Regulation of full-length and truncated growth hormone (GH) receptor by GH in tissues of lit/lit or bovine GH transgenic mice

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Iida, Keiji, Juan P. del Rincon, Dong-Sun Kim, Emina Itoh, Karen T. Coschigano, John J. Kopchick, and Michael O. Thorner. Regulation of full-length and truncated growth hormone (GH) receptor by GH in tissues of lit/lit or bovine GH transgenic mice. Am J Physiol Endocrinol Metab 287:E566–E573, 2004.—Two truncated isoforms of growth hormone (GH) receptor (GHR) were identified in mice and in humans. The proteins encoded by these isoforms lack most of the intracellular domain of the GHR and inhibit GH action in a dominant negative fashion. We have quantified the mRNAs encoding the GHR isoforms in mouse tissues by use of real-time RT-PCR and examined the effect of GH excess or deficiency on regulation of mRNA levels of the GHR isoforms in vivo. In the liver, the truncated GHR mRNAs (mGHR-282 and mGHR-280) were 0.5 and <0.1%, respectively, the level of full-length GHR (mGHR-fl). In skeletal muscle, the values were 2–3 and 0.1–0.5% of mGHR-fl, respectively, and in subcutaneous fat, the values were 3–5 and 0.1–0.5% of mGHR-fl. The bovine GH transgenic mice showed a significant increase of mGHR-fl in liver but a significant decrease in skeletal muscle, with no difference in subcutaneous fat when compared with control mice. The lit/lit mice showed a significant decrease of mGHR-fl in liver, no difference of mGHR-fl in muscle, and a significant increase of mGHR-fl in subcutaneous fat when compared with lit/+ mice. The mRNA of mGHR-282 was regulated in parallel with mGHR-fl in all tissues of all mice examined, whereas that of mGHR-280 was not changed in either GH-excess or GH-deficient states. In conclusion, two truncated isoforms of GHR mRNAs were detected in liver, skeletal muscle, and subcutaneous fat of mice. The ratio of GHR-tr to GHR-fl mRNA was tissue specific and not affected by chronic excess or deficiency of GH.

Isoform; alternative splicing; dominant negative inhibition

The actions of growth hormone (GH) are mediated by binding to a specific, high-affinity cell surface receptor (GHR) (3). Expression of GHR is a requirement for cellular responsiveness to GH. The GHR is a member of the cytokine receptor superfamily and consists of an extracellular, a single transmembrane, and an intracellular domain (23). The GHR gene includes nine protein-coding exons from exon 2 to exon 10 (15). Exon 2 encodes a secretory signal sequence, exons 3–7 the extracellular ligand-binding domain, exon 8 the transmembrane domain, and exons 9–10 the intracellular domain, including the conserved box 1 region, which is critical for JAK2 association and subsequent signal transduction (30).

In humans, two mRNA transcripts produced by alternative splicing within or around exon 9 result in truncated GHRs (GHR-tr), named hGHR-279 and hGHR-277 (8, 28). GHR-279, consisting of 279 amino acids, is produced by deletion of the first 26 bp of exon 9 of the full-length receptor (GHR-fl). On the other hand, GHR-277 is produced by complete skipping or “splicing” of exon 9 of the GHR-fl. Both of these alternatively spliced mRNAs encode a translational frame shift that results in a premature stop codon. Thus GHR-279 and GHR-277 possess intact extracellular and transmembrane domains but lack >97% of the intracellular domain, including the box 1 region. Functional studies confirmed that these receptor variants could form heterodimers with GHR-fl and inhibit GHR signaling in a dominant negative fashion (1, 19, 28). Two clinical case reports of the GH insensitivity syndrome (GHIS) strongly support the significance of GHR-tr as a negative regulator of GHR signaling in vivo (1, 18). All other reported cases of GHIS possess biallelic mutations, in other words, homozygous (27) or compound heterozygous (21) mutations in the GHR gene, whereas two cases (1, 18) show GHIS with heterozygous mutation producing GHR-tr. The mutations of the GHR gene in these cases were identified at the splice-acceptor (1) and splice-donor (18) sites of exon 9, respectively. Both of the mutations cause splicing abnormality and complete skipping of exon 9, resulting in frame shift and production of hGHR-277 from mutant allele, the structure of which is identical to one of the isoforms produced by alternative splicing from the GHR gene. Because the mutation is heterozygous in these cases, GHR-fl is also produced from the other wild-type allele. The relevant finding from two cases is that both of the probands with the splice site mutation of exon 9 showed GHIS despite having a normal allele. These cases strongly suggest that hGHR-277 fully inhibits the function of GHR-fl in vivo, if the expression of hGHR-277 is comparable to that of hGHR-fl. We hypothesized that GHR-tr, produced by alternative splicing in physiological conditions, may also play a role as a negative regulator of GHR signaling in vivo, even without a rare mutation of the GHR gene. It is likely that GHR-tr may function in the situation that GHR signaling is excessive or reduced, as observed in patients with acromegaly or GH deficiency, respectively. The expression levels of GHR-fl may also play a role to regulate GHR signaling. Therefore, we attempted to clarify the significance of
GHR-tr as well as GHR-fl produced by alternative splicing in vivo by use of two mouse models that have altered GHR signaling.

Mice that express the bovine GH transgene are useful models for investigating the effect of chronic GH excess in vivo (7, 9). These giant mice show high circulating levels of GH as well as IGF-I (7). The lit/lit mice are dwarf mice with isolated GH deficiency due to an inactivating mutation of the GH-releasing hormone receptor gene (14).

We studied 1) the expression of mouse GHR-tr as well as GHR-fl in liver, skeletal muscle, and subcutaneous fat of mice and 2) the effects of GH excess and deficiency on regulation of GHR isoforms in mice with real-time RT-PCR.

MATERIALS AND METHODS

Animals and tissues. All studies were performed using 3-mo-old male mice. Two different strains of mice, referred to as bGH (giant transgenic mice that express bovine GH) (7) and lit/lit (dwarf mice with an inactivating mutation of the GH-releasing hormone receptor gene) (14) were used in this study. Nontransgenic littermates for bGH mice are approximately twice and 20–25% of normal, respectively (7, 10). Liver, skeletal muscle (quadriceps for bGH and control littermates; gastrocnemius for lit/+ and lit/lit) and subcutaneous white fat (inguinal) from the mice (n = 5–6/group) were collected and flash-frozen in liquid nitrogen and then stored at −80°C for subsequent mRNA analysis. All animal protocols were approved by the Institutional Animal Care and Use Committees of the University of Virginia and Ohio University.

Total RNA preparations. The RNA extraction was performed as described previously (17). The quantity of extracted total RNA was determined using the RiboGreen RNA Quantitation Kit (Molecular Probes, Eugene, OR) with a Genios multi-detection plate reader (Phenix Research Product, Hayward, CA).

Primer design. All PCR primers were purchased from Qiagen (Valencia, CA). As shown in Fig. 1A, we designed three sets of specific primers to amplify GHR isoforms. The reverse primer (mGHR-R) was produced complementary to the sequences within exon 10 of the mouse full-length GHR (mGHR-fl) gene. We designed the forward primer to detect the mouse homologs of hGHR-279 and hGHR-277, which lack the first 26 bp of exon 9 and all of exon 9, respectively. The forward primers specific for mGHR-fl, mGHR-282, and mGHR-280 were produced complementary to the sequences, including the first 26 bp of exon 9, the boundary of exon 8, and the partially deleted exon 9, or the boundaries of exon 8 and exon 10 of mGHR-fl cDNA, respectively. The sequences of PCR primers used were as follows: GH-fl, forward, 5′-GATTTTACCCCAGTCCAGTCTCTTCA-TCCACA-3′; reverse, 5′-GACCCCTAGTCTCTTTCATCCACA-3′; GHR-282, forward, 5′-TCAAAAGCAAGAAGTCCAGTCTCTTCCACA-3′; reverse, 5′-GACCCCTAGTCTCTTTCATCCACA-3′; GHR-280, forward, 5′-TCAAAAGCAAGAAGTCCAGTCTCTTCCACA-3′; reverse, 5′-GACCCCTAGTCTCTTTCATCCACA-3′; IGF-I, forward, 5′-GTTGGGACCGAGGCTTCTTACTTCTC-3′, reverse, 5′-GCTTCAGTGGGGGACGTACTTCTC-3′; 18S rRNA, forward, 5′-TCAAGAAGACAAATCGCAGG-3′, reverse, 5′-GGACACCTAAGGCGACATCACA-3′. Deduced structures of translated products of mGHR isoforms are shown in Fig. 1B.

PCR with isoform-specific primers. The iCycler iQ Real-Time PCR detection system (Bio-Rad Laboratories, Hercules, CA) was used for sample cDNA quantification. The detail of the material and of the real-time PCR protocol was described previously (17). SYBR Green I (Molecular Probes) was used for detection of PCR products. The iCycler iQ Real-Time PCR detection system (Bio-Rad Laboratories, Hercules, CA) was used for sample cDNA quantification. The detail of the material and of the real-time PCR protocol was described previously (17). SYBR Green I (Molecular Probes) was used for detection of PCR products.
assess PCR specificity, melting curves from 55–95°C in 0.5°C steps of 10 s each were generated. PCR products of each assay were also subjected to agarose gel electrophoresis to further confirm amplification specificity. The nucleotides of PCR products were confirmed by sequencing with a DNA sequencer (model 3100, Applied Biosystems, Foster, CA). PCR efficiencies of all reactions were between 95 and 100%. All measurements were performed in triplicate in individual assays, and the average value of the triplicate was used for analysis. The assays were performed on two or more occasions to confirm the reproducibility. Specific quantities were corrected for the amount of 18S rRNA amplified. All of the data presented were representative of data performed in one assay.

Quantification by real-time RT-PCR. A standard curve was generated by amplifying serial dilutions of a known quantity of plasmid DNA. The standards and cDNA samples were then coamplified in the same reaction plate. The standard curve displayed a linear relationship between cycle threshold (Ct) values and the logarithm of input plasmid copy number. The dynamic range of the standard curve spanned at least five orders of magnitude. The amount of product in a particular sample was determined by interpolation from a standard curve of Ct values generated from the plasmid dilution series.

Statistical analysis. The body weights of mice are expressed as means ± SD. Results of gene expression are expressed as means ± SE. Differences were determined by unpaired t-test (body weights of mice and Figs. 3–5) or two-way ANOVA (see Table 1). P < 0.05 was considered significant.

RESULTS

The body weights of bGH mice were 42.7 ± 3.9 g, whereas those of control mice were 29.9 ± 2.4 g (P < 0.0005 between bGH and control mice). The body weights of lit/lit mice were 13.0 ± 1.5 g, whereas those of lit/+ mice were 24.7 ± 4.2 g (P < 0.005 between lit/lit and lit/+ mice).

We detected the PCR products with isoform-specific primers (Fig. 2A) and confirmed them as mGHR-fl, mGHR-282, and mGHR-280 by sequencing (Fig. 2B).

In liver, the mGHR-fl mRNA levels in bGH mice were 507% of those of control mice (P < 0.01), and those of lit/lit mice were 51% of lit/+ mice (P < 0.05). The mGHR-282 mRNA levels in bGH mice were 440% of those of control mice (P < 0.01), and those of lit/lit mice were 58% of lit/+ mice (P < 0.05). The mGHR-280 mRNA levels of bGH mice or lit/lit mice were comparable to those of control mice or lit/+ mice, respectively (Fig. 3).

In skeletal muscle, the mGHR-fl mRNA levels in bGH mice were 56% of those of control mice (P < 0.05), and those of lit/lit mice were comparable to those of lit/+ mice. The mGHR-282 mRNA levels in bGH mice were 69% of those of control mice (P < 0.05), and those of lit/lit mice were comparable to those of lit/+ mice. The mGHR-280 mRNA levels in bGH mice or lit/lit mice were comparable to those of control or lit/+ mice, respectively (Fig. 4).

In subcutaneous fat, the mGHR-fl mRNA levels of bGH mice were comparable to those of control mice, and those of lit/lit mice were 178% of those of lit/+ mice (P < 0.05). The mGHR-282 mRNA levels of bGH mice were comparable to those of control mice, and those of lit/lit mice were 257% of lit/+ mice (P < 0.05). The mGHR-280 mRNA levels of bGH mice or lit/lit mice were comparable to those of control or lit/+ mice, respectively (Fig. 5).

Table 1 reveals the relative abundance of mGHR-tr to mGHR-fl (mGHR-tr/mGHR-fl) in each animal group. mGHR-282 compared with mGHR-fl was reduced in liver but relatively elevated in skeletal muscle and subcutaneous fat. The differences of mGHR-282/mGHR-fl between liver and muscle (P < 0.01) or between liver and fat (P < 0.01) were statistically significant, whereas there was no significant difference between muscle and fat. mGHR-280 compared with mGHR-fl was extremely low in liver but also reduced in skeletal muscle and subcutaneous fat. The differences of mGHR-280/mGHR-fl...
between liver and muscle \((P < 0.01)\) or between liver and fat \((P < 0.05)\) were statistically significant, whereas there was no significant difference between muscle and fat. Although there were tissue-specific differences in mGHR-tr/mGHR-fl, no difference was observed among wild-type, bGH, \(lit/+\), and \(lit/lit\) mice.

Finally, we examined the IGF-I mRNA levels as a biological marker of GHR signaling in tissues. Hepatic IGF-I mRNA levels of bGH mice were 315% of those of control mice, whereas levels of \(lit/lit\) mice were 12% of \(lit/+\) mice. In skeletal muscle, IGF-I mRNA levels of bGH mice were 230% of those of control mice, whereas \(lit/lit\) mice were 40% of \(lit/+\) mice. In subcutaneous fat, IGF-I mRNA levels of bGH mice were 283% of control, whereas \(lit/lit\) mice were 26% of \(lit/+\) mice (Fig. 6).

**DISCUSSION**

In this study, we examined the expression of GHR isoforms in mouse tissues, including liver, skeletal muscle, and subcutaneous fat, three primary target tissues for GH. Furthermore, we studied the effect of chronic excess and deficiency of endogenous GH on mRNA levels of GHR isoforms by use of bGH transgenic and \(lit/lit\) mice. We believe this is the first report concerning the abundance of GHR-tr in mouse tissues in vivo. A relevant finding in this study was the tissue-specific regulation of mGHR-fl and mGHR-282 in a state of GH excess or deficiency in vivo. We also demonstrated that the relative abundance of GHR-tr to GHR-fl was tissue specific in mice, although the GHR-tr/GHR-fl ratio was not changed in a state of either GH excess or deficiency in any tissues we examined. Our data indicate that tissue-specific expression and regulation of both GHR-fl and GHR-tr may, at least in part, play a role in regulating GHR signaling in a tissue-specific manner.

The mGHR-282/mGHR-fl ratio was lower in liver than in skeletal muscle or subcutaneous fat. The mGHR-280/mGHR-fl ratio showed the same trend but was extremely low. This tissue-specific expression of GHR-tr is in agreement with that in human tissues \((2, 12)\). Ballesteros et al. \((2)\) showed that hGHR-279, corresponding to mGHR-282, was expressed at levels <4% in liver and 7–10% in adipose tissue and muscle of hGH-fl. They also demonstrated that hGHR-277, corresponding to mGHR-280, was expressed at levels of 0.5% in liver and...
allow for the estimation of the true GHR-tr/GHR-fl ratio. Their results demonstrated that long-term glucocorticoid treatment caused downregulation of GHR-fl at both mRNA and protein levels without affecting the GHR-tr expression in human hepatoma cells, suggesting that the protein level of GHR-tr as well as GHR-fl correlates with mRNA levels in that situation.

The physiological significance of tissue specificity of GHR-tr/GHR-fl in vivo is still unclear. However, there is evidence that GHR signaling in the liver is more relevant than IGF-IR signaling in terms of growth of tissues. The previous study by Behringer et al. (4) indicated that GH, and not IGF-I, is the major regulator of liver growth, whereas IGF-I plays an important role for growth of most other tissues. The low ratio of mGHR-tr to mGHR-fl in the liver compared with that in other tissues may result in more efficient GHR signaling in the liver to maintain the liver growth. In addition, low GHR-tr expression in the liver may favor efficient hepatic production of serum IGF-I, because hepatic IGF-I is the major source of serum IGF-I.

Variable precursor mRNA splicing has been documented for a variety of transcriptional products by cytokines (25) or hormones (6, 22) and has physiological significance. In the GHR, a previous in vitro study (20) demonstrated that GH differentially regulated the mRNA expression of GHR isoforms in 3T3-L1 cell lines. There is also in vivo evidence demonstrating that human GHR-fl and GHR-tr are regulated in different manners. Fisker et al. (12) reported that gene expression of GHR-fl in abdominal subcutaneous adipose tissue of GH-deficient adults was not altered, whereas the expression of GHR-tr increased significantly after GH administration. In contrast, expression of GHR-fl in muscle increased significantly, whereas mRNA levels for GHR-tr decreased after GH treatment of GH-deficient patients. We hypothesized from these in vitro and in vivo results that differential expression between GHR-fl and GHR-tr might control GHR signaling in a chronic state of GH excess or deficiency. However, the results in this study showed that chronic excess or deficiency of GH in mice influenced GHR pre-mRNA processing but did not appear to alter the mGHR-tr/mGHR-fl ratio.

Human GHR truncation variants expressed at low levels in transfected cells showed inhibition of GHR-fl-mediated signal transduction (1, 19). Functional studies using a reporter gene containing the STAT5 (signal transducer and activator of transcription 5)-binding element demonstrated that GHR-tr could suppress the action of GHR-fl even when the ratio of the transfected cDNA was 1/10 for GHR-tr to GHR-fl (28). They also demonstrated that GHR-tr could form heterodimers with GHR-fl, suggesting a dominant negative effect of GHR-tr by forming a heterodimer with GHR-fl. In mice in vivo, however, our results demonstrate that the mGHR-282/mGHR-fl ratio

<0.1% in fat or muscle of hGHR-fl. Our results in mice showed that the expression levels of mGHR-282 and mGHR-280 in liver were 0.5 and <0.1% of the levels of mGHR-fl, respectively; the values in muscle were 2–3 and 0.1–0.5% of mGHR-fl, respectively; and the values in subcutaneous fat were 3–5 and 0.1–0.5% of mGHR-fl, respectively, suggesting that the expression of GHR-tr is not only tissue specific but also species specific. Vottero et al. (32) successfully detected the GHR-tr at the protein level by use of the fluorescence-activated cell sorter system with unique specific antibodies against both GHR and GHR-tr, although their methods did not

Table 1. Relative abundance of GHR-tr to GHR-fl in tissues

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<th>mGHR-282/mGHR-fl</th>
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<td>WT</td>
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<td>Liver</td>
<td>0.52±0.06</td>
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<td>Muscle</td>
<td>2.4±0.7*</td>
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<td>Fat</td>
<td>3.8±0.8*</td>
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Values for the ratios of truncated to full-length mouse growth hormone receptors (mGHR-tr to mGHR-fl) are expressed in %, bgH, bovine GH; muscle, skeletal muscle (quadriceps); Fat, subcutaneous fat; WT, wild-type control littermates for bgH mice. *P < 0.01, †P < 0.05 vs. value of liver in the same groups.

Fig. 5. mRNA levels of mGHR isoforms in subcutaneous fat. Values are means ± SE; n = 5–6 mice/group. *P < 0.05 vs. respective control mice.

The ratio of mGHR-tr to mGHR-fl in the liver compared with that in other tissues may result in more efficient GHR signaling in the liver to maintain the liver growth. In addition, low GHR-tr expression in the liver may favor efficient hepatic production of serum IGF-I, because hepatic IGF-I is the major source of serum IGF-I.

Variable precursor mRNA splicing has been documented for a variety of transcriptional products by cytokines (25) or hormones (6, 22) and has physiological significance. In the GHR, a previous in vitro study (20) demonstrated that GH differentially regulated the mRNA expression of GHR isoforms in 3T3-L1 cell lines. There is also in vivo evidence demonstrating that human GHR-fl and GHR-tr are regulated in different manners. Fisker et al. (12) reported that gene expression of GHR-fl in abdominal subcutaneous adipose tissue of GH-deficient adults was not altered, whereas the expression of GHR-tr increased significantly after GH administration. In contrast, expression of GHR-fl in muscle increased significantly, whereas mRNA levels for GHR-tr decreased after GH treatment of GH-deficient patients. We hypothesized from these in vitro and in vivo results that differential expression between GHR-fl and GHR-tr might control GHR signaling in a chronic state of GH excess or deficiency. However, the results in this study showed that chronic excess or deficiency of GH in mice influenced GHR pre-mRNA processing but did not appear to alter the mGHR-tr/mGHR-fl ratio.

Human GHR truncation variants expressed at low levels in transfected cells showed inhibition of GHR-fl-mediated signal transduction (1, 19). Functional studies using a reporter gene containing the STAT5 (signal transducer and activator of transcription 5)-binding element demonstrated that GHR-tr could suppress the action of GHR-fl even when the ratio of the transfected cDNA was 1/10 for GHR-tr to GHR-fl (28). They also demonstrated that GHR-tr could form heterodimers with GHR-fl, suggesting a dominant negative effect of GHR-tr by forming a heterodimer with GHR-fl. In mice in vivo, however, our results demonstrate that the mGHR-282/mGHR-fl ratio
was low, that no change was observed even in bGH transgenic or lit/lit mice, and that the mGHR-280/mGHR-fl ratio was extremely low. Therefore, this lack of change in the ratio of truncated to full-length GHR isoforms, despite the extreme differences in GH action in the mouse models studied, does not support the theory that the GHR-tr isoforms play a relevant role in controlling GHR signaling in the situation of chronic GH excess or deficiency. It is likely that tissue-specific changes of mGHR-fl, rather than mGHR-tr, play a relevant role to regulate GHR signaling. We cannot exclude, however, the possibility that GHR-tr may play a physiological role in other species or other situations, such as obesity, diabetes, malnutrition, or changes in glucocorticoid action, which affect the GH-IGF-I axis. Further investigations are required to clarify these issues.

Our novel findings are that bGH transgenic mice showed a significant increase of mGHR-fl in liver but a significant decrease of mGHR-fl in skeletal muscle and no difference in subcutaneous fat compared with levels of control mice. In addition, in lit/lit mice, we confirmed the previous reports demonstrating that GHR-fl mRNA levels were decreased in liver (24) and unchanged in muscle (5) of GH-deficient rodents. However, our results showed increased levels of GHR-fl in subcutaneous fat of lit/lit mice compared with lit/+ mice. Previous reports using hypophysectomized rats indicated that GHR mRNA in adipose tissue is decreased in GH-deficient hypophysectomized rats and is restored by GH treatment (13, 31). The discrepancy between our current results and previous reports is unclear. However, one possible explanation is that the expression of GHR mRNA may be different in hypophysectomized animals having multiple pituitary hormone deficiencies compared with that in lit/lit mice, which have isolated GH deficiency. Alternatively, there might be species-specific regulation of GHR. Thus, although there was an increase in GHR mRNA in a rat model after GH treatment (13, 31), human GH-deficient patients showed no changes of GHR-fl mRNA in subcutaneous fat (12).

Besides its growth-promoting action, GH can exert metabolic effects, and the tissue-specific pattern of GHR-fl expression may reflect the pathways involved for each tissue. Excessive GH action in skeletal muscle plays a role in the development of insulin resistance. Liver-specific IGF-I gene-disrupted mice, which have extremely low serum IGF-I levels, high serum GH concentrations, and no resistance to GH in peripheral tissues, demonstrated muscle insulin resistance (33). It is possible that the lower expression of mGHR-fl and relatively higher mGHR-tr/mGHR-fl ratio in skeletal muscle of bGH mice, demonstrated in our studies, may be a compensatory mechanism to maintain the normal GHR signaling and normal insulin action in muscle. In adipose tissues, GH causes lipolysis. The lit/lit mice demonstrated excessive fat accumulation (10), suggesting that low GHR signaling is associated with reduced lipolytic action. Therefore, it is also possible that higher expression of mGHR-fl in subcutaneous fat of lit/lit mice may be a compensatory reaction to prevent an unfavorable state, e.g., fat accumulation. To evaluate GHR signaling in a state of GH excess or deficiency, we examined the IGF-I mRNA levels as a marker of GHR signaling in tissues. IGF-I mRNA levels in liver, skeletal muscle, and subcutaneous fat of bGH mice were 315, 230, and 283%, respectively, of those of control mice (Fig. 6). Downregulation of mGHR-fl without change of the mGHR-tr/mGHR-fl ratio in skeletal muscle may be responsible for less increase of IGF-I mRNA in skeletal muscle of bGH mice compared with liver or subcutaneous fat. A low ratio of GHR-tr to GHR-fl in the liver is also compatible with this result. On the other hand, IGF-I mRNA levels in liver, skeletal muscle, and subcutaneous fat of lit/lit mice were 12, 40, and 26% of those of lit/+ mice, suggesting that in a state of GH deficiency, not only GHR expression but also other factors may play a relevant role to regulate GHR signaling, especially in skeletal muscle, where the reduction of IGF-I mRNA level in lit/lit mice was not severe. In this regard, Sadowski et al. (29) demonstrated the activation of mitogen-activated protein kinase (MAPK) or phosphatidylinositol 3-phosphate kinase (PI3K) induced by GH-inhibited, rather than GH-stimulated, IGF-I mRNA expression in C2C12 myoblast cell lines, in contrast to cells from other tissues. The same study also reported that the induction of SOCS (suppressor of cytokine signaling) proteins in C2C12 was different from that observed in 3T3-F442A adipocytes, suggesting that the difference of postreceptor pathways involved in GHR signaling in a given tissue may explain the tissue-specific difference of IGF-I induction by GH.

Fig. 6. mRNA levels of IGF-I in liver (A), skeletal muscle (B), and subcutaneous fat (C). Values are means ± SE; n = 5–6 mice/group. **p < 0.01 vs. respective control mice.
The previous reports suggest that a possible explanation of tissue-specific regulation of GHR-fl is the use of a different promoter of the GHR gene. There are several distinct 5′-untranslated region (UTR) (exon 1) variants in mouse GHR (11). Expression of each transcript is regulated in a tissue-specific manner (11, 26). Alternatively, tissue-specific recruitment of the DNA-binding protein complex at the promoter of the GHR gene may explain the tissue-specific regulation of GHR as observed in diabetic mice (16). Gowri et al. (16) demonstrated that the repressosome complex at the L2 promoter of the GHR gene played a role for differential regulation of GHR between liver and kidney in the insulinopenic diabetic state. Furthermore, there are five 5′UTRs in the mouse GHR gene, whereas nine 5′UTRs are identified in the human GHR gene (11). The difference between 5′UTR and its promoter may explain the species-specific regulation of GHR.

In conclusion, two truncated isoforms of GHR were detected in liver, skeletal muscle, and subcutaneous fat of mice. The absolute mRNA expression of GHR-fl and GHR-282 was affected by the GH state in a tissue-specific manner. The GHR-tr/GHR-fl ratio was tissue specific but not affected by the chronic excess or deficiency of GH in mice.

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