Disruption of growth hormone receptor gene causes diminished pancreatic islet size and increased insulin sensitivity in mice

Jun-Li Liu,1 Karen T. Coschigano,2 Katie Robertson,1 Mark Lipsett,3 Yubin Guo,1 John J. Kopchick,2 Ujendra Kumar,1 and Ye Lauren Liu1

1Fraser Laboratories, Department of Medicine, and 2Department of Surgery, McGill University, Montreal, Quebec H3A 1A1, Canada; and 3Edison Biotechnology Institute and Department of Biomedical Sciences, College of Osteopathic Medicine, Ohio University, Athens, Ohio 45701

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Liu, Jun-Li, Karen T. Coschigano, Katie Robertson, Mark Lipsett, Yubin Guo, John J. Kopchick, Ujendra Kumar, and Ye Lauren Liu. Disruption of growth hormone receptor gene causes diminished pancreatic islet size and increased insulin sensitivity in mice. Am J Physiol Endocrinol Metab 287: E405–E413, 2004. First published May 11, 2004; 10.1152/ajpendo.00423.2003.—Growth hormone, acting through its receptor (GHR), plays an important role in carbohydrate metabolism and in promoting postnatal growth. GHR gene-deficient (GHR−/−) mice exhibit severe growth retardation and proportionate dwarfism. To assess the physiological relevance of growth hormone actions, GHR−/− mice were used to investigate their phenotype in glucose metabolism and pancreatic islet function. Adult GHR−/− mice exhibited significant reductions in the levels of blood glucose and insulin, as well as insulin mRNA accumulation. Immunohistochemical analysis of pancreatic sections revealed normal distribution of the islets despite a significantly smaller size. The average size of the islets found in GHR−/− mice was only one-third of that in wild-type littermates. Total β-cell mass was reduced 4.5-fold in GHR−/− mice, significantly more than their body size reduction. This reduction in pancreatic islet mass appears to be related to decreases in proliferation and cell growth. GHR−/− mice were different from the human Laron syndrome in serum insulin level, insulin responsiveness, and obesity. We conclude that growth hormone signaling is essential for maintaining pancreatic islet size, stimulating islet hormone production, and maintaining normal insulin sensitivity and glucose homeostasis.

GROWTH HORMONE, acting through its receptor (GHR) and various signal transduction pathways, promotes postnatal growth and regulates fuel homeostasis (22, 24). Normally, it stimulates insulin and glucagon secretion, pancreatic islet cell proliferation, and hepatic glucose production, and it decreases glucose uptake by extrahepatic tissues, thereby increasing blood glucose concentration (11, 35, 37, 38). In primary cultures of pancreatic islet cells, growth hormone stimulates β-cell proliferation, insulin gene transcription, and insulin secretion (36). These effects are physiologically important, because neutralization of the biological activity of growth hormone by specific antibodies resulted in enhanced insulin sensitivity in rats (40). Some early studies have indicated that growth hormone deficiency in humans is associated with increased insulin sensitivity, decreased insulin secretion, and decreased fasting glucose concentrations (3, 11, 20). More recently, deletions or mutations in the GHR gene resulted in dysfunction of the receptor and caused Laron syndrome, which exhibits growth retardation, trunkal obesity, insulin resistance, and hyperinsulinemia (28, 29). GHR gene-deficient (GHR−/−) mice exhibit severe growth retardation, proportionate dwarfism, and greatly decreased serum insulin-like growth factor I (IGF-I) concentration, representing largely the human Laron syndrome (6, 46). Furthermore, GHR−/− mice have been found to have decreased fasting glucose and insulin levels, increased insulin sensitivity, as well as decreased glucose tolerance (9, 18, and observations made by Coschigano KT, Riders ME, Belush LL, and Kopchick JJ, at the 1999 Endocrine Society Annual Meeting). At the molecular level, insulin receptor abundance and insulin-stimulated receptor phosphorylation are elevated in the liver of GHR−/− mice (9). These observations strongly suggest that growth hormone signals are involved in β-cell growth, insulin production, and insulin actions and make it necessary to further characterize GHR−/− mice in glucose homeostasis and pancreatic islet structure/function. In particular, we studied the ontogeny of altered pancreatic islet function in relation to postnatal growth retardation in these mice. We demonstrated diminished pancreatic islet size due to decreased islet cell replication and growth, as well as decreased serum insulin level and insulin mRNA accumulation in adult GHR−/− mice. These defects occurred as early as 10 days postpartum. Although they were developed to mimic the human Laron syndrome, GHR−/− mice are clearly distinct in their lack of obesity, elevated insulin sensitivity, and decreased serum insulin level.

MATERIALS AND METHODS

Animal production and genotype. GHR−/− mice carry a targeted disruption of exon 4 of the mouse GHR/binding protein (BP) gene, as previously reported (46). Offspring derived from heterozygous (GHR+/−) mating pairs on a hybrid 129/Ola-BALB/c-C57BL/6 background were used in experiments. To determine genotype, genomic DNA was isolated from tail clips with standard methods. Primers In4–1 (5′-CCC TGA GAC CTC CTC AGT TC), In3+1 (5′-CCT CCC AGA GAG ACT GGC TT), and Neo-3 (5′-GCT CGA CAT TGG GTG GTA ACA T) were used in PCR reactions, which yield a 390-base band for the wild-type allele and 290/200 double bands for the knockout allele, as previously reported (4). The animals were maintained in 12:12-h dark-light cycles at room temperature with free access to food and water or, when indicated, were food deprived for 24 h with free access to water. At the desired age, the mice were killed.

Address for reprint requests and other correspondence: J.-L. Liu, Fraser Laboratories, M3–15, Royal Victoria Hospital, 687 Pine Ave. West, Montreal, QC H3A 1A1, Canada (E-mail: jun-li.liu@mcgill.ca).

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by decapitation, blood was collected for serum preparation, and pancreata were rapidly removed for biochemical or histological analysis. All animal handling procedures were approved by the McGill University Animal Care Committee.

**Blood chemistry and in vivo procedures.** Serum concentrations of insulin and glucagon were determined using RIA kits obtained from Linco Research (St. Charles, MO). Blood glucose levels were measured using a SureStep blood glucose meter and strips (LifeScan Canada, Burnaby, BC, Canada). The Clinical Chemistry Department of the McGill University Health Centre performed blood biochemistry analyses, including measurement of calcium, uric acid, creatine kinase, and -glutamyltransferase levels in serum. Insulin tolerance tests were performed on animals in the random-fed state. Animals were injected with human insulin (1 IU/kg ip, Sigma-Aldrich, St. Louis, MO), and blood glucose levels were measured at 0, 20, 40, and 60 min after the injection.

**RNA preparation and analysis.** Total RNA was isolated from fresh tissues by acid guanidinium isothiocyanate-phenol-chloroform extraction (5). RNA concentration was determined by spectrophotometry at 260/280 nm. For Northern blot analysis, 5–30 g of total RNA were subjected to electrophoresis on 1.5% agarose formaldehyde gels and transferred to Hybond N membranes (Schleicher & Schuell, Keene, NH), and the RNA blots were hybridized for 18 h at 60°C in a solution of 50% formamide (vol/vol). 32P-labeled antisense RNA probes were transcribed from a mouse insulin I cDNA (Rsal/EcoRI fragment, extending from 48 to 725 bp) (42) and from the pTRI- 3,4,000

**RESULTS**

**Decreased blood levels of insulin, glucagon, and glucose.** As previously reported (9, 18, 46), adult 2-mo-old GHR−/− mice exhibited severe growth retardation and decreased blood glucose (80% of control) and serum insulin concentrations (40% of control) compared with their wild-type littermates under random-fed status (Fig. 1, B and C, 3rd time points). Additionally, serum glucagon levels (pg/ml) were reduced significantly, although to a lesser degree than those of insulin [wild-type (WT): 148 ± 10, n = 7 vs. GHR−/−: 116 ± 9, n = 6, P = 0.04]. When the mice were fasted for 24 h, the decrease in blood glucose levels in GHR−/− mice was still significant, compared with their wild-type littermates (Fig. 1C). GHR−/− littermates exhibited marginal growth retardation (10% reduction in mean body wt vs. WT; not significant) and no change in blood glucose levels when random-fed or 24-h-fasted mice were measured (data not shown).

**Decreased insulin and IGF-I gene expression.** Consistent with decreased serum insulin concentration, insulin mRNA levels in the pancreas (determined by Northern blots) were markedly reduced to 40% in GHR−/− mice vs. their wild-type littermates (Fig. 2). Because IGF-I mediates many of the growth hormone actions in target tissues, and secretion of IGF-I is virtually abolished in GHR−/− mice (6, 46), the expression of IGF-I mRNA in liver and pancreas was also studied. Pancreatic IGF-I mRNA levels (determined by RNase protection assay) were reduced to 38% of that of wild-type littermates, to a similar extent as the insulin message. Liver IGF-I mRNA levels, determined by Northern blots, which exhibit a 0.7-kb major form and a 7-kb minor form as reported (31), were reduced to 37 and 24% of those of wild-type littermates, respectively (Fig. 2).

**Increased insulin sensitivity.** Insulin tolerance tests were performed in 7-mo-old mice under random-fed status. GHR−/−
mice exhibited hypoglycemia compared with their wild-type littermates. Insulin injection caused a significant 26–40% reduction in blood glucose levels from 20 to 60 min in wild-type littermates (Fig. 3). GHR$^{-/-}$ mice exhibited further decreased blood glucose levels of 60 and 67% at 40 and 60 min, significantly lower than those of control mice, thus exhibiting elevated sensitivity to insulin.

Diminished pancreatic islet size. To investigate the phenotype of pancreatic islets of GHR$^{-/-}$ mice, we performed immunohistochemistry by use of insulin antibody in 2-mo-old mice. The size of insulin-staining islets was measured in both wild-type and GHR$^{-/-}$ mice. In wild-type mice, there was an

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**Fig. 1.** Age-dependent changes in body wt and insulin and glucose levels in growth hormone receptor-deficient (GHR$^{-/-}$) mice. Mice were studied at the ages of 3, 10, 60, and 180 days. Serum levels of insulin and blood glucose concentrations were determined in random-fed GHR$^{-/-}$ mice and their wild-type (WT) littermates. As indicated, some glucose assays were also performed on 24-h-fasted mice. Data are expressed as means ± SE. Nos. of mice in each group are indicated in parentheses. $P$ values are derived from comparison with WT littermates by unpaired $t$-tests. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. WT littermates.

**Fig. 2.** Changes in the level of insulin and IGF-I gene expression in GHR$^{-/-}$ mice. WT littermates and GHR$^{-/-}$ mice were killed at 2–3 mo of age, and pancreas (Pan.) and liver were removed to prepare total RNA. Pancreatic insulin and liver IGF-I mRNA levels were analyzed using Northern blot hybridization. Pancreatic IGF-I mRNA levels were measured using an RNase protection assay. All relative intensity values were expressed as means ± SE after correction for abundance of $\beta$-actin mRNA. No. of measurements is indicated in parentheses. ***$P < 0.001$ vs. WT littermates by unpaired $t$-tests.

**Fig. 3.** Increased insulin sensitivity in adult GHR$^{-/-}$ mice. Insulin tolerance tests were performed in 7-mo-old mice. WT and GHR$^{-/-}$ mice of both sexes in random fed state were injected with insulin (1 IU/kg ip). Blood glucose levels were measured from tail blood at 0, 20, 40, and 60 min after injection. Percentage values relative to time 0 are expressed as means ± SE. **$P < 0.01$, ***$P < 0.001$ vs. WT mice in unpaired $t$-test. Similar results were obtained in a separate group of 3-mo-old mice.
even distribution of pancreatic islet sizes, from small to "giant" islets that differ 5- to 10-fold in diameter (Fig. 4, A and B). In GHR−/− mice, this distribution pattern was shifted toward the smaller islets, without the presence of "giant" ones (Fig. 4, A and B). As a result, the average islet size for GHR−/− mice decreased by 68% (P < 0.0001) compared with that in wild-type littermates (pancreatic islet size: WT 84.5 ± 11.5, n = 33 vs. GHR−/− 29.0 ± 4.9, n = 35; units expressed as ×10^3 μm^2). The distribution of islets per unit pancreas area, measured in nonoverlapping ×25 images of the pancreatic sections, was unchanged (data not shown).

Total β-cell mass, determined by insulin antibody staining, was 4.5-fold lower in GHR−/− than in wild-type animals (Table 1). β-Cell mass-to-body weight ratios were also 50% lower in knockout than in wild-type mice, indicating that the reduced β-cell mass in knockout mice is due not only to their decreased body size. Pancreas mass-to-body weight ratios exhibited no significant decrease. In contrast, total α-cell mass (in mg) was unchanged in GHR−/− dwarf mice, as shown in Table 1 by a 2-fold increase in ratio of α-cell mass to body weight. There was no sexual dimorphism in these parameters.

**Early onset of abnormalities in glucose homeostasis.** To exclude the possibility that pancreatic islet growth in GHR−/− mice was proportionally reduced as a result of general growth retardation and to explore how early one can detect defects in pancreatic islet function and glucose homeostasis, we studied young pups 3 and 10 days after birth (before onset of the peripubertal growth spurt in the 3rd wk). At 3 days of age, GHR−/− pups were already retarded by 15% in mean body weight (WT 2.31 ± 0.09 g vs. GHR−/− 1.97 ± 0.08 g; n = 6–17, P < 0.05), although serum insulin and glucose concentrations remained normal (Fig. 1, 1st time points). At 10 days of age, GHR−/− pups showed further growth retardation (by 22% in mean body weight), and blood glucose and serum insulin levels were markedly reduced, to a similar extent as found in adult GHR−/− dwarfs (Fig. 1, 2nd time points). Measurement of pancreatic islet size in 10-day-old pups by use of immunohistochemistry with insulin antibody revealed a significant 28% reduction in GHR−/− islet size (pancreatic islet size: WT 12.8 ± 1.3, n = 18 vs. GHR−/− 9.2 ± 1.0, n = 23, P = 0.03; units expressed as ×10^3 μm^2). Eventually the growth retardation of GHR−/− mice, as reflected by body weight, reached 51% in 2-mo-old adults (Fig. 1, 3rd time points).

**Table 1. Changes in pancreatic tissue and islet β- and α-cell mass in GHR−/− mice**

<table>
<thead>
<tr>
<th></th>
<th>WT (9)</th>
<th>GHR−/− (7)</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>32.3 ± 0.6</td>
<td>14.4 ± 0.7</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Pancreas mass, g</td>
<td>0.277 ± 0.025</td>
<td>0.107 ± 0.008</td>
<td>0.00005</td>
</tr>
<tr>
<td>β-Cell mass, mg</td>
<td>1.70 ± 0.26</td>
<td>0.37 ± 0.07</td>
<td>0.0006</td>
</tr>
<tr>
<td>β-Cell mass/body wt, mg/kg</td>
<td>53 ± 8</td>
<td>25 ± 3</td>
<td>0.007</td>
</tr>
<tr>
<td>Pancreas mass/body wt, %</td>
<td>0.86 ± 0.07</td>
<td>0.75 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>α-Cell mass, mg</td>
<td>0.032 ± 0.002</td>
<td>0.029 ± 0.013</td>
<td>NS</td>
</tr>
<tr>
<td>α-Cell mass/body wt, mg/kg</td>
<td>0.9 ± 0.1</td>
<td>1.8 ± 0.3</td>
<td>0.022</td>
</tr>
</tbody>
</table>

Wild-type (WT; 4 male, 5 female) and growth hormone receptor-deficient (GHR−/−; 3 male, 4 female) 10- to 11-mo-old mice were analyzed. Values are means ± SE; nos. of mice are indicated in parentheses. P values are derived from comparison with WT littermates by unpaired t-tests. NS, nonsignificant.
Decreased cell proliferation and growth in pancreatic islets.
To investigate how lack of growth hormone signaling may affect pancreatic islet growth, we studied cell proliferation with immunofluorescence and a Ki67 replication marker in pancreatic sections prepared from 3-day-old mice. As shown in Fig. 5, in wild-type mice (left) the proliferating cell nuclei (Ki67 in green) were easily detectable in endocrine islets double-labeled with insulin antibody (in red). On the other hand, in islets of GHR−/− mice (right), the distribution of Ki67-positive cells was more scattered. Islet cells that are undergoing replication, measured by Ki67-positive nuclei per given area of the pancreatic islets, exhibited a 68% reduction in GHR−/− vs. wild-type littermates (WT 4.8 ± 0.6 cells per 1,000 μm² of islet area, n = 9 vs. GHR−/− 1.5 ± 0.2, n = 8, P < 0.001). This indicates that a lower percentage of islet cells were undergoing replication in the GHR−/− mice at this age, although insulin and glucose levels were perfectly normal (see above). Attempts in older mice (14-day and 4-mo) were unsuccessful, because the replication rate in wild-type mice was too low for a meaningful comparison (data not shown).

To reflect changes in individual cell growth caused by lack of growth hormone signaling, we further measured islet cell size in pancreatic sections. In normal adults (2 mo old), the average islet cell size in GHR−/− mice (843 ± 32 μm²/cell, n = 10) was significantly reduced by 20% compared with wild-type littermates (1,061 ± 40 μm² per cell; n = 10, P = 0.0004), suggesting decreased cell growth in GHR−/− islets.

Blood biochemical profile. Alterations in insulin responsiveness and pancreatic islet function in GHR−/− mice might be part of a more profound change in general metabolism. To detect other possible abnormalities, we analyzed 17 biochemical parameters in the blood of 3- to 4-mo-old, normally fed mice. Although GHR−/− mice had normal lipid profiles, they exhibited significant elevations in serum levels of chloride, albumin, urea, alanine aminotransferase, and creatine kinase. Other parameters were not significantly altered (Table 2).

Are GHR−/− mice obese? Unlike humans with Laron syndrome, which is accompanied by marked trunkal obesity (1, 28), GHR−/− mice were not obviously obese up to 4 mo of age. The mean body mass index (derived by dividing the body weight in kilograms by the square of the body length in meters, from nose to anus) of GHR−/− mice was not increased (Table 3). Blood biochemistry assays revealed normal lipid profiles (serum cholesterol, triglyceride, and HDL cholesterol levels) (Table 2). Nevertheless, careful measurement of the relative weight (to total body wt) of 3 fat pads revealed a selective enlargement in subcutaneous fat mass. The relative weight of the subcutaneous, lateral abdominal fat increased 2.5-fold compared with wild-type littermates. Other visceral fat pads found in the abdomen and surrounding the kidney were virtually unchanged (Table 3). As expected, GHR−/− mice exhibited a significant 22% decrease in relative weight of their livers (to total body wt) (Table 3) (41, 46). These changes exhibited no

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**Fig. 5.** GHR−/− mice exhibit decreased proliferation in pancreatic islet cells. Pancreatic sections taken from 3-day-old GHR−/− mice (right) and their WT littermates (left) were double-labeled with insulin (red) and Ki67 antibodies (green fluorescence). From a total of 8–9 islets examined in each genotype group, representative islet images (in ×400) are presented. Top: anti-insulin only; middle: anti-Ki67 only; bottom: computer-merged images.
of growth hormone signals caused diminished pancreatic islet size and β-cell mass, accompanied by elevated sensitivity to insulin. Islet hypotrophy is likely a primary cause of decreased pancreatic insulin mRNA and serum insulin levels. Glucagon production was also affected, although to a lesser extent and with no decrease in α-cell mass. The islet change was greater in proportion than the body growth retardation of the GHR−/− mice and was detectable as early as 10 days of age (islet replication decrease seen in 3-day-old mice), before the onset of the peripubertal growth spurt, suggesting specific effects independent of growth retardation. Double-stained immunofluorescence experiments suggest that decreased cell replication might contribute to the islet hypotrophy. Notably, GHR−/− mice exhibited the opposite characteristics of insulin secretion and sensitivity, as well as of fat mass, compared with human Laron syndrome patients.

Results of this study, demonstrating diminished pancreatic islet size and insulin mRNA accumulation in GHR−/− mice, indicate that the normal growth of the pancreatic islets and the level of insulin production were both affected by the lack of growth hormone signals. Consequently, basal levels of serum insulin under random-fed status or after 24 h of fasting were proportionally diminished. The decrease in pancreatic islet size seems to be disproportionately greater than the general growth retardation in GHR−/− mice, because, as body weight (mass) represents a 3-dimensional measurement (assuming mass represents volume when the object is homogeneous), its reduction to 49% of that of wild-type littermates (at 2 mo of age) would have caused reductions to 79% in 1-dimensional (such as body length) and 62% in 2-dimensional measurements (such as pancreatic islet area). [If \[x^3 = 0.49\], then \[x = (0.49)^{1/3} = 0.79\], and \[x^2 = 0.62\], where \(x\) represents 1-dimensional reduction.] In fact, GHR−/− mice exhibited an islet size of 32% of wild-type mice, significantly smaller than the calculated proportion of 62%, indicating a greater than proportional reduction in size. Our age-dependent studies, e.g., the relationships of body

Table 2. Changes in blood chemistry in GHR−/− mice

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>GHR−/−</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>GHR−/−</td>
</tr>
<tr>
<td>Sodium, mM</td>
<td>148±1</td>
<td>149±1</td>
</tr>
<tr>
<td>Potassium, mM</td>
<td>6.8±0.3</td>
<td>7.2±0.4</td>
</tr>
<tr>
<td>Chloride, mM*</td>
<td>115.1±0.5</td>
<td>117.8±0.8</td>
</tr>
<tr>
<td>Albumin, g/l*</td>
<td>14.7±0.4</td>
<td>17.2±0.4</td>
</tr>
<tr>
<td>Total protein, g/l</td>
<td>46.8±0.8</td>
<td>44±1.2</td>
</tr>
<tr>
<td>Albumin/total protein</td>
<td>0.315±0.008</td>
<td>0.391±0.007</td>
</tr>
<tr>
<td>Creatinine, μM</td>
<td>21.9±0.9</td>
<td>23.5±1.3</td>
</tr>
<tr>
<td>Urea, mM*</td>
<td>7.2±0.6</td>
<td>12.2±1.6</td>
</tr>
<tr>
<td>HDL cholesterol, mM</td>
<td>2.47±0.13</td>
<td>1.99±0.15</td>
</tr>
<tr>
<td>Triglyceride, mM</td>
<td>1.24±0.1</td>
<td>1.00±0.11</td>
</tr>
<tr>
<td>Cholesterol, mM</td>
<td>2.56±0.13</td>
<td>2.17±0.14</td>
</tr>
<tr>
<td>Potassium, mM</td>
<td>6.8±0.4</td>
<td>5.8±0.6</td>
</tr>
<tr>
<td>Calcium, mM</td>
<td>2.38±0.02</td>
<td>2.34±0.04</td>
</tr>
<tr>
<td>Uric acid, μM</td>
<td>132±9</td>
<td>154±11</td>
</tr>
<tr>
<td>Creatine kinase, IU/l*</td>
<td>848±122</td>
<td>1556±290</td>
</tr>
<tr>
<td>γ-Glutamyl transferase, IU/l</td>
<td>2.4±0.3</td>
<td>1.6±0.4</td>
</tr>
</tbody>
</table>

Sera were prepared from GHR−/− mice and their WT littermates at 3–4 mo of age, both males and females, at random-fed status. Values are means ± SE; n, nos. of mice. P values are derived from comparison with WT littermates using unpaired t-tests. *Independent parameters exhibiting significant changes in GHR−/− mice.

Table 3. Changes in fat pad weight in GHR−/− mice in relation to liver and body weights

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>GHR−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (6)</td>
<td>Female (8)</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>30.2±1.2</td>
<td>23.6±0.7$^*$</td>
</tr>
<tr>
<td>Body length, cm</td>
<td>9.5±0.2</td>
<td>9.2±0.1</td>
</tr>
<tr>
<td>Liver wt, % body wt</td>
<td>4.21±0.14</td>
<td>5.03±0.23$^*$</td>
</tr>
<tr>
<td>Body mass index</td>
<td>3.3±0.2</td>
<td>2.8±0.1$^*$</td>
</tr>
<tr>
<td>Fat wt, % body wt</td>
<td>Visceral</td>
<td>1.14±0.3</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td>0.37±0.02</td>
</tr>
<tr>
<td></td>
<td>Renal</td>
<td>0.40±0.24</td>
</tr>
</tbody>
</table>

Three fat pads and the liver were dissected from 4-mo-old GHR−/− mice and their WT littermates. Total body wt, wet tissue wt, and body length (nose to anus) were measured. Data are expressed as means ± SE; nos. of mice/group are indicated in parentheses. P values are derived from comparison with WT littermates by unpaired t-tests ($^*P < 0.05$, $^P < 0.001$, female vs. male WT; $\dagger P < 0.01$, $\ddagger P < 0.001$ vs. same sex WT littermates).

DISCUSSION

The classical form of Laron syndrome is caused by deletions or mutations in the GHR gene, resulting in dysfunction of the receptor and thus growth retardation, truncal obesity, insulin resistance, and hyperinsulinemia (28, 29). When or mutations in the GHR gene, resulting in dysfunction of the GHR

GHR

Table 3.

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weight and islet size in 10-day-old pups, and body weight/length in 4-month-old GHR−/− mice vs. their wild-type littermates, further support this notion. More directly, our measurement of β-cell mass (Table 1) demonstrates a net deficit of the endocrine pancreas, significantly greater than proportionate growth retardation. Reductions in pancreatic islet size and serum insulin levels were observed as early as 10 days in GHR−/− mice, before onset of the profound peripubertal growth retardation. Growth hormone is known to promote islet cell growth and to prevent apoptosis in monocytes and tumor cells (15, 16). Diminished pancreatic islet size in adult GHR−/− mice might be attributed to either decreased proliferation or increased apoptosis of pancreatic islet cells. One of the primary changes was decreased islet cell proliferation, possibly due to lack of growth hormone signaling and/or concomitant lack of IGF-I production. The size of individual islet cells in adult GHR−/− mice was also significantly reduced by 20%, which further supports a reduced cell growth and might also contribute to a decreased islet cell mass.

Growth hormone maintains glucose utilization and hepatic glucose production, decreases responsiveness of target tissues to insulin, and diminishes the conversion of glucose to fat, all contradictory to insulin effects. Children and adults with growth hormone deficiency exhibit fasting hyperglycemia (decreased hepatic production of glucose), increased insulin sensitivity, and diminished insulin secretion (22). Rodent models of chronic growth hormone excess are useful tools to investigate the mechanism by which growth hormone induces insulin resistance. Decreased insulin receptor (IR), IRS-1, and IRS-2 tyrosyl phosphorylation in response to insulin was found in skeletal muscles, whereas a chronic activation of the IRS-phosphatidylinositol 3-kinase pathway was found in the liver of growth hormone transgenic mice (11). In contrast, both growth hormone-deficient Ames dwarf and GHR−/− mice exhibit a state of hypersensitivity to insulin, associated with increased insulin receptor abundance and receptor phosphorylation activity in hepatocytes (9–11). Prolactin and its receptor (PRLR) are highly homologous to growth hormone and GHR, and PRLR−/− mice exhibited very similar results, i.e., decreased islet cell mass, insulin mRNA level, islet insulin content, and glucose tolerance (13). Under basal conditions in GHR−/− mice, the reductions in glucose concentration likely reflect a reduction in hepatic glucose production (due in part to lack of growth hormone signals). Under randomized fasted status with reduced insulin levels, GHR−/− mice are still hypoglycemic, probably due to increased insulin sensitivity, which overcompensates for the reduced insulin secretion.

IGF-I mediates many growth-promoting effects of growth hormone (30). GHR−/− mice exhibit lack of both growth hormone action and IGF-I production (46). Growth hormone has been shown to stimulate β-cell proliferation, glucose-stimulated insulin release, and insulin biosynthesis in cultured rat islets (35). These actions on islet cells are not necessarily all mediated through IGF-I expression (7), because IGF-I stimulates β-cell proliferation but inhibits glucose-stimulated insulin secretion and insulin biosynthesis (19, 45). Recent reports of pancreatic islet β-cell-specific gene targeting demonstrated that lack of IGF-I receptor on β-cells caused no change in normal islet growth and β-cell mass, reduced expression of GLUT2 and glucokinase genes, and impaired insulin secretion upon stimulation (26, 44). It remains to be determined whether the islet hypotrophy observed in GHR−/− mice is due directly to a lack of growth hormone signal or indirectly to a lack of IGF-I production. We have attempted to rescue islet defects by islet-specific IGF-I overexpression by use of a rat insulin promoter IGF-I transgene (using rat insulin promoter 1). Although the transgene itself caused no change in general growth and pancreatic islet development, it increased islet cell mass 3.8-fold and essentially restored it to wild-type level, supporting the notion that IGF-I mediates the growth hormone effects on islet cells (Guo Y, Lu Y, Coschigano KT, Kopchick JJ, Tang Z, Robertson K, and Liu JL presented at the American Diabetes Association 64th Scientific Sessions in June 2004). On the other hand, diminished insulin biosynthesis seems to have been caused by the lack of growth hormone signaling and cannot be explained by IGF-I deficiency. Furthermore, because growth hormone and IGF-I affect insulin sensitivity in opposite ways, increased insulin sensitivity in GHR−/− mice suggests a direct effect of growth hormone deficiency.

GHR−/− mice resemble human Laron syndrome patients in growth retardation and other key elements (25, 46). As part of this study, we clearly demonstrate that these mice are oversensitive to insulin, hypoinsulinemic, and not obviously obese. In contrast, Laron syndrome patients are known to exhibit hyperinsulinemia, insulin resistance (27), and truncal obesity (1, 28). These important distinctions might reflect a difference in species as well as in the etiology of defects. Rodents are born at a developmental stage corresponding to ~26 wk of human gestation (23). A human embryo deficient in GHR gene expression would be influenced significantly during the “prolonged” intrauterine growth in the third trimester. As another well-documented example, humans lacking insulin receptors show severe intrauterine growth retardation and hypoglycemia, in contrast to insulin receptor-deficient mice (23). Moreover, the Laron syndrome is caused by heterogeneous GHR mutations (usually partial defects) vs. complete gene inactivation in GHR−/− mice. Finally, downstream mediators of growth hormone action, such as the interplay of IGF-I and IGF-II, might also contribute differently in human and mouse. For instance, IGF-II production is maintained throughout life in humans but virtually ceases after birth in rodents (8, 17, 33).

Our results, extending those of previous reports, demonstrate that in addition to causing general growth retardation, GHR gene deficiency induces diminished pancreatic islet size (and β-cell mass), insulin gene expression, and serum levels. Hepatic as well as pancreatic expression of the IGF-I gene is also drastically reduced. Blood glucose and serum glucagon levels are significantly reduced. GHR−/− mice exhibit increased insulin sensitivity. The abnormalities in glucose homeostasis occur as early as 10 days after birth, when growth retardation in GHR−/− mice was relatively mild. Diminished pancreatic islet mass appears to be related to decreases in proliferation and cell growth. Finally, GHR−/− mice are different from the patients with Laron syndrome in serum insulin level, insulin responsiveness, and obesity. We conclude that growth hormone signaling is essential for maintaining pancreatic islet size, stimulating islet hormone production, and maintaining normal insulin sensitivity and glucose homeostasis.
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