Acute and Short-Term Effects of Growth Hormone on Insulin-Like Growth Factors and Their Binding Proteins: Serum Levels and Haptic Messenger Ribonucleic Acid Responses in Humans*

HANS OLIVECRONA†, AGNETA HILDING†, CHRISTINA EKSTRÖM†, HANS BARLE, BJÖRN NYBERG, CHRISTER MÖLLER, PATRICK J. DELHANTY, ROBERT C. BAXTER, BO ANGELIN, TOMAS J. EKSTRÖM, AND MICHAEL TALLY

Gastroenterology Center (H.O., H.B., B.N.), Department of Surgery, Molecular Nutrition Unit (H.O., B.A.), Center for Nutrition and Toxicology, Center for Metabolism and Endocrinology (B.A.), Department of Medicine, Karolinska Institute at Huddinge University Hospital, S-141 86 Huddinge, Sweden; Endocrinology and Diabetes Unit (A.H., C.M., M.T.), Department of Molecular Medicine, Department of Clinical Neuroscience (C.E., T.J.E.), Center for Molecular Medicine, Karolinska Hospital, S-171 76 Stockholm, Sweden; and Kolling Institute of Medical Research (P.J.D., R.C.B.), University of Sydney, Royal North Shore Hospital, St. Leonards, NSW 2065, Australia

ABSTRACT

We investigated the acute (4–5 h) and short-term (5 days) effects of GH treatment on hepatic messenger RNA (mRNA) levels of the genes for the insulin-like growth factors (IGFs), insulin-like growth factor binding protein-1, -2, and -3 (IGFBPs), and the acid labile subunit (ALS), as well as serum levels of these proteins in humans. At the mRNA level, we observed an increase in IGF-1 transcription (+173%) following GH treatment in the acute group, which remained elevated in the short-term treatment group. IGFBP-2 mRNA decreased after short-term GH treatment, without changes in IGFBP-1 or -3 expression. The ALS transcript level increased after 5 days. In serum, we found increased levels of IGF-I and insulin, and decreased levels of IGF-II, in the short-term treatment group. IGFBP-1 decreased in both treatment groups, whereas IGFBP-2 was reduced after 5 days treatment. ALS increased in the short-term group. We observed increased IGFBP-3 serum levels after 5 days of GH treatment, likely due to increased formation of the ternary complex. Our results show that the metabolic effects by GH on the IGF axis are complex. In addition to a direct stimulation of IGF-I and ALS expression, GH inhibits IGFBP-1 serum levels and IGFBP-2 expression in an indirect manner, possibly facilitating enhanced IGF bioavailability to target tissues. (J Clin Endocrinol Metab 84: 553–560, 1999)

G H ACTS ON several tissues throughout the body, and one of the major target tissues is the liver. The somatomedin hypothesis implies the importance of insulin-like growth factor I (IGF-I) as the mediator of GH action (1). Besides its own specific effects, GH regulates IGF-I and the IGF-binding proteins, acting in both direct and indirect fashions. Accordingly, GH-treatment of GH-deficient patients normalizes circulating levels of several of these peptides (2–4). IGF-I and IGF-II are both important factors for growth and differentiation, and they both bind to the IGF type 1 receptor, which confers the mitogenic signals to the cell nucleus. In addition, IGF-II binds to the mannose-6-phosphate/IGF type 2 receptor, which in most cells seems to be a scavenger for IGF-II (5). The homeostasis of the IGFs is regulated at a multitude of levels. The transcriptional regulation is complex, involving several promoters (6), giving rise to a number of transcripts that, at least for IGF-II, are subject to differential and growth-dependent translatability (7). Post-translational processing also occurs, and finally, the synthesis and actions of the IGF-binding proteins will affect the serum levels of the IGFs and their ability to confer their signals to the cell nucleus. The liver is of direct importance in maintaining serum levels of several of the insulin-like growth factor binding proteins (IGFBPs), with a key role in the regulation of serum concentrations of free IGFs and the production of IGFBPs. The binding proteins maintain the serum concentrations of IGFs through protection from degradation, and also function as regulators of IGF-action at the cellular level (8). IGFBP-1 is thought to be a direct regulator of free IGF-I and IGF-II (1) and increases during different catabolic states (9). IGFBP-2 has been reported to be elevated in cancer patients (10, 11). IGF-I and IGF-II form a ternary complex with IGFBP-3 and the acid-labile subunit (ALS), which binds over 90% of IGFs in the circulation (12). GH treatment in vitro (13) as well as in vivo (14, 15) increase serum concentrations of IGFBP-3 and ALS. Different pathological and catabolic conditions, GH deficiency, trauma, cancer, starvation, but also...
pregnancy, result in an increased proteolytic serum activity directed towards IGFBP-3, thereby possibly altering the balance between bound and free IGF-I (16, 17). In addition to its known function of binding the cell survival factor IGF-II, IGFBP-3 was recently shown to mediate TGFβ effects on p53 independent apoptosis (18).

It is thus obviously of great importance to characterize the integrated response of IGFs and their binding proteins to GH. The present study had two major objectives: 1) to compare the effects of GH on serum concentrations of IGF-I, IGF-II, IGFBP-1, IGFBP-2, IGFBP-3, and ALS with the changes in the expression of the respective genes at the messenger RNA (mRNA) level in liver tissue from the same individuals; and 2) to investigate the acute effects of GH, following a single injection of 12 IU, in comparison with the effects of a short-term treatment for 5 consecutive days. Our data demonstrate an acute in vivo effect by GH on the mRNA levels of IGF1, whereas short-term treatment is required to observe an increase in ALS and a decrease in IGFBP-2 mRNA levels. Our results also indicate that the increased serum levels of IGFBP-3 seen after short-term GH treatment are not due to an increased gene transcription but rather to a stabilization effect by increased serum ALS levels.

Materials and Methods

Subjects and experimental procedure

The study comprised 31 patients submitted for laparoscopic cholecystectomy. None of the patients were markedly obese, and none had any clinical or laboratory signs of hepatic, renal, or thyroid disease. Informed consent was obtained from each patient, and the ethical aspects of the study were approved by the ethics committee of the Karolinska Institute.

The patients were subdivided into three groups. In 11 of the patients (short-term GH treatment group), baseline fasting blood samples were drawn 1 week before the operation. These patients were treated with human recombinant GH (12 IU daily sc [Genotropin, Pharmacia & Upjohn, Inc., Sweden] at 0800 h for 5 consecutive days, with a bolus injection on the night before surgery 12 h preoperatively. Ten patients (acute GH treatment group) received a single injection of GH (12 IU) after blood sampling in the morning, approximately 4.5 h before surgery. The remaining 10 patients comprised the control group. Serum (post-treatment or control) was drawn immediately before the induction of anesthesia in all patients. After introduction of the laparoscopic instruments in the abdomen, a liver biopsy (200–300 mg) was taken from the left liver lobe. The biopsies were immediately frozen and kept in liquid nitrogen until analyzed (19). Cholecystectomy was then performed without complications.

RNA isolation

Total RNA was extracted from parts of the liver biopsies according to the guanidinium isothiocyanate/acid phenol procedure (20).

Probe generation and RNase protection analysis

For RNase protection analysis (RPA), antisense cRNA probes were generated using T3, T7, and Sp6 RNA polymerase (Promega Corp., Madison, WI, USA) with α-32P-UTP (Amersham, Buckinghamshire, UK), according to the manufacturer’s protocol.

From the human IGFBP-1 complementary DNA (cDNA) (21), a 250-bp PstI-Apal fragment was cloned into the pGem 3Z vector (Promega Corp.). This fragment corresponds to exon 2 and part of exon 3, a region with low homology (∼45%) to other known human IGFBPs. For making the cRNA probe, the insert was linearized with HindIII and transcribed with T7 RNA polymerase.

For IGFBP-2 specific transcripts, an EcoRI-HindIII fragment (22) cloned into the pBluescript SK vector (Stratagene, La Jolla, CA) was used.

This clone was linearized with HhaI to generate a 204-bp specific template, which was transcribed using T7 RNA polymerase.

For IGFBP-3 specific transcripts, a HindIII-EcoRI fragment (23) in the pBluescript SK vector was used. This fragment was linearized with RsaI to generate a 168-bp specific template and transcribed with T3 RNA polymerase.

Total IGF2 transcripts were detected by using the antisense RNA probe generated from the XhoI linearized phIGF2 clone (covering bases 131–260 in the human IGF2 exon 9, accession number X0 7868 in GenBank) and Sp6 RNA polymerase. This sequence is contained in all known IGF2 transcripts.

IGF1 transcripts were detected by using a probe made from a 169-bp DdeI fragment of the human IGF1A cDNA, covering the translated region of bases 362–520 (24), blunt cloned into the Smal site of pGEM3Z vector (Promega Corp.). The construct was linearized with BanHI before being transcribed with T7 RNA polymerase.

ALS cDNA (25) in the pGEM plasmid (Promega Corp.) was cut with PvuII to generate a 126-bp template and transcribed with T7 RNA polymerase.

As an internal control, a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) clone (Tri-GAPDH, Ambion, Inc., Austin, TX) was transcribed with T3 RNA polymerase to generate a probe giving a 316-bp RNase protected fragment.

The specific activity of the 32P-UTP used was 400 Ci mmol⁻¹ for IGF1, IGF2, IGFBP-1, IGFBP-2 and IGFBP-3. For ALS, a specific activity of 800 Ci mmol⁻¹ was used. For GAPDH the specific activity of the 32P-UTP was 20 Ci mmol⁻¹.

The RNase protection analysis was performed with 10 μg of total RNA using the RPA II kit from Ambion, Inc. according to the manufacturer’s protocol. RPA was performed with several probes combined. The probes for IGFBP-1, IGFBP-2, IGFBP-3, and GAPDH were combined; the IGF1, IGF2, and GAPDH probes were combined; the ALS and GAPDH probes were combined. Figure 1 shows representative RNase protection analysis with the various probes alone and combined using liver RNA.

For quantitation of the various transcripts, phosphor imager analysis (BAS-1000 Fuji Photo Film Co., Ltd.) was performed using GAPDH as internal control.

RIAs

GH concentrations in serum were measured by a commercial assay (DELFIA, Wallac, Turku, Finland), based on the dissociation-enhanced lanthanide fluorescence immunoassay principle, detection limit, 0.04 μg/L.

IGF-I was determined in serum by RIA after separation of IGFs from IGFBPs by acid-ethanol extraction and cryoprecipitation. To minimize interference of remaining IGFBPs, des(1–3)-IGF-I was used as radioligand (26). The intra- and interassay CVs were 4% and 11%, respectively. Serum levels of IGF-I are age dependent, declining with age. Thus, IGF-I values were also expressed as so scores calculated from the regression line of the values in 247 healthy adult subjects (27).

Immunoreactive IGF-II levels were determined by RIA using a polyclonal rabbit antibody, after serum samples had been fractionated over Bio-Gel 10 columns (Bio-Rad Laboratories, Inc., Hercules, CA) according to (28), followed by lyophilization of a sample from the eluate. In the RIA, 25 ng/ml IGF-I was added in order to displace IGF-II from interfering binding proteins, and this IGF-I concentration did not interfere in the RIA.

IGFBP-2 and ALS were measured by RIA as previously described (10, 29).

IGFBP-3 in serum was measured by RIA using a commercially available RIA kit (DSL 6700, Diagnostic Systems Laboratories, Webster, TX). The mean and normal range was 3.6 and 2.1–5.0 mg/L in men and 3.8 and 2.3–5.3 mg/L in women.

IGFBP-1 concentrations in serum were determined according to the method of Pövoa et al. (30). The sensitivity of the RIA was 3 μg/L, and the intraassay and interassay CVs were 3% and 10%, respectively.

The geometrical mean and range of IGFBP-1 were 34 and 12–91 μg/L in healthy subjects, aged 20–66 yr. (31).

Insulin was measured using guinea pig antiserum and charcoal addition to separate bound and free insulin (32). The intraassay CV was 5%,
FIG. 1 Examples of RNase protection analysis of IGFBP-1, -2, and -3; ALS; GAPDH; and total IGF1 and IGF2 on liver RNA. RNase protection assay was performed as described in the Materials and Methods section. a, RNase protected bands of IGFBP-1, IGFBP-2, IGFBP-3, and GAPDH. Lanes 2–5 show the protected bands of the individual probes. Lane 1 is a negative control using all probes combined. Lane 1 is a negative control using all probes combined with yeast RNA. b, RNase protection analysis of GAPDH and ALS. Lanes 2 and 3 show the probes used individually and lane 4 combined. Lane 1 is a negative control with combined probes using yeast RNA. c, Total IGF1 and IGF2 RNA probes were used individually in lanes 1 and 2, respectively. GAPDH individually is shown in lane 3, and all three combined in lane 4.

Statistics

Data are described as mean ± SEM or geometric mean ± SEM range, unless otherwise stated. Levels of mRNA were quantitated in relation to GADPH mRNA and the values are expressed as percent of the mean in the control group. Peptide variables with nonnormal distribution, GH, IGF-I and insulin were log transformed before analysis in order to get a more closely approximated Gaussian distribution. One-way ANOVA or Kruskal-Wallis ANOVA on ranks was employed to evaluate differences between groups with normal and nonnormal distribution, respectively. When a significant difference between the groups was detected, pair-wise comparisons between means or medians were performed by the Student-Newman-Keuls test. Difference within groups, without vs. with treatment, was analyzed by paired t test. The value of acceptance for statistical significance was set at P < 0.05. Statistical analysis was performed using SigmaStat for Windows (Jandel Scientific GmbH, Erkrath, Germany). For the purpose of calculation, undetectable GH levels were assigned a value of 0.04 μU/mL.

Results

The relatively high doses of GH administered to the patients in the study were well tolerated, and no adverse side effects were noted during the 5 days of treatment. As expected, routine laboratory results gave evidence of some degree of fluid retention because both hemoglobin and albumin concentrations tended to decrease. There was no laboratory evidence of hepatic dysfunction. Table 1 summarizes the numeric data from serum analyses and shows statistical evaluation of GH effects within the acute and short-term treatment groups separately, using Student’s paired t test. As expected, serum levels of GH were significantly increased in both treatment groups. There was no difference between the groups in serum insulin before treatment. Insulin levels were unchanged after GH injection in the acute group, whereas it increased 2-fold (P < 0.01) in the short-term treatment group.

IGF-I

mRNA for IGF-I was significantly higher in both groups of patients receiving GH before operation as compared with controls (Fig. 2a, upper panel). The percentage difference was 173% (P < 0.001) in the acute treatment group and 100% (P < 0.01) in the short-term treatment group.

Serum levels of IGF-I in the group receiving acute treatment did not differ from those in the controls but were 3-fold higher (P < 0.001) in the short-term treatment group (Fig. 2a, lower panel). Before treatment, serum IGF-I in sp score showed normal age related levels in all groups, and the patients treated for 5 days increased to almost 5sd above normal levels (Table 1).

IGF-II

The expression of IGF-II mRNA did not differ between the groups as shown in Fig. 2b, upper panel.

Serum levels of IGF-II in the acute treatment group were similar compared with the control group. However, after short-term treatment with GH, serum IGF-II was 33% (P < 0.01) lower compared with controls (Fig. 2b, lower panel). There was also a highly significant 26% (P < 0.001) treatment-related decrease compared with baseline, in serum IGF-II within the short-term treatment group (Table 1).

ALS

Expression of the acid-labile subunit in liver tissue, measured as mRNA, was 85% (P < 0.05) increased in the short-term treatment group of patients. However, no significant change was found after one injection only (Fig. 3a, upper panel).

Serum levels of ALS were 1.5-fold (P < 0.001) higher in short-term treated patients compared with controls, whereas a single injection of GH was without effect in the acute treatment group (Fig. 3a, lower panel). The short-term group was furthermore the only one with a significant increase when compared with values before treatment (P < 0.001) (Table 1).

IGFBP-3

GH administration had no effect on the expression of IGFBP-3, measured as mRNA, in liver tissue in either the
acute or short-term treatment group, as compared with the control group (Fig. 3b, upper panel).

Serum levels of IGFBP-3 was 44% (P, 0.01) higher in the short-term treatment group, but no difference was found in the acutely treated group (Fig. 3b, lower panel). Before treatment, no differences in serum IGFBP-3 were found between the groups. Comparison within groups showed that only the short-term treatment group had a significant increase (Table 1).

**IGFBP-1**

Acute or short-term GH treatment had no significant effect on the expression of IGFBP-1 mRNA in liver tissue (Fig. 4a, upper panel).

In serum, however, IGFBP-1 was significantly lower (35%, P < 0.05) in both GH-treated groups compared with controls (Fig. 4a, lower panel). When levels at time of operation were compared with levels before treatment, a highly significant decrease was confirmed (P < 0.01). No correlation was found between serum levels of IGFBP-1 and insulin in neither of the groups.

**IGFBP-2**

No difference was found in expression of IGFBP-2 mRNA in acutely treated patients, whereas short-term treatment resulted in 65% (P < 0.01) lower levels compared with controls (Fig. 4b, upper panel).

No significant effect of GH was found on circulating levels of IGFBP-2 when compared between groups, at time of operation (Fig. 4b, lower panel). However when the comparison was made within groups, a highly significant (40%, P < 0.001) decrease in IGFBP-2 was found in the short-term treatment group (Table 1).

---

**TABLE 1.** Serum levels of various peptides before and after GH treatment and values for the control group.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 10)</th>
<th>Acute GH treatment (n = 10)</th>
<th>Short-term GH treatment (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Female/male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>41 (4)</td>
<td>53 (4)</td>
<td>49 (4)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.3 (1.1)</td>
<td>26.8 (1.3)</td>
<td>24.4 (1.0)</td>
</tr>
<tr>
<td>GH (µg/L)</td>
<td>0.37 (0.21–0.64)</td>
<td>23.1 (18.8–28.4)</td>
<td>4.47 (3.63–5.50)</td>
</tr>
<tr>
<td>IGF-I peptide (µg/L)</td>
<td>200 (181–220)</td>
<td>162 (156–168)</td>
<td>562 (513–616)</td>
</tr>
<tr>
<td>IGF-I SD score</td>
<td>-0.01 (0.3)</td>
<td>-0.1 (0.2)</td>
<td>4.8 (0.3)</td>
</tr>
<tr>
<td>IGF-II peptide (µg/L)</td>
<td>1119 (46)</td>
<td>959 (75)</td>
<td>752 (58)</td>
</tr>
<tr>
<td>IGFBP-3 peptide (mg/L)</td>
<td>4.32 (0.18)</td>
<td>3.97 (0.20)</td>
<td>6.24 (0.3)</td>
</tr>
<tr>
<td>ALS peptide (mg/L)</td>
<td>28.4 (1.6)</td>
<td>26.4 (1.4)</td>
<td>43.8 (1.9)</td>
</tr>
<tr>
<td>IGFBP-1 peptide (µg/L)</td>
<td>51 (6)</td>
<td>31 (47)</td>
<td>32 (6)</td>
</tr>
<tr>
<td>IGFBP-2 peptide (µg/L)</td>
<td>293 (28)</td>
<td>363 (49)</td>
<td>260 (22)</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>20 (17–21)</td>
<td>19 (16–24)</td>
<td>45 (37–54)</td>
</tr>
</tbody>
</table>

M (SEM) or a geometric mean (SEM range).

b P < 0.001 vs. before treatment, within group.

c P < 0.01 vs. before treatment, within group.

---

*Fig. 2.** Hepatic expression (upper panels) and serum levels (lower panels) of (a) IGF-I and (b) IGF-II in the control group, acute GH treatment group, and short-term GH treatment group, respectively, at the time of operation. Upper panels, Box plots indicate the median and lower and upper quartiles; whiskers indicate 10th and 90th percentiles. The mean values are depicted as dashed lines. Lower panels, Data are shown as mean (±SEM). ANOVA analysis, *, P < 0.05; **, P < 0.01; ***, P < 0.001.
Discussion

Our knowledge of how GH regulates gene expression in the liver is mainly derived from experiments in animal models. In this open study we have been able to compare mRNA expression in human liver tissue with observed changes in serum levels in the same individuals. This approach has enabled us to describe changes in several of the peptides thought to be directly involved in GH-regulated events and also in modulators of secondary actions of GH regarding growth and general metabolism.

The hepatic IGF-I mRNA levels were significantly higher in the two GH-treated groups compared with controls. IGF-I mRNA was nearly 3-fold elevated above controls, already 4–5 h after a single GH injection of 12 IE. This finding confirms that GH has a direct effect on hepatic IGF-I expression as previously shown in animal studies (33, 34). The somewhat lower IGF-I mRNA found after 5 days of GH treatment is most likely due to the longer interval between GH injection and time of operation, as indicated by the lower levels of GH in serum. It is possible that GH could mediate a negative
feedback effect of GH receptor expression, similar to other cytokine receptors (35). No increase in IGF-I was observed in the circulation 4–5 h after a single injection of 12 IE GH, which is in accordance with observations in GH-deficient patients, where serum IGF-I levels start to rise 4 h after a single GH injection (36). A 3-fold increase of serum IGF-I levels was observed after treatment with GH for 5 days. The mean IGF-I concentration reached levels corresponding to approximately +5sd of age matched controls as expected with daily doses of 12 IE. We also analyzed the IGF type 1 receptor mRNA levels using RNase protection analysis, but no significant differences were found between the groups (data not shown).

Our findings thus indicate that GH has a rapid effect on the transcription of the IGF-I gene, and that there is a delay before the peptide is accumulated in the circulation in the high molecular ternary complex IGF-I/IGFBP-3/ALS. The rise in total serum IGF-I levels is dependent on the availability of ALS. The onset of the GH-induced transcription of the ALS gene was delayed in comparison to the IGF-I gene because no difference was observed in ALS mRNA 4–5 h after GH injection. After 5 days of GH administration, ALS mRNA was higher, with a 2-fold increase, similar to IGF-I mRNA, compared with control subjects. This delay in stimulation of ALS expression is not due to GH-induced IGF-I production because IGF-I administration previously has been shown to suppress GH production and induce a rapid decline in serum ALS levels (37). A direct GH effect on ALS gene transcription was recently shown in hypophysectomized rats (14). Furthermore, Fielder et al. (38) could show an effect of GH on circulating levels of ALS in serum from hypophysectomized rats.

In addition to serum IGF-I and ALS, serum concentrations of IGFBP-3 are presently used as an index of GH effects during replacement therapy with GH in both adults and children (39–44). The IGFBP-3 mRNA content in the liver did not differ between subjects treated with GH and controls. Studies in rats have revealed that IGFBP-3 is not expressed in hepatocytes but in other cells present in the liver (45), and it is unclear if GH can stimulate IGFBP-3 production in these cells. Neither GH itself nor the increased IGF-I levels stimulated hepatic IGFBP-3 expression. However, serum levels of IGFBP-3 were significantly higher in the group treated for 5 days, with an increase of 34%. The source of the IGFBP-3 elevation in the circulation remains unclear, and it cannot be excluded that GH stimulates IGFBP-3 expression and release from other tissues, such as endothelial cells. Furthermore, IGF-I has been shown to release IGFBP-3 attached to cell surfaces (46). The most likely explanation for the rise in serum IGFBP-3 after GH administration is that increased production of ALS and IGF-I promotes the recruitment of IGFBP-3 attached to cell surfaces. It has previously been shown that increased formation of the ternary complex (IGF-I/IGFBP-3/ALS) prolongs the half-life of IGFBP-3 in the circulation (47).

GH is not thought to be a regulator of IGF-II. Accordingly, the expression of total IGF-II mRNA in liver tissue did not differ from controls. Surprisingly, we found that the short-term treatment group had a highly significant decrease in circulating IGF-II. A similar decrease in IGF-II levels has previously been observed in patients with active acromegaly (30), and serum levels of proIGF-II decreased after the administration of IGF-I at high doses (48). The most obvious explanation to these results is that GH-induced IGF-I competes for the ternary complex, thus making IGF-II more susceptible to proteolytic degradation and/or clearance.

It was previously shown that GH can suppress serum levels of IGFBP-2 in humans (49). In this study, hepatic IGFBP-2 mRNA in the short-term group was only 27% of the levels in the control group. This novel finding reflects a late GH event and may be secondary to other effects induced by GH. The IGFBP-2 levels decreased concomitantly with the elevation of the ternary complex, and the decrease in total IGF-II levels after 5 days with GH. In accordance with this, it was reported that high serum IGFBP-2 levels are found in patients with nonislet cell tumor hypoglycemia (NICTH), who have high levels of free IGF-II and proIGF-II, as well as low insulin levels (50). Therefore, IGFBP-2 expression has been proposed to be stimulated by IGF-II and suppressed by insulin. Furthermore, the findings of lower IGFBP-2 in obese subjects (51), and the elevated IGFBP-2 levels during malnutrition and starvation, when insulin levels are low, tend to support the concept that insulin can act as a suppressor of IGFBP-2 production. It has also been shown that increased occupancy of IGFBP-2 by increasing concentrations of IGF-I or IGF-II may increase the binding of IGFBP-2 to endothelial cells in peripheral tissues (52). This does not, however, explain the fact that levels of mRNA were approximately 70% lower compared with the control group. The possibility exists, therefore, that GH confers a direct effect on IGFBP-2 regulation.

No significant difference in hepatic IGFBP-1 mRNA was found between the groups. A lower IGFBP-1 was expected in the short-term treatment group due to the higher insulin levels induced by GH, and in fact serum IGFBP-1 levels were lower. It is well established that insulin suppresses hepatic IGFBP-1 expression at the transcriptional level, as well as its release, both in vitro and in vivo (19, 53). The decrease in IGFBP-1 levels after 5 days of GH could therefore be attributed to the increased insulin levels, although we could not show this correlation significantly. However, this does not explain the 35% lower values seen in samples taken 4–5 h after a single GH injection during fasting conditions. IGFBP-1, mainly derived from the liver, has a short half-life in the circulation and displays rapid changes in serum levels and diurnal rhythm. During fasting conditions, a morning decline in IGFBP-1 is not expected. It cannot be excluded, however, that GH induced a transient increase in insulin that was not detected by RIA in serum samples. Infusion of GH in the physiological range, without elevation of insulin levels, was without effect on IGFBP-1 pattern (54). However, the diurnal variation of IGFBP-1 levels in GH-deficient patients, similar to healthy subjects, is inversely correlated to insulin, although values are elevated relative to insulin (27). GH has been reported to inhibit IGFBP-1 expression in cultured rat hepatocytes, but this effect could not be confirmed in human Hep G2 cells (55). It is still possible, but not proven, that the IGFBP-1 gene similar to the IGF-I and ALS genes belongs to the group of GH-regulated genes.

In conclusion, the present study reveals that the metabolic
effects of GH on the IGF axis in man are complex. Besides stimulating IGF-I and ALS expression, GH also inhibits IGFBP-1 and IGFBP-2, possibly facilitating enhanced IGF bioavailability to target tissues.

Acknowledgments

The authors wish to thank professor Kerstin Hall for fruitful discussions regarding interpretation of results.

References


