Dexamethasone decreases serum and liver IGF-I and maintains liver IGF-I mRNA in parenterally fed rats

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Dexamethasone decreases serum and liver IGF-I and maintains liver IGF-I mRNA in parenterally fed rats. Am J Physiol Regulatory Integrative Comp Physiol 282: R528–R536, 2002; 10.1152/ajpregu.00085.2001.—Insulin-like growth factor-I (IGF-I) gene expression is regulated by nutritional and hormonal factors. High-dose glucocorticoids decrease food intake, and this confounds studies addressing glucocorticoid effects on IGF-I gene regulation. We investigated alterations in the hepatic IGF-I endocrine system induced by a catabolic dose of dexamethasone (Dex) in rats given adequate nutrition by continuous infusion of total parenteral nutrition (TPN) solution with or without IGF-I administration. The four TPN groups included control, +Dex, +IGF-I, and +IGF-I + Dex (n = 9–11/group). Dex induced a 12% loss of body weight in association with a 50% decrease in hepatic immunoreactive IGF-I, a 10% decrease in serum IGF-I, and no change in steady-state liver IGF-I mRNA or growth hormone (GH) receptor binding. Exogenous IGF-I increased serum IGF-I, attenuated Dex-induced catabolism, and did not reduce hepatic levels of IGF-I and IGF-I mRNA despite decreased serum GH. These data suggest that Dex-induced catabolism is associated with downregulation of the hepatic IGF-I endocrine system at the translational or posttranslational level when adequate nutrition is provided.

supraphysiological levels of glucocorticoids, whether endogenous or exogenous, are associated with growth retardation (14, 33). Patients with elevated levels of glucocorticoids due to catabolic illnesses often show a poor anabolic response, despite provision of adequate nutrition. A balance between nutritional, hormonal, and metabolic factors contributes to anabolism. Of particular interest is the anabolic hormone insulin-like growth factor-I (IGF-I), which mediates many of the actions of growth hormone (GH) and whose synthesis is reduced by malnutrition and catabolism. GH stimulates hepatic production of IGF-I, the major contributor to serum IGF-I (38). Once secreted into blood, ~90% of IGF-I circulates bound to IGF binding protein (IGFBP)-3, and only 5% of IGF-I is free in circulation (35). The IGFBPs modulate the availability of IGF-I to various target tissues, and after hepatic secretion into the circulation, IGF-I acts in an endocrine fashion (13). We refer to this as the hepatic IGF-I endocrine axis.

We hypothesize that the synthetic glucocorticoid dexamethasone (Dex) downregulates the hepatic IGF-I endocrine system and that this contributes to whole body catabolism and growth suppression. Dex may alter IGF-I production via three modes of action: effects secondary to altered GH levels, direct effects on the IGF-I axis, or effects secondary to altered nutrition. Dex-induced growth retardation in rat models has been shown to have varying effects on GH production, including decreased GH production (24, 37), normal GH mRNA levels (10, 28), reduced GH receptor (GHR) expression (7), and altered GHR mRNA (9, 10) and GH binding proteins (GHBPs; Ref. 7). Dex has been shown to decrease (21, 23) or not change (16) circulating IGF-I levels, decrease (16) or not change (20) hepatic IGF-I mRNA, suppress IGF-I receptor response (10), and alter IGF-I receptor mRNA (26) and IGFBP concentrations (15, 17). In addition, Dex has been shown to uncouple receptor signaling transduction mechanisms (27), regulate eukaryotic translation initiation factors (29), and stimulate production of factors responsible for mRNA degradation (25). Thus Dex may modulate IGF-I gene expression and/or protein processing/secretion.

Previous studies assessing Dex effects on the IGF-I system have not adequately controlled for nutrition, and this may contribute to conflicting results. High-dose Dex results in decreased appetite and food intake (19, 24). The nutrient-gene interaction with IGF-I is well established (35), although the mechanisms are not completely defined; thus many findings are actually reports of simultaneous effects due to a combination of Dex and altered nutrition. Studies of Dex using a model with strict nutritional control, such as total parenteral nutrition (TPN), provide a more precise approach to study regulation of the IGF-I system.

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The purpose of this experiment was to investigate how a catabolic dose of Dex affects the hepatic IGFI-1 endocrine axis using a rat model of continuous infusion of TPN solution. We designed an experiment to determine if the whole body catabolism associated with Dex downregulates the hepatic IGFI-1 endocrine axis and if administration of IGFI-1, which has been shown to attenuate glucocorticoid-induced catabolism (39), modulates this response when adequate nutrition is provided.

MATERIALS AND METHODS

Animals

Animal facilities and protocols were approved by the University of Wisconsin-Madison Animal Care and Use Committee. Male Sprague-Dawley rats, –250 g, were housed individually in stainless steel cages while acclimated to a 12:12-h light-dark cycle at 22°C with free access to semipurified diet and water for 4 days. Forty-four rats were fasted 18 h before placement of jugular catheters on day 0. Infusion of TPN solution began on day 0 and provided the only source of nutritional intake for all treatment groups; water was consumed ad libitum. Body weight and grams of TPN solution infused were recorded daily.

Experimental Design

The purpose of this study was to assess the alterations in the hepatic IGFI-1 endocrine axis induced by a catabolic dose of Dex and to determine if attenuation of catabolism by exogenous IGFI-1 alters Dex effects on the hepatic IGFI-1 endocrine axis. This model mimics a similar stress response observed in severe catabolic illness, which includes elevated glucocorticoid levels, insulin resistance, and nitrogen wasting. These symptoms are often observed in clinical situations where TPN is used. This experiment utilizes a 2-by-2 factorial design (+IGF-1, –Dex). The four TPN treatment groups included: TPN control (–IGF-1 – Dex), Dex control (–IGF-1 + Dex), IGF-1 (+IGF-1 – Dex), and IGF-1 + Dex. After 7 days of TPN, 91% of the animals survived for a final sample size of 9–11 rats/group. After catheterization of the jugular vein, TPN infusion was gradually increased to provide adequate nutrition by day 2 continuing through day 7 (day 0: 19.4 ± 0.2 g/day, day 1: 41.1 ± 0.3 g/day, days 2–7: 57.1 ± 0.3 g/day). We defined adequate TPN as 231–245 nonprotein kcal/kg body wt (171–182 kcal nonprotein energy/kg body wt) (34). This level of nutrition is equivalent to the work of Tao (39). On day 7, TPN rats were anesthetized by intravenous injection of 18 mg ketamine/kg body wt and then were exsanguinated. Blood was collected and centrifuged to isolate serum. Liver was collected, weighed, and immediately frozen in liquid nitrogen.

TPN Solution Preparation

TPN solution was prepared aseptically using commercial preparations of amino acids plus electrolytes (8.5% Travalos, Baxter, Deerfield, IL), 60% dextrose (Baxter), 20% long-chain triglyceride lipid emulsion (Intralipid, Pharmacia, Clayton, NC), vitamins (Astra USA, Westborough, MA), and trace elements (Multitrace-4, American Regent Laboratories, Shirley, NY) as shown in Table 1. The TPN solution provided (in g/l) 43 amino acids, 216 dextrose, and 20 lipid, providing 80 and 20% of nonprotein energy from carbohydrate and fat, respectively.

Table 1. Composition of total parenteral nutrition solution

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>43 g/l</td>
</tr>
<tr>
<td>Dextrose</td>
<td>216 g/l</td>
</tr>
<tr>
<td>Lipid</td>
<td>100 ml/l</td>
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<tr>
<td>Nonprotein energy</td>
<td>3,905 kJ/l</td>
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<tr>
<td>Ascorbic acid</td>
<td>50 mg/l</td>
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<tr>
<td>Retinol</td>
<td>50 µg/l</td>
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<tr>
<td>Ergocalciferol</td>
<td>2.5 µg/l</td>
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<tr>
<td>dl-rrac-a-Tocopherol acetate</td>
<td>5 mg/l</td>
</tr>
<tr>
<td>Thiamin-HC</td>
<td>1.5 mg/l</td>
</tr>
<tr>
<td>Riboflavin</td>
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</tr>
<tr>
<td>Pyridoxine-HCl</td>
<td>2 mg/l</td>
</tr>
<tr>
<td>Niacinamide</td>
<td>20 mg/l</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>7.5 mg/l</td>
</tr>
<tr>
<td>Follic acid</td>
<td>200 µg/l</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>2.5 µg/l</td>
</tr>
<tr>
<td>Biotin</td>
<td>30 µg/l</td>
</tr>
<tr>
<td>Choline (choline citrate)</td>
<td>200 mg/l</td>
</tr>
<tr>
<td>Zinc</td>
<td>3 mg/l</td>
</tr>
<tr>
<td>Copper</td>
<td>1.2 mg/l</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.3 mg/l</td>
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<tr>
<td>Iodine as KI</td>
<td>12 µg/l</td>
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<tr>
<td>Sodium</td>
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<tr>
<td>Potassium</td>
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<tr>
<td>Calcium</td>
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<tr>
<td>Phosphorus</td>
<td>15 mmol/l</td>
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<tr>
<td>Magnesium</td>
<td>2.5 mmol/l</td>
</tr>
<tr>
<td>Chloride</td>
<td>84 mmol/l</td>
</tr>
<tr>
<td>Acetate</td>
<td>65 mmol/l</td>
</tr>
</tbody>
</table>

*Travalos 8.5% with electrolytes (Baxter Healthcare, Deerfield, IL); +60% dextrose injection (Baxter Healthcare); +20% soybean oil emulsion, Intralipid (Kabi Pharmacia, Clayton, NC); “M.V.I., 2.5 ml vial 1 and 2.5 ml vial 2 (Astra Pharmaceutical Products, Westborough, MA); “M.T.E. concentrate, chloride salts in 0.9% NaCl, 5 ml (Abbott Laboratories, Chicago, IL); “selenious acid (Luitpold Pharmaceuticals, Shirley, NY); “Sigma Chemical (St. Louis, MO); solution sterilized by passage through 0.22-µm filter; *additional potassium added as KCl, 2 mmol/ml (Taylor Pharmacal, Decatur, IL); *calcium gluconate, USP (Luitpold Pharmaceuticals, Shirley, NY).
Serum IGFs were removed from binding proteins by high-pressure liquid chromatography under acidic conditions. Total IGF-I concentrations were determined by a double-antibody radioimmunoassay (RIA; Ref. 6). Materials included rhIGF-I as a standard, $^{125}$I-rhIGF-I (Amersham, Arlington Heights, IL), polyclonal antibody to human IGF-I (National Hormone and Pituitary Program, Baltimore, MD) as the primary antibody, and goat anti-rabbit IgG and normal rabbit serum (Antibodies, Davis, CA). Serum GH concentrations were determined using a RIA kit (Amersham Pharmacia Biotech, Piscataway, NJ).

RNase Protection Assays

Liver RNA was extracted (TRIzol Reagent, GIBCO BRL Life Technologies, Grand Island, NY), and integrity was confirmed by ethidium bromide staining of 28S and 18S rRNAs on an agarose-formaldehyde gel. Ten micrograms of liver total RNA and nontarget yeast RNA was utilized in the RNase protection assays (RPAs; HybSpeed RPA Kit, Ambion) with saturating amounts of probe to determine liver IGF-I and GHR/GHBP mRNA levels. RPAs were performed in duplicate gels with 3-4 samples/treatment on each gel. The final sample size was 6-8 rats/treatment group. Band intensities were quantified by phosphorimaging (Packard Instruments, Meriden, CT). Levels of IGF-I mRNA transcript were normalized for 18S rRNA and expressed as a fold difference relative to the TPN control. Fold differences in band intensities for GHR/GHBP mRNA were calculated relative to the TPN control.

IGF-I. An exon-2-derived cDNA probe was used to generate an antisense RNA probe to detect liver IGF-I mRNA as previously described (31). The exon-2 probe yielded a doublet at 305 nt and bands at 290 and 238 nt (31). Ribosomal 18S RNA antisense template (Ambion, Austin, TX) was transcribed and used for a control and appeared as a doublet at 100 and 80 nt.

GHR/GHBP. A plasmid (T7T318) containing rat GHR/ GHBP cDNA sequence (18, 40), courtesy of Lawrence Mathews and Gunnar Norstedt, was linearized with BamHI, and then an antisense RNA probe (449 bp) was transcribed using T7 polymerase. RPA reveals protected bands at 439 and 298 nt, corresponding to GHR mRNA and GHBP mRNA, respectively.

Immunoreactive IGF-I

Liver was homogenized in 0.1 M ammonium formate (pH = 7.0) (1 g/5 ml) and spun at 14,000 g for 15 min. The pellet was reextracted with 0.5 ml 10% formic acid and centrifuged, and the supernatants were combined. A C2 Bond Elut column (Varian, Harbor City, CA) was prewashed with methanol and 0.1 M ammonium formate. The supernatant (0.5 ml) was applied to the column and then washed with 1 ml 7% acetic acid and 1 ml 20% acetonitrile-0.1% trifluoroacetic acid (30). Samples were allowed to gravitate through the column; if necessary, minimal vacuum was applied using the Vac Elut (Varian) system. Immunoreactive IGF-I was extracted in 2 ml of 45% acetonitrile-3% trifluoroacetic acid. A 20-ml fraction was used in the IGF-I assay described previously. Recovery of $^{125}$I-IGF-I and $^{125}$I-IGF-I with 0.5 ml supernatant through the Bond Elut column was 71 and 68%, respectively. Recovery of pure rhIGF-I and rhIGF-I with 0.5 ml supernatant through the Bond Elut column and IGF-I assay was 92 and 89%, respectively. To assess that IGFBPs were eliminated from the fraction from which IGF-I was assayed, extracts were freeze-dried and subjected to Western ligand blot as described in Serum IGFBP Concentration.

GH Binding Assay

Crushed, frozen liver tissue was placed in 2.5 ml homogenization solution (25 mM Tris, 10 μM benzamidine, 3 mM aminocaproic acid, 300 mM sucrose, 1 mM phenylmethylsulfonyl fluoride, 106 kallikrein inhibitory units (KIU)/l aprotinin, pH 7.8) per gram of tissue and homogenized for 1 min. The homogenate was centrifuged at 1,500 g for 20 min at 4°C, and then the resulting supernatant was centrifuged at 100,000 g for 90 min at 4°C. The resulting microsomal pellet was resuspended in 4 M MgCl2 (2.5 ml/g initial liver wt) and incubated on ice for 20 min to strip the microsomes of endogenously bound hormone. The membranes were pelleted (100,000 g for 35 min, 4°C) and resuspended in cold 25 mM HEPES buffer, pH 7.6. The microsomes were centrifuged a second time (100,000 g for 35 min, 4°C) and resuspended in 25 mM HEPES, pH 7.6, with 106 KIU/l aprotinin. Protein concentrations were determined using the Pierce bicinchoninic acid (BCA) assay (Rockford, IL). Membranes were stored at −70°C.

The GH binding assays were performed in triplicate using polyethylene microfuge tubes. Each tube contained 200 μg membrane protein and 0.2 ng/ml $^{125}$I-rhGH [Nutropin (Genentech, South San Francisco, CA) iodinated at the University of Wisconsin-Madison using the iodogen method] with a specific activity of 120 μCi/μg. Two-hundred micrograms of liver microsomes was used because it was within a linear range for specific binding. The assay buffer contained 50 mM Tris, pH 7.4, 20 mM MgCl2, and 0.5% BSA.

The radioligand binding was competed with 0–10−11 M bovine GH (bGH; Monsanto, Chesterfield, MO). The final volume of the assay was 500 μl/tube. Reactions were incubated 16 h overnight at room temperature, and then binding buffer was added to each tube and mixed. The bound and free hormone were separated by centrifugation and washing as described previously (22). Pellets were quantitated with the Wallac gamma counter (Turku, Finland). Nonspecific binding was measured in the presence of 9.1×10−8 M bGH and was not significantly different between treatment groups. Total binding was measured in the absence of cold hormone. Specific binding was determined as a difference in total and nonspecific binding. The specificity of binding was confirmed by competing $^{125}$I-rhGH with bGH, porcine follicle-stimulating hormone, porcine leuteinizing hormone, rhIGF-I, and porcine insulin (cold excess 9.1×10−8 M). Receptor number (R0) and binding affinity (K0) were determined using the LIGAND iterative curve-fitting program, which generated and analyzed Scatchard plots. The GH binding data were best described using a single-site model.

Serum IGFBP Concentrations

Serum IGFBP-3 was determined using the Far Western ligand blotting procedure, a modified Western ligand blot (11). In short, 2 μl of serum was subjected to SDS-PAGE (12.5% resolving gel, 4% stacking gel), and proteins were transferred to nitrocellulose by electroblotting. Incubation of the nitrocellulose with 3% Nonidet P-40, 1% BSA, and 0.1% Tween 20 was necessary for quenching before hybridization with $^{125}$I-IGF-I (Amersham, Arlington Heights, IL) overnight. The nitrocellulose was washed in 0.1% Tween 20 and
saline buffer and exposed to film. Bands present in the 38- to 43-kDa range, showing as a triplet, correspond to variations in glycosylation of IGFBP-3 (13). The bands were quantified by phosphorimaging (Packard Instruments).

Liver Protein, DNA, and Fat Analyses

Liver homogenates were used to assay protein (BCA colorimetric assay, Pierce Chemical, Rockford, IL) and DNA content (12). Total fat was measured using a gravimetric procedure described by Folch et al. (5). Approximately 1 g of liver tissue was weighed, recorded, and then homogenized in 20 ml of Folch reagent (chloroform-methanol, 2:1). This was filtered, and 4 ml 0.74% KCl was added to the eluant. The sample was centrifuged at 1,800 rpm for 10 min, and the upper phase was discarded. Chloroform-methanol-H2O (3:48:47) was then added, the sample was spun, and the upper phase was discarded three times. The sample was dried under nitrogen in a heated water bath, and then the fat residue was resuspended in 5 ml Folch reagent. An aliquot under nitrogen in a heated water bath, and then the fat residue was resuspended in 5 ml Folch reagent. An aliquot was taken, dried down, and weighed to determine fat content.

Statistical Analysis

Treatment groups were compared using two-way ANOVA that determined the independent effects or interaction between IGF-I and Dex (version 8.0, SAS Institute, Cary, NC). Individual differences between groups were determined by one-way ANOVA (P < 0.05) as followed by the protected least significant differences test. Data are presented as means with standard errors. Means with unlike superscripts indicate a significant difference. Statistics were performed on log-transformed data for serum hormone levels because residual plots of these data sets indicated that there was unequal variance between groups. Pearson correlations were performed.

RESULTS

Whole Body Growth

There were no significant differences in initial body weights on day 0. Final body weights (day 7) were significantly different between all treatment groups. Rat group mean final body weights from highest to lowest were IGF-I > TPN control > IGF-I + Dex > Dex control (Table 2). Body weight changes (day 7 − day 0) reflected the same statistical pattern with non-Dex-treated rats gaining 30 g (IGF-I) and 17 g (TPN control) and Dex-treated rats losing 12 g (IGF-I + Dex) and 32 g (Dex control). IGF-I had a main effect to increase body weight (P = 0.0001), whereas Dex had a main effect to decrease body weight (P = 0.0001). Dex induced a significant negative nitrogen balance, and IGF-I attenuated Dex effects and significantly improved nitrogen retention (TPN control, 388 ± 93; Dex control, −665 ± 86; IGF-I, 662 ± 31; IGF-I + Dex, −196 ± 83 mg nitrogen/7 days; P = 0.0001 among all groups) as previously demonstrated (11). These data demonstrate that intraperitoneal injections of 70 μg Dex/day resulted in whole body catabolism consistent with loss of lean body mass.

<table>
<thead>
<tr>
<th>Initial Body Wt, g</th>
<th>Final Body Wt, g</th>
<th>Body Wt Change, g/7 days</th>
<th>Serum GH, μg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPN control</td>
<td>326 ± 3</td>
<td>17 ± 2</td>
<td>27 ± 7</td>
</tr>
<tr>
<td>Dex control</td>
<td>212 ± 3</td>
<td>−32 ± 24</td>
<td>38 ± 5</td>
</tr>
<tr>
<td>IGF-I</td>
<td>275 ± 3</td>
<td>30 ± 1</td>
<td>32 ± 5</td>
</tr>
<tr>
<td>IGF-I + Dex</td>
<td>230 ± 2</td>
<td>−12 ± 3</td>
<td>12 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Means with different letters are significantly different. Data were analyzed by 1-way and 2-way ANOVA for unequal variance between groups. Pearson correlations were performed. Two-way ANOVA was used to detect significant main effects and interaction between treatment factors of IGF-I and Dex (P < 0.05).

Serum IGF-I, Hepatic Immunoreactive IGF-I, and Liver IGF-I mRNA

Whole body catabolism that occurs with Dex administration may be due, in part, to changes in the hepatic IGF-I endocrine axis. The administration of IGF-I significantly increased serum IGF-I levels above TPN and Dex controls by ~100%; this effect was independent of Dex treatment (IGF-I main effect, P = 0.0001). Dex showed a trend to decrease serum IGF-I (P = 0.067). Dex and IGF-I exhibited no significant interaction (see Fig. 1A).

Dex had a significant main effect to decrease liver immunoreactive IGF-I by ~50%. This response was consistent when data were expressed as nanograms IGF-I per gram liver (Fig. 1B), total nanograms IGF-I per liver, nanograms IGF-I per gram fat-free liver, or milligrams of DNA. Treatment with IGF-I without Dex resulted in immunoreactive IGF-I levels not significantly different from respective TPN controls.

Liver IGF-I mRNA was assessed by RPA (see Fig. 2). In this model of well-controlled nutrition, neither Dex nor exogenous IGF-I administration had a significant main effect on hepatic IGF-I mRNA levels (main effects: Dex, P = 0.53; IGF-I, P = 0.54) nor was there significant Dex-by-IGF-I interaction (P = 0.12). In our model, no significant effect of Dex on hepatic IGF-I mRNA was observed.

Correlation of Hepatic IGF-I Endocrine Axis and Body Weight

We assessed correlations in the hepatic IGF-I axis and body weight. Final body weight and nitrogen balance were significantly correlated with liver immunoreactive IGF-I (r = 0.83, P = 0.0001 and r = 0.81, P = 0.0001, respectively) and serum IGF-I (r = 0.39, P = 0.0001) by 1-way and 2-way ANOVA for unequal variance between groups. Pearson correlations were performed. Two-way ANOVA was used to detect significant main effects and interaction between treatment factors of IGF-I and Dex (P < 0.05).
0.0120 and \( r/H_{11005} 0.41 \), \( P/H_{11005} 0.0092 \), respectively), but not liver IGF-I mRNA (\( P/H_{11005} 0.41 \) and \( P/H_{11005} 0.71 \)). Liver immunoreactive IGF-I was significantly correlated with serum IGF-I when IGF-I-treated rats were removed from the analysis (\( r/H_{11005} 0.73 \), \( P/H_{11005} 0.0013 \)). Hepatic IGF-I mRNA was not significantly correlated with serum or liver immunoreactive IGF-I. Overall, the strongest positive correlations were noted between liver immunoreactive IGF-I and final body weight (\( r/H_{11005} 0.83 \), \( P/H_{11005} 0.0001 \)), cumulative nitrogen balance (\( r/H_{11005} 0.81 \), \( P/H_{11005} 0.0001 \)), and serum IGF-I (\( r/H_{11005} 0.38 \), \( P/H_{11005} 0.0435 \)).

Serum GH and Hepatic GHR/GHBP mRNA

Serum GH concentrations are presented in Table 2. Serum GH was significantly suppressed in IGF-I + Dex rats compared with the Dex and IGF-I groups (\( P/H_{11005} 0.0291 \), 1-way ANOVA). The decrease in GH levels in the IGF-I + Dex group may reflect feedback inhibition of GH production due to increased levels of IGF-I. When Dex was given without IGF-I treatment, GH levels were increased compared with TPN controls. Increased serum GH levels in Dex rats are consistent with decreased feedback inhibition of GH release due to reduced circulating levels of IGF-I.

Dex administration resulted in a 40 and 54% decrease in liver GHR and GHBP mRNA levels, respectively (see Fig. 3). IGF-I did not have a significant main effect on either GHR or GHBP mRNA expression.

Despite large differences in body weight gain or loss, no significant changes in hepatic GHR binding, affinity, or GHR number were observed (Table 3). The significant 40% decrease in abundance of GHR mRNA due to Dex treatment does not correlate systematically with the liver GH binding profile, showing no significant differences in GHR binding or GHR number.

**IGFBPs**

IGFBP-3, the major carrier of serum IGF-I, is known to modulate IGF-I availability, so we assessed serum IGFBP-3 using Western ligand blot (Fig. 4). Two-way ANOVA shows that in the presence of Dex, IGF-I significantly increased serum IGFBP-3 levels (Dex × IGF-I interaction, \( P/H_{11005} 0.0157 \)). IGF-I or Dex treatment alone did not alter serum IGFBP-3 levels compared with the TPN control. These data support that IGF-I modulates IGFBP-3 levels in the presence of Dex.

**Liver Weight and Cellularity**

Dex-induced whole body catabolism (average daily wt loss of 4.2 g) resulted in hepatomegaly (Table 4) as previously noted in parenterally (11) and orally fed (36)
The gels were performed in triplicate or quadruplicate, repeating the parental feeding of the rats. Dex increased the absolute and relative mass of liver by 55% compared with TPN controls. We measured hepatic contents of fat, water, protein, and DNA to determine if the hepatomegaly induced by Dex was consistent with cellular hypertrophy, possibly due to increased fat content, and cellular hypoplasia, based on a decrease in total hepatic content of DNA from 105 ± 3 to 93 ± 3 mg/liver (P = 0.0097). These data suggest that Dex-induced hepatomegaly (11, 36) results in hepatic steatosis, which may reflect increased lipolysis and hepatic uptake of fatty acids (2), and a reduced number of hypertrophic hepatocytes.

Dex showed significant interaction with IGF-I. IGF-I attenuated Dex-induced increases in liver mass (Dex × IGF-I interaction, P = 0.0001). The ability of IGF-I to attenuate Dex-induced hepatomegaly was associated with partial normalization of the aberrations in hepatic composition. Treatment with IGF-I and Dex significantly reduced hepatic mass and lipid content, increased protein and DNA concentrations, and decreased the ratio of protein to DNA compared with treatment with Dex alone. These changes suggest that IGF-I attenuated the hepatic steatosis and abnormal hepatic cellularity induced by a catabolic dose of Dex.

There was no evidence that TPN-induced abnormal liver composition in the current study based on comparison of the TPN control groups with orally fed, normal reference rats. Orally fed rats showed similar liver mass (4.23 ± 0.11 g/100 g body wt) and concentrations of fat (22.0 ± 1.5 mg/g liver), protein (151 ± 2 mg/g liver), and water (71 ± 1%) compared with TPN controls.

**DISCUSSION**

IGF-I gene expression is regulated by hormonal and nutritional factors (35). The liver is the primary source of circulating IGF-I; thus hepatic IGF-I gene expres-
Table 4. Liver absolute and relative weight with concentrations of protein and DNA, protein-to-DNA ratio, fat concentration, and percent water

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Liver, g</th>
<th>Liver, g/100 g body wt</th>
<th>Protein, mg/g liver</th>
<th>DNA, mg/g liver</th>
<th>Protein: DNA</th>
<th>Fat, mg/g liver</th>
<th>%Water</th>
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<tr>
<td>TPN control</td>
<td>10</td>
<td>10.16 ± 0.26</td>
<td>3.88 ± 0.19</td>
<td>146 ± 2</td>
<td>10.4 ± 0.3</td>
<td>14.1 ± 0.3</td>
<td>19.6 ± 2.0</td>
<td>73 ± 1</td>
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<tr>
<td>Dex control</td>
<td>10</td>
<td>16.20 ± 0.51</td>
<td>7.65 ± 0.21</td>
<td>138 ± 2</td>
<td>5.8 ± 0.3</td>
<td>24.2 ± 1.2</td>
<td>76.1 ± 11.9</td>
<td>68 ± 3</td>
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<tr>
<td>IGF-I</td>
<td>11</td>
<td>9.97 ± 0.17</td>
<td>3.63 ± 0.06</td>
<td>158 ± 4</td>
<td>10.8 ± 0.3</td>
<td>14.7 ± 0.3</td>
<td>18.3 ± 1.7</td>
<td>72 ± 1</td>
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<tr>
<td>IGF-I + Dex</td>
<td>9</td>
<td>12.51 ± 0.44</td>
<td>5.46 ± 0.22</td>
<td>145 ± 1</td>
<td>7.3 ± 0.3</td>
<td>20.3 ± 0.9</td>
<td>35.0 ± 5.7</td>
<td>68 ± 1</td>
</tr>
</tbody>
</table>

P value (1-way ANOVA)  
IGF-I 0.0001  
Dex 0.0001  
IGF-I × Dex 0.0001

P value (2-way ANOVA)  
IGF-I 0.0001  
Dex 0.0001  
IGF-I × Dex 0.0001

Values are means ± SE. Data were analyzed by 1-way and 2-way ANOVA for TPN rats (+IGF-I, +Dex). P values are generated from the F-test. After 1-way ANOVA, the protected LSD test was performed. Means with different letters are significantly different. Two-way ANOVA determined significant main effects and interaction between treatment factors of IGF-I and Dex ($P < 0.05$).

Our data show that Dex-induced catabolism is associated with a 50% decrease in hepatic IGF-I, a 10% decrease in serum IGF-I, and no change in liver mRNA, suggesting that Dex regulation of hepatic IGF-I gene expression occurs at a translational or posttranslational level. This result is a significant departure from prevalent literature that establishes regulation by glucocorticoids at physiological concentrations at the level of mRNA abundance. Our results disagree with Luo and Murphy (16), who report decreased liver IGF-I mRNA in Dex-treated rats, but agree with Miura et al. (20), who demonstrate no significant changes in IGF-I secretion or IGF-I mRNA levels in primary cultures of rat hepatocytes treated with Dex.

Two main factors help explain our observations. First, it is likely that conflicting results are partially attributed to nutritional confounding because high-dose Dex decreases ad libitum food intake 30–50% (19, 24, 36). Pair feeding does not adequately control nutrition because it stimulates meal feeding rather than nibbling and imposes undesirable metabolic alterations (1). Although continuous infusion of a high-dextrose TPN solution presents physical and metabolic challenges, it provides strict nutritional control of adequate energy and models the feeding method used for critically ill patients with elevated endogenous glucocorticoids who cannot eat.

Second, our research used high-dose Dex administration to TPN rats, resulting in a 12% loss of body weight over 7 days. Unlike low-dose Dex administration, which has been shown to be associated with muscle cell proliferation and activation of muscle IGF-I receptor tyrosine phosphorylation (8), high-dose Dex administration induces gluconeogenic pathways, resulting in the breakdown of body protein and fat for energy as is associated with whole body catabolism (14). This suggests a possible association between up-regulation of gluconeogenic flux and maintenance of hepatic IGF-I mRNA.

The study design allowed us to isolate the main effects of Dex on the hepatic IGF-I endocrine axis. Dex did not have a significant main effect on liver IGF-I mRNA abundance. Our studies did not measure mRNA half-life, as this is difficult in whole animal models. However, we measured IGF-I in serum and in liver after tissue extraction of IGF-I. IGF-I peptide levels in serum and liver were decreased by 10 and 50%, respectively, with Dex treatment. The lack of correlation of liver IGF-I mRNA with liver immunoreactive IGF-I ($r = 0.0066$, $P = 0.98$) or serum IGF-I ($r = -0.2254$, $P = 0.33$) suggests that modulation of hepatic IGF-I gene expression is occurring posttranscriptionally. The essence of this is that if steady-state liver IGF-I mRNA abundance is unchanged, yet peptide levels are changed, this is prima facie evidence for translational or posttranslational control.

Recent literature supports our findings and is consistent with the notion that glucocorticoids regulate IGF-I gene expression at a translational or posttranslational level. A report by Shah et al. (29) demonstrates that Dex negatively modulates the activation of a subset of the protein synthetic machinery, resulting in inhibition of translation initiation. Singleton et al. (32) have shown that glucocorticoid-induced myopathy is due to the ability of Dex to inhibit the antiapoptotic effects of IGF-I by impairing activation of phosphatidylinositol-3-kinase. Cultured hepatocytes, treated with Dex, show decreased IGF-I production but no change in IGF-I mRNA levels (20). These works extend the common notion that glucocorticoids modulate transcription to include translation.

IGF-I administration has been shown to attenuate Dex catabolism (11, 36, 39), which we have confirmed in the present study. We evaluated the ability of exog-
The inhibitory effect of Dex on liver IGF-I peptide levels cannot be attributed to GH resistance at the level of GHR binding as liver GHR binding was not altered by Dex. Similarly, the decrease in liver IGF-I due to Dex cannot be attributed to serum GH levels, which tended to increase with Dex treatment. We observed a significant decrease in abundance of GHR mRNA by Dex, but this does not correlate systematically with the liver binding data. Thus these data support our primary conclusion of translational or posttranslational regulation of hepatic IGF-I production by Dex. These data differ compared with GH binding in rats fasted for 3 days (3). Fasted rats showed an 80% decrease in serum IGF-I, little change in serum GH, and decreased GH binding due to a loss of high-affinity GHRs, suggesting that hepatic resistance to GH at the level of GHR binding contributes to decreased liver IGF-I production during fasting.

In conclusion, these data support our hypothesis that, under conditions of adequate nutrition, Dex downregulates the hepatic IGF-I endocrine axis and is primarily responsible for whole body catabolism observed with Dex. Hepatic immunoreactive IGF-I was significantly, positively correlated with nitrogen balance, final body weight, body weight change, and serum IGF-I. Serum IGF-I was significantly but not as tightly correlated with these same parameters, and this suggests that serum IGF-I may be an indicator of clinical status (4). In addition, these data support the use of IGF-I as an anticytotoxic agent in critical illness.

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