Growth hormone receptor gene deficiency causes delayed insulin responsiveness in skeletal muscles without affecting compensatory islet cell overgrowth in obese mice

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Robertson, Katie, John J. Kopchick, and Jun-Li Liu. Growth hormone receptor gene deficiency causes delayed insulin responsiveness in skeletal muscles without affecting compensatory islet cell overgrowth in obese mice. Am J Physiol Endocrinol Metab 291: E491–E498, 2006. First published April 18, 2006; doi:10.1152/ajpendo.00378.2005.—Growth hormone (GH), acting through its receptor (GHR), is essential for somatic growth and development and maintaining metabolic homeostasis. GHR gene-deficient (GHR−/−) mice exhibit drastically diminished insulin-like growth factor-I (IGF-I) levels, proportional growth retardation, elevated insulin sensitivity, and reduced islet β-cell mass. Unlike the liver, which is mostly unaffected by changes in IGF-I level, skeletal muscles express high levels of IGF-I receptor (IGF-IR). The net result of a concurrent deficiency in the actions of both GH and IGF-I, which exert opposite influences on insulin responsiveness, has not been evaluated. We studied insulin-stimulated early responses in the insulin receptor (IR), insulin receptor substrate-1 (IRS-1), and p85 subunit of phosphatidylinositol 3-kinase. Upon in vivo insulin stimulation, skeletal muscles of GHR−/− mice exhibit transient delayed responses in IR and IRS-1 phosphorylation but normal levels of p85 association with IRS-1. This is in contrast to normal/elevated insulin responses in hepatocytes and indicates tissue-specific effects of GHR gene deficiency. In addition to stimulating normal islet cell growth, GH may participate in islet cell overgrowth, which compensates for insulin resistance induced by obesity. To determine whether the islet cell overgrowth is dependent on GH signaling, we studied the response of male GHR−/− mice to high-fat diet (HFD)-induced obesity. After 17 wk on a HFD, GHR−/− mice became more significantly obese than wild-type control mice and exhibited increased β-cell mass to a slightly higher extent. These data demonstrate that GH signaling is not required for compensatory islet growth. Thus, in both muscle insulin responsiveness and islet growth compensation, normal levels of GH signals do not seem to play a dominant role.

gene-disrupted mice; insulin receptor; phosphorylation; β-cell mass; high-fat diet

GROWTH HORMONE (GH), acting through its receptor (GHR), is essential for somatic growth and development and maintaining metabolic homeostasis (20, 21). Concerning insulin production and action, GH is known to stimulate islet cell growth and insulin secretion (31–34). On the other hand, GH counteracts insulin’s actions and causes insulin resistance in insulin target tissues. Consequently, GHR gene-deficient (GHR−/−) mice exhibit proportional growth retardation, elevated insulin sensitivity partly due to increased insulin receptor (IR) level and receptor activation in hepatocytes, reduced islet β-cell mass due to decreased islet cell proliferation, and selective increases in fat pad weights (5, 7, 26, 46). GHR gene deficiency also causes a concurrent decrease in the production of insulin-like growth factor-I (IGF-I), which also plays a role in islet cell growth, insulin secretion, and maintaining insulin sensitivity (13, 17, 28, 43, 45). Unlike the liver, which is mostly unaffected by IGF-I, skeletal muscles express high levels of IGF-I receptor (IGF-IR) (13). Both tissues are prime targets of GH actions. The net result of a concurrent deficiency in the actions of both GH and IGF-I, which normally exert opposite influences on insulin responsiveness, has not been evaluated in the GHR−/− mouse. Maintaining islet β-cell mass and adequate insulin secretion to meet metabolic demands is crucial to avoid the development of type 2 diabetes (1, 19, 36, 38). In this aspect, in contrast to human Laron syndrome, where GH insensitivity is characterized by hyperinsulinemia, insulin resistance, and truncal obesity, GHR−/− mice exhibit decreased serum insulin levels and increased insulin sensitivity and are not grossly obese (7, 24, 25). This may be caused by species-specific characteristics of GH physiology but more likely by the fact that mice are not exposed to the “buffet-type” human diet, which favors obesity and insulin resistance. In addition to stimulating normal islet cell growth, GH may participate in islet cell overgrowth, which compensates for insulin resistance induced by obesity. To our knowledge, a response of GHR−/− mice to diet-induced obesity and its possible effects on insulin responsiveness and islet compensation have not been reported. To extend our early studies on GHR−/− mice in the two key aspects, i.e., increased insulin sensitivity and decreased islet cell growth, the current study was designed to explore the interplay of GH and IGF-I in regulating insulin responsiveness of the skeletal muscles and the response of GHR−/− mice to high-fat diet-induced obesity, insulin resistance, and islet overgrowth.

MATERIALS AND METHODS

Materials. The reagents and apparatus for SDS-PAGE and immunoblotting were obtained from Bio-Rad (Richmond, CA). The monoclonal anti-phosphotyrosine antibody (αPY; PY99) and the polyclonal anti-IR β-subunit antibody (αIR; C-19) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-rat carboxy-terminal insulin receptor substrate-1 (IRS-1; αIRS-1 antibody) and the anti-

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body to the p85 subunit of phosphatidylinositol 3-kinase (PI3K; op85 antibody) were purchased from Upstate Biotechnology (Lake Placid, NY). The insulin antibody (H-86) used in immunohistochemistry was obtained from Santa Cruz Biotechnology.

Animal procedures. GHR−/− mice carry a targeted disruption of exon 4 of the mouse GHR/binding protein (GHR/BP) gene, as previously reported (46). Offspring (wild types as controls and GHR−/−) derived from heterozygous (GHR+/−) mating pairs on a hybrid 129/Ola-BALB/c-C57BL/6 background were used in experiments.

To determine genotype, we isolated genomic DNA from tail clips with standard methods. Primers Inl-4 (5′-CCC TGA GAC CTC CTC AGT TC), Inl-3 (5′-CCC CCC CCC AGA GAG ACT GGC TT), and Neo-3 (5′-GCT CGA CAT TGG GTG GAA ACA T) were used in PCR reactions, which yield a 390-base band for the wild-type allele and 290/200-base double bands for the knockout allele, as previously reported (5).

The animals were maintained in 12:12-h 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. For the study of insulin response, GHR−/− mice and their wild-type littermates (6 mo of age) were fasted for 24 h and anesthetized by intraperitoneal administration of a cocktail of ketamine-xylazine-acetamizone. The mice were injected with insulin (10 IU/kg ip) or saline; after 5 or 15 min, they were killed and their soleus and gastrocnemius muscles were removed along with the liver to prepare cell lysate. All animal-handling procedures were approved by the McGill University Animal Care Committee. Serum concentrations of insulin (Linco Research) were determined using a radioimmunoassay kit.

Immunoprecipitation and Western blots. The tissues were homogenized in 5 ml of protein extraction buffer containing 150 mM NaCl, 10 mM Tris, pH 7.4, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.5% Nonidet P-40, 100 mM NaF, 10 mM sodium pyrophosphate, 10 mM sodium orthovanadate, and protease inhibitors (Complete Mini EDTA-free; Roche, Indianapolis, IN) at 4°C (43). The samples were incubated on ice for 30 min and centrifuged at 600 rpm for 20 min at 4°C, and then the supernatant was removed and centrifuged at 13,000 rpm for 45 min at 4°C in a Beckman JA20–1 rotor. The Bradford assay (Bio-Rad) was used to measure protein concentration, and the sample was aliquoted and stored at −80°C.

Tissue lysates containing 2 mg of total protein were immunoprecipitated with the antibodies αIR or αIRS-1 (2 μg/ml final concentration) overnight at 4°C. The next day, 40 μl of protein G-Sepharose (Roche) were added, and samples were incubated for another 1.5 h on a rocking platform at 4°C and centrifuged at 14,000 rpm for 2 min at 4°C. The precipitate was washed three times with the protein extraction buffer. The final precipitate was boiled for 5 min in 40 μl of Laemmli sample buffer (Bio-Rad).

Samples were loaded onto a 7% SDS-polyacrylamide gel with the use of a Mini Protean apparatus (Bio-Rad). Transfer of proteins from the gel to 0.2-μm nitrocellulose (Trans-Blot transfer medium; Bio-Rad) was performed for 1.5 h at 100 V by using the Bio-Rad mini transfer apparatus in a transfer buffer consisting of 25 mM Tris, 192 mM glycine, and 20% methanol. The membranes were blocked for 1 h at room temperature to reduce nonspecific binding in a TBS-T buffer (composed of 10 mM Tris·HCl, 150 mM NaCl, and 0.1% Tween 20, pH 7.6) containing 3% BSA for phosphotyrosine detection or 2% ECL Advance blocking agent (Amersham Biosciences, Amersham, UK) for protein detection. The membranes were incubated overnight at 4°C with αPY, αIR, or αIRS-1 (all at 1:1,000) each and diluted in corresponding blocking buffer. The membranes were washed, incubated with secondary antibodies, and detected by chemiluminescence using the ECL Advance Western blotting detection kit (Amersham).

To detect the amount of p85 associated with IRS-1, the blots that were used to detect αIRS-1 were stripped with Re-Blot Plus (Chemicon International), washed in TBS-T for 5 min, blocked for 30 min, and then probed with op85 antibody (1:1,000) and detected using chemiluminescence. Images were captured using a Fluorchem 8900 imager (Alpha Innotech, San Leandro, CA), and densitometry was carried out using AlphaEase software (Alpha Innotech).

High-fat diet-induced obesity. Male GHR−/− mice and their male wild-type littermates (3.5 mo of age) were fed for 17 wk with a high-fat diet (HFD; Research Diets, New Brunswick, NJ). Their body weight was measured once a week, and blood glucose levels every 3 wk, using a OneTouch blood glucose meter (LifeScan, Burnaby, BC, Canada). At 15 wk, an insulin tolerance test was performed. Animals were injected with recombinant human insulin (0.75 IU/kg ip; Roche), and blood glucose levels were measured at 0, 20, 40, and 60 min afterward. At 17 wk, the mice were anesthetized by intraperitoneal administration of a cocktail of ketamine-xylazine-acetamizone, and then body length and weight were measured and fat pads were removed and weighed. The mice were killed by cervical dislocation, and then blood was collected for serum preparation and pancreas were rapidly removed for histochemical analysis.

Immunohistochemistry and islet cell mass measurement. Pancreatic sections were stained with an insulin antibody (H-86; Santa Cruz Biotechnology) using diaminobenzidine substrate, which resulted in a brown immunoreactive signal with a hematoxylin counterstain (blue) of cell nuclei. The β-cell mass and average cell size were determined as previously reported (2, 26).

Statistical analysis. Data are expressed as means ± SE. Student’s t-test (unpaired and paired) was performed using InStat software version 3 (GraphPad Software, San Diego, CA).

RESULTS

A general characterization of GHR−/− mice was reported by our group recently (16, 26). Because of a lack of GH signaling, the body weight of adult GHR−/− mice is only one-half that of their wild-type littermates, confirming severe growth retardation; blood glucose and serum insulin levels are significantly reduced to 80 and 40% of the normal values, respectively; serum level of the total IGF-I is reduced to one-half because of a lack of GH stimulation; and GHR−/− mice exhibit significantly increased sensitivity to insulin tolerance tests (16, 26).

Most of these characteristics have also been reported by other groups (7, 11, 12, 46) and were unchanged in the animals used in this study (data not shown).

Delayed and diminished insulin receptor phosphorylation in skeletal muscle. GHR−/− mice exhibit elevated insulin responsiveness, including increased IR levels and activation in the hepatocytes (11, 46). To study whether this phenomenon is tissue specific, we have tested insulin-stimulated early responses in the skeletal muscles and liver. GHR−/− mice and control littermates were injected with insulin (10 IU/kg ip) or saline, and muscle or liver homogenates were subjected to immunoprecipitation with the use of an antibody against the IR β-subunit (αIR), followed by immunoblotting with the same antibody and that against the tyrosine phosphorylation (αPY). After densitometry analysis was completed, the amount of tyrosine phosphorylation of the IR β-subunit was normalized using the total receptor levels. As shown in Fig. 1, in skeletal muscles of wild-type mice, 5 min of insulin treatment caused a significant 3.5-fold increase in IR phosphorylation (A and B, left) over that of untreated animals. However, upon insulin stimulation of GHR−/− mice, IR phosphorylation was only marginally elevated 1.3-fold [not significant (NS)], significantly lower than in wild-type mice. In contrast, in the liver (Fig. 1, A and B, right), insulin treatment caused significantly increased phosphorylation in both groups of mice, e.g., 7.5- and 6.8-fold in wild-type and GHR−/− mice, respectively, compared with untreated controls. Later, at 15 min, wild-type
mice maintained a significant elevation in IR phosphorylation (4.3-fold; Fig. 1, C and D, left), which was only marginally elevated 1.9-fold in GHR−/− mice (P < 0.05), lower than in wild-type mice mostly because of an elevated basal activity. As a control, in the liver (Fig. 1, C and D, right), insulin treatment caused fourfold significantly increased phosphorylation in both the wild-type and GHR−/− mice compared with untreated controls. The IR responses in the liver of GHR−/− mice, almost identical to that of wild-type mice, were slightly lower than reported previously (11).

Delayed IRS-1 phosphorylation in skeletal muscle. The same sets of muscle/liver proteins were used to determine the level of IRS-1 phosphorylation. The muscle or liver homogenates were subjected to immunoprecipitation with an antibody against IRS-1 (αIRS-1), followed by immunoblotting with the same antibody and another against the tyrosine phosphorylation (αPY). The data were normalized with the amount of total IRS-1 levels (Fig. 2). In skeletal muscles of wild-type mice, 5 min of insulin treatment caused a significant increase in IRS-1 phosphorylation (2.1-fold) that was virtually abolished in the GHR−/− mice (Fig. 2, A and B, left). As a positive control for insulin action in Fig. 2, A and B, right, IRS-1 phosphorylation in liver samples displayed a 1.5-fold increase in both wild-type and GHR−/− mice compared with untreated control. Later, at 15 min, the muscles of both wild-type and GHR−/− mice showed comparable and significant elevations of IRS-1 phosphorylation of 2.0- and 1.6-fold, respectively (Fig. 2, C and D, left). The liver samples (Fig. 2, C and D, right) exhibited an increase in IRS-1 phosphorylation of 1.7- or 3.5-fold in wild-type or GHR−/− mice, respectively. The IRS-1 responses in the liver of GHR−/− mice were slightly higher than reported previously (11). The results of IR and IRS-1 phosphorylation in the skeletal muscles are in contrast to that reported previously and to what we have demonstrated in the hepatocytes, which exhibited increased IR protein level, normal or slightly increased (rather than delayed or decreased) receptor activation, and IRS-1 phosphorylation in GHR−/− mice (11).

Normal p85 stimulation in skeletal muscle. PI3K is activated after insulin stimulation and IRS-1 phosphorylation. In this study, the PI3K activation was measured as the amount of p85 protein associated with IRS-1 in an immunoprecipitate with the use of anti-IRS-1. With the same muscle or liver homogenates that were immunoprecipitated with αIRS-1 (Fig. 2, C and D), the proteins were immunoblotted with an antibody against p85 (αp85). The values were normalized by the total IRS-1 levels (Fig. 3B). In the muscle, p85 levels were increased similarly (2.6- and 2.2-fold) in both wild-type and GHR−/− mice when stimulated by insulin; i.e., the signaling pathway leading to the activation of the PI3K by insulin was unaltered in the skeletal muscle of GHR−/− mice. As positive controls, increased p85 association with IRS-1 was demonstrated in the livers of both wild-type and GHR−/− mice, as reported previously (11).

HFD-induced obesity in GHR−/− mice. To study whether GH signaling is involved in the compensatory growth of pancreatic islets in response to obesity-induced insulin resistance, we challenged male GHR−/− mice and their wild-type littermates with a HFD for 17 wk. Their body weights were measured once a week (Fig. 4A) and their blood glucose levels every 3 wk (data not shown). Both wild-type and GHR−/− mice gained significant weight with the HFD, 21 and 31% of their initial body weights, respectively (Fig. 4B). To further demonstrate increased fat mass and reveal potential depot-specific effect, various fat pads were excised, weighed, and corrected for total body weight. As shown in Fig. 4C, both the
GHR−/− mice and their wild-type littermates significantly increased the weight of each fat pad (except the renal in the GHR−/−), indicating that GHR−/− mice are not resistant to HFD-induced obesity. Normally, GHR−/− mice are hypersensitive to insulin, in contrast to human Laron patients. To determine whether the mice have decreased insulin responsiveness as a result of obesity, we performed an insulin tolerance test on all animals, on either HFD or normal diet, 2 wk before the end of the study. In both wild-type and GHR−/− mice, a 15-wk HFD failed to cause a significant change in insulin sensitivity due to obesity. Serum insulin levels, another indirect indicator of insulin resistance, were unaffected as well (data not shown). Thus HFD for 17 wk created obesity, but not insulin resistance, in both wild-type and GHR−/− mice.

Evaluation of islet cell overgrowth due to obesity. To study whether the HFD-induced obesity can cause a compensatory islet overgrowth in the GHR−/− mice, we measured β-cell mass at 17 wk from pancreatic sections stained with insulin. As shown in Fig. 4D, consistent with our previous report (26), GHR−/− mice on the normal diet had only 45% of the β-cell mass compared with wild-type littermates; after the HFD, however, both types of mice displayed similar extents of islet compensation, e.g., 2.8-fold in wild-type and 3.3-fold in GHR−/− mice, respectively. Representative islets are illustrated in Fig. 4E. In both wild-type and GHR−/− mice, HFD caused a significant enlargement of the islet size. The islet compensation was likely caused by cell hyperplasia, because the average cell size (representing hypertrophy) and islet density per tissue area (representing islet neogenesis) were unaffected (data not shown). Thus obese GHR−/− mice exhibited a normal, compensatory overgrowth of islet cells.

Fig. 2. Skeletal muscles exhibit a delayed response in insulin-stimulated insulin receptor substrate-1 (IRS-1) phosphorylation in GHR−/− mice. Mice fasted for 24 h were injected with insulin (10 IU/kg ip) for 5 (A and B) or 15 min (C and D) before being killed, and their muscles or livers were removed to prepare cell lysate. A and C: lysates were precipitated with IRS-1 antibody and probed with the same antibody as well as with anti-phosphotyrosine (IRS-1-P-tyr) in Western blots. A representative blot is illustrated from experiments of n = 8 at 5 min and n = 5 at 15 min for skeletal muscles and n = 3 for liver. B and D: densitometric quantification of the IRS-1 phosphorylation levels corrected by total IRS-1 protein level. *P < 0.05; **P < 0.01 vs. untreated controls.

Fig. 3. Insulin-stimulated p85 association with IRS-1 in skeletal muscles of GHR−/− mice and their wild-type littermates. Mice fasted for 24 h were injected with insulin (10 IU/kg ip) for 15 min before being killed, and their muscles or livers were removed to prepare cell lysate. A: lysates (same as in Fig. 2C) were precipitated with IRS-1 antibody and probed with the same antibody, stripped, and then probed with p85 antibody in Western blots. A representative blot is illustrated from experiments of n = 5 for skeletal muscles and n = 3 for liver. B: densitometric quantification of the p85 levels corrected by total IRS-1 protein level. *P < 0.05 vs. untreated controls.
DISCUSSION

Using GHR−/− mice, our group recently demonstrated that GH signaling is essential for maintaining pancreatic islet growth, stimulating islet hormone production, and maintaining normal insulin sensitivity and glucose homeostasis (26). Through islet-specific overexpression of IGF-I, we were able to rescue some of the islet defects, suggesting that IGF-I mediates some GH actions on islet growth (16). In the current study, we have further characterized GHR−/− mice in insulin responsiveness and in islet cell growth. Specifically, we have shown that GH signaling does not play a dominant role in either insulin responsiveness in the skeletal muscles or in islet growth compensation due to obesity. Antagonizing insulin’s actions, GH decreases glucose uptake, maintains hepatic glucose production, decreases responsiveness of target tissues to insulin, and diminishes the conversion of glucose to fat. Conversely, GHR−/− mice are clearly hypersensitive to insulin’s actions (11, 26). As for specific target tissues affected, it has been reported (11) that in the hepatocytes of GHR−/− mice, both the basal levels of IR protein and the response in IR phosphorylation are elevated. As another important target tissue of insulin action, the role of skeletal muscles has not been evaluated in GHR−/− mice. In a similar system, GH-deficient Ames dwarfs, also hypersensitive to insulin, exhibit reduced...
insulin-stimulated phosphorylation of IR and IRS-1 in the skeletal muscles (10). Our results are thus consistent with those of Ames mice, perhaps because of a common deficiency in endogenous IGF-I production.

As a primary insulin target, skeletal muscles express high levels of receptors for insulin, GH, and IGF-1 (14). Insulin causes IR autophosphorylation, which recruits IRS-1 and other SH2-containing docking molecules. Among other signaling pathways, insulin stimulation causes dissociation of the p85 subunit from PI3K and, thereby, activation of the p110 subunit, which is a major mediator of insulin’s actions (39, 40). These early responses cause activation of downstream molecules such as Akt/PKB, recruitment of the glucose transporter GLUT4 into the plasma membrane, and increased glucose uptake into the muscle cells. The current study was designed to reveal possible changes in early insulin responses in the skeletal muscle and to compare them with those of hepatocytes in GHR\(^{-/-}\) mice. In contrast to the elevated or normal insulin responsiveness exhibited in the liver of GHR\(^{-/-}\) mice (11), our results indicate that upon insulin stimulation, there is no elevated insulin response; i.e., skeletal muscles do not contribute to increased insulin sensitivity in GHR\(^{-/-}\) mice. If anything, there were transiently delayed and/or diminished responses in the early phase of IR or IRS-1 phosphorylation. One of the underlying differences is that hepatocytes do not express IGF-I receptor (29) and are unaffected by secondary deficiency of the underlying differences is that hepatocytes do not express IGF-I receptor (29) and are unaffected by secondary deficiency of the underlying differences is that hepatocytes do not express IGF-I receptor (29) and are unaffected by secondary deficiency of the underlying differences is that hepatocytes do not express IGF-I receptor (29) and are unaffected by secondary deficiency of the underlying differences is that hepatocytes do not express IGF-I receptor (29) and are unaffected by secondary deficiency of the underlying differences is that hepatocytes do not express IGF-I receptor (29) and are unaffected by secondary deficiency of the underlying differences is that hepatocytes do not express.

IGF-I does not bind to hepatocytes or adipocytes, and therefore its primary insulin-like action is believed to be mediated through the skeletal muscles (6). Indeed, in newborn IR-deficient mice, IGF-I directly activates PI3K and, presumably, glucose uptake in the muscles, because it corrects the hyperglycemia (8). It has been established that in the skeletal muscles, GLUT4 translocation to the cell membrane is stimulated by both insulin and IGF-I through their cognate receptors, a crucial process in postprandial glucose disposal. The importance of this mechanism is clearly demonstrated by muscle-specific ablation of the GLUT4 gene, which causes severe insulin resistance and glucose intolerance (47). Moreover, muscle-specific inactivation of both IR and IGF-IR in MKR mice, by overexpressing a dominant negative protein, creates an even more severe phenotype by causing an early onset of diabetes (13). To understand the relative contributions of either IR or IGF-IR to this defect, note that muscle-specific inactivation of IR gene alone (using Cre/loxP system) is insufficient to cause significant insulin resistance or glucose intolerance (4), indicating that IGF-IR, or IGF-IR in conjunction with IR, plays a potent role in stimulating glucose uptake in the skeletal muscles. Of course, for more conclusive proof one would have to create a specific ablation of IGF-IR gene alone in the muscle cells. In the meantime, our results indicate that GH signaling is not dominant in countering insulin’s actions in the muscles and that the major site at which GH antagonizes insulin’s actions is the liver (6). This supports tissue-specific influences of GH on in vivo insulin responsiveness.

There is no doubt that GH has an insulin-counterregulatory role in skeletal muscle, and our results are, in general, consistent with other related models of GH deficiency or excess. Transgenic mice overexpressing GH antagonist (GHa) exhibit elevated insulin sensitivity and decreased blood glucose and serum insulin levels, similar to GHR\(^{-/-}\) and Ames dwarfs, although their growth retardation is much milder (44). They maintain a normal rate of glucose uptake in skeletal muscles and brown adipose tissues and exhibit normal (rather than elevated) insulin responses in IR, IRS-1, and Akt in the skeletal muscles, similar to our GHR\(^{-/-}\) mice (44). The enhanced insulin sensitivity in GHa mice seems to be caused by significantly improved glucose uptake in white adipose tissues, resembling that occurring in the liver of GHR\(^{-/-}\) mice. In this study, slightly differently from that in GHa mice, the delayed insulin responsiveness in the skeletal muscles was transient and limited to IR and IRS-1 only. By 15 min after insulin stimulation, all parameters including PI3K were normalized. Whether this phenomenon has any physiological implications needs to be addressed in future studies. For instance, as a primary organ for glucose disposal, insulin-stimulated glucose uptake could be affected, albeit transiently. Even then, an accumulated effect may consequently contribute to the phenotypes such as longevity of these mice (10). This study and the reports of insulin responsiveness in the liver of GHR\(^{-/-}\) mice and in the muscles of Ames dwarfs suggest that normal levels of GH signals do not antagonize insulin responsiveness in the skeletal muscles (10, 11). This is not necessarily contradictory to the finding that GH antagonist corrects the insulin resistance in LID mice, because the latter finding only implies that excessive GH secretion causes insulin resistance in skeletal muscles (44).

In addition to effects on insulin action, GH is an important growth factor for islet cells (42, 45). Downstream of the GHR/Jak2 interaction, signal transducer and activator of transcription 5 activation and consequent induction of cyclin D2 are essential for the mitogenic effect of GH on \(\beta\)-cells (15, 31). In nonislet cells, GH increases the activity or protein level of Foxa-2 and hepatocyte nuclear factor-1\(\alpha\), key molecules in \(\beta\)-cell growth (30, 41). These factors likely mediate GH-stimulated islet cell growth. However, it is unclear whether GH signals are involved in compensatory overgrowth of islet cells such as in pregnancy or obesity. In this aspect, GH secretion is markedly diminished due to obesity (35), which may contribute to the eventual failure of \(\beta\)-cells. Thus we were interested in studying whether GHR\(^{-/-}\) mice would be able to increase \(\beta\)-cell growth in response to obesity. On the other hand, GHR\(^{-/-}\) mice are not grossly obese, unlike human Laron syndrome (26). Another question was whether GHR\(^{-/-}\) mice would be resistant to obesity itself. For these purposes, we have successfully induced obesity in two sets of mice at 3–4 and 9–10 mo of age. Under the experimental conditions, the obesity was not severe enough to cause significant changes in insulin tolerance, let alone diabetes. Nevertheless, our results clearly demonstrate that GHR\(^{-/-}\) mice respond more efficiently to the HFD in becoming obese and exhibit a significant increase in islet cell growth, slightly higher than wild-type mice. Thus GH signals are not essential for the compensatory growth of islet cells in response to obesity. The causes of enlarged \(\beta\)-cell mass in nondiabetic obese humans and rodents include increased islet cell replication, neogenesis, and cellular hypertrophy (9, 18, 27). Currently, there are many other factors for islet cell growth that are potentially involved in islet compensation. They include cyclins D1 and D2 (23), nutrients such as glucose, hormones such as a combination of epidermal
INSULIN RESPONSIVENESS AND ISLET GROWTH IN GHR-/– MICE

E497

growth factor and gastrin (37), glucagon-like peptide-1, and several growth factors including fibroblast growth factor and hepatocyte growth factor (3, 22). As we wrote this report, we were unaware of any positive involvement of these factors in obesity-induced islet compensation.

In summary, in response to in vivo insulin stimulation, the skeletal muscles of GHR-/– mice exhibit transient delayed and/or diminished responses in IR and IRS-1 phosphorylation. This finding is in contrast to elevated or normal insulin responses in hepatocytes, perhaps because of a concurrent decrease in IGFI-I effect. When challenged with a HFD, GHR-/– mice became more significantly obese, in contrast to the human Laron syndrome of GH insensitivity, which displays default obesity over the normal population. As a consequence of obesity, GHR-/– mice displayed an enhanced β-cell compensation, slightly greater than that of wild-type mice, demonstrating that GH signals are not required for compensatory islet growth. Thus, in both muscle insulin responsiveness and islet compensation, GH does not seem to play a dominant role.

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REFERENCES


