Regulation of the Growth Hormone (GH) Receptor and GH-Binding Protein mRNA (43755)

HIDESUKE KAJI,[1] SHIN-ICHIROU OHASHI, HIROMI ABE, AND KAZUO CHIHARA
Third Division, Department of Medicine, Kobe University School of Medicine, Kobe 650, Japan

Abstract. **In fasting rats, a transient increase in growth hormone-binding protein (GHBP) mRNA levels was observed after 1 day, in muscle, heart, and liver, but not in fat tissues. The liver GH receptor (GHR) mRNA level was significantly increased after 1 day (but not after 5 days) of bovine GH (bGH) treatment in fed rats. Both the liver GHR mRNA level and the net increment of plasma IGF-I markedly decreased after 5 days of bGH administration in fasting rats. These findings suggest that GHR and GHBP mRNAs in the liver are expressed in a different way and that the expression of GHBP mRNA is regulated differently between tissues, at least in rats. The results also suggest that refractoriness to GH in a sustained fasting state might be beneficial in preventing anabolic effects of GH. In humans, GHR mRNA in lymphocytes, from subjects with either GH-deficiency or acromegaly, could be detected by the reverse transcription-polymerase chain reaction method. In one patient with partial GH insensitivity, a heterozygous missense mutation (P561T) was identified in the cytoplasmic domain of GHR.** [P.S.E.B.M. 1994, Vol 206]

Growth hormone-binding protein (GHPB) is an alternatively spliced product of the GH receptor (GHR) gene in the rat and the mouse (1, 2). It appears to be affected by proteolytic cleavage of GHR in humans and rabbits (3). In addition, since GHR and GHBP are known to have a significant interaction in determining the biological activity of GH, it is important to elucidate whether GHR and GHBP are coordinately regulated or not.

It is generally known that plasma GHBP and IGF-I levels, as well as hepatic GHR levels, decrease in the fasting state. However, the precise mechanism by which the plasma IGF-I level decreases during fasting still needs clarification. In the present study, the effects of fasting and GH administration on GHR and GHBP mRNA levels were examined in the rat liver, as well as extrahepatic tissues, by Northern blot analysis.

In order to clarify the mechanism of GH action in humans, we also attempted to detect human GHR mRNA from liver obtained at autopsy or from the lymphocytes of patients with GH-deficiency or acromegaly, by the reverse transcription-polymerase chain reaction (RT-PCR) method. In addition to the extracellular domain of GHR or GHBP, it is also important to know the role of the cytoplasmic domain of GHR on the signal transduction system. We have recently identified the point mutation of the GHR gene encoding the cytoplasmic domain in a patient with partial GH insensitivity.

Materials and Methods

Animal Study. Male Sprague-Dawley rats (Keari Co. Ltd., Osaka, Japan), aged 6 weeks, were housed under controlled temperature (22°C) and light conditions (lights-on at 06:00 hr; lights-off at 18:00 hr), with standard food and water *ad libitum,* for several days before the experiments.

RT-PCR and preparation of probes for GHR and GHBP mRNA in rats. Two primer pairs were synthesized specifically for portions of GHR or GHBP cDNA. Total RNA was transcribed into cDNA by AMV reverse transcriptase and amplified by the PCR method, as described previously (4). After DNA amplification, the PCR mixtures were electrophoresed in 3% agarose gels, and the amplified GHR and GHBP cDNA fragments were visualized as a single band after

[1] To whom requests for reprints should be addressed at Third Division, Department of Medicine, Kobe University School of Medicine, Kobe 650, Japan.

0037-9727/94/2063–0257$10.50/0

ethidium bromide staining. Purified GHR and GHBP cDNA fragments were subcloned into pBluescript II SK+ and their sequences were confirmed by the dideoxy-chain termination method (4). Subcloned cDNA fragments were used as cDNA probes for GHR and GHBP after digestion with EcoRI and BamHI, respectively. The EcoRI fragment of pILaT1 was used for hybridization of α-tubulin mRNA (5). GHR, GHBP and α-tubulin cDNA probes were labeled with α-[^{32}P] dCTP, with a modification of the random priming method.

Northern blot analysis. Rat tissues were homogenized and the total RNAs were isolated with a slight modification of the method used by Chomczynski (6). Northern blot analysis was performed as described previously (7), also with a slight modification. After confirming that no residual radioactivities were left on the filters by autoradiography, subsequent hybridization and an α-tubulin cDNA probe was performed as described above. The bands of autoradiograms were quantified by an automated image processing system. Normalization of each mRNA abundance was achieved by division with the quantity of the α-tubulin mRNA.

RIAs. Plasma GH and IGF-I concentrations were measured by respective specific RIA (7, 8).

Human Study. For RT-PCR detection of GHR mRNA, liver tissue was obtained at autopsy from a patient with nonendocrine disease; lymphocytes were taken from three patients with adult onset GH deficiency, and one patient with active acromegaly, whose plasma basal GH was higher than 10 ng/ml. For structural analysis of the GHR gene, blood was obtained from a 12-year-old female of short stature (−6.17 SD), presenting with no acute response of plasma IGF-I to GH injections (0.15 IU/kg, sc).

RT-PCR of human GHR mRNA. RNA was extracted as described above from liver or lymphocytes separated by ficoll-hypaque. Partial sequence of GHR mRNA was amplified by the RT-PCR method as described.

Sequence analysis of human GHR gene. Genomic DNA or RNA were extracted from blood cells. Exon 10, encoding most of the cytoplasmic domain of GHR, was amplified from genomic DNA by PCR. Amplified DNA was subcloned into pT7 Blue T-vector. Plasmid containing insert was prepared from several clones and sequenced by the dideoxy chain termination method. Exon 2 through 9, encoding the other region of GHR, were amplified by PCR after RT from lymphocyte RNA and sequenced in a similar manner.

Results

Tissue Distribution of Rat GHR and GHBP mRNA. Use of Northern blot analysis or the RT-PCR method show that GHR and GHBP mRNA are most abundantly expressed in the rat liver, and widely distributed in the cartilage, fat, muscle, kidney, testis, etc. GHR, but not GHBP mRNA expression, could be clearly identified in the rat pituitary, cerebral cortex, and cerebellum by the RT-PCR method.

Effect of Fasting and GH on Rat GHR and GHBP mRNA Levels. Body weight gradually but significantly decreased in fasting rats compared with control rats. Plasma GH levels decreased after 1 day of fasting and remained low until the 7th day. Plasma IGF-1 levels decreased by 62% after 1 day of fasting, and by 80% after 3 days. Hepatic GHR mRNA levels did not significantly change after 1 or 3 days of fasting but significantly decreased after 7 days. On the other hand, 1 day of fasting caused a transient but significant increase in the hepatic GHBP mRNA level, followed by a gradual decrease on Day 3 and 7.

The effect of fasting on GHBP mRNA levels was also examined in extrahepatic tissues (Fig. 1). Fasting for 1 day caused a significant increase in GHBP mRNA levels in muscle and heart but not in fat tissue. In the heart, a significant increase in the GHBP mRNA level was also observed after 3 days fasting.

Bovine GH (bGH, 1.4 IU/mg, ip) administration did not affect the change in body weight in rats fed *ad libitum* or in rats fasting for 1 and 5 days. Injection of bGH for 1 day caused a net increase of 296 ng/ml in plasma IGF-I level of fed rats. The same treatment in fasting rats was still effective and increased the plasma IGF-I level by 284 ng/ml. bGH injections for 5 days resulted in a smaller net increase of plasma IGF-I level, 234.2 ng/ml, than that for 1 day, and in a slight decrease in GHR mRNA levels of fed rats (Fig. 2). In fasting rats, daily bGH injections for 5 days dramatically down-regulated liver GHR mRNA levels (Fig. 2). The net increase of plasma IGF-I level was only 37 ng/ml after 5 days bGH treatment in fasting rats. bGH injection for either 1 or 5 days failed to change GHBP mRNA levels in fed rats (Fig. 2) and tended to decrease the GHBP mRNA level in fasting rats, although the difference was not statistically significant.

Human GHR mRNA Detection by RT-PCR. GHR mRNA is abundant in the human liver obtained at autopsy and also identified in lymphocytes from patients with GH deficiency and active acromegaly by the RT-PCR method (Fig. 3).

Sequence Analysis of GHR Gene in a Patient with GH Insensitivity. When six subclones containing amplified DNA encoding most of the cytoplasmic domain of GHR were analyzed, the sequences of three subclones were normal, while substitution of ACU (threonin) for CCU (proline) at codon 561 was observed in three subclones (Fig. 4, upper panel). To confirm this observation, amplified DNA was digested

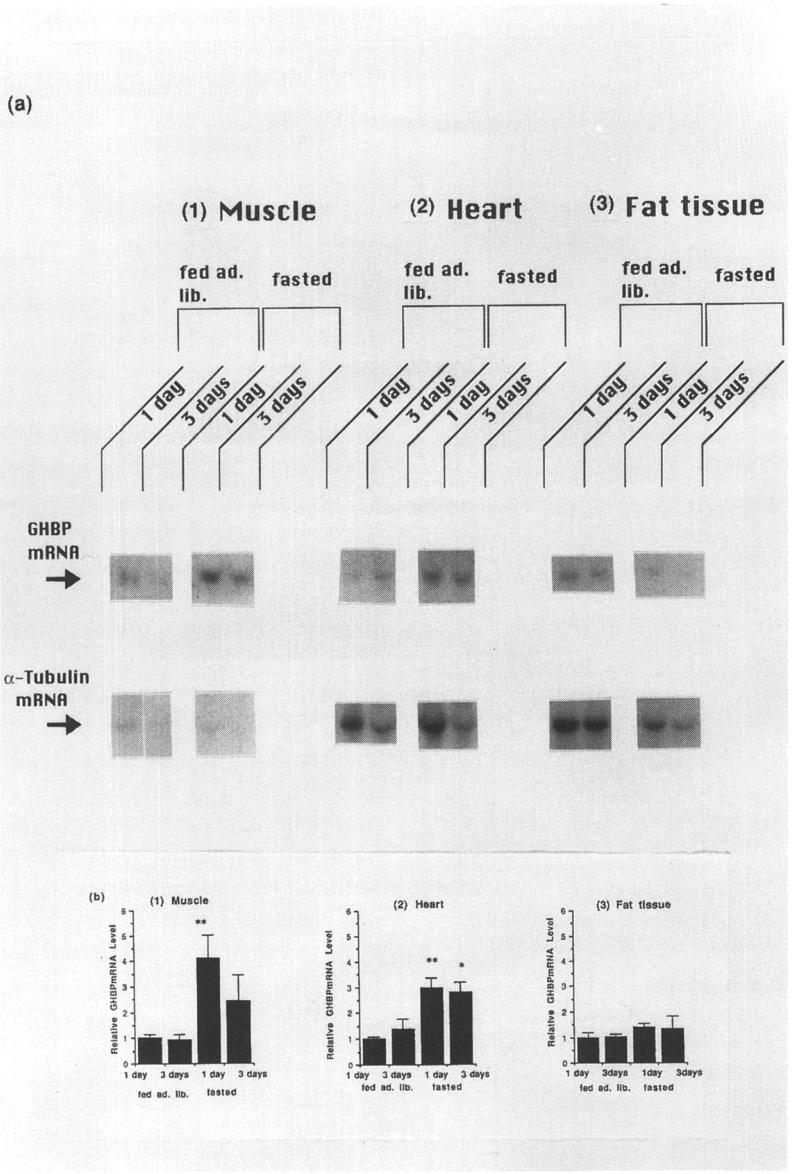


Figure 1. Time-dependent effect of fasting on GHBP mRNA levels in rat muscle, heart, and fat tissues by Northern blot analysis (upper panel). Relative intensity of GHBP mRNA levels normalized by α-tubulin mRNA levels were compared among different tissues (lower panel). Each bar represents mean ± SE of four determinations. Asterisks indicate statistical significance of the difference (**$P < 0.01$, *$P < 0.05$ vs fed rats).

with restricted enzyme Stu-I, which was able to digest as Position 1778 of GHR cDNA. As shown in Fig. 4, amplified DNA from a normal subject was almost entirely digested with Stu I, whereas only half of the amounts of DNA from the patient appeared to be digested, suggesting the heterozygous missense mutation (P561T) (Fig. 4, lower panel).

Discussion

The present study demonstrated that hepatic GHR mRNA and GHBP mRNA are discordantly regulated by fasting and GH administration in rats. The reason why fasting causes a transient increase in GHBP mRNA, but not GHR mRNA, is unknown. We cannot completely exclude the possibility that both mRNAs were coordinately increased, but that GHR mRNA is more rapidly degraded than GHBP mRNA, because of the presence of destabilizing sequences (ATTT) in the 3′-untranslated region of the rat GHR cDNA. To determine the mechanism by which 1 day of fasting causes an increase in GHBP mRNA, and considering the fact that plasma GH levels decreased in fasting rats, we examined the effect of GH replacement on GHBP mRNA in fasting rats. However, GH administration failed to completely restore GHBP mRNA level. Thus, an increase in GHBP mRNA during an early period of fasting is not entirely due to the decrease in plasma GH level.

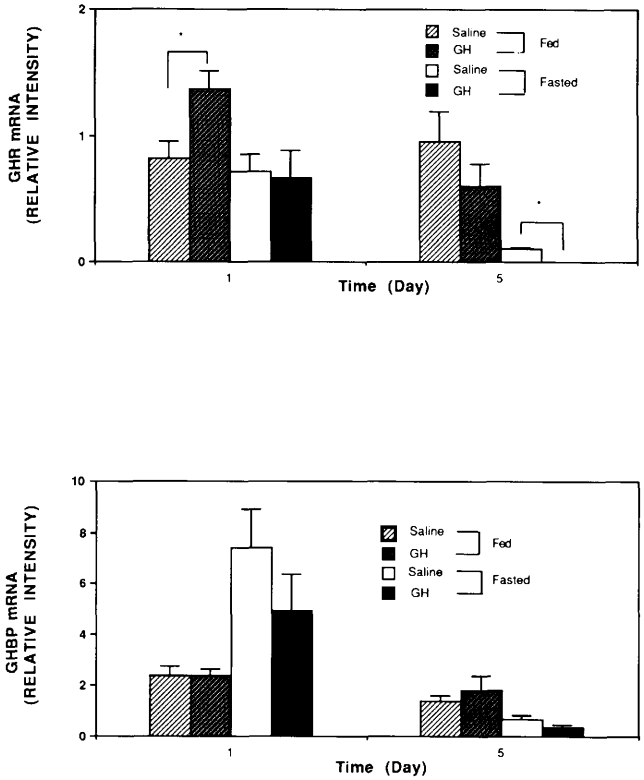


Figure 2. Time-dependent effect of GH administration on liver GHR mRNA (upper panel) and GHBP mRNA levels (lower panel) in fasting and fed rats by Northern blot analysis. Each bar represents the mean ± SE of four determinations of relative mRNA levels normalized by α-tubulin mRNA levels. An asterisk indicates the statistical significance of the difference ($^*P < 0.05$ vs corresponding saline-injected rats).

Plasma IGF-I levels began to decrease immediately after fasting, as previously reported (9), whereas GHR mRNA level was not completely parallel with the IGF-I level, at least during an initial period after the start of fasting. On the first day of fasting, the decreased IGF-I level was partially recovered by GH administration, suggesting that it is probably not due to GH resistance, but rather due to the reduced GH release caused by fasting. In rats fasting for 5 days, exogenous GH administration failed to increase plasma IGF-I levels, indicating the development of resistance to GH. Refractoriness to GH in sustained fasting rats may be explained by the decrease in the number of GHRs, since the hepatic GHR mRNA level was significantly decreased and GH binding to liver membrane was also reduced in the prolonged fasting state (10). However, postreceptor impairment in the fasting state cannot be excluded, since Thissen *et al.* (11) have reported that plasma IGF-I responded poorly to GH injection in protein-restricted rats, even after the decrease in GH binding to liver membranes was recovered by continuous infusion of GH. It has also been reported that IGF-I synthesis is more sensitive to nutritional factors than to GH in steer (12).

GH administration differentially affects hepatic GHR mRNA levels in fed and fasting rats. In fed rats, GH administration caused a significant increase in GHR mRNA levels after 1 day but failed to change after 5 days. In agreement with the change of GHR mRNA levels in fed rats, it was reported that repeated injections of GH caused a small increase in GH binding to hepatic membrane in lambs on a high nutrition diet (12). This GH-induced increase in GHR mRNA levels might be explained not only by the direct action of GH, but also by autocrine or paracrine action of IGF-I, or other local factors induced by GH. In fasting rats, however, GH administration failed to change GHR mRNA levels after 1 day of fasting but caused a dramatic decrease in GHR mRNA levels after 5 days of fasting. The mechanism by which the decline of GHR mRNA by GH is more exaggerated in the fasting state remains to be solved. As mentioned previously, fasting causes a decrease in GH release in rats, and the regulation by GH of hepatic GHR mRNA expression may be sensitive to exogenous GH in GH-deficient state.

It is also important to know whether the regulation of GHR mRNA or GHBP mRNA is tissue specific. Transient elevation of GHBP mRNA by fasting was also observed in the liver, muscle, and heart but not in fat tissue. Previous studies revealed a variation in the contents of both mRNAs in liver, kidney, lung, and ileum during somatic development (13). Another study has also demonstrated the increase in GHR mRNA by hypophysectomy in liver and muscle, but a decrease in fat tissue (14).

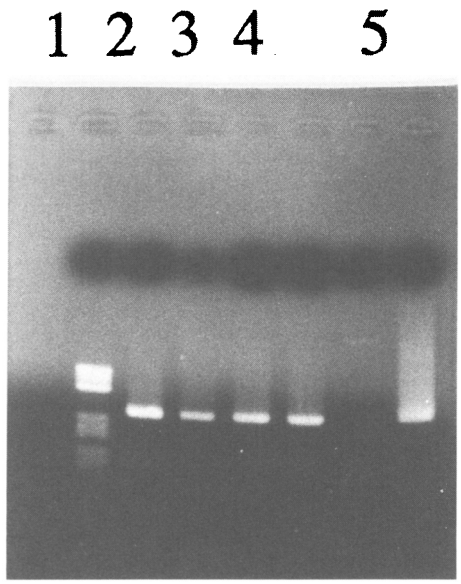
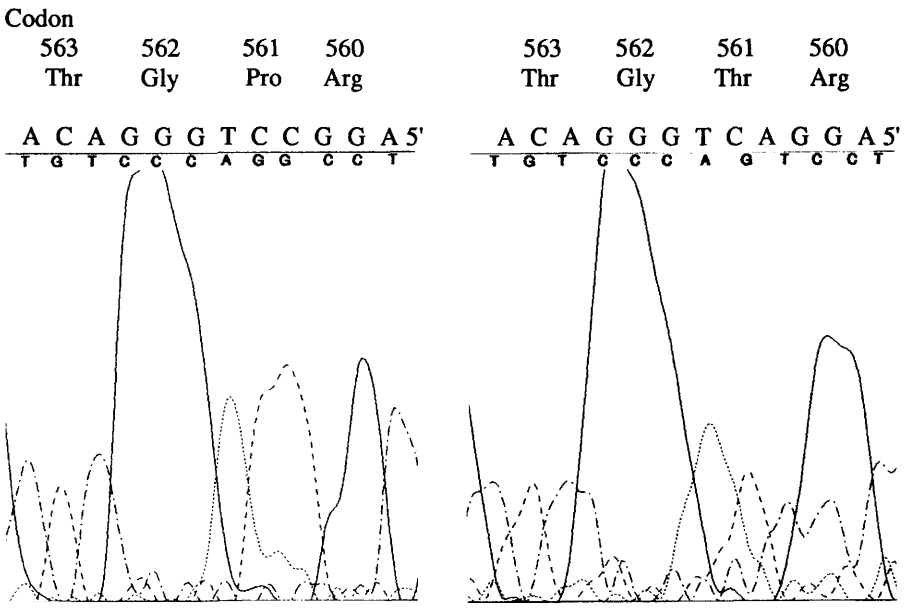
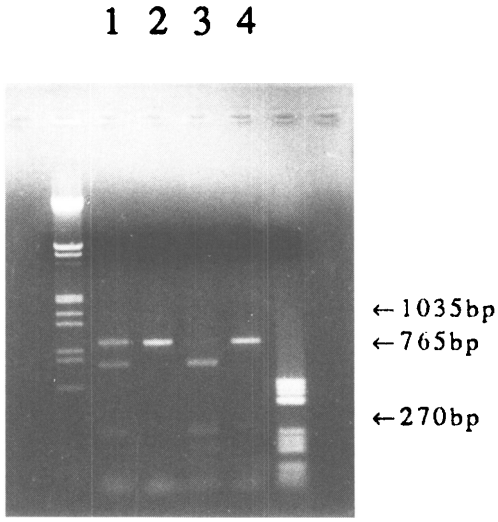


Figure 3. Detection by RT-PCR method of a partial sequence of human GH receptor mRNA from liver obtained at autopsy (Lane 5), or from lymphocytes of patients with GH deficiency (Lanes 1, 2, and 4) and active acromegaly (Lane 3). PCR products were electrophoresed on agarose gel and stained with ethidium bromide.





1; cut with Stu I (patient YA)
2; uncut
3; cut with Stu I (control)
4; uncut

Figure 4. (Upper panel), the region corresponding to nucleotides 1775–1786 of hGH receptor cDNA is shown. When PCR products derived from a patient genomic DNA are subcloned and sequenced, three of six clones were wild type (left panel), while another three clones demonstrated C to A transition at Position 1778 (right panel). (Lower panel), analysis of point mutations in the cytoplasmic GH receptor gene by PCR-RFLP. A 1035 base pair band is seen in PCR products from the patient (Lane 2) and control subjects (Lane 4). Stu I is able to digest at Position 1777 of GHR cDNA. PCR products from the control subject were almost completely digested by Stu I (Lane 3), whereas only half the amount of PCR products from the patient could be digested with Stu I (Lane 1).

It is still not known whether the regulation of GHR and GHBP is different among species. In humans, regulation of GHR mRNA is still unclear. In the present study, GHR mRNA could be identified in human lymphocytes by the RT-PCR method. GHR mRNA levels in lymphocytes appeared to be much less than those in

liver, but similar in the GH-deficient and GH-excess states, although the results are semiquantitative and subjects are limited. We also demonstrated a heterozygous missense mutation (P561T) in the cytoplasmic domain of GHR in a female Japanese patient with partial GH insensitivity, whose diagnosis appeared to be Noonan syndrome. The same mutation in a male Caucasian with Laron syndrome was recently reported by Kou *et al.* (15). It would be interesting to clarify the functional importance of this mutation.

In summary, we have demonstrated here the discordant regulation of hepatic GHR and GHBP mRNA by fasting as well as GH administration, and the tissue-specific regulation of GHBP mRNA in fasting rats. We also identified GHR mRNA in human lymphocytes from subjects with GH-deficiency and acromegaly, and heterozygous missense mutation of cytoplasmic domain of GHR in a patient with GH insensitivity.

We are grateful to Dr. A. F. Parlow and the National Hormone and Pituitary Agency for supplying bovine GH and IGF-I RIA kit. This work was supported in part by a Grant-in-Aid for scientific research from the Japanese Ministry of Education, Science and Culture, the Japanese Ministry of Health and Welfare, and the Growth Science Foundation.

1. Baumbach WR, Horner DL, Logan JS. The growth hormone-binding protein in rat serum is an alternatively spliced form of the rat growth hormone receptor. Genes Dev **3**:1199–1205, 1989.
2. Smith WC, Kuniyoshi J, Talamantes F. Mouse serum growth hormone (GH) binding protein has GH receptor extracellular and substituted transmembrane domains. Mol Endocrinol **3**:984–990, 1989.
3. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, Wood WI. Growth hormone receptor and serum binding protein: Purification, cloning and expression. Nature **330**:537–543, 1987.
4. Kaji H, Takahashi Y, Chihara K. The regional distribution of thyrotropin-releasing hormone receptor messenger ribonucleic acid in the brain. Neurosci Lett **151**:81–84, 1993.
5. Lemischka IR, Farmer S, Racaniello VR, Sharp PA. Nucleotide sequence and evolution of a mammalian a-tubulin messenger RNA. J Mol Biol **151**:101–120, 1981.
6. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem **162**:156–159, 1987.
7. Kaji H, Hinkle PM. Attenuation of thyroid hormone action by 1,25-dihydroxy-vitamin D_3 in pituitary cells. Endocrinology **124**:930–936, 1989.
8. Okimura Y, Chihara K, Kita T, Kashio Y, Sato M, Kitajima N, Abe H, Takahashi K, Fujita T. Discordance between growth hormone (GH) responses after GH-releasing hormone and insulin hypoglycemia in myotonic dystrophy. J Clin Endocrinol Metab **67**:1074–1079, 1988.
9. Straus DS, Takemoto CD. Effect of fasting on insulin-like growth factor-I (IGF-I) and growth hormone receptor mRNA levels and IGF-I gene transcription in rat liver. Mol Endocrinol **4**:91–100, 1990.
10. Postel-Vinay MC, Cohen-Tanugi E, Charrier J. Growth hormone receptors in rat liver membranes: Effects of fasting and refeeding and correlation with plasma somatomedin activity. Mol Cell Endocrinol **28**(3):657–669, 1982.
11. Thissen JP, Triest S, Underwood LE, Maes M, Ketelslegers JM. Divergent responses of serum insulin-like growth factor-I and liver growth hormone (GH) receptors to exogenous GH in protein-restricted rats. Endocrinology **126**:908–913, 1990.
12. Breier BH, Gluckman PD, Bass JJ. Influence of nutritional status and oestradiol-17B on plasma growth hormone, insulin-like growth factor-I and -II and the response to exogenous growth hormone in young steers. J Endocrinology **118**:243–250, 1988.
13. Walker JL, Moats-Staats BM, Stiles AD, Underwood LE. Tissue-specific developmental regulation of the messenger ribonucleic acids encoding the growth hormone-binding protein in rat fetal and postnatal tissues. Pediatr Res **31**:335–339, 1992.
14. Orian JM, Snibson K, Stevenson JL, Brandon MR, Herington AC. Elevation of growth hormone (GH) and prolactin receptors in transgenic mice expressing ovine GH. Endocrinology **128**:1238–1246, 1991.
15. Kou K, Lajara R, Rotwein P. Amino acid substitutions in the intracellular part of the growth hormone receptor in a patient with the Laron syndrome. J Clin Endocrinol Metab **76**:54–59, 1993.