Regulation of the acid-labile subunit in sustained endotoxemia

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The acid-labile subunit (ALS) of the 150-kDa insulin-like growth factor-binding protein (IGFBP) complex is an 85-kDa glycoprotein that is thought to regulate the bioavailability of IGFs in the serum, and possibly other tissue compartments, by sequestering them in a ternary complex with either IGFBP-3 or IGFBP-5 (2, 6, 13, 22, 48). Prolonged critical illness is characterized by severe wasting of lean body mass that has been attributed partly to suppression of the IGF axis (3, 5, 47, 50). Concordantly, serum levels of ALS and other ternary complex components have been found to be decreased in critically ill patients (2, 5, 11, 49).

Circulating levels of ALS have been found to correlate strongly with IGFBP-3 and IGFBP-5 serum concentrations (7) and consequently would be expected to affect the bioavailability and metabolic activity of the IGFs in serum. ALS and IGF-I are expressed predominantly in the liver, and their expression is regulated at the level of transcription by GH (12, 23, 39). Additionally, we have found that insulin modulates hepatic expression of ALS (17, 18). Altered hepatic sensitivity to GH and insulin would be expected to modulate circulating levels of ALS and, consequently, other components of the ternary complexes.

The effects of sustained endotoxia on ALS expression have not yet been studied. Sustained endotoxemia in rats provides a model of inflammation-induced catabolism different from endotoxemic shock and very acute responses measured within hours of administration of a potentially lethal dose of lipopolysaccharide. We have used the model of sustained endotoxemia described by Peisen et al. (42) to examine both acute and more chronic effects of sublethal doses of endotoxin on ALS and other IGFBP ternary complex components in relation to alterations in expression of GH receptor (GHR), the inhibitors of GH signaling (28), suppressor of cytokine signaling (SOCS-3) and cytokine-inducible SH2-containing protein (CIS), and markers of hepatic insulin action IGFBP-1 and phospho pyruvate carboxykinase (PEPCK). This model uses juvenile rats that respond to endotoxin treatment by rapidly losing weight, suggesting a lesion in the somatotropic axis. Similar prolonged exposure to endotoxin has also been demonstrated to suppress insulin postreceptor signaling pathways (38). We have used pair-feeding throughout the study to preclude the confounding nutritional effects on ALS serum levels, which we have previously demonstrated (17). We find that the major acute effect of endotoxemia on regulation of ALS is through suppressed nutrient intake, with significant augmentative effects of endotoxin. However, sustained endotoxin treatment is characterized by increased ALS and ternary complex component expression relative to pair-fed controls, which coincides with differential regulation of markers of insulin sensitivity and glucose homeostasis in the liver.
MATERIALS AND METHODS

Animal Preparation and Experimental Protocol

Juvenile male Sprague-Dawley rats were individually housed at a constant temperature and 12:12-h light-dark cycle; they had free access to rat chow and water for 5 days before the study commenced. Experiments were conducted with the approval of the Royal North Shore Hospital Animal Care and Ethics Committee.

Once acclimatized, animals were divided into 11 groups of similar mean weight (84.2 ± 12.7 SD g for the entire group). The groups consisted of 7 untreated animals culled immediately after the acclimatization period (n = 7), 2 endotoxin-treated animals culled at 6 (n = 6), 12 (n = 6), 24 (n = 7), 36 (n = 6), and 48 h (n = 6) after the initial injection, and 3 respective pair-fed controls culled at 6 h (n = 6) and 12, 24, 36, and 48 h (n = 5 each). Animals in the endotoxin-treated groups received intraperitoneal injections of 2.5 mg/kg Escherichia coli lipopolysaccharides (LPS, serotype O127:B8; 0.5 mg/ml in 0.9% saline; Sigma, St Louis, MO) every 12 h for 48 h (see Ref. 42). Control pair-fed groups were injected with nonpyrogenic 0.9% saline. The food intake of the endotoxin groups was measured at 12-h intervals, and subsequently the mean weight of rat chow eaten by the endotoxin groups was given to the pair-fed groups in which the protocol was started on the following day. This meant that the level of food intake was identical in both endotoxin- and saline-treated animals during each of the 12-h periods studied (see Fig. 1A). The endotoxin-treated animals did not eat during the first 6 h after treatment, and so their respective pair-fed group was fasted. All of the food provided to the pair-fed animals was eaten. The body weights and food and water intake of the animals were recorded at 12-h intervals throughout the study period. After anesthesia, blood was collected by cardiac puncture, and liver tissue was quickly removed, snap-frozen in liquid nitrogen, and stored at −80°C until analysis. Serum samples were stored at −20°C until analysis.

RIAs

All samples were analyzed in duplicate within the same assay unless otherwise stated. Rat ALS and IGFBP-5 were measured by specific RIAs, which have been described previously (4, 7). Rat IGFBP-1 was measured by a disequilibrium-based modification of a previously described method to enhance sensitivity (36). Briefly, serum samples were incubated overnight at room temperature with the primary antibody. IGFBP-1 tracer was added on the following morning and incubated for 7 h at room temperature. Samples were then treated as previously described. Rat IGF-I was measured in acid-ethanol-extracted and -neutralized whole sera by use of an IGF-1-specific RIA utilizing des(1–3) IGF-I as tracer (16). Interleukin-1β (IL-1β) was measured using a rat-specific ELISA kit (Cytoscreen, BioSource International, Camarillo, CA). A rat insulin RIA kit (Linco Research, St. Charles, MO) was used to measure serum insulin levels.

RNA Extraction, Cloning of cDNAs, and Northern Blotting

Total RNA was extracted from snap-frozen rat livers with the guanidine isothiocyanate-cesium chloride extraction method, as described previously (20). The RNA pellets were briefly dried, resuspended in RNase-free water, and stored at −80°C. Total RNA samples (20 μg) from all animals were electrophoresed in 1% agarose gels containing 2.2 M formaldehyde. The integrity of the ethidium bromide-stained RNA samples was confirmed on an UV light box. RNA was then transferred by capillary blotting to Nitroprobe GT membranes (Bio-Rad, Richmond, CA) and cross-linked by heat treatment at 80°C in a gel-drying apparatus for 2 h.

Rat cDNA probes for GHR, SOCS-3, CIS, and PEPCK were generated by RT-PCR from rat liver total RNA. The cDNAs were sequenced to confirm their identity. The ALS cDNA has been described previously (26), and the IGFBP-1 cDNA was kindly provided by Dr. S. Shimasaki (UCSD, San Diego, CA). Complementary DNAs were labeled with a Ready-To-Go random-priming kit (AMRAD-Pharmacia, Upsalla, Sweden) and [32P]dCTP (AMRAD-REN, Upsalla, Sweden). Northern blot analyses for each gene were performed sequentially, as described previously (26). Northern blots were quantified with a PhosphorImager and the ImageQuant computer software package (Molecular Dynamics, Sunnyvale, CA). Expression of the genes was normalized relative to 36B4 mRNA levels. Two different gels were needed to run all samples from the study, and care was taken to distribute samples from all groups onto each gel.

GH-Binding Studies

Microsomal membrane fractions were prepared from 6- and 36-h pair-fed (n = 6) and endotoxin-treated (n = 6) liver tissue. Briefly, liver tissue was homogenized in 0.25 M sucrose and then fractionated by differential centrifugation at 4°C, as previously described (8). Two hundred micrograms of membrane protein were incubated with 20,000 cpm 125I-labeled hGH (70–100 Ci/g) for 16 h at 22°C in 25 mM Tris-HCl-10 mM CaCl2 (pH 7.4) in 0.5% bovine serum albumin (BSA) in a total volume of 300 μl. After the incubation period, bound and free GH were separated by adding 1 ml of ice-cold 25 mM Tris-HCl-10 mM CaCl2 (pH 7.4) in 0.5% BSA and centrifuged for 30 min at 4°C. The supernatants were discarded, and membrane pellets were counted. Nonspecific binding was determined in the presence of excess unlabeled hGH (1 μg/tube).

Statistics

Comparative data were analyzed by one-way ANOVA with Statview v. 5 (SAS, Cary, NC). Linear regression analysis was used to assess the relationship between the measured variables in the pair-fed and LPS-treated groups separately. All data are provided as means ± SE, and P values <0.05 were considered statistically significant.

RESULTS

Effect of LPS Treatment on Body Weight and Food Intake

During the study period, control and LPS-treated rats were pair-fed to assess nutritional effects of endotoxin treatment (Fig. 1A). However, it is important to note that from 6 to 24 h, animals ate little food (<3 g). As a result, the greatest body weight loss was observed in LPS-treated rats at 24 h (12.2 ± 2.3% of initial body wt, P = 0.056), which was followed by a recovery at 36 h to pair-fed control levels (Fig. 1B). Correspondingly, endotoxin treatment caused marked diarrhea and lethargy, especially during the first 12 h of treatment, but the endotoxin dose caused no deaths. Control animals also consistently lost weight during the course of the experiment but generally less so than LPS-treated rats, suggesting that endotoxin had an addi-
tional effect on weight gain (4.7–6.1% of initial body wt with a maximum loss at 48 h of 8.2%). No significant differences in the final body weight were observed among the pair-fed controls and LPS-treated and freely fed animals at 36 and 48 h. Water intake was not significantly altered by LPS treatment during the course of the protocol (data not shown).

Effect of LPS Treatment on Ternary Complex Components

We examined the expression of ALS and other components of the ternary complexes during the first 12 h after endotoxin treatment to obtain “baseline” data on the initial effects of endotoxemia, to which we could then relate the effects of sustained treatment with endotoxin.

ALS expression. Hepatic ALS gene expression was suppressed by LPS-induced inflammation (Fig. 2, A and B), reaching its nadir at 12 h after the initial LPS treatment, and was significantly lower than that of pair-fed controls (50% of controls, \( P = 0.0006 \)). Similar to the effect on hepatic gene expression, the concentration of serum ALS was significantly decreased in LPS-treated rats (40% of controls, \( P = 0.004 \) at 24 h) compared with their respective pair-fed controls (Fig. 2C). However, this followed a delayed time course, with serum levels reaching their nadir 12 h after that of hepatic gene expression. Subsequently, ALS gene expression in LPS-treated animals returned to pair-fed control levels and by 36 h surpassed them (150% of controls at 36 and 48 h, \( P = 0.0007 \) and \( P = 0.01 \), respectively). Again, similar to hepatic gene expression albeit with a delay, ALS serum levels recovered to control levels by 36 h and rose above them by 48 h (210% of controls, \( P = 0.01 \)). Levels of ALS hepatic gene expression and serum protein in pair-fed groups were significantly suppressed by 6 and 12 h compared with the 0-h group (untreated, freely fed animals). Correspondingly, ALS serum levels were rapidly suppressed in both the pair-fed group (~60% of untreated levels by 6 h) and the LPS-treated group. However, in the pair-fed group then stabilized at approximately this level until 36 h, whereas the LPS group reached a nadir at 30% of levels in untreated animals.

IGF-I expression. Endotoxemia causes acute suppression of hepatic IGF-I gene expression and circulating levels that appear to be dependent on reduced sensitivity of the liver to GH (19). However, the effects of sustained endotoxin treatment have not been examined. Analysis of expression of all hepatic IGF-I transcripts demonstrated a significant and sustained suppression to 76–83% of pair-fed control levels lasting from 6 to 24 h after the initial injection of LPS (Fig. 3A). By 12 h, total IGF-I mRNA steady-state levels were significantly suppressed to ~40% of untreated control levels and then recovered partially to ~50% of controls at 48 h. However, relative to pair-fed controls, significant differences were observed only at 6–12 h.
Because we postulated that IGF serum levels are dependent on circulating ALS as well as on hepatic GH sensitivity, we assessed circulating levels by specific RIA. We used des(1–3) IGF-I tracer to prevent interference from IGFBPs, and we demonstrated a rapid fall in IGF-I serum levels in both pair-fed and LPS-treated animals. No significant differences were observed between experimental groups until 48 h, when IGF-I levels in LPS-treated animals were ~210% of control levels (Fig. 3B), corresponding temporally with increased circulating ALS.

Although little overall difference in serum IGF-I regulation between pair-fed and LPS-treated groups was observed, the relationship between IGF-I and ALS in the serum differed between the two groups. ALS was found to correlate relatively strongly with IGF-I in the pair-fed group (r² = 0.408, P = 0.0001) but not in the LPS-treated group (r² = 0.041, P = 0.29), suggesting an overall differential regulation of IGF-I and ALS by inflammation.

**Circulating IGFBP-5.** Because the pattern of expression of circulating ALS relative to IGF-I appeared differently regulated in pair-fed and endotoxemic animals, we also assessed the profile of another ternary complex component, IGFBP-5, using a newly developed RIA (7). IGFBP-5 levels followed those of IGF-I, being acutely and markedly suppressed by both food restriction and endotoxin treatment, and then recovering toward untreated control levels by 36 h (Fig. 4). IGFBP-5 correlated well with ALS (r² = 0.285, P = 0.002) and IGF-I (r² = 0.516, P = 0.0001) in pair-fed animals but less well in endotoxin-treated animals (ALS, not significant; IGF-I, r² = 0.173, P = 0.043). This effect corresponds with the parallel increases in IGF-I and IGFBP-5 after 12 h of endotoxin treatment and the response of ALS, whose rise is delayed by ~6 h relative to these other ternary complex components. IGFBP-5 mRNA has been demonstrated to be undetectable in rat liver (51).

**Effect of LPS Treatment on Hepatic GHR and Inhibitors of GH Signaling**

Because ALS has been demonstrated to be markedly GH dependent (18, 39), we examined the effects of sustained endotoxemia on hepatic GHR mRNA levels and GH binding to liver microsomal membranes. Also, it has been shown that SOCS-3 mediates IL-1β suppression of GH-regulated ALS gene expression (14) and that SOCS-3 and CIS are negative regulators of the GH-signaling pathway (43). SOCS-3 and CIS are rapidly induced after endotoxin treatment, probably mediated by cytokines (15, 41), and their expression is associated with acute loss of hepatic GH sensitivity (37). Although the effect of sustained endotoxin treatment has not been examined, hepatic SOCS-3 and CIS gene expression was raised in sepsis 18 h after cecal ligation and puncture (32). For these reasons, we were interested to know how the expression of SOCS-3 and CIS was modulated during prolonged exposure to endotoxin.

**GHR expression.** Hepatic steady-state levels of GHR mRNA were rapidly suppressed relative to pair-fed controls after LPS treatment, falling to ~30% of untreated control levels within 6 h, and these levels were maintained until 12 h (Fig. 5, A and B). These levels correspond to 47 and 55% of pair-fed control levels at 6 and 12 h (P < 0.0001 at both times). Correspondingly, GH binding to hepatic microsomal membrane fractions was suppressed by 44% (P < 0.03) in the LPS-treated group relative to that of pair-fed animals at 6 h (Fig. 5C). By 24 h, GHR gene expression had normalized relative to pair-fed controls (50% of untreated control levels), decreased slightly relative to control levels at 36 h, and returned to control levels at 48 h. At 36 h, GH binding to microsomal membranes in endotoxemic rats had normalized, whereas pair-fed animals showed a relative 30% suppression, although this was not statistically significant.

GHR gene expression in the pair-fed group was found to correlate significantly with both ALS gene expression (r² = 0.765, P < 0.0001) and ALS serum levels (r² = 0.208, P = 0.0087). However, in the LPS-treated experimental group, GHR mRNA correlated significantly only with hepatic ALS gene expression (r² = 0.759, P < 0.0001), not with serum levels, possibly due to the long half-life of ALS in the serum. GHR levels are means ± SE, *P < 0.05.

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**Fig. 3.** Insulin-like growth factor I (IGF-I) gene expression and serum levels. A: PhosphorImager analysis of hepatic total IGF-I mRNA transcripts normalized for 36B4 mRNA (% of 0-h controls). B: IGF-I serum levels. ■, Pair-fed controls; ○, endotoxin-treated animals. Values are means ± SE; *P < 0.05.

**Fig. 4.** Serum levels of IGF-binding protein (IGFBP)-5 measured by specific RIA. ■, Pair-fed controls; ○, endotoxin-treated animals. Values are means ± SE; *P < 0.05.
mRNA expression correlated well with IGF-I gene expression (combined transcripts) in both LPS ($r^2 = 0.672, P < 0.0001$) and pair-fed groups ($r^2 = 0.436, P < 0.0001$). GHR mRNA expression was also associated with serum IGF-I in both groups (LPS: $r^2 = 0.396, P = 0.0003$; pair-fed: $r^2 = 0.397, P = 0.0002$). Like serum ALS, GHR correlated with IGFBP-5 only in the pair-fed group ($r^2 = 0.118, P < 0.03$).

**Hepatic SOCS-3 and CIS.** There was an approximately eightfold induction of SOCS-3 hepatic gene expression in LPS-treated animals (Fig. 6, A and B) compared with control levels at $6 \text{ h} (P < 0.0001)$, which was maintained at threefold pair-fed control levels at $12 \text{ h} (P < 0.0001)$. By $24 \text{ h}$, SOCS-3 gene expression had returned to pair-fed (and untreated) control levels. Hepatic CIS gene expression was also induced by 1.5-fold ($P = 0.0024$) in LPS-treated rats at $6 \text{ h}$ but was not sustained, like SOCS-3, and returned to untreated control levels by $12 \text{ h}$ (Fig. 6, A and C). Subsequently, hepatic mRNA levels of both SOCS-3 and CIS remained close to untreated control levels in both endotoxemic and pair-fed animals until $48 \text{ h}$. Because IL-1$\beta$ has been demonstrated to suppress ALS expression through a SOCS-3-mediated mechanism, we also measured circulating IL-1$\beta$ levels. IL-1$\beta$ was acutely and transiently upregulated to $\sim 80 \text{ pg/ml}$ at $6 \text{ h}$ after the start of endotoxin treatment, corresponding with SOCS-3 and CIS induction (data not shown). However, during sustained endotoxin treatment, the level of this cytokine remained at undetectable levels. No IL-1$\beta$ was detectable in pair-fed control sera for the duration of the experiment.

**Expression of Hepatic Markers of Insulin Action**

PEPCK and IGFBP-1 are strongly suppressed by insulin at the mRNA level in liver (9, 27, 40), and increased expression of these genes can be attributed,
in part, to reduced hepatic sensitivity to insulin. The induction of IGFBP-1 in endotoxemia is also mediated by the proinflammatory cytokines IL-1, tumor necrosis factor-α, and IL-6 (34), which are acutely but transiently induced immediately after endotoxin treatment. Conversely, PEPCK expression is suppressed by endotoxin (29), and its levels of expression during this acute phase may depend on a balance between its responses to insulin (and hepatic insulin sensitivity) and proinflammatory cytokines. The expression of ALS, at the posttranscriptional level in particular, appears to be dependent on insulin, and changes in insulin sensitivity of the liver could affect ALS regulation (18). Therefore, we assessed the expression of PEPCK and IGFBP-1 as surrogate markers of hepatic insulin action during sustained (as opposed to acute) endotoxemia.

PEPCK mRNA levels were transiently induced within 6 h of endotoxin treatment but returned to baseline by 12 h (Fig. 7A). However, in pair-fed animals, PEPCK mRNA levels were markedly induced above those of endotoxemic animals and remained raised above baseline for the duration of the protocol. We also measured IGFBP-1, a marker of insulin sensitivity in the liver. IGFBP-1 gene expression followed a very similar pattern to that of PEPCK, although it rose more slowly during the initial period of fasting in pair-fed animals (Fig. 7B). With use of a rodent-specific RIA for the first time in this type of model, IGFBP-1 serum levels in the LPS-treated group were rapidly, transiently, and considerably induced relative to untreated controls (136-fold, \( P < 0.0001 \)) at 6 h. However, unlike hepatic gene expression, serum levels in pair-fed animals were not significantly raised until 48 h (Fig. 7C).

Overall, serum insulin levels were extremely variable in endotoxin-treated animals, making it difficult to assess the relative impact of circulating levels vs. hepatic sensitivity on expression of PEPCK and IGFBP-1 (Fig. 7D). However, the acute induction of both PEPCK and IGFBP-1 correlated temporally with the suppression of insulin below detectable levels at 6 h, and also with the peak in serum IL-1β, described earlier. Insulin subsequently remained low until 36 h, when it peaked to 250% of untreated control levels (\( P = 0.0125 \)) and eventually normalized at 48 h. Levels of insulin in pair-fed animals remained relatively stable during the course of the experiment, with no significant variations from untreated control values. Serum glucose was acutely suppressed at 6 h in endotoxic rats but returned to pair-fed levels by 12 h, after which glucose levels were generally higher in endotoxic rats at 24 and 48 h (Fig. 7E).

DISCUSSION

The animal model on which this study was based demonstrated the neuroendocrine role of IL-1β in inhibiting the somatotropic axis in rapidly growing juvenile rats treated sequentially with endotoxin (42). A number of studies have also demonstrated the acute suppression of hepatic GH sensitivity by endotoxin, although none have examined longer-term effects on regulation of components of the GH-signaling pathway and downstream markers of sensitivity. Our aim was to examine the regulation of ALS, a relatively specific marker of hepatocyte GH sensitivity and serum GH levels, during sustained endotoxin-induced stress.
Acute (0–12 h) Effects of Endotoxemia

To determine baseline effects of not only endotoxin but also restricted nutrition, we examined their acute effects at 6 and 12 h. During the first hours of endotoxin treatment, there was a rapid modulation of both gene expression and activation of members of the GH-signaling pathway (37). However, during this acute inflammatory phase, the main influence on expression of ALS appears to be the change in nutritional intake, which also suppresses hepatic GHR expression. This finding contrasts with an earlier study in which, by immunoblotting, levels of ALS were determined to be relatively unaffected by decreased nutrition during the acute inflammatory phase (1). This may be related to differences in methodological approach; however, the correlation between suppressed GHR gene expression/GH binding and ALS gene expression in our study lends credence to the rapid suppression of ALS serum levels that we have observed by a quantitative immunoassay. Despite this, after 12 h of LPS treatment, ALS gene expression is significantly suppressed to between 40 and 50% of pair-fed control values, suggesting an additional effect of endotoxin. This is reflected in the significant suppression of GH binding to hepatic microsomal membrane fractions 6 h after the start of the protocol. Although mirrored by a similar incremental effect on IGF-I gene expression, serum levels of IGFBP-5 and IGF-I were not significantly suppressed below pair-fed control levels by endotoxin, perhaps indicating that ALS was still in functional excess during this acute period. Temporally correlating with this transient suppression of ALS relative to pair-fed controls is the acute, if transient, increase in SOCS-3 and CIS gene expression. SOCS-3 has recently been shown to be upregulated in hepatocytes by IL-1β (14, 15) and may constitute one mechanism to explain our earlier finding that ALS is suppressed in a GH-dependent manner by this cytokine (21). IL-1β has also been demonstrated to suppress hepatic GHR and IGF-I gene expression (19, 46). The strong nutritional effect we have observed on ALS gene expression seems to be related very closely to GHR expression and not to SOCS-3 or CIS expression, which remained at close to undetectable levels throughout the protocol in the pair-fed controls. Nutritional effects on GH postreceptor signaling have also recently been described in juvenile rats, although only after 48 h of complete withdrawal of nutritional intake (10). Serum levels of ALS responded to endotoxin more slowly than hepatic gene expression, taking 24 h to reach their nadir. This time lag of ~12 h may be related to the long half-life of ALS in the circulation (35). Both hepatic steady-state mRNA and serum levels of ALS reach 20–25% of their control values after endotoxin treatment, suggesting regulation of serum levels by hepatic gene expression and output.

Circulating insulin was acutely suppressed by endotoxin, and this could potentially impact on posttranscriptional regulation of ALS release by the liver (18). IGFBP-1 serum and mRNA were acutely and tran-
siently increased at 6 h, consistent with undetectable serum insulin and induction of proinflammatory cytokines, as has been described previously (25). PEPCK gene expression was also acutely induced, but to a lesser extent than IGFBP-1, perhaps because of the suppressive effects of cytokines on the expression of this gene (30).

Effects of Sustained (12- to 48-h) Endotoxemia

We found that, during sustained endotoxin treatment, hepatic ALS gene expression eventually increased above that of pair-fed controls, perhaps in response to the increased baseline mass and pulsatility of circulating GH observed by Peisen et al. (42) at this time. A similar recovery in IGF-I levels was also observed that did not appear to be completely related to increased hepatic gene expression and may be consistent with increased stability within the ternary complex due to increased levels of ALS. Similarly, we found that IGFBP-5 serum levels recovered during this phase, in accord with the observed increase in circulating IGFBP-3 with long-term endotoxin treatment (44). This evidence suggests their regulation in the serum through the induction of ALS and subsequent sequestration and stabilization in ternary complexes. However, unlike IGF-I, IGFBP-5 levels recover up to untreated control levels within 48 h in endotoxin-treated animals, possibly demonstrating a separate level of regulation that may be related to its extrahepatic site of expression. IGFBP-3 probably occurs in much higher circulating concentrations than IGFBP-5 in ternary complexes, and its regulation may partially account for the discrepancy in serum profiles between IGF-I and IGFBP-5. However, we currently have no direct means of quantifying rat IGFBP-3 to assess this proposition.

GH mRNA levels in the endotoxin-treated animals did not overshoot those of the pair-fed group in the same way as ALS, suggesting that modulation of the serum GH profile may be of more importance in regulating ALS during this period. During sustained endotoxin treatment, there does not appear to be the same hepatic resistance to GH observed in the acute phase as described by others (37), because GHR mRNA levels and GH binding return toward those of the control group at 24–36 h, matching the raised level of ALS gene expression. Furthermore, SOCS-3 and CIS gene expression is reduced to pair-fed control levels. Our finding agrees with observations of prolonged critical illness in humans. Such patients are not markedly GH resistant, since administration of GH secretagogues restores circulating levels of components of the GH-dependent ternary complex toward normal levels (49). In humans, it appears that alterations in GH-secretory profile may impact most upon circulating levels of ALS and IGF-I.

There is evidence that rats develop resistance to endotoxin after repeated treatment, for example as evidenced by a reduced cytokine secretory response and hypothermic reaction to a subsequent injection of endotoxin (33, 52). However, it was shown in animals...
treated sequentially with endotoxin that, during the first 24 h, serum GH is suppressed to undetectable levels, whereas during the second 24 h of treatment, GH release is elevated and pulsatile (42). Furthermore, altered acute effects on glucose kinetics could still be demonstrated in rats treated for 2 days with endotoxin (33). These findings suggest that endotoxin-induced modulation of the GH axis and hepatic insulin resistance remain up to ≥48 h of endotoxin treatment. Although endotoxin tolerance could partly explain our findings, the remarkable recovery in circulating levels of the ternary complex components in endotoxin-treated relative to the pair-fed controls is less easy to explain, because both groups experienced the same degree of food restriction.

Surprisingly, we found that nutritional restriction had a more significant effect on hepatic insulin-regulated genes in saline-injected than in endotoxin-injected animals, with continuously raised levels of PEPCK and IGFBP-1 gene expression in the pair-fed animals. Unlike pair-fed controls, between 12 and 48 h, PEPCK and IGFBP-1 mRNA in endotoxin-treated animals rapidly returned to untreated control levels after an acute transient peak. Furthermore, after an acute transient period of hypoglycemia, serum glucose levels return to the pair-fed range. PEPCK and IGFBP-1 have been characterized to be acutely induced by endotoxin, but this is the first study to examine the effects of sustained endotoxemia on their expression. Sustained endotoxin treatment has been demonstrated to suppress insulin signaling through suppression of total levels of insulin receptor and insulin receptor substrates-1 and -2 (38) and would thus be expected to induce continued raised levels of these genes. However, the reported development of a highly pulsatile (but erratic) profile of serum GH release in rats with sustained endotoxemia may alter the sensitivity of these genes to insulin (42), since it has been found that acute but not prolonged GH treatment promotes the suppressive effect of insulin (31). The effects of sustained endotoxemia on circulating ALS coincide with this apparent counterregulatory influence of endotoxin on the effects of reduced nutritional intake, suggesting a role for modulated insulin signaling in regulating components of the ternary complex.

Our results suggest that ALS may be buffering the IGF axis against the effects of inflammation, in that IGF-I levels are not significantly altered relative to those of their pair-fed controls. This may be a consequence of a relative excess of ALS in the circulation, even in endotoxin-treated rats. This agrees with recent studies in catabolic conditions, in which infused IGF-I-IGFBP-3 complexes are capable of forming ternary complexes with ALS in septic rats (45). Moreover, IGF-I infused in this way retains its anabolic activity in these catabolic animals, presumably because its half-life in the circulation is increased by its sequestration into ternary complexes. There is additional evidence that the clearance rate of IGF-I is unaffected in endotoxin-treated animals relative to controls (24). The kinetics in this study described a two-compartment model, and the amount of IGF-I compartmentalizing into the slow-release fraction, presumably ternary complexed forms, was similar in control and endotoxin-treated rats.

Overall, our data support a role for suppressed hepatic GH sensitivity in regulating the gene expression and circulating levels of ALS in acute inflammatory states. However, we found that an important component to this regulation was nutritional, probably mediated by decreased levels of hepatic GHR. In contrast, decreased food intake can fully explain the acute suppression in circulating IGF-I and IGFBP-5 levels, which fall together with those of their pair-fed controls. This occurs despite a significant endotoxin effect on IGF-I gene expression. During the sustained period of endotoxin treatment, ALS expression appeared to be independently of changes in the hepatic GHR and possibly related more closely with altered GH release into the circulation. Another regulatory mechanism may involve modulation of insulin signaling in the liver, although more work is needed to demonstrate a direct link.

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