GH insensitivity induced by endotoxin injection is associated with decreased liver GH receptors

DOMINIQUE DEFalQUE, NATHALIE BRANDT, JEAN-MARIE KETELSLEGERS, AND JEAN-PAUL THISSEN
Unité de Diabétologie e Nutrition, School of Medicine
The University of Louvain, B-1200 Brussels, Belgium

Defalque, Dominique, Nathalie Brandt, Jean-Marie Ketelslegers, and Jean-Paul Thissen. GH insensitivity induced by endotoxin injection is associated with decreased liver GH receptors. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E565–E572, 1999.—Sepsis induces a state of growth hormone (GH) resistance associated with a decrease of circulating insulin-like growth factor (IGF) I, a GH-dependent anabolic hormone mainly produced by the liver. To address the mechanisms that might trigger GH insensitivity in sepsis, we investigated the regulation of liver GH receptor (GHR) and its gene expression by endotoxin. Endotoxin injection in rats decreased serum IGF-I and liver GH-binding sites after 10 h. In contrast to liver GHR, circulating GH-binding protein (GHBP) levels were not significantly reduced after endotoxin injection. The parallel decrease in IGF-I and GHR and in their corresponding liver mRNAs suggests that decreased serum IGF-I and liver GHR were likely to result from decreased liver synthesis. Although GH administration in control animals significantly enhanced serum IGF-I, it did fail to prevent the decline in serum IGF-I and liver GH-binding sites in endotoxemic rats. In this study, we showed that endotoxin injection induces a state of GH insensitivity associated with decreased liver GHR. This decline in GHR, which cannot be prevented by exogenous GH, might contribute to the GH insensitivity observed in sepsis.

CRITICAL ILLNESS such as sepsis, trauma, and burns cause a decrease in lean body mass associated with increased morbidity and mortality. This loss of body protein is mainly mediated by an accelerated rate of protein catabolism (35). Insulin-like growth factor (IGF) I is a growth hormone (GH)-dependent growth factor that inhibits protein breakdown (28, 29). Therefore, a decrease in circulating IGF-I concentrations might be involved in the development of these catabolic states. Indeed, critically ill patients have low levels of IGF-I (26, 34). Septic patients, however, failed to raise serum IGF-I in response to exogenous GH (6), suggesting that sepsis is associated with a state of GH insensitivity. In rats, endotoxin injection, which simulates a septic state, acutely decreased circulating GH and IGF-I (10, 25). However, as in humans, chronic GH infusion in these animals did not reverse the endotoxin-induced reduction of serum IGF-I (16). The mechanisms by which sepsis in humans and endotoxin in rats cause a state of GH insensitivity are still unknown.

The purpose of the present study was to investigate the cellular mechanisms that might be responsible for the GH insensitivity induced by endotoxin. To address the potential role of liver GH receptors (GHR) in this resistance, we assessed the GH-binding sites and GHR gene expression in response to endotoxin injection. In addition, we also compared the effects of exogenous GH on circulating IGF-I and liver GHR in control and endotoxemic rats.

MATERIALS AND METHODS

Animals
All experimental procedures were carried out in compliance with the appropriate institutional and national ethical guidelines for work with laboratory animals. Fifty-two 3-wk-old female Wistar rats (45–55 g) were obtained from the Katholieke Universiteit Leuven (Heverlee, Belgium). They were housed for 7 days under conditions controlled for temperature (22 ± 2°C) and lighting, with a cycle of 12 h of light (7:00 AM to 7:00 PM) followed by 12 h of darkness. Body weights were recorded every morning. Food access was limited to the period between 7:00 PM and 9:00 AM, whereas access to tap water was allowed without any restriction.

Endotoxin and Hormone Preparations
Escherichia coli lipopolysaccharide (LPS; serotype O127:B8 phenol extract), obtained from Sigma Chemical (St. Louis, MO), was resuspended in sterile endotoxin-free saline to obtain 400 µg/ml and 1.2 mg/ml solutions. The injected volume of LPS ranged between 440 and 530 µl. Bovine GH, kindly provided by Monsanto (St. Louis, MO), was resuspended in glycine buffer (pH 9.6) and then diluted in sterile, endotoxin-free saline to obtain a 1 mg/ml solution. The injected volume of GH was between 140 and 170 µl.

Experimental Protocols
After a 7-day adaptation period, rats received LPS, GH, or saline injections between 8:00 AM and 10:00 AM and were killed 5 or 10 h later. We collected blood into glass tubes and centrifuged it at 1,800 rpm for 10 min at 4°C to collect serum. We then stored serum at −20°C until IGF-I, rat GH (rGH), and GH-binding protein (GHBP) determination. Livers were removed, flash-frozen in liquid nitrogen, and stored at −80°C until homogenate preparation and RNA extraction.

Experiment 1: Effect of LPS on plasma IGF-I and liver GHR. After the 7-day adaptation period, 4 of the 28 rats were killed in the morning (control rats). The remaining 24 rats were divided into three groups (8 rats/group). LPS (750 µg/100 g body wt ip) was administered to the first group; the second group received LPS at a lower dosage (250 µg/100 g body wt ip), and the third group was given intraperitoneal...
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Experiment 2: Effect of LPS on IGF-I response to GH. After the 7-day adaptation period, 24 rats were divided into 4 groups (6 rats/group). The first group received one injection of LPS (750 µg/100 g body wt) and one injection of saline (sc), the second group received one injection of GH (200 µg/100 g body wt sc) and one injection of saline (ip), the third group received LPS and GH, and the fourth group received two injections of saline. All rats were killed 10 h after injection.

RIA

Serum IGF-I extraction and RIA. We extracted serum samples using octadecasilicylic acid columns (Waters C18 Sep-Pak cartridges; Millipore, Milford, MA) and eluted bound proteins with 7% acetic acid and IGF-I with 100% methanol (7). We measured concentrations of IGF-I using the RIA procedure as previously described (5). We used the antibody at a dilution of 1:15,000; the assay buffer consisted of 0.03 mol/l sodium phosphate (pH 7.5), 0.05% Tween 20, 10 mmol/l EDTA, 0.2% protamine sulfate, and 0.02% NaN3. The RIA had a sensitivity of 0.2 ng/ml, and intra- and interassay coefficients of variation were 5 and 13%, respectively.

Serum rGH RIA. The RIA procedure for serum rGH determination has been described previously (21). Results are expressed in terms of the RP-2 standard. The sensitivity was 2 ng/ml, and intra- and interassay coefficients of variation were 8 and 12%, respectively.

Liver and Serum GH-Binding Studies

We determined liver somatogenic binding by incubating liver homogenates with a tracer amount of 125I-labeled bovine GH, as described previously (19). Total somatogenic binding sites were determined on liver homogenates treated with 4 M MgCl2 to remove the endogenous or exogenous GH bound to the binding sites (the "desaturation" process). Free available somatogenic sites were determined on homogenates treated in parallel with water. The bovine GH, kindly provided by Paladini (Buenos Aires, Argentina), was labeled with the lactoperoxidase method and purified on a Sephadex G-75 column (30 × 1 cm). We performed the desaturation of the somatogenic binding sites (22) using a modification of the method described by Kelly et al. (13). Briefly, 100-µl aliquots of liver homogenate were mixed in 12 × 75-mm glass tubes with 3.5 M cold assay buffer (25 mM Tris·HCl, pH 7.4, 10 M CaCl2, 0.1% BSA; 4°C) and centrifuged at 2,500 for 30 min. After the supernatants were aspirated, the pellets were resuspended in 100 µl assay buffer and either 0.5 ml water or 0.5 ml 4 M MgCl2 (dissolved in deionized distilled water). After incubation for 5 min at room temperature, 3.5 ml cold assay buffer were added, the samples were centrifuged at 2,500 g for 30 min, and the supernatant was removed. We washed the pellets again with 3.5 ml cold assay buffer and, after centrifugation, resuspended them in 100 µl assay buffer for binding studies or in 100 µl distilled water for protein determination. The binding assays were performed as follows. The treated pellets (0.40–0.90 mg protein) were incubated at 0°C with 100 µl of a tracer amount of 125I-labeled bovine GH [40,000 counts/min (cpm)] corresponding to 0.4 ng in the absence or presence of excess unlabeled hormone (1 µg/tube). The specifically bound labeled hormone (B) was expressed as a percentage of the total labeled hormone (T) incubated with the liver homogenates, and the binding was corrected for the amount of protein present in the incubation medium.

Serum GHBP levels were determined on serum (10 µl) incubated at room temperature with 125I-labeled bovine GH (50,000 cpm corresponding to 0.5 ng in 50 µl) in the absence (total binding) or presence of an excess of unlabeled hormone (1 µg/tube, nonspecific binding) (23). Bound and free hormones were separated by gel filtration at 4°C on Ultrogel AcA34 columns (1 × 40 cm), and the specific binding of bovine GH to serum was expressed as a percentage of the total radioactivity (B/T × 100). In our conditions, a clear resolution between free bovine GH and bovine GH bound to GHBP was obtained. The specificity and affinity of the GHBP for bovine GH have been established in our laboratory (23).

RNA Extraction and Northern Blot Analysis

Total liver RNA was extracted using the guanidine thiocyanate and cesium chloride method (32). Total RNA (20 µg) was denatured in formaldehyde-3-(N-morpholins) neoponesulfonic acid and subjected to electrophoresis on a formaldehyde-agarose gel. We assessed the homogeneity of RNA loading by ultraviolet transillumination of the gels after staining with ethidium bromide. The RNA was transferred to nylon membranes (Hybond; Amersham, Little Chalfont, UK) by vacuum blotting (Vacugene; Pharmacia, Uppsala, Sweden). Levels of IGF-I mRNA and GHR-GHBP mRNAs were determined by hybridization with specific riboprobes. A 32P-labeled 18S probe was used as a control probe to verify uniform loading. A 194-bp Aval I-Hinfl cDNA rat IGF-I exon 4 fragment was ligated into the plasmid vector Bluescript (Strategene, La Jolla, CA) and linearized by HindIII (4). This probe included only the coding region for the mature IGF-I peptide. 32P-labeled RNA transcripts, as visualized by Northern blot analysis, presented a complex picture consisting of a large (7.5 kb) discrete transcript, a group of transcripts ranging from ~0.8 to 1.2 kb, and two additional minor transcripts of ~1.7 and 4 kb. The two major mRNA size classes (7.5 and 0.8–1.2 kb) displayed the same length of the 3′ untranslated region (UTR). The 7.5-kb mRNAs have an unusually long 3′-untranslated region (~6 kb). Because all these transcripts may potentially be desributed into IGF-I precursors, we performed a densitometric analysis of the four bands visible on the blot. The densitometric results corresponded to the sum of all IGF-I mRNA transcripts. The 900-bp Bgl II fragment of the rGH cDNA, subcloned into the vector pT7, was linearized with BamH I. The sequence used as a probe encodes part of the extracellular domain of the receptor identical to a part of the GHBP (24). Hybridization of liver RNA with this probe reveals two major bands corresponding to the GHR mRNA (4.3 kb) and the GHBP mRNA (1.3 kb), as indicated by previous investigations (1). According to this, the GHR mRNA and GHBP levels were assessed by the densitometric analysis of the 4.3-kb and 1.3-kb bands, respectively. Specifically, 32P-labeled RNA antisense probes were generated from linearized plasmids using uridine 5′-32P-triphosphate (activity 800 Ci/mmol; Amersham) and T7 RNA polymerase. The probe used for metallothionein was an oligomer (21 bp) obtained from GIBCO (Life Technologies, Merelbeke, Belgium). For the 18S ribosomal RNA, a 23-mer oligonucleotide was synthesized on a DNA synthesizer (Millipore, Brussels, Belgium). Both oligonucleotides were end-labeled with 32P using adenosine-5′-32P-triphosphate (activity 3,000 cpm).
Ci/mmol; Amersham) and a T4 polynucleotide kinase (Amersham). We quantified the mRNA levels by densitometric scanning of the hybridization signal (LKB Ultroscan XL laser densitometry, Bromma, Sweden) using Gel Scan software (Pharmacia). The mRNA levels were expressed as a percentage of the mean value observed in control rats.

Statistical Analysis

Experimental data are presented as means ± SE. In experiment 1, to determine the respective influence of the two independent factors (dose of LPS and time after injection), data were analyzed by two-way ANOVA followed by the Scheffé test. In experiment 2, data were analyzed by one-way ANOVA followed by the Newman-Keuls test. Statistical significance was set at P < 0.05.

RESULTS

Effects of LPS on IGF-I and GH

A single injection of LPS rapidly decreased serum IGF-I concentrations. This diminution was time and dose dependent (P < 0.001 for both; Fig. 1A). Five hours after injection of a low dose of LPS (250 µg/100 g body wt), the serum IGF-I had already declined by 20% vs. saline-injected rats (P < 0.05). After 10 h, we observed a 37% decrease compared with time-matched values in saline-injected rats (P < 0.01). The IGF-I decrease was even more pronounced with the high dose of LPS. In response to 750 µg/100 g body wt of LPS, the IGF-I concentrations declined by 36% after 5 h and by 61% after 10 h compared with saline-treated rats (P < 0.001 for both). This decrease in serum IGF-I was associated with reduced liver IGF-I gene expression. Indeed, 10 h after injection, the liver IGF-I mRNA levels were decreased by 49% in response to the low dose (P < 0.01) and by 66% in response to the high dose (P < 0.001) of LPS (Fig. 1B). Despite the decline in circulating IGF-I, the rGH concentrations were not significantly reduced by LPS injection (Table 1).

Effects of GH on IGF-I Response to LPS

To investigate the possibility of a state of GH insensitivity responsible for the decrease in IGF-I after LPS injection, we compared the IGF-I response to a single injection of GH in saline- and LPS-treated rats. As previously shown (Fig. 1A), acute LPS injection decreased serum IGF-I concentrations (−30%, P < 0.05) in comparison with control animals (Fig. 2A). As expected, GH injection in saline-injected rats increased circulating IGF-I (33%, P < 0.01 vs. controls). In contrast, GH injection in LPS-treated rats did not prevent the endotoxin-induced decrease of circulating IGF-I. Indeed, the IGF-I concentrations in these rats (242 ± 7 ng/ml) averaged those observed in rats treated by LPS without GH (236 ± 31 ng/ml; not significant (NS)). The liver IGF-I mRNA changes paralleled changes in circulating IGF-I peptide (Figs. 2B and 3). Although GH injection increased liver IGF-I mRNA by 51% in saline-treated rats compared with controls (P < 0.001), it did not prevent the endotoxin-induced decrease of IGF-I liver mRNA in LPS-treated rats.

Effects of LPS on Liver GHR and GHBP Levels

To determine whether decreased GHR might be one of the mechanisms responsible for the absence of IGF-I response to GH in LPS-treated animals, we measured the liver GH binding after LPS injection. The liver GH-binding sites were dramatically reduced by LPS injection (Fig. 4A). This decrease was already marked 5 h after the low dose of LPS (−50%, P < 0.01 vs. time-matched controls). The maximal decrease of GH-binding sites was observed 10 h after the large dose of LPS (−73%, P < 0.001). The decline of GH binding in response to LPS was therefore faster and more pronounced than the decline of serum IGF-I (Fig. 1A). This reduction in liver GH binding was associated with
Table 1. Serum rGH concentrations, serum GHBP levels, and liver GHBP mRNA levels

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<th>0 h</th>
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<tr>
<td>Serum rGH, ng/ml</td>
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<tr>
<td>Control</td>
<td>13 ± 6</td>
<td>7 ± 3</td>
<td>4 ± 1</td>
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<td>LPS (250 µg/100 g)</td>
<td>8 ± 3</td>
<td>5 ± 3</td>
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<td>LPS (750 µg/100 g)</td>
<td>3 ± 1</td>
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| Serum GHBP, B/T X 100
| Control              | 12.3 ± 1.7 | 12.0 ± 0.8 | 10.6 ± 1.0 |
| LPS (250 µg/100 g)   | 10.2 ± 2.5 | 7.7 ± 0.6 |         |
| LPS (750 µg/100 g)   | 10.4 ± 1.0 | 7.6 ± 0.7 |         |
| Liver GHBP mRNA, % of control |         |         |         |
| Control              | 100 ± 5 | 90 ± 11 | 102 ± 10 |
| LPS (250 µg/100 g)   | 64 ± 9† | 63 ± 5‡ |         |
| LPS (750 µg/100 g)   | 70 ± 4* | 49 ± 7‡ |         |

Values are means ± SE; n = 4 rats/group. Serum rat growth hormone (rGH) concentrations, serum growth hormone-binding protein (GHBP) levels, and liver GHBP mRNA levels 5 and 10 h after lipopolysaccharide (LPS) injection (250 µg/100 g body wt and 750 µg/100 g body wt) vs. saline. B, bound labeled hormones; T, total labeled hormones. *P < 0.05, †P < 0.01, and ‡P < 0.001 vs. time-matched controls.

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Fig. 2. Serum IGF-I and liver IGF-I mRNA response to growth hormone (GH) in saline- and LPS-treated rats. A: serum IGF-I concentrations 10 h after LPS and/or GH injection. LPS (750 µg/100 g body wt) was administered to rats with or without GH injection (200 µg/100 g body wt sc). Values represent means ± SE of 6 rats/group. *P < 0.05, **P < 0.01 compared with controls. B: liver IGF-I mRNA levels 10 h after LPS and/or GH injection. LPS (750 µg/100 g body wt ip) was administered to rats with or without GH injection (200 µg/100 g body wt sc). Values represent means ± SE of 6 rats/group. *P < 0.05, ***P < 0.001 compared with controls.

Fig. 3. Northern blot analysis of liver IGF-I mRNA (A), GH receptors (GHR)/GH-binding protein (GHBP) mRNAs (B), and 18S ribosomal RNA (C) 10 h after administration of LPS (750 µg/100 g body wt) and/or GH (200 µg/100 g body wt). Northern blot analysis was performed as indicated in MATERIALS AND METHODS; n = 3 rats/group.

Effects of GH on Liver GHR and GHR mRNA Response to LPS

In an attempt to prevent the decline in GHR induced by LPS, we assessed the effect of exogenous GH on liver GH binding in animals treated with LPS. Consistent with our previous experiment (Fig. 4A), total liver GH-binding sites were reduced by 76% in the LPS-treated group (P < 0.01 vs. controls; Fig. 5A). Exogenous GH did not affect total liver GH binding but decreased free binding (P < 0.05 vs. controls) as a consequence of the occupation of the receptors. Despite GH administration, the decline of liver GH binding caused by LPS was not prevented. Changes in liver GH binding induced by LPS were reflected by changes in liver GHR mRNA levels (–43% vs. controls, P < 0.001; Figs. 3 and 5B). However, although GH administration decreased GHR gene expression (Fig. 4B). Indeed, GHR mRNA levels were already reduced 5 h after injection with the high dose (–37%, P < 0.01) and even with the low dose (–29%, P < 0.05) of LPS. As for the GH-binding sites, a further decrease was observed in GHR mRNA levels after 10 h, by –42% with the low dose and –57% with the high dose (P < 0.001 for both), in comparison with time-matched, saline-injected rats. Serum GHBP levels were reduced by LPS injection (–28%, 10 h after 750 µg LPS vs. time-matched controls; NS) albeit not significantly (Table 1), despite a marked decrease in GHBP mRNA levels (–51%, 10 h after 750 µg LPS vs. time-matched controls, P < 0.001; Table 1).
in LPS-treated rats did not affect liver GH binding, exogenous GH partially normalized GHR mRNA levels in these animals (83% of control levels for LPS + GH vs. 57% of control levels for LPS, \( P < 0.01 \), and LPS + GH vs. controls; NS). Similarly, liver GHBP mRNA levels were reduced by LPS (−43% vs. controls, \( P < 0.001 \)) and partially restored to control levels by GH (LPS + GH: −24% vs. controls, \( P < 0.05 \); Fig. 3).

**Effects of LPS on Metallothionein mRNA**

To demonstrate the induction of an acute inflammatory response after LPS injection, we determined the expression of metallothionein, a positive acute-phase reactant. Five hours after injection, metallothionein mRNA levels were increased 7-fold by a low dose (\( P < 0.05 \) vs. saline) and 20-fold by a high dose (\( P < 0.001 \) vs. saline) of LPS (Fig. 6).

**DISCUSSION**

Our results show that the GH resistance caused by LPS injection in rats is associated with decreased liver GHR, a mechanism potentially responsible for the decrease of circulating IGF-I in response to LPS. The parallel reduction in liver GHR gene expression and GH binding suggests that a decrease in GHR synthesis contributes to the decline in GH binding caused by LPS. The failure of GH to prevent the decrease in liver GHR indicates that this decrease is not caused by the decline in GH secretion.

Several groups observed a reduction in GH secretion and circulating IGF-I levels in response to acute LPS injection in rats (10, 25). Given the cardinal role of GH in the stimulation of IGF-I production by the liver, decreased GH secretion has been considered responsible for the decline of IGF-I. However, observations in humans suggest that decreased IGF-I might result
from a state of GH insensitivity (8). Indeed, Ross et al. (26) reported low circulating IGF-I levels in critically ill patients despite elevated GH secretion. Dahn et al. (6) made the observation that exogenous GH was unable to stimulate IGF-I production in septic patients. More recently, Fan et al. (10) showed that after a single injection of LPS in rats, plasma IGF-I levels remained low despite the fact that GH levels had returned to normal values. In agreement with these authors, our study supports the possibility that reduced GH secretion is not the only cause for the decline in IGF-I concentrations after LPS injection. First, in our model we observed no significant decrease in serum concentrations of GH in LPS-treated rats. This observation must be interpreted cautiously, however, because single blood samples taken at the time that the rats were killed may not reflect integrated pulsatile GH secretion (30). Second, our observation that GH treatment did not increase the IGF-I levels in LPS-treated rats allows us to confirm that LPS induces a state of GH insensitivity. This observation is consistent with the results of Liao et al. (16), who observed that constant GH infusion with osmotic minipumps did not reverse the endotoxin-induced reduction of IGF-I. Taken together, these observations clearly establish the acute inflammatory state caused by LPS injection as a new cause of GH insensitivity.

To unravel the mechanisms of LPS-induced GH resistance, we investigated whether liver GHR might be decreased in response to endotoxin. Our data show for the first time that liver GHR are downregulated by endotoxin in vivo. Indeed, liver GH binding was reduced by 75% 10 h after LPS injection. Although this dramatic decrease of GH binding is similar in amplitude to changes observed in fasting, the kinetic of decrease is much faster in response to LPS than to fasting (20). Indeed, in fasting, a 75% reduction of liver GH binding is only reached after 72 h, whereas after LPS a reduction of the same amplitude is already observed after 10 h. This major loss in liver GH binding might mediate the GH resistance induced by LPS. Our results do not allow us to conclude whether this reduction in binding was due to changes of receptor number. Nevertheless, the concomitant reduction of liver GHR mRNA levels suggests that decreased GH binding after LPS results at least partially from decreased GHR synthesis. In the rat, GHBP is synthesized from an alternatively spliced mRNA in which exons encoding the transmembrane and cytosolic domains of GHR are replaced with an exon that encodes a hydrophilic peptide (1). After LPS, GHBP mRNA levels were markedly reduced in contrast to GHRBP, which was not significantly decreased. The reason for this discrepancy is unknown but might be due to differences in the half-life between the GHBP and its mRNA. The very slight reduction of GHBP levels after LPS strongly contrasted with the marked decrease in GHR. The reason for the discrepancy between changes in GHR and changes in GHBP may therefore reside in the longer half-life of the serum GHBP (2.4 h) (12) by comparison with the liver GHR (30–40 min) (2).

The mediators causing the LPS-induced GH resistance are unknown. Anorexia induced by LPS might contribute to the GH resistance, as nutritional deprivation of calories, proteins, and micronutrients (zinc) is a well-recognized cause of GH resistance (31). However, the design of our experiment allows us to exclude such a hypothesis. Because control and LPS-treated animals did not have access to food between the time of LPS injection and death, the decrease in GHR and the GH resistance that we observed cannot be mediated by nutritional deprivation. The in vivo biological activities of LPS are largely mediated by the production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6. These cytokines are largely responsible for the acute inflammatory reaction induced by LPS, which is illustrated in our experiment by the marked stimulation of the metallothionein hepatic gene expression. The role of these cytokines in the decline of GHR caused by LPS is suggested by our recent observation showing that IL-1β downregulates the GHR gene expression in primary cultured hepatocytes (33). Our experiments also showed that IL-1β, and to a lesser extent TNF-α, can blunt the IGF-I mRNA response to GH in these hepatocytes. Furthermore, the role of IL-1β and TNF-α in the decline of GHR induced by LPS injection has been observed in vivo. Indeed, neutralization of IL-1β and TNF-α actions by specific antagonists, such as IL-1 receptor antagonist and antiserum directed against TNF-α, partially blunts the decrease in serum IGF-I caused by LPS (9, 15). Further studies remain to be done to establish whether these cytokines also mediate the decrease of GHR caused in vivo by LPS, as suggested by our in vitro
results. Taken together, these observations support the major role of enhanced production of IL-1β and TNF-α in the LPS-induced decline in IGF-I.

LPS injection stimulates the activity of the hypothalamic-pituitary-adrenal axis, resulting in an increase in circulating glucocorticoids. Excess of glucocorticoids might also contribute to the state of GH resistance and, therefore, to the decline in IGF-I caused by LPS. Indeed, previous experiments have shown that dexamethasone blunts the IGF-I mRNA response to GH in hypophysectomized rats (18) and in primary cultured hepatocytes (3). In addition, incubation with dexamethasone decreases the GH binding to 3T3-F442A cells (14) and the GHR mRNA levels in hepatocytes (3). Furthermore, administration of the glucocorticoid receptor antagonist RU 486 can prevent the IL-1-induced reduction in plasma IGF-I concentrations (11). All of these observations support the role of excess glucocorticoids in the decline of IGF-I caused by LPS.

In summary, our results demonstrate that endotoxin injection induces a state of GH insensitivity potentially mediated by the loss of liver GHR. As suggested by in vitro observations, glucocorticoids and proinflammatory cytokines are probably involved in the mechanisms of this GH insensitivity. A better understanding of the mechanisms of GH insensitivity in sepsis and critical illness would allow the development of new strategies to restore the anabolic actions of GH in these situations (27).

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Address for reprint requests and other correspondence: J.-P. Thissen, Unité de Diabetologie et Nutrition, UCL/IAAB 5474 Ave. Hippocrate, 54, B-1200 Brussels, Belgium (E-mail: thissen@iabidlab. ucl.ac.be).

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