Disruption of the growth hormone receptor gene in adult mice increases maximal lifespan in females

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Growth hormone (GH) and insulin like growth factor I (IGF-I) are important for a variety of physiological processes including growth, development and aging. Mice with reduced levels of GH and IGF-I have been shown to live longer than wild type controls. Our laboratory has previously found that mice with a GH receptor gene knockout (GHRKO) from conception exhibit low rates of cancer, resistance to diet-induced diabetes, and extension of lifespan. The GHRKO mouse as well as other mouse lines with reduced GH action display low IGF-I levels, smaller body size, increased adiposity and increased longevity. To date, nearly all of these mouse strains carry germline mutations. Importantly, the effect of a long-term suppression of the GH/IGF-I axis during adulthood, as would be considered for human therapeutic purposes, has not been tested. The goal of this study was to determine if temporally controlled *Ghr* gene deletion in adult mice would affect metabolism and longevity. Thus, we produced adult-onset GHRKO mice (aGHRKO) by disrupting the *Ghr* gene at 6 weeks of age. We found that aGHRKO mice replicate many of the beneficial effects observed in long-lived GHRKO mice. For example, aGHRKO mice, like GHRKO animals, displayed retarded growth and high adiposity with improved insulin sensitivity. Importantly, female aGHRKO animals showed an increase in their maximal lifespan, while the lifespan of male aGHRKO mice was not different from controls.

Background

Evidence from worms to mammals indicates that reduced insulin/insulin-like growth factor-I (IGF-I)-like action increases lifespan (1). In mice and other mammals, the IGF-I pathway is tightly coupled to the activity of growth hormone (GH). GH binds to GH receptor (R) on cells in many tissues and initiates intracellular signaling cascades that ultimately result in the regulation of many physiological processes. In addition to stimulation of longitudinal growth, GH has several other roles, including: 1) promotion of lipolysis and inhibition of lipogenesis, 2) inhibition of insulin's action and 3) stimulation of IGF-I production by the liver and most other tissues (2). While IGF-I is an important mediator of GH action, GH and IGF-I have unique as well as overlapping activities (3). Several mouse lines with reduced action of the GH/IGF-I axis have extended longevity (4). We have previously disrupted the mouse *Ghr* gene (GHRKO) and found that these mice have a striking increase in lifespan (5). In fact, one GHRKO mouse lived for almost 5 years and currently holds the record for the longest-lived laboratory mouse (6). Although the GHRKO mice have been extensively investigated, the precise mechanisms behind their lifespan extension are not yet fully elucidated.

GHRKO mice are dwarf with low levels of IGF-I and absent GH activity from conception. They are obese, have increased insulin sensitivity and are less prone to several age-associated disorders including cancer and diabetes than controls (7–9). A similar phenotype is found in patients with GHR deficiency, namely individuals with Laron Syndrome (LS). These individuals lack functional

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GHR and are therefore insensitive to GH (10, 11). They have greatly reduced IGF-I levels and increased GH levels, are obese, and a cohort from Ecuador have been found to have reduced rates of cancer and diabetes (10–12). Longevity has not been formally studied in LS patients, but there are two human populations with congenital GH deficiency (GHD), where some longevity data are available. Patients with a mutation in the GH releasing hormone receptor in Itabaianinha, Brazil are dwarf and experience some early deaths, but individuals who reach 20 years of age have a normal lifespan (13). The 'little people of Krk' in Croatia do not die prematurely but seem to have a normal lifespan even though they lack GH and other pituitary hormones (14).

In contrast to congenital GHD, both childhood- and adult-onset GHD lead to an increased risk for cardiovascular disease and higher rates of mortality (15, 16). In these patients, the GH dysfunction usually occurs after birth and is typically associated with traumatic brain injury (TBI), pituitary adenoma growth or brain irradiation. At the other physiologic extreme is acromegaly, a condition of excess GH. Untreated individuals with acromegaly have a decreased lifespan, an outcome which is improved by treatment that normalizes GH and IGF-I levels (17, 18). Similar to humans, mice with excess GH, and therefore excess IGF-I, are giant, lean, insulin resistant and have a shorter lifespan than control littermates (19).

Extending lifespan and/or improving healthspan through inhibition of the GH/IGF-I axis is interesting from a human health perspective. However, the impact of inhibiting this axis after childhood/pubertal development is not known. In the current study, our aim was to determine the effects of suppression of the GH/IGF-I axis in adult mice. Thus, we generated adult-onset GHRKO (aGHRKO) mice by disrupting the *Ghr* gene at 6 weeks of age when the mice were fertile and their growth rate had peaked. The consequences of this gene disruption on growth, metabolic parameters and lifespan are revealed and discussed.

Materials and Methods

Generation and maintenance of inducible aGHRKO mice

The mice carrying a 'floxed' *Ghr* allele (FFxx) were generated by the Knockout Mouse Project (KOMP) and have been described elsewhere (20, 21). B6.129-Gt(ROSA)26Sortm1(cre/ERT2)Tyj/J mice (22) (ffCC) were purchased from the Jackson Laboratory. These mice express an inducible ubiquitous Cre recombinase gene, transcription of which is driven by the ROSA26 gene promoter/enhancer (ROSA26-Cre-ERT2). The ROSA26 locus has been shown to be ubiquitously expressed in cells of all tissues (22–24). Mice for this study were bred to homozygosity for both the floxed *Ghr* and the Cre alleles (FFCC) in a C57BL/6 genetic background. To induce the *Ghr* gene disruption, 6-week old mice received 100 μl IP injections of 10 mg/ml tamoxifen dissolved in peanut oil (25) once per day over five consecutive days, for a total of 5 mg of tamoxifen (26). This dosing regimen gave better gene disruption than either a single injection or three daily injections of the same daily dose (data not shown). Controls received identical injections of peanut oil. Mice were divided into experimental (n = 20 per sex) and longevity (n = 35 per sex) groups. Both female and male mice were included in each study. Mice in the experimental group were used to study body composition and size, metabolic parameters and RNA expression, whereas only body weights and lifespan were recorded in the longevity group in order to reduce stress. All results apart from longevity and Supplemental Figure 1 are based on the experimental group. Mice were housed at 22°C under a 14-hour light/10-hour dark cycle, 3–4 mice per cage and ad libitum access to water and standard laboratory chow (ProLab RMH 3000). Dissections of the experimental group were performed at 9 and 19 months of age and took place in the morning after a 12-hour overnight fast followed by exposure to CO_2 until they became unconscious. Then blood was collected from the orbital sinus and they were sacrificed by cervical dislocation. All experiments were approved by the Ohio University Institutional Animal Care and Use Committee.

Assessment of body composition and growth parameters

All mice were weighed every two to five weeks until death. In the experimental group, monthly body compositions were measured using a Bruker LF50 Minispec system as previously described (27). At sacrifice, body length as well as weights of brain, liver, heart, kidney, spleen, lung, gastrocnemius, soleus, quadriceps, interscapular brown adipose tissue (BAT) and perigonadal, inguinal, retroperitoneal and mesenteric white adipose tissue (WAT) depots were recorded.

Validation of GRH gene disruption

Ghr and *Igf1* mRNA levels were determined in liver, heart, kidney, white adipose tissue (perigonadal, inguinal, retroperitoneal and mesenteric depots) and skeletal muscle (gastrocnemius and quadriceps) in 9 month old mice as previously described (20, 21, 28, 29). *Ghr* expression was also studied at 19 months in the same tissues although soleus was investigated as a representative skeletal muscle instead of gastrocnemius and quadriceps. Briefly, tissues were collected at sacrifice, frozen in liquid nitrogen and stored at –80°C. For RNA isolation, frozen tissues were homogenized using a Precellys 24-Dual homogenizer, after which RNA was isolated using TRIzol Reagent (Ambion/Life Technologies) following manufacturer's instructions. A Maxima First Strand cDNA Synthesis Kit for RT-qPCR was used for cDNA synthesis and Maxima SYBR Green/Fluorescein qPCR Master Mix (Thermo Scientific) for real-time quantitative PCR (qPCR). Samples were run on a Bio-Rad iCycler (Bio-Rad Laboratories). Depending on tissue, expression of *Ghr* and *Igf1* was normalized to two or three of the following reference genes: *Actb*, *B2m*, *Eef2*, *Eif3f*, *Hprt*, *Rpl38* and *Rps3*. More details and a list of used primers and their sequences are included in Tables 1–3 in Supplemental data. Expression data were normalized using the

qBase Plus software (Biogazelle) and GraphPad Prism version 5.01 (GraphPad Software) was used for statistical analysis as previously reported (29).

Glucose metabolism

Insulin tolerance tests (ITT) and glucose tolerance tests (GTT) were performed at 5, 9 and 13 months of age (n = 10–20), similar to previous procedures performed in our laboratory (20, 30). ITTs were started at 9:00 AM in a fed state. Recombinant human insulin (Humulin-R; Eli Lilly & Co) was diluted in sterile 0.9% NaCl solution for a final concentration of 0.075 U/ml, of which the mice received 0.01 ml/g body weight as an IP injection. Blood glucose was measured before the insulin injection and 15, 30, 45, 60, 90 and 120 minutes after the injection. For GTTs, mice were fasted for 12h before testing was started at 9:00 AM. A 10% glucose solution was prepared in sterile PBS, and the mice received 0.01 ml glucose solution/g body weight as an IP injection. Blood glucose was measured before injections and 15, 30, 45, 60, 90 and 120 minutes after injections. All glucose measurements were done using tail tip blood and OneTouch Ultra glucose strips and glucometers (Lifescan).

Blood biochemistry

Serum was collected at both 9 and 19 month time points prior to dissection. GH and IGF-I were measured by ELISA (Mouse/Rat - Growth Hormone, #22-GHOMS-E01, and Mouse/Rat - IGF-1 ELISA, #22-IG1MS-E01, ALPCO Diagnostics) in samples from both time points. Total adiponectin was measured in 9-month dissection samples by a Mouse HMW & Total Adiponectin ELISA (#47-ADPMS-E01, ALPCO Diagnostics). Insulin, c-peptide, leptin, resistin, IL-6 and MCP-1 as well as IGFBP1–3 and 5–7 were measured in the 9-month samples by Mouse Metabolic (#MMHMAG-44K) and IGF Binding Protein Panels (#MIGFBPMAG-43K) on a Milliplex 200 Analyzer (Millipore). Manufacturers' instructions were followed for all assays.

Liver triglycerides

Liver triglycerides were determined in the cohort of mice dissected at 9 months as previously described (31). Briefly, 50–100 mg pieces of liver were digested for 1 hour at 37°C in 3M KOH/65% EtOH and then neutralized with 2M Tris-HCl. Triglycerides (GPO) reagent (#T7532, Pointe Scientific) was used to determine triglyceride content.

Statistics

Values are reported as mean ± SD. All statistical analyses were performed using GraphPad Prism version 5.01 (GraphPad Software). Student's t-tests were used for analyses between two groups whereas time-dependent data was analyzed by two-way ANOVA with repeated measures combined with a Bonferroni posttest. A log-rank test was used for comparison of survival data. For maximal lifespan, a contingency table with number of animals above the 90th percentile for the respective sex was generated and analyzed by Fischer's exact test (32). P values < 0.05 were considered significant.

Results

GHR knockout verification and changes in the GH/IGF-I axis

In order to generate aGHRKO mice, *Ghr* was disrupted by tamoxifen injections in 6-week old mice homozygous for a floxed *Ghr* and an inducible ubiquitous Cre recombinase gene, ROSA26-Cre-ERT2. *Ghr* levels were determined at 9 and 19 months of age, with controls set to 100%. *Ghr* mRNA levels were significantly reduced in most tissues analyzed at both 9 and 19 months of age (Figure 1). Liver *Ghr* mRNA was > 99% decreased in aGHRKO mice of both sexes at both time points. In inguinal, mesenteric, retroperitoneal and perigonadal WAT depots, the decreases in *Ghr* expression varied from 54 to 92%, depending on depot, age and sex. These differences were statistically significant for all depots except female retroperitoneal fat tissue. Kidney *Ghr* expression was reduced 46% in male and 63% in female aGHRKO at 9 months, and 67% and 92%, respectively, at 19 months. Female but not male aGHRKO heart tissue had significantly decreased *Ghr* RNA levels with a 55% decrease at 9 months and a 65% decrease at 19 months. At 9 months, quadriceps and gastrocnemius had 37% and 30% respective decreases in male and 60% and 50% decreases in female aGHRKO mice. A different skeletal muscle, soleus, was investigated at 19 months, and a 38% decrease in *Ghr* expression was found in female aGHRKO mice while no significant decrease was observed in males.

Serum GH and IGF-I levels were determined at 9 as well as 19 months of age in order to confirm long-term maintenance of the *Ghr* knockout. Significantly increased GH levels were accompanied by significantly reduced IGF-I levels in male and female aGHRKO mice at both time points (Figure 2A and 2B). In order to determine whether some tissues were compensating for the lack of circulating IGF-I and/or responding to the high circulating GH, we measured *Igf1* mRNA levels in several tissues at 9 months of age (Figure 2C). As expected, *Igf1* expression was very low in the aGHRKO livers of both sexes: >99% decreased compared to controls. Inguinal fat depot showed a strong trend for decreased *Igf1* expression, whereas significant decreases of 29 to 57% were found in retroperitoneal and perigonadal fat depots of both sexes. A trend for decreased *Igf1* expression was observed in mesenteric fat of female but not male aGHRKO mice. Kidney *Igf1* expression was decreased by 36% in male and by 37% in female aGHRKO mice. In heart, no difference in *Igf1* expression was observed in either sex. A prominent increase in *Igf1* mRNA was found in male but not female skeletal muscle; *Igf1* expression was increased by 157% in quadriceps and by 221% in gastrocnemius. The aGHRKO mice displayed several changes in circulating IGFBP levels (Figure 2D). Whereas IGFBP-1, –2 and 6 levels were significantly increased in both sexes, a significant decrease in IGFBP-3 was observed in both male and female mice.

Reduced size and altered body composition of aGHRKO mice

Following tamoxifen injections at 6 weeks of age, reduced weight gain was observed in both sexes of aGHRKO mice compared to mice that received vehicle (Figure 3A and 3B). By two-way ANOVA, the overall effect of treatment was significant in both sexes: $P = .0003$ in females and $P = .0001$ in males. In males, this difference was significant starting at 2 weeks after injections whereas females displayed a significant difference starting 4 weeks after injections. These significant differences persisted at most time points that were assessed. Weights of the longevity group can be found in Supplemental Figure 1. Smaller body size was also observed at the time of each dissection, ie, at 9 and 19 months of age, as a significant decrease in body weight (Figure 3C) and length (Figure 3D) of aGHRKO mice of both sexes. More details of body size at the time of dissection can be found in Supplemental Tables 5 and 6.

As demonstrated in Figure 4A, aGHRKO mice are small with high adiposity. At the first body composition measurement following tamoxifen injections (8 weeks of age), aGHRKO males and females showed a significant increase in relative fat mass and decrease in relative lean mass (Figure 4B-E). A significant ($P < .0001$) effect of treatment was also observed on relative fat and lean mass in both sexes. All tissue and organ weights were normalized to lean body mass (as determined by the body composition measurements) since the conventional normalization to total body weight would exaggerate the differences between the fat aGHRKO and controls. Much of the increase in fat mass at 9 months of age was due to the significantly enlarged inguinal fat depot in both sexes (Figure 5A). In male aGHRKO, the perigonadal fat depot also showed a significant increase in size while no significant differences were found for mesenteric or retroperitoneal fat. Other changes were also observed at 9 months. Brain weight was higher in aGHRKO males and females than in controls whereas the weights of liver in males and spleen in both sexes were significantly decreased. An increase in the weight of gastrocnemius muscle was found in male but not female aGHRKO, while the other investigated skeletal muscles (soleus and quadriceps) showed no changes. An increase in BAT weight was observed in females but not in males. At 19 months (Figure 5B), inguinal and mesenteric fat mass was increased in male aGHRKO while no other significant differences were found in WAT. On the other hand, relative weights of all studied organs – liver, kidney, heart, spleen and lung – were decreased in both sexes of aGHRKO mice in comparison to controls. Relative weight of quadriceps was also decreased in both sexes. Absolute tissue weights at 9 and 19 months can be found in Supplemental Tables 5 and 6.

Improved insulin sensitivity but poor glucose tolerance

Both female and male aGHRKO mice had significantly improved insulin sensitivity at 9 months of age (Figure 6A-C). On the other hand, their glucose tolerance was impaired in comparison to control mice (Figure 6D-F). Similar results for ITT as well as GTT were obtained at 5 and 13 months of age (Supplemental Figure 2). We also observed changes in fasted and nonfasted glucose levels (Figure 7A). Fasted glucose levels were increased in both sexes at 5 months of age and in female aGHRKO at 9 months of age. Nonfasted glucose levels were increased in males at 9 and 13 months of age. In agreement with improved insulin sensitivity, insulin and C-peptide levels were significantly decreased in aGHRKO males although not in females (Figure 7B and 7C; Supplemental Table 4).

Changes in adipokine, cytokine and liver triglyceride levels

Adipokine and cytokine levels as well as liver triglycerides were determined at 9 months of age. Adiponectin was increased in both sexes of aGHRKO (Figure 8A) whereas leptin levels did not differ from controls (Figure 8B). Resistin was significantly elevated in female aGHRKO mice (Figure 8C). There was a trend for increased IL-6 (Figure 8D) and MCP-1 (Figure 8E) levels in the aGHRKO mice, but only IL-6 in female aGHRKO was significantly increased. Despite the high adiposity, aGHRKO mice do not show increased liver triglyceride accumulation (Figure 8F). To the contrary, female aGHRKO have significantly lower liver triglyceride levels than control mice.

An increased maximal lifespan in female aGHRKO mice

Maximal survival was 151 weeks for both control and aGHRKO males while female aGHRKO mice had a significant increase in maximal lifespan ($P = .0246$). The last female control mouse died at 150 weeks of age while the last aGHRKO female survived until 177 weeks of age. Median survivals of control and aGHRKO males were 123 and 119 weeks, respectively (Figure 9). In females, median survival was 125.5 weeks in controls and 124 weeks in aGHRKO mice. No significant differences were observed in mean lifespans using log-rank test; that is, male controls lived 120 ± 20 and aGHRKO 119 ± 16 weeks whereas female controls lived 122 ± 17 and aGHRKO 128 ± 26 weeks.

Discussion

The GH/IGF-I axis plays an important role in metabolism and longevity as demonstrated in multiple species. An increased lifespan is observed in many mouse lines in which this axis has been suppressed. GHRKO mice, which were generated in our laboratory, have a remarkable increase in lifespan and an uncommon phenotype of excessive adiposity accompanied by high insulin sensitivity. These mice inspired longevity studies in mice with tissue-specific ablation of the *Ghr* in liver (21), fat (20) and muscle (28) in order to better understand the mechanisms of their lifespan extension. Results of these studies did not completely recapitulate the extended lifespan found in the global GHRKO mice although one cohort of male muscle-specific GHRKO (MuGHRKO) displayed an increased longevity (28). This result suggests a role for muscle GHR in affecting lifespan.

In the current study, we disrupted the *Ghr* during adulthood and determined the metabolic phenotype and lifespan of these animals. In order to do this, we generated mice with a conditional tamoxifen-inducible disruption of the *Ghr*. Using ROSA26-Cre-ERT to drive Cre expression, we found the knockdown of *Ghr* to be most effective in liver and fat, whereas knockdown in muscle and heart were less effective. The changes in *Ghr* expression had a substantial impact on maximal lifespan in females; however, no effect was observed in males. This sex-specific effect on longevity is of interest and warrants further study.

As found in germline GHRKO mice, circulating IGF-I levels of aGHRKO mice were strongly suppressed while serum GH levels were highly increased. High circulating GH in combination with varied *Ghr* expression across tissues caused large differences in local *Igf1* expression in aGHRKO mice. Whereas liver *Igf1* mRNA was barely detectable, *Igf1* expression was higher in skeletal muscle of male aGHRKO mice than in controls. Even so, aGHRKO mice displayed an abrupt reduction of postpubertal growth rate when compared to control animals. Nasal-anal length of aGHRKO was also significantly reduced. Additionally, significant differences were observed in body composition already two weeks after disruption. In particular, aGHRKO mice had an increase in relative fat mass and a decrease in relative lean mass. This dwarf and fat phenotype of aGHRKO mice is similar but less extreme than that seen in the germline GHRKO, which are less than half the size of controls and have even higher amounts of relative fat than aGHRKO.

Since aGHRKO mice have extremely low liver *Ghr* and *Igf1* levels while some *Ghr* and *Igf1* expression was present in other tissues, it is of interest to compare these mice to the liver-specific GHR knockout mice, namely LiGHRKO (21) and GHRLD (33), as well as liver-specific IGF-I knockout mice, namely LI-IGF-I-/- (34) and LID (35). Of these mouse lines, IGF-I levels are lowest in GHRLD and LiGHRKO mice with 6%–13% of levels found in controls while LI-IGF-I-/- and LID mice have 25% of control IGF-I levels. Surprisingly, only the LiGHRKO mice are smaller than their respective controls, which is possibly due to short duration of the other studies. That is, LI-IGF-I-/- mice were studied for 7 weeks after induction of knockout, which was done at 4 weeks of age, and LID mice were studied for only 6 weeks with knockout present from birth. The GHRLD study does not indicate the age of the mice but, based on endpoint mouse weights, we can estimate that these mice were studied for approximately 8 weeks. LiGHRKO mice did not show a significant decrease in body weight until 26 weeks of age in males and 17 weeks in females. In aGHRKO mice, IGF-I levels were similar to both liver-specific GHRKO mouse lines: 2%–14% of control IGF-I levels. However, we observed a significant decrease in body weight at 2 weeks after induction of *Ghr* knockout in males and 4 weeks after induction in females. This growth pattern is likely due to the reduced *Ghr* and *Igf1* expression in several tissues in addition to the liver and, therefore, less compensation by local IGF-I production even though such compensation is seen in skeletal muscle of male aGHRKO mice.

Increased insulin sensitivity is a common shared feature of animals with extended lifespan (1, 4). In aGHRKO mice, we observed an interesting combination of improved insulin sensitivity and impaired glucose tolerance. A similar situation is seen in the germline GHRKO mice (36, 37) as well as GHA mice that express a GHR antagonist (30, 38). We also found increases in fasted and nonfasted blood glucose levels of aGHRKO mice. The germline GHRKO mice have low to normal glucose levels and low insulin levels throughout their lifespan (39) whereas GHA mice, which have a normal lifespan, exhibit normal glucose and insulin levels except for old males that develop hyperinsulinemia (38). GH suppresses insulin signaling (40) but has also been suggested to stimulate pancreatic β-cell proliferation and insulin production (possibly together with IGF-I) (36, 41, 42). Therefore, the glucose intolerance seen in aGHRKO, GHRKO and GHA mice is possibly due to decreased β-cell mass resulting in a reduced ability to secrete sufficient insulin in response to an artificially high exogenous bolus of glucose during a GTT. This is supported by a study showing that local *Igf1* expression in islet cells of germline GHRKO mice results in normal glucose tolerance and normalizes their otherwise reduced β-cell mass (37). A β-cell-specific GHRKO mouse, βGHRKO, displays a lower insulin response to glucose

without a change in GTT or in β-cell mass (41). Based on studies using an adult-onset GH deficiency model (AOiGHD mice), Cordoba-Chacon et al suggest that there is something other than β-cell mass affecting insulin response in GH-altered mice, such as lipotoxicity (43), but further studies are needed. We did not focus on the pancreas in our studies and do not know to what extent *Ghr* was ablated in any of the pancreatic cell types. The observed changes in glucose metabolism could simply be a consequence of improved insulin sensitivity, and therefore, no need for a rapid response to unusually high glucose levels. Studies on the pancreas of these different strains of mice are warranted.

Similar to aGHRKO, the aforementioned AOiGHD mice (44) are a model of adult-onset reduction of GH action. In AOiGHD, GH deficiency is induced at 10 weeks of age using diphtheria toxin directed to the GH producing somatotrophs. This results in an approximately 50% reduction in circulating GH as well as IGF-I levels (only males studied). Thus, aGHRKO mice have much lower serum IGF-I but on the other hand much higher GH levels to which some tissues with low or moderate *Ghr* ablation can respond. AOiGHD mice have a modest reduction in body weight and an increase in relative body fat with enlarged inguinal and retroperitoneal fat depots. Relative liver weight of male AOiGHD mice is decreased, which we also observed in aGHRKO mice as well as in GHRKO and GHA mice (5, 27, 38). In AOiGHD mice, decreased liver size can be explained by a decrease in liver TAGs, but normal liver TAG levels are found in GHRKO (27) and GHA (38) as well as the male aGHRKO mice. Female aGHRKO do not show a significant decrease in liver weight at 9 months and actually have a decreased TAG content at this age. It is possible that GH directly regulates liver growth but enlarged livers observed in LiGHRKO mice (21) do not support this. Another possibility is that liver glycogen storages are smaller in mice with improved insulin sensitivity. Measuring liver glycogen levels or determining cell size could help answer these questions in future studies.

Our most remarkable finding is the increased maximal lifespan of female aGHRKO mice. In nearly all investigated tissues, *Ghr* gene disruption was more efficient in female than male mice and males seemed to compensate for decreased liver *Igf1* expression by increased *Igf1* expression in skeletal muscle. These differences in *Ghr* and *Igf1* expression offer a potential explanation for the increase in female but not male maximal lifespan. In addition, a sex-dependent increase in mean lifespan has been found in three other models of altered GH/IGF-I axis, namely the LI-IGF-I-/- (45), IGF-1R+/- (46), and IGFBP-2 (47) transgenic mice, all of which show an increased lifespan for females but not males. There is no obvious explanation for the sexual dimorphism observed in these studies. Interestingly, similar sex-dependency is also found in a human population where certain IGF-IR mutations correlate with more female centenarians (48).

Collectively, aGHRKO mice share several features with the long-living germline GHRKO mice as well as with the GHA mice with normal lifespan. We also see many similarities with AOiGHD mice but longevity studies have not yet been performed in these mice. Berryman et al (38) have tried to answer the question why germline GHRKO mice have an extended lifespan but GHA mice do not, and have found a possible explanation in the adipose tissue. In germline GHRKO, the increase in fat mass is largely due to a larger inguinal fat depot, a depot which has been shown to be beneficial for metabolism (49, 50). GHA males reach control weight at one year of age due to high fat accumulation in all adipose depots. This increase in total fat mass is accompanied by a hyperinsulinemic state at older ages. In contrast, GHA females remain smaller than controls, do not display the extreme adiposity and only show an increase in the inguinal adipose depot, thus strongly resembling germline GHRKO mice. In both sexes of aGHRKO mice, the increase in adiposity is largest in, but not limited to, the inguinal depot. Given the similar phenotype of GHA females and the germline GHRKO, it is rather surprising that GHA mice have a normal lifespan while aGHRKO females show an increase in maximal lifespan. GHA females actually show a trend for increased median lifespan and maximal survival but the changes are not significant (5). Due to the increases in fat mass of aGHRKO mice, changes in adipokine levels are expected. Yet, we found no change in leptin levels, whereas they are increased in GHA mice (38) and, to some extent, in male germline GHRKO (39) mice. Adiponectin is typically negatively correlated with adiposity but also associated with increased insulin sensitivity (51), which is often considered a marker of extended longevity (4). Increased adiponectin levels are typical for mice with decreased GH activity; germline GHRKO as well as GHA mice display increased adiponectin levels despite high adiposity (38, 39), and adiponectin was also elevated in aGHRKO mice.

Results from our mouse study show that adult *Ghr* inactivation can improve insulin sensitivity and, in females, longevity. However, there are limitations to the current work. Most importantly, the *Ghr* gene was not disrupted in a similar fashion in all tissues. As a result, a condition of excessive GH action existed in some tissues while others, such as the liver, were GH insensitive. A higher and more ubiquitous degree of *Ghr* knockout by using a different Cre mouse line could overcome some of the limitations of this study. For example, CAGGCre-ER

mice express the *Cre* gene ubiquitously (52–56) and may provide more efficient *Ghr* deletion. If further studies confirm that blocking GH action in adulthood provides successful health outcomes, GHR-targeted therapeutics could be considered for improving health and longevity in humans.

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