was 52%, 40%, and 36%, respectively.

Cardiomyopathy and atrial thrombosis.—Data on cardiomyopathy and atrial thrombosis are shown on Table 1. In the control group, one rat had Grade 3 cardiomyopathy and the myocardium was severely destroyed by infiltration of leukemic cells. The percentage of rats with Grade 1 lesion in the control, low-dose GH, and high-dose GH groups was 82.6%, 85%, and 91.3%, respectively. The occurrence of atrial thrombosis in the control, low-dose GH, and high-dose GH groups was 13%, 30%, and 12.5% respectively.

Bile duct hyperplasia and fatty change.—Data on bile duct hyperplasia are shown on Table 1. The percentage of rats with Grade 2 lesion or higher in the control, low-dose GH, and high-dose GH groups was 26%, 10%, and 15%, respectively. As shown on Table 1, no rat had fatty change lesion more severe than Grade 1.

Neoplasms.—The data on the occurrence of neoplasms are shown on Table 1. Testicular interstitial cell tumor was distributed through all ages, and the prevalence of the tumor in the control, low-dose GH, and high-dose GH groups was 78.2%, 84.2%, and 82.6%, respectively. The percentage of animals with adrenal pheochromocytoma in the control, low-dose GH, and high-dose GH groups was 19%, 29%, and 20%, respectively, and the percentage with pituitary adenoma was 27.2%, 10%, and 16.6%, respectively. The occurrence of leukemia in the control, low-dose GH, and high-dose GH groups was 39%, 42%, and 52%, respectively. Other neoplasms detected include Islet cell adenoma, C-cell adenoma, bronchioalveolar adenoma, sarcoma, fibroma, fibroadenoma, bronchioalveolar carcinoma, papillary cyst adenoma of the thyroid, and metastatic neoplasm. As shown in Table 1, there were no significant group differences in the occurrence of these neoplasms.

Probable cause of death.—Probable cause of death was classified as due to non-neoplastic, neoplastic, and a combination of both lesions. The number of rats that were suspected to have died from the different lesions in the different groups is shown in brackets as follows. Control group: non-neoplastic [4], neoplastic [7], combined [2], out of 23 rats; low-dose GH group: non-neoplastic [11], neoplastic [5], combined [5], out of 21 rats; high-dose GH group: non-neoplastic [14], neoplastic [4], combined [0], out of 18 rats. Diagnosis of cause of death could not be made on 2 rats from the low-dose GH group and 6 rats from the high-dose GH group due to severe postmortum autolysis of tissues. Analysis of the frequency of lesions or grade of lesions with Fisher's exact test (25) revealed no significant differences between the three groups.

Power and Sample Size Estimation

A conservative estimate of noncentrality parameter (magnitude of mean differences) is used to calculate power and sample size (31) for each of Experiments 1 to 5. With the number of animals used in Experiments 1 to 5, we have 80% power to detect mean differences in survival of 2.6, 3.9, 5.4, 4.9, and 4.2 months, respectively.

Discussion

The biggest challenge in initiating this study was determining the appropriate dose of GH to administer to rodents in a long-term study. This is because there is no precedence of well-controlled studies in which recombinant human GH was given intermittently to rodents from adulthood until they died spontaneously. As a result, in this study the determination of the appropriate dose of GH for long-term therapy in rodents was incorporated into the first experiment. The appropriateness of the doses we settled on is evident from the data on serum IGF-I. Aging decreased serum IGF-I levels as has been previously reported in humans and rodents (1,32,33), and GH therapy reversed the decrease, with the 50-μg dose returning the level to that found in three-month-old animals. In addition, in the female F344 rats, but not in female mice that started receiving the hormone at 22 months of age, the decline in body weight that occurred with aging was prevented in the animals that received GH. Although GH therapy had similar salutary effects on body weight in male mice, GH did not protect against age-related decline in body weight in male rats,

Table 1. Lesions in Control and GH-Treated F344 Male Rats That Died Spontaneously

	18 to 26 months			26 to 30 months			> 30 months		
Type of Lesions	C (n = 9)	L (n = 9)	H (n = 9)	C (n = 9)	L (n = 9)	H (n = 9)	C (n = 5)	L (n = 6)	H (n = 6)
Nephropathy									
Grade 0	1	1	2	0	0	0	0	0	0
Grade 1	2	4	0	1	0	1	0	1	0
Grade 2	6	3	5	0	2	4	1	1	2
Grade 3	0	1	2	5	1	1	3	2	1
Grade 4	0	0	0	3	2	1	1	2	3
Grade E	0	0	0	0	0	0	0	0	0
Cardiomyopathy									
Grade 0	0	1	0	1	0	0	0	0	0
Grade 1	7	8	8	8	6	8	4	3	5
Grade 2	1	0	1	0	0	0	1	2	1
Grade 3	1	0	0	0	0	0	0	0	0
Atrial thrombosis	2	1	1	0	3	2	1	2	0
Bile duct hyperplasia									
Grade 0	2	2	2	4	0	1	1	2	3
Grade 1	4	6	4	3	4	5	3	4	2
Grade 2	3	1	1	2	1	0	1	0	1
Grade 3	0	0	0	0	0	1	0	0	0
Fatty change									
Grade 0	8	8	6	9	4	2	4	5	2
Grade 1	1	1	1	0	0	2	1	0	3
Grade 2	0	0	0	0	0	0	0	0	0
Grade 3	0	0	0	0	0	0	0	0	0
Leukemia									
Grade 0	3	5	4	8	1	4	3	5	2
Grade 1	2	1	4	1	3	1	2	0	3
Grade 2	1	2	0	0	0	0	0	0	0
Grade 3	3	1	1	0	0	1	0	1	1
Bronchioalveolar tumor	0	1	0	1	0	0	1	0	0
Testicular interstitial cell tumor	6	7	6	9	5	7	3	4	6
Pheochromocytoma	1	2	0	2	0	1	1	3	2
Pituitary adenoma	1	0	2	4	1	0	1	1	0
Pancreas Islet cell adenoma	1	0	0	0	0	0	0	0	0
Thyroid C-cell adenoma	1	1	0	0	0	0	0	0	1
Thyroid papillary adenoma	0	0	0	0	0	1	0	0	0
Sarcoma	0	0	1	1	0	0	0	0	0
Fibroma	0	1	1	1	1	0	0	2	0
Keratoacanthoma	0	1	0	0	0	0	1	0	0
Fibroadenoma	0	0	0	0	0	0	0	0	1
Metastatic tumor	0	1	0	1	0	0	0	0	0
Number of tumor-bearing rats	8	8	7	9	6	7	5	6	6

Notes: C, L, and H refer to control, low-dose GH, and high-dose GH groups, respectively. Fisher's exact test analysis (Siegel S. *Nonparametric Statistics for the Behavioral Sciences.* New York: McGraw Hill: 1956:175–179) revealed no significant difference between groups.

even though in these animals GH reversed the age-related decline in serum IGF-I levels and returned the serum IGF-I concentration of aged rats to the levels found in 3-month-old rats. Prolonged administration of a higher dose of GH (2.5 mg/kg body weight three times per week) increased body weight as well as food intake in rats (Orhii and Kalu, personal observation). The doses of GH used in the current studies range from 10 μg–1 mg/kg body weight two to three times per week. The protective effects of GH on age-related decline in body weight observed in some of the experiments were not due to alterations in food intake as there was no significant difference in the amounts of food ingested by control and GH-treated animals in all five studies. In earlier studies with bovine GH, it was reported that in rodents, GH administration did not prevent the loss of body weight due to aging (34,35). Our findings indicate that the effects of GH therapy on body weight in aging rodents are complex and may relate in part to gender, species, and the age at which GH therapy is initiated.

Although in Experiment 1 female F344 rats received 10 and 50 μg of GH per kg body weight three times per week for most of the experimental period, in Experiment 2 male F344 rats were given 50 μg and 1.0 mg of GH, and in the rest of the experiments, male and female mice received 1.0

mg of GH per kg body weight two times per week. The 1.0-mg dose was based on an earlier study in which Khansari and Gustad (22) gave 30 μg of GH per mouse two times per week, which is about 1.0 mg of GH per kg body weight in mice weighing approximately 30 g. This GH regimen was reported to increase longevity in male Balb/c mice when GH therapy was begun at 17 months of age (22). As a result of the enormous implications of such a finding, it was important to reproduce the Khansari-Gustad (22) study as closely as possible while excluding its shortcomings. There were at least four shortcomings: First, GH was available to Khansari and Gustad (22) in limited amounts and as a result, during the experimental period, GH administrations were given or suspended depending on availability. Second, mice were withdrawn periodically from the treatment groups to provide tissues for immunologic investigations which were the primary focus of their study. Third, a complete longevity study was not done and conclusions on extension of life span were based on partial data. Fourth, the average life span of the male Balb/c mice in the facility used in the Khansari-Gustad (22) study was only 21 months. All the animals in their control group had died by the age of 23.3 months. The short life span indicates that the mice in the Khansari-Gustad study were dying early, probably as a result of problems unrelated to aging per se and that GH might have modulated this problem rather than aging. In our current study with male Balb/c mice, their mean life span, median life span, 10th percentile life span, and maximum life span were 24.7, 22.6, 32.6, and 34.3 months, respectively.

In contrast to the findings of Khansari and Gustad (22), in our studies GH therapy had no significant effect on mean life span, median life span, 10th percentile life span and maximum life span in male and female Balb/c mice and male and female F344 rats given various doses of GH, including the dose given by Khansari and Gustad (22). However, it is of note that in the male Balb/c mice, which is the same strain and gender studied by Khansari and Gustad (22), the median life span of GH-treated animals was 3.6 months longer than that of control animals, but the difference was not statistically significant.

The lack of a significant effect of GH on longevity in our study is in contrast to several reports that sustained high levels of GH in transgenic mice shorten their life span (16–20). In these studies on transgenic animals, the levels of GH were excessively elevated lifelong, and in some animals to levels several orders of magnitude higher than normal, resulting in a variety of degenerative diseases (16–20). Transgenic and other animals models with excessive GH secretion are not appropriate for studying the role of GH in normal physiology or for studying the effects of intermittent low-dose GH as is used for therapy in humans. Although the rodent models we described in this study are more relevant to GH therapy in humans than transgenic animals overexpressing GH, long-term administration of recombinant human GH to rodents may lead to the formation of antibodies to the human GH and the abrogation of its biologic actions. Although we did not measure GH antibody titers in our studies, several findings indicate that the GH we administered remained bioactive. For instance, in many of our studies, GH-treated animals maintained higher body weights than controls for most of their life span. In addition, in the studies where serum IGF-I was measured, its levels were elevated in a dose-dependent manner in GH treated animals during the terminal part of the life span of the rats when serum IGF-I levels are normally decreased (32,33). The action of GH on the liver must have been sustained in these long-term studies, because most of the circulating IGF-I derive from the stimulatory action of GH on hepatic IGF-I production (36). In the study where GH failed to prevent age-related loss of body weight of male rats, IGF-I levels measured late in the life span were elevated, indicating that the lack of effect on body weight and on life span was not due to the neutralization of GH bioactivity by antibodies.

The perception that GH therapy may have a positive influence on life span derives, at least in part, from the following observations. First, hypophysectomy decreases the life span of rodents and replacement therapy with GH reduces mortality in such animals (35). Second, aging decreases GH secretion and circulating IGF-I levels and is associated with altered physiologic functions and body composition; some of these alterations are reversed by GH therapy (1,5). Third, GH therapy has been reported to improve psychological well-being (37,38). The above findings have contributed to the perception that maintaining levels of circulating GH or IGF-I in elderly people may delay aging and extend life span. However, experimental proof that GH or IGF-I therapy extends life span in humans or in experimental animals is lacking.

At 26 months of age the appearance of the skin of the control male Balb/c mice seemed deteriorated, with scanty fur in comparison to the GH-treated male mice of the same age (data not presented). In contrast, the physical appearance of the skin of the control aged female Balb/c mice was not as deteriorated as that of control aged male mice, and there were no visual differences in the skin and fur of the control and GH-treated female mice. These findings may be related to a stimulatory action of GH on skin connective tissue synthesis, but the reasons for the gender differences are unknown.

Sustained high levels of plasma GH in humans and experimental animals are associated with diverse side effects. Glomerulosclerosis, hepatocellularmegaly, myocardial fibrosis, and splanchnomegaly (39–41) have been reported in transgenic mice with high levels of plasma GH, and mammary epithelial proliferation was observed in primates following GH therapy (42). It has been suggested that the pathologic disorders are due to GH per se rather than to IGF-I (41,42), which is generally considered to mediate many of the actions of GH (43,44). In our study, GH was administered intermittently, and the resultant increase in serum IGF-I did not exceed the levels found in young normal rats. There are currently no reports on the pathologic evaluation of animals that died spontaneously following long-term therapy with low doses of GH in the range used for therapy in humans, as was used in the current study. We observed that in control, solvent-treated animals, the main pathologic lesions were nephropathy, cardiomyopathy, leukemia, and testicular interstitial cell tumor. These lesions differ from those commonly associated with chronic, elevated GH in transgenic ani-

mals, but they are similar to lesions that were reported to be prevalent in aged F344 male rats that died spontaneously (45). The prevalence of the lesions found in control animals was not significantly altered by GH therapy in this study. However, GH therapy was associated with decreased incidence of leukemia and pituitary adenoma, but the difference from controls was not statistically significant. Although GH therapy has been reported to increase the risk of leukemia (46,47), a cause-and-effect relationship between the two has not been established. Furthermore, in our study, analysis of the frequency and grade of other lesions revealed no significant difference between the GH-treated and control animals.

In conclusion, we have described rodent models for investigating the long-term effects of low-dose GH therapy that is relevant to long-term GH therapy in humans in clinical trials. Our pilot studies indicate that intermittent administration of GH in the range of 10 μg to 1.0 mg per kg body weight two to three times per week can be given long-term to rodents with no overt deleterious effects on longevity and pathology.

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