Two-Year Body Composition Analyses of Long-Lived GHR Null Mice

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Growth hormone receptor gene–disrupted (GHR−/−) mice exhibit increased life span and adipose tissue mass. Although this obese phenotype has been reported extensively for young adult male GHR−/− mice, data for females and for other ages in either gender are lacking. Thus, the purpose of this study was to evaluate body composition longitudinally in both male and female GHR−/− mice. Results show that GHR−/− mice have a greater percent fat mass with no significant difference in absolute fat mass throughout life. Lean mass shows an opposite trend with percent lean mass not significantly different between genotypes but absolute mass reduced in GHR−/− mice. Differences in body composition are more pronounced in male than in female mice, and both genders of GHR−/− mice show specific enlargement of the subcutaneous adipose depot. Along with previously published data, these results suggest a consistent and intriguing protective effect of excess fat mass in the subcutaneous region.

Key Words: Body composition—Growth hormone—Obesity—Adipose depots—Gender differences.

DECREASED growth hormone (GH)/insulin-like growth factor-I (IGF-I) signaling is known to extend life span (as reviewed in (1)). For example, several studies using mouse models with disruptions to the GH/IGF-I axis, including the Ames dwarf (1), Snell dwarf (2), and growth hormone receptor/GH-binding protein gene–disrupted (GHR−/−) dwarf (3), have all reported extended longevity. Unfortunately, in the Ames and Snell dwarf mice, pituitary deficiencies in prolactin- and thyroid-stimulating hormone production exist in addition to GH; therefore, it is not possible to determine which of the absent hormones are responsible for the increased longevity (4–9). Because the GHR−/− mice have a disruption specifically in the *GHR* gene, these mice are useful for studying the impact of GH on aging (3). Caloric restriction (CR) also has been shown to enhance longevity in flies, mice, and most recently in nonhuman primates (10). Interestingly, CR of GHR−/− mice does not further extend life span (11), suggesting GH resistance/insensitivity and CR work via similar mechanisms for life-span extension even though gene expression profiling suggests that several genes are differentially expressed (2,12–14).

Attempts to determine mechanisms responsible for the increased life span in GHR−/− mice are ongoing. These mice remain dwarf throughout life and have elevated circulating GH and markedly reduced IGF-I levels (15). GHR−/− mice have a slight decrease in fasting glucose levels at younger ages (up to 10 months) (15–21), although no significant differences are found in older male mice (15,22,23). Insulin levels are significantly lower when compared with controls, independent of gender or age, and have a concomitant improvement in insulin sensitivity (11,15,16,18–25). Reduced levels of thyroid hormone and lower core body temperature in these mice have also been reported (21). Although less consistent, lipid levels are typically improved with total cholesterol and low-density lipoprotein cholesterol decreased in male GHR−/− mice (17,20,25). Finally, these mice are protected from fatal neoplasia (26). Collectively, these characteristics are generally considered favorable for the health of the mice and likely contribute to improving their longevity.

Based on the known lipolytic/antilipogenic actions of GH, it is expected that an absence of GH action will result in increased adipose tissue in GHR−/− mice. Indeed, several reports in which whole-body composition analyses were performed consistently show that male GHR−/− mice are relatively obese in comparison to littermate controls (18,20,27,28), with the only exception in one report in which young mice (6–7 weeks) did not show significant differences in percent whole-body fat (28). Furthermore, despite the significantly reduced body size and weight of the male GHR−/− mice, the absolute weight of their total fat mass is comparable to that of littermate controls in 6-month-old male mice (18). Thus, whole adipose tissue mass appears to be one of the few tissues not reduced in adult male mice. Importantly, the accumulation of fat mass is not uniform among different depots; in particular, the subcutaneous depot is disproportionately enlarged (16,18,27,29). Increases in adiposity are often associated with decreases in life span and impaired insulin sensitivity. Thus, these

long-lived mice offer a unique and counterintuitive situation in which obesity is associated with improved life span and many health parameters.

Previous studies that have assessed body composition in GHR−/− mice have not used both genders and have assessed body composition at a particular age. Although one study did report body composition in both genders at several time points (28), this study used a separate cohort of mice for only three ages and had a wide range of ages to represent young, adult, and aged mice. No studies have tracked body composition in the same cohort of mice over their life span. Because earlier studies in bovine GH transgenic mice and growth hormone releasing hormone receptor–deficient lit/lit mice show clear age- and gender-dependent effects on body composition (30,31), there is a need to evaluate the patterns of body composition changes throughout the life span in these GHR−/− mice. This might offer insight as to how relative obesity can be accompanied by improvements in life span. Thus, the purpose of this study was to assess systematically the changes in body composition more than 2 years in male and female GHR−/− mice in order to provide a better understanding of the metabolic dysfunction surrounding adiposity and its contribution to longevity.

Materials and Methods

Animals

Male and female GHR−/− in a C57BL/6J background and wild-type (WT) littermate controls were used in this study. The generation of this gene-disrupted mouse and subsequent backcrossing into the C57Bl/6J strain have been described previously (3). Although the body composition measurements were initiated with more mice, ultimately only six male GHR−/−, six male WT, eight female GHR−/−, and eight female WT mice were used for the final analyses due to death of several mice during the 2-year measurement period. For bone mineral density and liver triglyceride (TAG) measurements, tissues from these same mice were assayed; however, an additional cohort of age-matched mice was used to confirm the results. In this additional cohort of mice, there were 10 male GHR−/−, 10 male WT, 9 female GHR−/−, and 6 female WT mice. Mice were bred and housed within the animal facility at Ohio University with a 14-hour light/10-hour dark cycle in a temperature-controlled environment (21°C–23°C). Mice were housed up to four per cage with ad libitum access to food and water. Mice were weaned at 28 days of age onto a standard rodent diet (ProLab RMH 3000; PMI Nutrition International, Inc., St. Louis, MO; 14% of kilocalories from fat, 16% from protein, and 60% from carbohydrates). Mice were maintained on the standard rodent diet throughout the study. All procedures were approved by the Ohio University Institutional Care and Use Committee and fully complied with federal, state, and local policies.

Weight and Body Composition Measurements

Body weights were measured for each animal in duplicate just prior to body composition measurements using a standard scale, and the mean body weight was used for analysis (32). The Bruker Minispec (Bruker Optics, The Woodlands, TX), which employs nuclear magnetic resonance (NMR) technology to estimate the fat, lean, and fluid mass of the animals, was used to assess body composition. Weight and body composition measurements of all mice were taken every 2 weeks from 6 weeks of age until 16 weeks of age (measured at 6, 8, 10, 12, 14, and 16 weeks). Starting at 16 weeks, measurements were taken every 4 weeks until 104 weeks of age (or 2 years). The percent fat mass, percent lean mass, and percent fluid mass of each animal were calculated at each time point using fat, lean, and fluid mass divided by body weight, respectively. Additionally, a single body composition measurement was taken at 104 weeks for the second cohort of mice.

Organ Weights

Tissues were harvested at 105 weeks of age after sacrificing the mice by cervical dislocation. Several tissues were weighed and collected, including liver, heart, spleen, kidney, and four adipose depots (subcutaneous, epididymal/parametrial, retroperitoneal, and mesenteric). Tissues were frozen in liquid nitrogen and stored at −80°C for future analysis.

Liver TAG Measurements

Liver samples were digested in a 3 M KOH in 65% ethanol solution overnight for the extraction and measurement of TAG levels, as described previously (33). A Triglycerides-GPO kit (Pointe Scientific, Canton, MI) was used to measure the glycerol content of the samples. TAG levels were established assuming that the average molecular weight of TAG is 885 g/mol.

Bone Length and Mineral Density

In order to obtain estimates of bone mineral density and femur length, the right hind limbs were first dissected at the hip following which most soft tissues were removed. Femora were then mounted in foam blocks and scanned using the GE eXplore Locus Small Animal MicroCT Scanner (GE Healthcare, London, Ontario, Canada) using a 20-µm voxel protocol with the following scan parameters: 80 kV, 450 µA, and 2000-milliseconds exposure time. Bone density was normalized using an acrylic calibration phantom that included densities equivalent to air, water, and bone. A mid-diaphyseal region of the femur was selected using a threshold of 800 HU in order to separate bone tissue from the background image. Femur length was measured between the proximal end of the greater trochanter and the distal edge of the intercondylar fossa. Bone mineral density of the selected tissue was then calculated using the bone analysis package
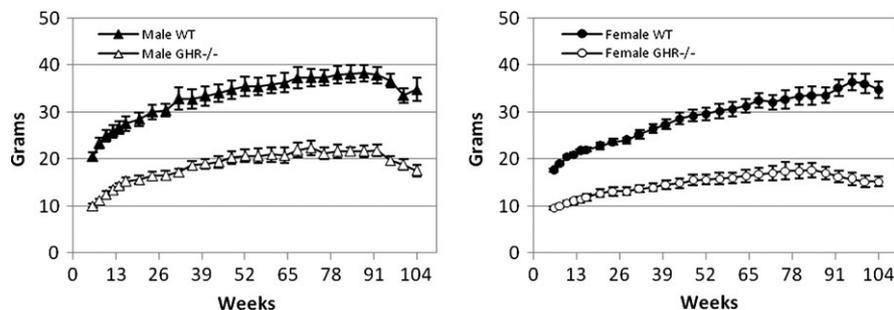
Figure 1. Body weight over time for male and female growth hormone receptor gene–disrupted (GHR−/−) mice and littermate controls. Weight measurements were taken every other week from 6 weeks of age until 16 weeks and taken monthly from 16 weeks until 104 weeks or 2 years of age. Data are expressed as mean ± *SEM*. Two-way repeated measures analysis of variance revealed a significant impact of genotype, $F(1,24) = 163.2$, $p = 2 \times 10^{-12}$, and gender, $F(1,24) = 14.2$, $p = .001$. WT, wild type.

in GE Microview 2.2.1. For the first cohort of mice that were used for body composition analyses, only four male GHR−/− femora were available for bone length and mineral density analyses. Because of the resultant low sample size for male GHR−/− mice in this original group, data from the second cohort of animals are provided in Figure 4; yet, results from statistical analyses for both groups are reported.

Statistical Analysis

Data are presented as mean ± *SEM*. Statistics were performed using the SPSS version 16.0 software (Chicago, IL). Comparisons of longitudinal data were done with two-way repeated measures analysis of variance (ANOVA). When the treatment by time interactions were significant, pairwise contrasts were conducted for specific weeks using a Bonferroni procedure. Data for other measurements were analyzed using the appropriate two-way ANOVA or Student's *t* test. All data generated with the second cohort of animals were analyzed separately. Differences were considered significant at $p < .05$.

Results

Body Weight

The mean body weights of both male and female GHR−/− mice were significantly less than their littermate controls at all time points measured (Figure 1). Even at the start of the study at 6 weeks of age, the weight of both genders of GHR−/− mice was 53% of corresponding littermate controls. By the last measurement at 2 years or 104 weeks of age, weights of male and female GHR−/− mice were 56% and 44%, respectively, of their littermate controls. Thus, GHR−/− mice were consistently about one half the weight of control mice. Both genders and genotypes exhibited accelerated pubertal growth rates up until ~16 weeks of age followed by a slower progressive increase in weight in subsequent weeks. Body weights for all mice except female WT peaked by or before 96 weeks of age and began to decline by the final measurement at 104 weeks of age.

Body Composition

Longitudinal measurements of fat mass and lean mass as well as fat and lean mass normalized to body weight showed markedly different trends by genotype and gender. For fat mass, there was very little difference between GHR−/− male mice and littermate controls, with the exception that fat mass gain in GHR−/− mice was more rapid and prominent between 10 and 28 weeks of age (Figure 2A). In contrast, female mice had no significant difference in fat mass until 92 weeks of age, at which point WT mice had higher fat mass through the remainder of the study (Figure 2B). Two-way repeated measures ANOVA revealed no significant difference based on genotype, $F(1,24) = 0.00$, $p = .994$, or gender, $F(1,24) = 4.2$, $p = .052$, although there was a significant interaction between genotype and gender, $F(1,24) = 5.38$, $p = 0.3$. Because of the extraordinary differences in body weight, it is also necessary to consider fat mass changes relative to body weight. Percent fat mass (Figure 2C) was markedly elevated at all time points in male GHR−/− relative to WT male mice. A similar trend was observed for female mice except that the difference between genotypes was not as large and the difference dissipated by 88 weeks of age (Figure 2D). Two-way repeated measures ANOVA revealed a significant difference based on genotype, $F(1,24) = 69.8$, $p = 1.5 \times 10^{-8}$, and gender, $F(1,24) = 5.4$, $p = .03$, as well as a significant interaction between genotype and gender, $F(1,24) = 12.2$, $p = .002$. All groups of mice experienced fat mass and percent fat mass loss as they approached 2 years of age. In males, fat mass peaked by 80 and 72 weeks of age for GHR−/− and control mice, respectively. The fat mass loss in females occurred a bit later with control mice reaching peak fat mass at 96 weeks of age and GHR−/− mice by 84 weeks of age.

Whereas absolute fat mass was similar between genotypes but significantly increased in GHR−/− mice when normalized to body weight, lean mass showed the opposite trend. That is, absolute lean mass was significantly reduced in GHR−/− mice (Figure 3A and B), whereas percent lean mass was relatively proportional to body size (Figures 3C and D). Specifically, two-way repeated measures ANOVA
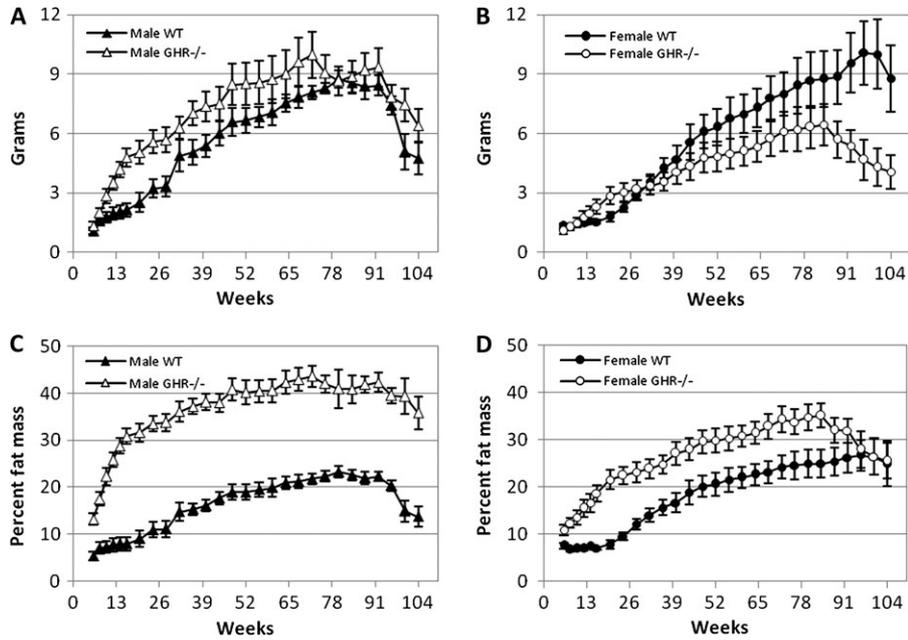
Figure 2. Absolute fat mass (**A** and **B**) and percent fat mass (**C** and **D**) for male (**A** and **C**) and female (**B** and **D**) growth hormone receptor gene–disrupted (GHR−/−) and littermate control (wild-type [WT]) mice. Data are expressed as mean ± *SEM*.

revealed a significant difference in absolute lean mass based on genotype, $F(1,24) = 434$, $p = 7.5 \times 10^{-17}$, and gender, $F(1,24) = 18.3$, $p = 0.00025$, as well as a significant interaction between genotype and gender, $F(1,24) = 8.9$, $p = .007$. For percent lean mass, the data revealed no significant difference based on genotype, $F(1,24) = 0.06$, $p = .82$, or gender $F(1,24) = 1.4$, $p = .24$ or interaction between genotype and gender, $F(1,24) = 1.9$, $p = .18$. All mice showed a more drastic increase in lean mass in early life (6 weeks until ~20 weeks) that remained relatively stable throughout the remainder of the study. An exception was found in control female mice in which lean mass gains were seen even during the final months of measurement. Much like lean mass, fluid mass, as predicted with this nuclear magnetic resonance technology, was proportional to body size (data not shown).
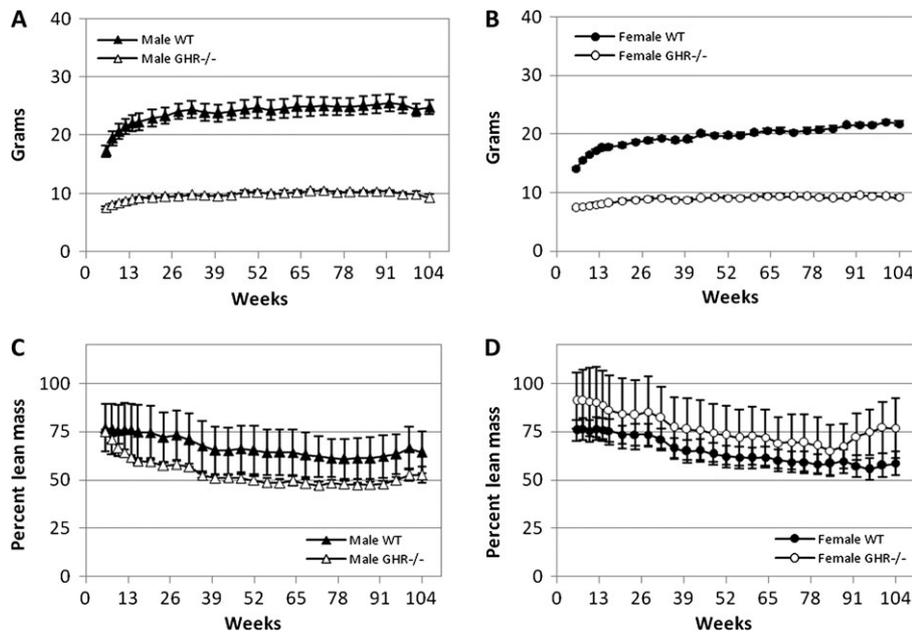

Figure 3. Absolute lean mass (**A** and **B**) and percent lean mass (**C** and **D**) for male (**A** and **C**) and female (**B** and **D**) growth hormone receptor gene–disrupted (GHR−/−) and littermate control (wild-type [WT]) mice. Data are expressed as mean ± *SEM*.
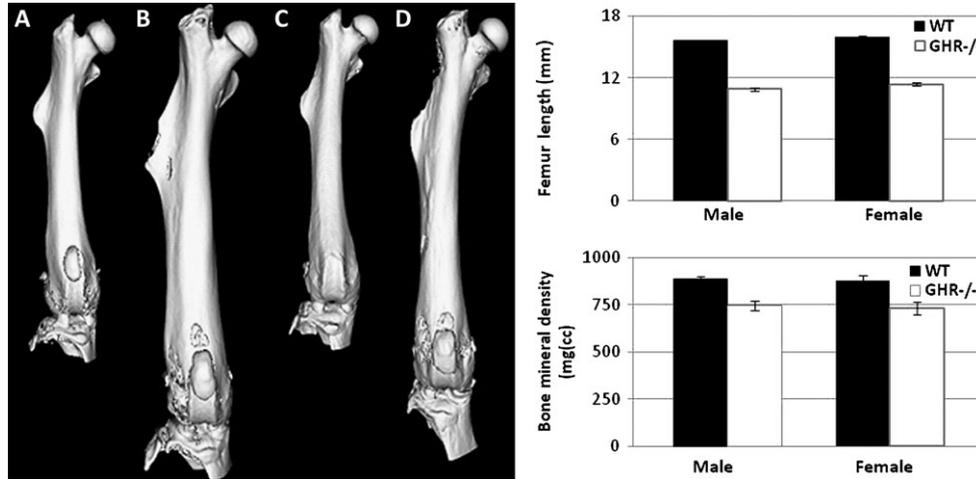
Figure 4. Femoral length (**B**) and bone mineral density estimates (**C**) of mid-diaphyseal cortex in male and female growth hormone receptor gene–disrupted (GHR−/−) and wild-type (WT) mice. Bone mineral density estimates were calculated subsequent to scanning on a GE eXplore Locus Small Animal MicroCT Scanner. Data are expressed as mean ± *SEM*, n = 10 (male GHR−/− and WT), n = 6 (female WT), n = 9 (GHR−/− female). Right femora (**A**–**D**) of 2-year-old mice were extracted, mounted in foam, and scanned using the following protocol: 20-μm voxel, 80 kV, 450 μA, and 2000-milliseconds exposure time.

Bone

Mean values for femoral length were 10.9 ± 0.1 for male GHR−/− mice, 11.4 ± 0.1 for female GHR−/− mice, 15.6 ± 0.1 for male WT mice, and 16.1 ± 0.07 for female WT mice. Femoral length exhibited significant differences between both genotype, $F(1,30) = 1531.8$, $p = 2.6 \times 10^{-27}$, and gender, $F(1,30) = 15.77$, $p = 0.004$ (Figure 4) with females having longer femur lengths at 2 years. However, there was no significant interaction between genotype and gender, $F(1,30) = 0.034$, $p = 0.8$. Although significant differences were noted in bone mineral density estimates between genotypes, $F(1,30) = 27.89$, $p = 0.000011$, no significant difference was identified between genders, $F(1,30) = 0.141$, $p = .71$ (Figure 4). Similar to femoral length, there was no

significant interaction effects between genotype and gender, $F(1,30) = 0.005$, $p = 0.94$. Data are shown for the second cohort of mice. The data generated with the first cohort of mice, used for the body composition analyses, showed a similar trend as the data shown for the second cohort of mice; however, femoral lengths for this set of mice did not reveal a statistically significant difference between genders, $F(1,22) = 1.6$, $p = .16$, possibly due to the smaller sample size.

Tissue Weights

Because of the significant difference in body weights for GHR−/− and WT mice, it is important to consider both absolute weight of tissues as well as tissue weights normalized to body weight (Figure 5). For absolute weight, most nonadipose
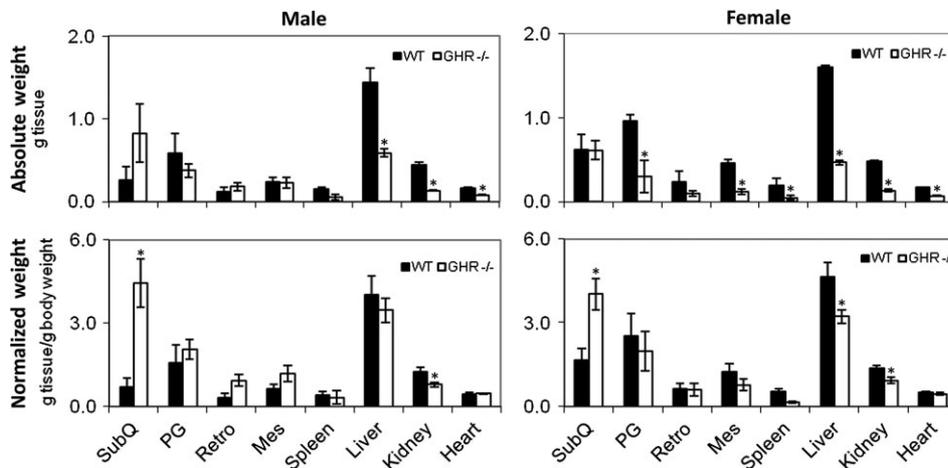

Figure 5. Absolute and normalized organ weights of male and female growth hormone receptor gene–disrupted (GHR−/−) and wild-type (WT) mice at 2 years of age. Weights of inguinal (SubQ), perigonadal (PG) for the epididymal fat pad in males and the parametrial fat pad in females, retroperitoneal (Retro), mesenteric (Mes), spleen, liver, kidney, and heart were in 105-week-old mice at the time of dissection. Data are expressed as mean ± *SEM*. Asterisk indicates GHR−/− samples were significantly different than corresponding WT mice.

tissues in GHR−/− mice were significantly smaller in size in comparison with WT mice, corresponding to the dwarf size of the former. That is, absolute weights of liver, heart, and kidney were decreased in size in comparison with control mice. Using two-way ANOVA to compare the tissue weights for each genotype and gender, there was an expected main effect of genotype for the absolute weights of spleen, liver, kidney, and heart (with F values ranging from 12.8 to 410, all with a $p < .001$) but no significant effect of gender or interaction between gender and genotype. Many of the significant differences observed in absolute mass of tissues were not maintained when normalized to body weight, suggesting that the lower tissue mass in GHR−/− mice is merely proportional to the smaller body size. One interesting exception for both male and female mice was the kidneys, which were decreased in GHR−/− mice for both normalized and absolute values. Using two-way ANOVA for normalized tissue weights, there was a main effect of genotype for only relative kidney weights, $F(1,25) = 27.0$, $p = .00003$, with no effect of gender or interaction between gender and genotype.

Adipose tissue showed a different trend. In contrast to other tissues, the absolute weights of all adipose depots in male GHR−/− mice were not significantly different from WT mice. Although not always significant, the trend for the absolute weights of most adipose depots was to be reduced in female GHR−/− compared with littermate controls, with the exception of the inguinal subcutaneous fat pad. Two-way ANOVA for absolute adipose tissue weight revealed only a significant main effect for the mesenteric fat, $F(1,25) = 5.5$, $p = .03$. When normalized to body weight, the only significant difference by genotype as determined by two-way ANOVA was in the subcutaneous fat pad, $F(1,25) = 27.6$, $p = 0.00003$, which had a value nearly double the WT mice for both male and female mice. Thus, the subcutaneous fat pad in both male and female mice was preferentially enlarged relative to body size in older mice as has been reported previously in younger male mice (18,28).

Liver TAG

The TAG concentration in livers of GHR−/− mice did not differ significantly from their WT littermates, and there was no significant difference between males and females of the same genotype. Specific values for male GHR−/−, male WT, female GHR−/−, and female WT were 24.7 ± 2.2, 23.5 ± 5.1, 37.5 ± 16.1, and 36.2 ± 7.5 mg/g tissue, respectively. Due to large standard errors and relatively small group sizes in the cohort from the body composition study, we repeated liver TAG measurements in the second cohort of 24-month-old mice. The mean concentrations were similar to the first group of mice analyzed (e.g., male WT was 25.6 ± 4.3 mg/g tissue in the second cohort vs 23.5 in the first group of mice). Again, no significant difference was found in any group.

Discussion

Because of their increased longevity, GHR−/− mice have been the focus of intensive examination. Interestingly, GHR−/− mice remain long lived despite reports of increased adiposity (18,20,27,28), a feature more commonly associated with reduced longevity. The present study is the first to monitor body composition longitudinally in the same cohort of GHR−/− animals to determine the lifelong changes in lean and fat mass of this long-lived mouse model. Overall, the data for GHR−/− mice show marked increases in percent body fat mass and no significant difference in percent lean mass throughout the majority of their life, as well as a decrease in bone mineral density at the end of the study. There are distinct declines in body weight and adiposity in later life for all groups of mice studied, which may serve as a unique indicator of specific aging processes or deterioration in health. Furthermore, the previously reported increase in the inguinal subcutaneous fat pad in younger male mice (16,18,27,29) is maintained in later life for male mice, and this is the first report of a similar depot-specific trend in female mice. Despite these consistencies with previous reports, there are some important gender- and age-specific differences between GHR−/− and WT groups.

Several previous studies have assessed body composition in GHR−/− mice. These studies reported consistently that GHR−/− male mice have higher percent fat mass at 3.5 months (20), 5 months (27), 6 months (18), 7–10 months, and 28–32 months (28) in comparison to controls. The only exception in the literature is with very young mice (6–7 weeks) in which it has been reported that there is no significant difference in percent fat mass (28). Importantly, these studies were conducted on mice with differing genetic backgrounds, suggesting that this trend is not strain specific in male mice. The present data for male mice are consistent with these previous reports except that increased percent fat mass was seen at all ages, even in male mice as young as 6 weeks old. Female GHR−/− mice have been less rigorously studied with only one previous study report, indicating that female mice are also relatively obese but only in older mice from 2–3 years of age; no increase in percent fat mass is noted in younger ages (6–7 weeks and 7–10 months) (28). Our data show significant increases in percent fat mass for female GHR−/− mice at all ages except in older (2-year-old) mice. The apparent discrepancy could be due to the differing background strain, to the cross-sectional versus longitudinal experimental design, or the difference in the methodology used to assess fat mass (Dual-Energy X-Ray Absorptiometry [DXA] vs NMR).

Surprisingly, there was no significant difference in absolute fat mass despite the extreme dwarf size of the male and female GHR−/− mice compared with littermate controls. Although many studies have provided data regarding percent fat mass, only one previous article had reported data on total absolute fat mass (27). This manuscript also reported that there was no significant difference in absolute fat mass.

Because absolute fat mass is unchanged, it is tempting to assume that adipose tissue is unscathed by the lack of GH action and develops to its normal size in these dwarf mice. However, a compelling argument against this theory is that the accumulation of adipose tissue in GHR−/− mice is not uniform, with different depots being disproportionately enlarged and others being proportional to their dwarf size. Specifically, multiple reports show a profound increase preferentially in the mass of subcutaneous white adipose depots in younger male and female GHR−/− mice from 4 to 6 months of age (16,18,27,34), in interscapular brown adipose tissue in male mice 3–4 months of age (20,29), and occasionally in retroperitoneal fat pad in 3- and 6-month-old male mice (20,27). Other models of GH deficiency, such as the Sma1 mice that have a missense mutation in the GH gene, show a similar preference in accumulation of fat in the subcutaneous region (35). In the present study using older mice, only the subcutaneous fat pad in GHR−/− mice is enlarged, with the absolute weight being similar to WT and with normalized weights being significantly elevated in both genders. It is interesting to note that the epididymal fat pad, a male depot commonly studied because of its ease of dissection, is reduced in terms of absolute mass and not significantly different when normalized to body weight, indicating that there is no specific accumulation of fat in this region. As such, studies reporting the impact of GH on adipose tissue that utilized solely this fat pad should be repeated using additional fat pads for comparison. Overall, there is little doubt that an adipose depot–dependent effect occurs in GHR−/− mice, which now appears to be maintained in later life in male and female mice, as demonstrated in this study. It should be noted that marked increases in subcutaneous adipose tissue have been reported for GH-deficient humans (36,37), although other depots may also be enlarged (38).

Lean mass shows a trend opposite of fat mass. That is, absolute levels of lean mass were significantly decreased in GHR−/− mice in both genders compared with controls. Lean mass was remarkably stable for male WT and GHR−/− mice as well as for female GHR−/− mice after a pubertal growth spurt in early life. In contrast, female WT mice continued to have a modest increase in lean mass even at the last time point measured. Completely opposite of fat mass, lean mass when normalized to body weight was not significantly different between genotypes or genders. Thus, lean mass was proportional to body weight. Taken together, these body composition data suggest that the increases in body weight throughout adulthood and the reduction in body weight seen in older mice are not due primarily to changes in lean mass but rather reflect alterations in body fat. Similar to these results, aging in humans is generally associated with increases in total adiposity over the adult life span (reviewed in (39)), until extreme old age when fat mass decreases (40,41). However, distinct from studies in human populations, a loss of lean mass also tends to accompany aging, with lean tissue reductions noted as early as 45 years of age that continue with advanced aging (42).

Other studies have tracked body composition changes over life span in mice. Two studies have utilized mice that have altered GH function (30,31). In 1993, Donahue and Beamer (31) used a chemical method to evaluate body composition in GH-deficient *lit/lit* male and female mice along with littermate controls up to 1 year of age. In another article, Palmer and colleagues (30) reported body composition for male and female bovine GH transgenic mice in the C57JBl/6J background compared with littermate controls also up to 1 year of age. Both articles reveal the importance of assessing body composition in both genders and over time as differing trends were noted in younger mice than in older mice as well as in males versus females. For example, bovine GH transgenic (bGH) male and female mice were shown to be relatively lean but only at older ages. At younger ages, the male bGH mice had more fat mass and the female bGH mice had similar fat mass as littermate controls. Another observation from this study was that WT male C57Bl/6J mice have a notable increase in fat mass throughout Year 1, a phenomenon that was also seen in female mice yet the increase in fat mass was delayed throughout the pubescent and early adult period. Finally, this study reported rapid increases in lean mass prior to 24 weeks that stabilized throughout the remainder of the study. Similar age- and gender-dependent trends were reported for the lit/lit study. Both previous studies only followed body composition out to 12 months of age for WT mice. The results of the present study have some similarities to these previous reports in that WT mice have a steady increase in fat mass throughout the first year of life, females had a delayed onset of fat mass gains, and both male and female WT mice had a rapid pubertal increase in lean mass. As the present study continued to track WT mice for another year, the current data add additional information as to the normal changes in body composition in this commonly studied strain of mice. WT mice continue to experience gains in body weight and fat mass, but by 2 years, both male and female WT mice experienced some loss in body weight, a weight loss that can almost exclusively be attributed to fat mass loss. Although it is possible that fat mass loss in later life could be a signal that the health is deteriorating, this same trend is noted for the long-lived GHR−/− mice.

Several studies have examined the skeletal phenotype associated with GHR−/− mice. The majority of these reports have been conducted in young male mice where profound effects have been noted. For example, GHR−/− mice exhibit a reduction in epiphyseal plate width of the proximal tibia by 20% (20 days old) to 30% (10 weeks old) compared with WT littermates (43,44). Tibial linear growth rate also is reduced by ~65% in GHR−/− mice between postnatal Days 20 and 40, likely a result of reduced proliferation and hypertrophy of chondrocytes (43). Bone mineral density and femoral length in 3.5-month-old male GHR−/− mice are

also decreased by 32% and 74%, respectively (45). Only one previous study has examined bone in older GHR−/− mice. Bonkowski and colleagues (28) compared several features of bone in three groups of mice: young (6–7 weeks), adult (7–10 months), and aged (28–32 months) male and female mice using DXA. They documented a reduction in total body bone mineral density, bone mineral content, and bone area in GHR−/− mice, regardless of age and that total body bone mineral density either increased or did not change as a function of age for all genotypes. Relevant for the study conducted herein, although Bonkowski and colleagues (28) reported that bone mineral density was reduced in GHR−/− mice, it was only female GHR−/− that showed further decreases in femoral bone mineral density (BMD) with advancing age, suggesting the presence of both age- and gender-dependent effects. Due to the two-dimensional nature of DXA scans (grams per square centimeter), compared with the three-dimensional capabilities of microCT (milligrams per cubic centimeter), microCT appears to be a more accurate method for measuring BMD; thus, the use of different scanning methods could account for the differences noted between the reports (46). Femur length is also decreased in GHR−/− mice in both genders in this and the study of Bonkowski and colleagues (28). Of note, there was a significant effect of gender on femur length with females exhibiting significantly larger femora than males of both genotypes. Bonkowski and colleagues (28) reported a similar trend that did not reach statistical significance.

High levels of TAG in nonadipose tissues are usually linked to insulin resistance. Therefore, it is somewhat surprising that the liver TAG content has been shown to be elevated in male 18-month-old Ames dwarf mouse, another model with increased longevity (47). Furthermore, CR appears to reduce the TAG content in the Ames dwarf to the levels seen in WT mice. In contrast, liver TAG content is reduced in male and female bovine GH transgenic mice, a model with excess GH action and physiologically opposite to the model studied in the present study (30,48). Based on these counterintuitive results from previous studies, we predicted that liver TAG content might also be elevated in the insulin-sensitive older GHR−/− mice. This prediction was not supported by the data in that no significant differences were identified in liver TAG content of older male or female GHR−/− mice compared with littermate controls. This finding does not rule out that differences may be present at younger ages as we have noted numerous other phenotypic effects related to lipid distribution that are dependent on age. However, by 2 years, this difference is no longer apparent in two separate groups of mice, which may also be a unique feature of the GHR−/− model as opposed to the Ames dwarf mice. Other notable differences between these models include the additional hormone deficiencies in the Ames dwarf mice and their differing responses to CR (1,11). Of note, patients with Laron syndrome, who have GH gene deletion comparable to that of GHR−/− mice, have also been reported to have nonalcoholic fatty livers (49).

The results generated using the GHR−/− mice demonstrate that mice can have a disproportionate amount of adipose tissue and remain relatively obese throughout much of their lives, yet still exhibit improved insulin sensitivity and longevity. This is an intriguing situation that defies much of what we consider dogma. Potentially, the unique and consistent pattern of fat deposition in the subcutaneous region along with the apparent normal levels of TAG accumulation in nonadipose tissue may offer insight as to why these mice live longer. Based on recent transplant studies that demonstrate inherent beneficial effects of subcutaneous fat even when transplanted in a visceral region (50), it is reasonable to assume that the unique depot distribution in GHR−/− mice would alter numerous physiological parameters, such as adipokine expression and glucose homeostasis, among other possibilities. Regardless, in this long-lived mouse model, it appears that the excess adipose tissue is "healthier" in the absence of the GHR. Further studies into the mechanisms of fat metabolism in this mouse model are needed to explore this phenomenon. Additionally, a better understanding of the distinctive features of the subcutaneous depot in GHR−/− mice in comparison to other depots also warrants further study and will undoubtedly provide clues as to how an obese state can be accompanied by improvements in many health parameters. Analyzing other long-lived models for depot-specific differences in fat mass may offer additional evidence to the unique protective nature of this particular depot.

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