Reduced Levels of Thyroid Hormones, Insulin, and Glucose, and Lower Body Core Temperature in the Growth Hormone Receptor/Binding Protein Knockout Mouse

STEVEN J. HAUCK,*[,1] WILLIAM S. HUNTER,* NATASHA DANILOVICH,† JOHN J. KOPCHICK,‡ AND ANDRZEJ BARTKE*

Department of Physiology, Southern Illinois University School of Medicine, Carbondale, Illinois 62901–6512; †Clinical Research Institute of Montreal, Montreal PQ H2W 1R7, Canada; ‡Edison Biotechnology Institute and Department of Biomedical Sciences, College of Osteopathic Medicine, Ohio University, Athens, Ohio 45701

The mechanisms that are responsible for the extension of lifespan in the mouse with targeted disruption (knockout [KO]) of the growth hormone (GH) receptor/binding protein (GHR-KO) are unknown. However, in the long-living Ames dwarf mouse, blood glucose and body core temperature (T_{co}) are consistently lower than in normal mice. In addition, insulin levels are reduced and corticosterone levels are elevated in male dwarfs. These functional alterations, similar to those seen in animals under caloric restriction, have not been proven to be causally related to the extension of lifespan, but they do provide some insight into what traits may be necessary for long life. Therefore, to investigate which of these parameters are similarly affected in two genetically unrelated, yet similarly long-living mouse models, we measured T_{co}, thyroid hormones (triiodothyronine [T_3] and thyroxine [T_4]), and insulin, in addition to morning and afternoon levels of glucose and corticosterone, in young adult male and/or female GHR-KO mice and their normal siblings. T_{co} in GHR-KO mice was numerically reduced throughout the 24-hr period; however, these differences were only significant 4 hr prior to lights-off (14:00 hr), immediately after lights-off (18:00 hr), and during the 3 hr preceding lights on (03:00 to 06:00 hr). GHR-KO mice had significantly reduced levels of T_3 and T_4, while the ratio of these hormones was similar to that in normal mice. Insulin levels in GHR-KO mice were lower than in normal mice; levels in male GHR-KO mice were below the detectable limits of the assay used. Glucose levels in GHR-KO mice (male and females) were lower than in normal mice in measurements taken in both morning and afternoon; however, these differences arose from consistent reductions in males, as morning glucose levels in GHR-KO females were similar to those of normal mice. Corticosterone levels measured in blood plasma collected under basal (nonstressed) conditions showed sex-related alterations. Basal corticosterone levels in female GHR-KO mice were similar to normal females, while those in male GHR-KO mice were higher than in normal males in the afternoon. Corticosterone levels in stressed GHR-KO females were similar to those measured in stressed normal females. These data show that the long-living GHR-KO mouse shares a reduction in glucose, insulin, thyroid hormones, and T_{co} with the Ames dwarf mouse. Reductions in these parameters may be important to the underlying mechanisms of delayed aging in these animals.

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The mechanisms that control the aging process in higher organisms are complex and poorly understood. While considerable evidence for the genetic control of aging has been accumulated in lower organisms (1–3), there are few identified genetic mutations that positively affect longevity in mammals. Evidence from different long-lived animal models (4, 5) suggests that deficiencies in the growth hormone- (GH) insulin-like growth factor 1 (IGF-1) axis arising from genetic mutations seem to confer a longevity advantage over genetically normal animals. Although the likelihood of controlling the aging process by genetic manipulation is currently difficult to evaluate, it is important to elucidate phenotypic characteristics common among long-living mutants. It is also of considerable interest to compare these characteristics with those of calorically restricted animals in which aging is delayed and the lifespan is extended (Table I).

One example of genetic mutations in the GH axis that extends lifespan is found in the Ames dwarf mouse. These

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[1] To whom requests for reprints should be addressed at Southern Illinois University School of Medicine, Carbondale, IL 62901–6512. E-mail: shauck@siumed.edu

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Table I. Effects of Mutations that Prolong Lifespan (Ames Dwarf, Prop-1^{df} and Snell Dwarf, Pit-1^{dw}) Versus Effects of Caloric Restriction in the Mouse

	CR	Dwarf mice
Growth and size	↓	↓↓
IGF-1	↓	↓↓↓
Fertility	↓	↓↓↓
Body temperature	↓↓	↓↓↓
Glucose	↓	↓
Insulin	↓	↓
Thyroid hormones	↓	↓↓
Corticosterone	↑	↑[a]

[a] Males only.

mice have significantly reduced body size due to a homozygous recessive mutation at the Prop-1 locus, which leads to a lack of differentiation of somatotrophic, lactotrophic, and thyrotrophic pituitary cells (6). As a result, these mice lack GH, prolactin (PRL), and thyroid stimulating hormone (TSH) (7). Thus, in addition to their diminutive size, Ames dwarf mice are also hypothyroid and most are infertile (7, 8). However, despite these apparent disadvantages, Ames dwarf mice live remarkably longer than their normal siblings (4).

Another long-living mouse model that has deficiencies in the GH axis is the Snell dwarf mouse (9). The Snell dwarf has a recessive mutation at the Pit-1 locus that is unrelated to the mutation producing the Ames dwarf (10). This mutation, however, also leads to failure of differentiation of GH-, TSH-, and PRL-secreting cells. Thus, the Snell dwarf has an identical endocrine phenotype as the Ames dwarf (6–8, 10), and has also recently been shown to have a longer lifespan than its normal littermates (5). Although the mechanisms responsible for delayed aging in both of these dwarf mice are yet to be elucidated, these animals share a number of phenotypic characteristics with animals subjected to caloric restriction (CR). As such, the alterations common to both dwarf mice and CR can be suspected as being important for delaying the aging process.

Among the common characteristics of dwarf mice and CR animals are differences in GH and body size. In rats placed on a CR diet from weaning, GH hormone levels and body size are significantly lower than in *ad libitum* fed controls (11), although long-term CR preserves pulsatile GH release and thus eventually reverses GH suppression (12). In addition, CR significantly reduces levels of IGF-1 (12). In dwarf mice, GH is entirely absent and IGF-1 is consequently severely reduced (8). A second common characteristic among long-living mouse models is a reduction in body core temperature (T_{co}). Ames dwarf mice have consistently reduced T_{co} both during rest and following mild stress (14). Snell dwarf mice have reduced T_{co} and reduced metabolic rate (15, 16). CR animals also have reduced body temperature; however, they apparently have only transiently reduced metabolic rates (17). Differences in metabolism and T_{co} are consistent with the lack of TSH and the resulting hypothyroidism. Studies of CR animals indicate that thyroid hormones are reduced (18), and it has been similarly shown that induction of hypothyroidism increases the mean lifespan of normal rats (19). Yet another characteristic common among these animals is the regulation of insulin and glucose. Both glucose and insulin levels in CR animals have been shown to be consistently reduced (11, 20, 21). In the Ames dwarf, plasma glucose levels are lower than in normal mice, while plasma insulin is similar to normal mice in female dwarfs and reduced in male dwarfs (22).

Despite these similarities, the multiple endocrine deficits of the Ames and Snell dwarf mice make it difficult to interpret which characteristics may be a requisite for increased longevity and which are merely correlates. Recently, however, a new mouse model of GH deficiency was developed. These mice have targeted disruption (knockout [KO]) of the GH receptor/GH binding protein gene, thus eliminating GH signaling and greatly reducing IGF-1 levels, though GH levels are elevated (23). Homozygous (–/–) GHR-KO mice are small in size and have delayed puberty, although unlike the Ames dwarfs, both sexes are fertile (23, 24). Due to the very recent development of this line, its importance to the study of aging has only recently been reported. GHR-KO mice, like the Ames and Snell dwarfs, live significantly longer than either their heterozygous normal (+/–) or homozygous normal (+/+) littermates (25). Thus, with this single endocrine deficiency, the GHR-KO mouse may be a potentially useful and simpler model of increased longevity in mammals and should allow more specific identification of the endocrine and metabolic alterations that are important to extending lifespan.

The objectives of this study were to measure several metabolic and endocrine parameters in the GHR-KO mouse that are known to be affected by CR or altered in long-lived dwarf mice. These included measurement of T_{co} and thyroid hormones 3′,3,5-L-triiodothyronine (T_3) and thyroxine (T_4). Nonfasted morning and afternoon glucose and morning insulin levels were also measured in these mice. Previous reports have shown that levels of glucose in males (26) and females (27) are reduced in GHR-KO mice following an overnight fast, but no data are available on glucose levels in these mice in the fed state or at different times of the day. In addition to these measurements, the level of corticosterone under nonstressed (morning and afternoon) and stressed conditions was determined. Corticosterone, which is an important component in the regulation of glucose levels, may also play a role in the aging process (11, 18, 20).

Materials and Methods

Animals. GHR-KO (–/–) mice and their normal (N, +/+ or +/–) siblings were produced by mating –/– males to +/– females in our breeding colony of these animals derived from the original GHR/BP KO (22). The original strain was crossed with C57BL/6 × C3H hybrids and thereafter bred in a closed colony. Animals were maintained on a 12:12-hr light:dark cycle at 20° to 24°C and were fed and watered *ad*

libitum in the Southern Illinois University animal facility. For measurements of hormones, glucose, and T_{co}, 4- to 5-month-old male and/or female KO and normal mice were utilized. All experiments were approved by the University Animal Care and Utilization Committee, and were conducted in accordance with NIH animal care guidelines.

T_{co} Measurement. T_{co} was determined as previously described (14). Briefly, six female GHR-KO and normal littermates were anesthetized and a temperature-sensitive radio transmitter (XM-FH, Minimitter, Sun River, OR) was implanted into the abdominal cavity. Due to a limited number of receivers (three total), the measurements were conducted in four sets, each set on a separate day. For measurement of T_{co}, two mice (one GHR-KO and one normal) were transported in their home cage to the laboratory, each cage was placed on one of two telemetry readers (RA 1010, Minimitter), and the animals were allowed to acclimate for approximately 24 hr. For each of four trials (one GHR-KO and two normal mice, or the converse), T_{co} was recorded every 15 min for a 24-hr time period and was averaged per mouse for each hour.

Hormones/Glucose Levels. Trunk blood was collected by decapitation or by cardiac puncture, following Isoflurane anesthesia, and was placed in tubes containing a 6% EDTA solution (50–100 μl depending on the size of the mouse), and was centrifuged at 5000 rpm at 4°C for 15 min. Plasma was collected and stored frozen until use.

For measurement of basal levels of glucose and basal corticosterone, mice of both sexes were separated two to three per cage on the evening prior to sacrifice. On the day of sacrifice, the mice were removed from the room where they were housed and were taken to an immediately adjacent separate area where they were sacrificed by decapitation. To minimize stress response, the time from initial cage disturbance to decapitation was less than 30 sec for each mouse. For this study, the animals were sacrificed starting either 1.5 hr after lights on (07:30 hr) or at 1.5 hr before lights off (16:30 hr) using eight GHR-KO and eight normal mice of each sex per time period. Plasma corticosterone was measured by radioimmunoassay (RIA) using a commercial kit (ICN Biomedicals, Costa Mesa, CA). Plasma glucose was measured using a colorimetric assay kit (Sigma, St. Louis, MO).

Corticosterone levels were also measured in blood plasma, collected at 09:00 hr, under stress conditions. For this study, 10 GHR-KO and 10 normal female mice were lightly anesthetized with Isoflurane, placed in a novel cage for 5 min, anesthetized again, and bled by cardiac puncture.

For measurement of thyroid hormones and insulin, female and male mice were sacrificed between 08:00 and 10:00 hr, using 10 GHR-KO and 10 normal mice of each sex. Plasma levels of T_3 and T_4 were measured in females only by commercial RIA kit (ICN Biomedicals). Insulin levels in females were measured by ICN RIA kit, while in males, insulin levels were measured by RIA kit from Diagnostic Products Corporation (DPC, Los Angeles, CA).

All RIA measurements were performed in duplicate within the same assay. Variation between duplicate samples of less than 5% was considered acceptable. The sensitivity for these assays were corticosterone, 25 ng/ml; T_3, 25 ng/dl; T_4, 1 μg/dl; insulin (ICN), 3 μIU/ml; and insulin (DPC), 4 μIU/ml.

Statistical Analysis. Glucose and hormone measurement data were analyzed by ANOVA and Fisher's PLSD *post hoc* test for comparisons of more than two group means, and by Student's *t* test for comparisons of two means. For T_{co}, body temperature over each hour of the 24-hr recording period was averaged per mouse and was analyzed by Student's *t* test for comparison of GHR-KO versus normal mice. $P < 0.05$ was considered significant, and data are presented as the means ± SEM.

Results

T_{co} Measurement. Throughout the 24-hr period of recording, T_{co} in GHR-KO mice was numerically lower than in normal mice (Fig. 1). Over this period, average T_{co} in GHR-KO mice was 0.4°C lower than normal mice (35.9° versus 36.3°C, respectively). However, these differences were statistically significant for only 6 hr during the 24-hr recording. These occurred during a single hour 4 hr prior to lights-off (14:00 hr), the initial hour after lights-off (18:00 hr), and during the 3 hr preceding lights on (03:00 to 06:00 hr).

Hormones/Glucose Levels. Plasma levels of T_3 in GHR-KO mice were approximately one-third lower than in the normal animals ($P < 0.0001$; Fig. 2a). Similarly, T_4 levels in GHR-KO mice were also reduced by approximately one-third ($P < 0.0005$; Fig. 2b). However, the ratio of T_3-to-T_4 in GHR-KO mice did not differ between the two groups ($P < 0.7974$; Fig. 2c).

Glucose levels in GHR-KO mice were reduced overall at both the morning and afternoon time periods when data

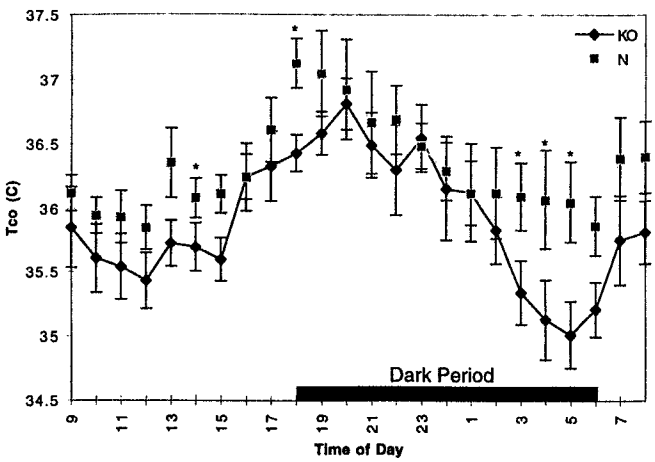

Figure 1. T_{co} plotted at 1-hr intervals of six female GHR-KO and normal mice. T_{co} in GHR-KO mice was reduced overall; however, these differences were significant only during two 1-hr periods at 1 and 4 hr before lights off and the 3 hr preceding lights on. Data reported are means ± SEM. *, Significantly different ($P < 0.05$).

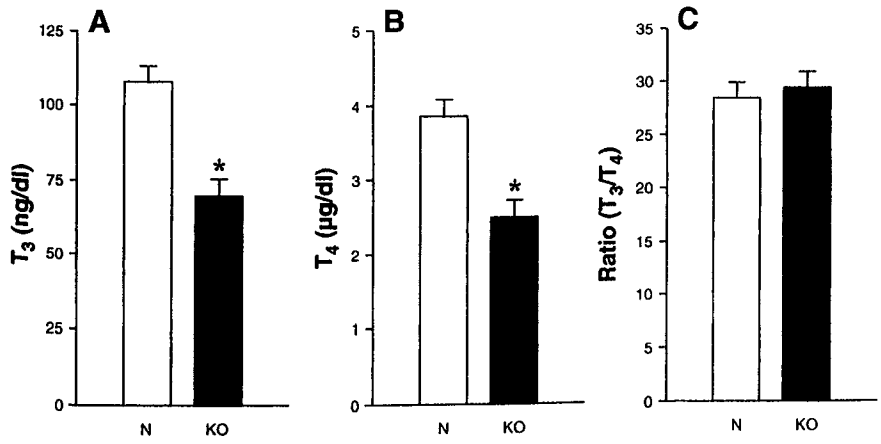

Figure 2. Thyroid hormones T_3 and T_4 in GHR-KO mice. GHR-KO mice had significantly reduced levels of T_3 (A) and T_4 (B) compared with normal mice, while the ratio of these hormones (C) was similar to normal mice. Data reported are means ± SEM. *, Significantly different ($P < 0.001$).

from males and females were combined ($P < 0.0008$ and < 0.0001, respectively; data not shown). However, these reductions were sex-dependent (Fig. 3). Glucose levels in normal mice tended to be higher in males than in females, while among GHR-KO mice these levels were similar between sexes. Male GHR-KO mice had reduced glucose levels when compared with normal males in both the morning and afternoon time measurements ($P < 0.0003$ and < 0.0001, respectively). Glucose levels in GHR-KO and normal male mice did not change significantly between the morning and afternoon determinations. In females, GHR-KO mice had normal levels of glucose in the morning ($P < 0.1345$). However, while glucose in normal females did not change significantly during the day, levels in GHR-KO females dropped significantly in the afternoon, such that this group had lower glucose than either afternoon normal females or morning GHR-KO females ($P < 0.0263$ and < 0.0009, respectively).

Plasma insulin levels in GHR-KO mice showed more consistent reductions. In males insulin levels in normal mice were 39.6 ± 9.19 µIU/ml, while insulin in GHR-KO males were below the delectability limit for the assay used (<4.0 µIU/ml; Fig. 4a). Normal females had apparently lower insulin levels than normal males (11.8 ± 2.95 µIU/ml). GHR-KO females had significantly lower insulin levels than normal females (3.964 ± 0.95 µIU/ml, $P < 0.0213$; Fig. 4b).

Corticosterone levels in GHR-KO mice were generally similar to the values measured in normal mice (Fig. 5a). As expected, in each group, plasma corticosterone levels were elevated in the afternoon versus morning; however, these apparent differences were significant only in GHR-KO males ($P < 0.0001$). Comparisons between genotypes revealed that corticosterone levels in females were similar between GHR-KO and normal mice, while in males, plasma levels tended to be higher in GHR-KO mice. However, this was significant only in the afternoon ($P < 0.0001$). Following moderate stress of repeated anesthesia and exposure to

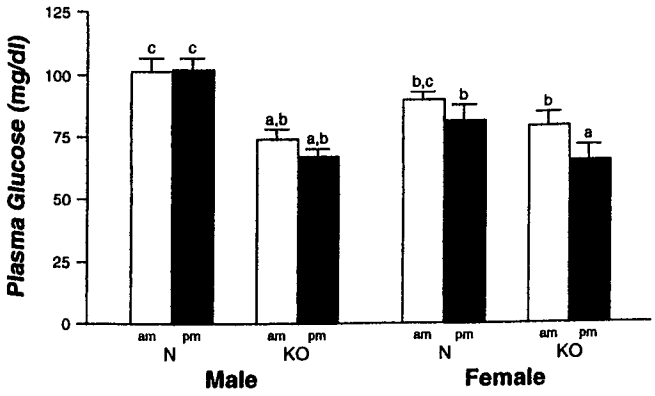

Figure 3. Morning and afternoon plasma glucose levels in male and female GHR-KO and normal mice. Glucose levels in GHR-KO mice (male and females) were lower than normal in measurements taken in both morning and afternoon; however, these differences arose from consistent reductions in the male, as morning glucose levels in GHR-KO females were similar to normal mice. Data reported are means ± SEM. a, b, and c: means that do not share a common superscript are significantly different ($P < 0.05$).

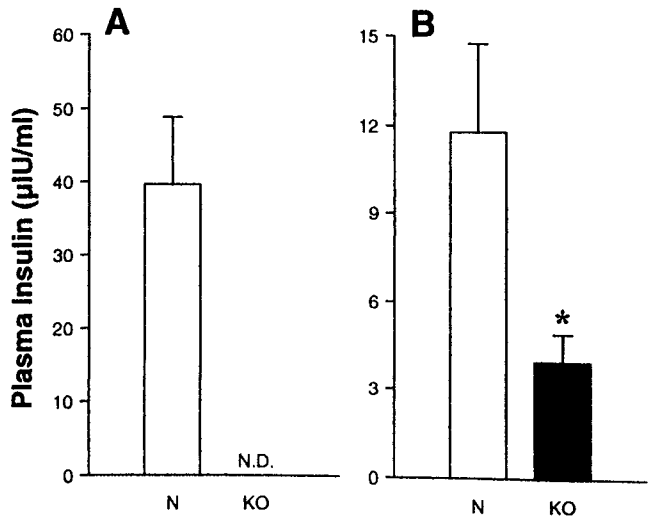

Figure 4. Plasma insulin levels in GHR-KO and normal male (A) and female (B) mice. Insulin in normal mice was significantly higher as compared to GHR-KO mice. Insulin levels in male GHR-KO mice failed to reach the limit of detection (4 µIU/ml) for the RIA kit used (Diagnostic Products Corp.). Normal female mice had apparently lower insulin levels than normal males, but significantly higher levels than GHR-KO females when using a more sensitive RIA (ICN Biomedicals). Data reported are means ± SEM. *, Significantly different ($P < 0.05$).

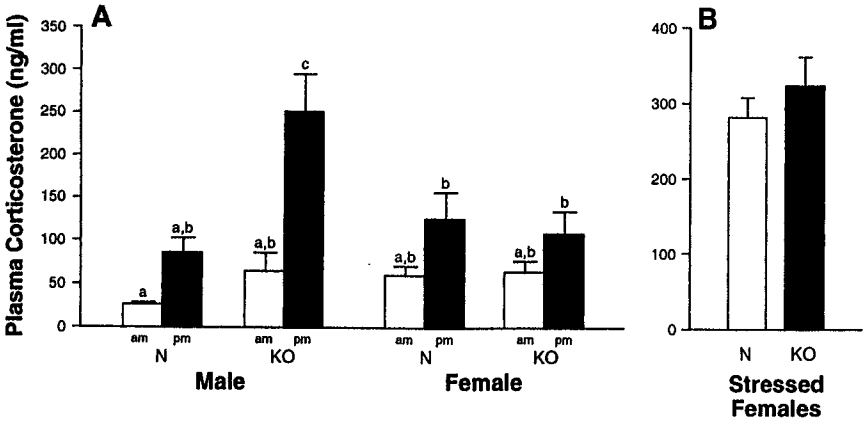

Figure 5. Corticosterone levels in GHR-KO and normal mice measured in males and females in both the morning and afternoon (A), and following moderate stress in females only (B). Data reported are means ± SEM. a, b, and c: means that do not share a common superscript are significantly different ($P < 0.05$).

a novel cage, GHR-KO and normal females responded with a substantial rise in corticosterone levels. Plasma corticosterone levels in stressed GHR-KO females were numerically higher than in stressed normal females, but this apparent difference was not statistically significant (Fig. 5b).

Discussion

Results of the present study reveal that GHR-KO mice share a number of characteristics with Ames dwarf mice and with animals subjected to CR. Since GHR-KO mice, Ames dwarf mice, and CR mice live substantially longer than the corresponding normal mice, common characteristics of these animals are of interest as putative mechanisms and/or markers of delayed aging and increased life expectancy. Among those common phenotypic parameters, GHR-KO mice have mild hypothyroidism, generally reduced body temperature, and reduced insulin and glucose levels. However, while the effects of GH resistance in the GHR-KO mice were qualitatively similar to the effects seen in the GH-, TSH-, and PRL-deficient Ames dwarf mouse and in CR animals, quantitatively, our findings in GHR-KO mice were often different from those in other models of increased longevity.

Results from measurement of T_{co} revealed that GHR-KO mice are only slightly hypothermic with respect to normal mice. While average T_{co} was significantly lower for six 1-hr intervals during the 24-hr recording period, during the remainder of the recording (i.e., 18 hr), T_{co} values measured in GHR-KO and normal mice were not significantly different. Presumably, reduced T_{co} is not among the key mechanisms of increased lifespan in GHR-KO mice. This contrasts with the findings in the Ames dwarf mouse in which T_{co} is consistently reduced (14). Lower body temperature resulting, perhaps, from reduced metabolic rate would potentially lead to the production of fewer free radicals, which are known to be related to the aging process (28). However, given the 30% to 50 % increase in lifespan seen in GHR-KO mice (26), the very moderate reductions in T_{co} reported here are quite unlikely to be responsible for such a dramatic effect.

Measurements of plasma T_3 and T_4 revealed that female GHR-KO mice are moderately hypothyroid when compared with their normal littermates. The consistent reductions in both thyroid hormones would suggest that there are no major alterations in thyroid function and that GHR-KO mice are only mildly hypothyroid. Thus, this long-lived model is again qualitatively similar to the Ames dwarf; however, quantitatively the phenotypic alteration is much milder in GHR-KO mice (29). These reductions are most likely secondary to GH resistance, since both GH and IGF-1 have facilliatory effects on the thyroid (8, 30). However, our results show that the presumably GH resistance-mediated reductions in thyroid hormone levels are mild when compared with those in the TSH and GH deficient Ames dwarf mouse.

The effects of GHR gene disruption on glucose metabolism and insulin levels measured in fed GHR-KO mice in the present study are not in complete agreement with reports in fasted GHR-KO mice (26, 27) or with results obtained in the Ames dwarf or CR animals (24, 13). Glucose levels in *ad libitum*-fed GHR-KO mice that were sacrificed in the morning were significantly reduced in males, but were only slightly and nonsignificantly lower in females when compared with normal mice. CR has consistently been shown to reduce glucose levels (11), and in the Ames dwarf, glucose levels are reduced in both sexes (22). However, the lack of reduced glucose levels in female GHR-KO mice in the morning may suggest a different diurnal pattern of glucose regulation in females than males, given the significant morning to afternoon reductions seen in female GHR-KO mice. Data on glucose levels in mice sacrificed in the afternoon, when the mice can reasonably be expected to have consumed little if any food during the immediately preceding light portion of the diurnal cycle, may be more comparable with the results obtained in fasted mice. Indeed, in the afternoon group, both male and female GHR-KO mice had significantly reduced glucose levels. Alternatively, other factors may have contributed to the differences between the results reported by Coschigano *et al.* (26) and those obtained in the present study, including the slightly different genetic background and use of both +/+ and +/− mice as normals, the assay method employed, or time and

method of blood collection. In contrast, insulin levels were consistently reduced in GHR-KO mice. Reduced insulin and glucose levels have been shown in CR mice (31). However, in young Ames dwarf mice, insulin is not reduced in either sex (22). Comparisons of data obtained in GHR-KO, dwarf, and CR mice suggest that increased responsiveness of plasma glucose levels to insulin is a common feature of these three types of long-living animals, while reductions in plasma glucose and insulin are dependent on nutritional state and are somewhat less consistently observed.

Corticosterone levels in GHR-KO mice were generally higher than in normal mice, with GHR-KO males having significantly elevated levels over normal males in the afternoon only. Ames dwarf males, like GHR-KO mice, have elevated corticosterone levels, while female dwarf mice have lower corticosterone than normal mice (22). Corticosterone has been reported to have a negative effect on neurons in the hippocampus, a brain area important for learning and memory as an animal ages (32). However, no deficits in learning and memory were detected in GHR-KO or in Ames dwarf mice; in fact, these animals exhibit a delay in memory decline with age (33). Chronic mild elevation of corticosterone is consistently observed in CR rats and mice and is suspected of having a role in mediating the beneficial "protective" effects of reduced dietary intake in these animals (34).

Thus, several phenotypic features shared by GHR-KO, dwarf, and CR mice emerge as possible mechanisms of increased lifespan in these animals. However, the primary mechanism that may ultimately be responsible for long life in these mice is likely to be the deficiency of GH signaling. In support of the possible importance of GH and IGF-1 in the context of aging, small body size within a species correlates strongly with increased longevity in mice (35), dogs (36), and apparently also in the human (37). It has been further suggested that genes that accelerate growth and reproductive fitness early in life also accelerate aging and reduce lifespan (38). Given the report that GHR-KO mice have an increased lifespan over their normal siblings (26), our findings implicate an important role of the GH-IGF-1 axis in the determination of lifespan in mammals. Therefore, mutations of genes in this axis may reduce growth and reproductive fitness and concomitantly delay aging and prolong life. While the many possible mechanisms that directly or indirectly link GH to aging have not been fully elucidated, it has been shown that mitogenic signaling, oxidative stress, and longevity in mammals may be very closely related. In mice with targeted disruption of the $p66^{shc}$ stress response gene, growth factor signaling is blocked, while resistance to reactive oxygen species (ROS)-induced stress and longevity are significantly increased (39). These suggest that the lack of GH signaling in GHR-KO mice may be a causal factor in their delayed aging that is, perhaps, related to the decreased production or increased removal of free radicals. More work is needed in order to investigate this intriguing possibility. Furthermore, these data show that while some similarities exist between GH-resistant, GH-deficient, and CR animals, some of their characteristics vary widely, and some parameters suspected of involvement in the aging process may be unchanged. This would seem to suggest that there is no single mechanism for increased longevity in these different models, and that multiple factors, genetic or nutritional, may interact to delay aging. We are currently investigating the effects of caloric restriction in dwarf mice to characterize some of these interactions.

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1. Ewbank JJ, Barnes TM, Lakowski B Lussier M Bussey H, Hekimi S. Structural and functional conservation of the *Caenorhabditis elegans* timing gene *clk-1*. Science **275:**980–983, 1997.
2. Arking R. Successful selection for increased longevity in *Drosophila*: Analysis of the survival data and presentation of a hypothesis on the genetic regulation of longevity. Exp Gerontol **22:**199–220, 1987.
3. Soliman MH, Lints FA. Longevity, growth rate and related traits among strains of *Tribolium castaneum*. Gerontologica **21:**102–116, 1975.
4. Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. Dwarf mice and the ageing process. Nature **384:**33, 1996.
5. Miller RA. Kleemeier award lecture: Are there genes for aging? J Gerontol **54:**B297–B307, 1999.
6. Sornson MW, Wu W, Dasen JS, Flynn SE, Norman DJ, O'Connell SM, Gukovsky I, Carriere C, Ryan AK, Miller AP, Zuo L, Gleiberman AS, Anderson B, Beamer WG, Rosenfeld MG. Pituitary lineage determination by the prophet of pit-1 homeodomain factor defective in Ames dwarfism. Nature **384:**327–333, 1996.
7. Bartke A. Histology of the anterior hypophysis, thyroid and gonads of two types of dwarf mice. Anat Rec **149:**225–235, 1964.
8. Bartke A. Genetic models in the study of anterior pituitary hormones. In: Shire JGM, Ed. Genetic Variation in Hormone Systems. Boca Raton, FL: CRC Press, pp113–126, 1979.
9. Snell GD. Dwarf, a new Mendelian recessive character of the house mouse. Proc Natl Acad Sci U S A **15:**733–734, 1928.
10. Li S, Crenshaw BE III, Rawson EJ, Simmons DM, Swanson LW, Rosenfeld MG. Dwarf locus mutants lacking three pituitary cell types results from mutations in the POU-domain gene pit-1. Nature **347:**528–533, 1990.
11. Weindruch R, Sohal SS. Caloric intake and aging. N Engl J Med **337:**986–994, 1997.
12. Sonntag WE, Xu X, Ingram RL, C'Costa A. Moderate caloric restriction alters the subcellular distribution of somatostatin mRNA and increases growth hormone pulse amplitude in aged animals. Neuroendocrinology **61:**601–608, 1995.
13. Breese CR, Ingram RL, Sonntag WE. Influence of age and long-term dietary restriction on plasma insulin-like growth factor 1 (IGF-1), IGF-1 gene expression, and IGF-1 binding proteins. J Gerontol **46:**B180–C187, 1991.
14. Hunter WS, Croson WB, Bartke A, Gentry MV, Meliska CJ. Low body temperature in long-lived Ames dwarf mice during rest and during stress. Physiol Behav **67:**433–437, 1999.
15. Schonholz DH, Osborn CM. Temperature studies in dwarf mice. Anat Rec **105:**605, 1949.
16. Gruneberg H. The Genetics of the Mouse. London: Cambridge University Press, 1943.
17. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. Science **273:**59–63, 1996.

18. Meites J. Anti-aging interventions and their neuroendocrine aspects in mammals. J Reprod Fertil **46**(Suppl):1–9, 1993.
19. Ooka H, Fujita S, Yoshimoto E. Pituitary-thyroid activity and longevity in neonatally thyroxine-treated rats. Mech Age Dev **22**:113–120, 1983.
20. Duffy PH, Feuers RJ, Hart RW. Effects of chronic caloric restriction on the circadian regulation of physiological and behavioral variables in old male B6C3F1 mice. Chronobiol Int **7**:291–303, 1990.
21. Masoro EJ. Dietary restriction. Exp Gerontol **30**:291–298, 1995.
22. Borg KE, Brown-Borg HM, Bartke A. Assessment of the primary adrenal cortical and pancreatic hormone basal levels in relation to plasma glucose and age in the unstressed Ames dwarf mouse. Proc Soc Exp Biol Med **210**:126–133, 1995.
23. Zhou Y, Xu BC, Maheshwari HG, He L, Reed M, Lozykowski M, Okada S, Cataldo M, Coschigano K, Wagner TE, Baumann G, Kopchick JJ. A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). Proc Nat Acad Sci U S A **94**:13215–13220, 1997.
24. Danilovich N, Wernsing D, Coschigano KT, Kopchick JJ, Bartke A. Deficits in female reproductive function in GH-R-KO mice: Role of IGF-1. Endocrinology **140**:2637–2640, 1999.
25. Choschigano KT, Clemmons D, Bellush LL, Kopchick JJ. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. Endocrinology **141**:2608–2613, 2000.
26. Coschigano KT, Riders ME, Bellush LL, Kopchick JJ. Glucose metabolism in growth hormone receptor/binding protein gene disrupted mice. 81st Annual Meeting of the Endocrine Society, San Diego, CA, **553**, 1999.
27. Dominici FP, Diaz GA, Bartke A, Kopchick JJ. Compensatory alterations of insulin signal transduction in liver of growth hormone receptor knockout mice. J Endocrinol **166**:579–590, 2000.
28. Harman D. The aging process. Proc Natl Acad Sci U S A **78**:7124–7128, 1981.
29. Brown-Borg HM, Borg KE, Bartke A. Introduction of bGH transgene to Ames dwarf mice compensates growth and produces a new model of isolated PRL deficiency. 77th Annual Meeting of the Endocrine Society, Washington, DC, **351**, 1995.
30. Jorgensen JOL, Ovesen P, Juul A, Hansen TK, Skekkebaek NE, Christiansen JS. Impact of growth hormone administration on other hormonal administration axes. Hormone Res **51**(Suppl 3):121–126, 1999.
31. Harris SB, Gunion MW, Rosenthal MJ, Walford RL. Serum glucose, glucose tolerance, corticosterone, and free fatty acids during aging in energy restricted mice. Mech Age Dev **73**:209–221, 1994.
32. Sapolsky RM. Why stress is bad for your brain. Science **273**:749, 1996.
33. Kinney BA, Coschigano KT, Kopchick JJ, Bartke A. Evidence that middle-aged growth hormone resistant GH-R-KO (Laron) mice have increased memory retention. Physiol Behav (in press) 2000.
34. Nelson JF, Karelus K, Bergman MD, Felicio LS. Neuroendocrine involvement in aging: Evidence from studies of reproductive aging and caloric restriction. Neurobiol Age **16**:837–843, 1995.
35. Roberts RC. The lifetime growth and reproduction of selected strains of mice. Heredity **16**:369–381, 1961.
36. Patronek GJ, Waters DJ, Glickman LT. Comparative longevity of pet dogs and humans: Implications for gerontology research. J Gerontol **52A**:B171–B178, 1997.
37. Samaras TT, Elrick H, Storms LH. Height, health and growth hormone. Acta Paediatr **88**:602–609, 1999.
38. Rollo CD. Phenotypes: Their Epigenetics, Ecology, and Evolution. London: Chapman and Hall, 1994.
39. Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, Lanfrancone L, Pelicci PG. The $p66^{shc}$ adaptor protein controls oxidative stress and life span in mammals. Nature **402**:309–313, 1999.