Dexamethasone inhibits small intestinal growth via increased protein catabolism in neonatal pigs

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Burbin, Douglas G., Timothy J. Wester, Teresa A. Davis, Marta L. Fiorotto, and Xiaoyan Chang. Dexamethasone inhibits small intestinal growth via increased protein catabolism in neonatal pigs. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E269–E277, 1999.—Our objective was to determine how dexamethasone (Dex) affects gastrointestinal protein metabolism and growth in neonatal pigs. Two-day-old pigs were given daily subcutaneous injections of either Dex (1 mg/kg body wt, n = 7) or saline (control, n = 6) for 7 days. In vivo protein synthesis was measured after 7 days with a bolus of [3H]phenylalanine. Tissue protein contents were measured in an initial control group of 2-day-old pigs and in control and Dex pigs after 7 days to estimate protein accretion and degradation. In control pigs, the protein accretion in the ileum was nearly sixfold greater than in the jejunum during the 7-day period. Dex nominally altered stomach growth but completely blocked the accretion of protein and DNA in the jejunum and ileum, with reduced villus height in the ileum. Dex increased the fractional protein degradation rate in the ileum (28%) and decreased the absolute protein synthesis rate in the jejunum and ileum by 17 and 21%, respectively. Dex resulted in a 40% lower total intestinal lactase activity compared with controls via reductions in both specific activity and tissue mass, especially in the ileum. Dex significantly decreased the circulating concentrations of insulin-like growth factor (IGF) I and IGF-binding protein (IGFBP)-1, -2, and -3. However, the tissue abundance of the IGF-I receptor in the stomach and ileum was greater in Dex pigs than controls. Our results suggest that Dex significantly inhibits small intestinal growth via both increased degradation and decreased synthesis of protein. Furthermore, the inhibition of intestinal growth resulted in significantly decreased lactose digestive capacity.

Studies based on measurements of whole body protein metabolism indicate that the primary mechanisms whereby Dex inhibits growth in neonates are increased protein catabolism (4, 15, 43) and reduced utilization of dietary protein (45). However, estimates of whole body protein metabolism cannot distinguish the relative turnover rates in individual tissues. Studies in animals have shown that Dex treatment increases protein catabolism via both increased degradation and decreased synthesis of protein in several tissues (12, 23); however, few studies have examined this in the intestine (35). The stimulation of tissue protein catabolism by Dex is associated with increased activity of proteolytic enzyme systems (19, 21), possibly via activation of the glucocorticoid receptor (2, 16). However, the stimulation of protein catabolism by Dex also may be mediated by a decrease in the circulating concentration (32) and local expression (1, 27) of insulin-like growth factor (IGF) I and the IGF-binding proteins (IGFBP) (27, 28, 44). Few studies have examined the effects of Dex on the circulating concentration of IGF-I and its IGFBP in vivo (40).

The objective of this study was to determine the effect of Dex on gastrointestinal growth in neonatal pigs. Previous studies that have examined the effects of Dex in both humans and animals are confounded by differences in the daily dosage and duration of Dex therapy. We administered Dex at a constant daily dose (1 mg/kg) that is at the low end of the range (0.2–5.0 mg/kg) used in previous animal studies (15, 13, 35) but is higher than the range of doses (0.2–0.6 mg/kg) reported in studies with human infants (4, 9, 47). We hypothesized that Dex decreases gastrointestinal growth through a stimulation of protein degradation. To establish whether any of the effects of Dex on intestinal growth are potentially related to IGF metabolism, we measured
the circulating levels of IGF-I and IGFBP and the abundance of the type I IGF receptor in gastrointestinal tissues.

MATERIALS AND METHODS

Animals and design. Two litters of conventional crossbred newborn pigs (Texas A & M University, College Station, TX) were obtained immediately after birth and weighed. After a 48-h suckling period, a 20-gauge Silastic catheter was surgically inserted into the jugular vein of each pig under general anesthesia. Pigs were allowed to recover for 6 h before the start of the experiment and were housed two to three per cage at an ambient temperature of 28°C. Pigs were assigned randomly to receive either Dex sodium at a daily dose of 1 mg/kg body wt (n = 7) or saline (control, n = 6) in two equal, subcutaneous injections at 0800 and 2000. To eliminate any possible confounding effects due to differences in voluntary food intake between control and Dex-treated groups, pigs were gavaged an equal amount of formula per unit of body weight during the 7-day period. Pigs were fed a liquid sow-milk replacer (Sowena, Merrick’s, Middleton, WI), which supplied 9 g protein and 325 kcal energy/kg body wt daily. During the 7-day treatment period, jugular blood (2 ml) was sampled daily for analysis of plasma IGF-I and IGFBP concentrations.

To estimate tissue growth and protein accretion, five pigs of similar initial body weight to that of the control and Dex-treated pigs were killed at the start of the 7-day treatment period, and samples of stomach, jejunum, and ileum were obtained for analysis as described in Tissue assays and histomorphometry. These pigs were allowed to suckle on sow for 12–28 h and then fed sow-milk replacer for 24 h before being killed to eliminate the confounding effect of retention of sow-milk immunoglobulin on intestinal protein content. The protocol was approved by the Animal Care and Use Committee of Baylor College of Medicine and was conducted in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals.

Measurement of in vivo protein synthesis. After 7 days of treatment, to ensure that they were in a fed state during the protein synthesis measurement, the pigs were given two formula feedings of 40 ml/kg body wt at 4 and 2 h before radioisotope infusion. In vivo fractional tissue protein synthesis was measured by flooding-dose methodology (5, 13). Briefly, pigs were injected with a bolus dose of L-[3H]phenylalanine (37 MBq/kg body wt; Amersham, Arlington Heights, IL) in a 150 mM phenylalanine solution at a dose of 10 ml/kg body wt via the jugular catheter. Thirty minutes after isotope injection, pigs were anesthetized with an intravenous dose of pentobarbital (50 mg/kg body wt) and exsanguinated. The abdomen was opened and bathed with ice-cold saline, and the small intestine from the ligament of Treitz to the ileocecal junction was removed, freed of mesenteric tissue, and placed in ice-cold saline; proximal and distal halves were designated as jejunal and ileal, respectively. Segments of the proximal jejunum and ileum were each placed in 10% buffered Formalin for histology, and the remainder was flushed with ice-cold saline, blotted dry, weighed, and frozen immediately in liquid nitrogen. The stomach was flushed with digesta with ice-cold saline, weighed, and frozen in liquid nitrogen. The specific radioactivity of [3H]phenylalanine in blood and tissue samples was determined by anion-exchange HPLC and liquid scintillation counting as described previously (5).

Tissue assays and histomorphometry. Tissue samples (100–200 mg) were homogenized in water, and aliquots were removed quickly for analysis of protein and DNA with bichonchonic acid (BCA; Pierce, Rockford, IL) and bis-benzimide (24), respectively. Aliquots of the tissue homogenate were analyzed for lactase and sucrase activity (10). The muscularis thickness, crypt depth, and villus height were measured on sections stained with hematoxylin and eosin. The mean values were determined from 20–30 well-oriented crypt-villus columns from multiple sections that were examined with a Zeiss Axioshot microscope in a blinded manner.

Plasma IGF-I and IGFBP analysis. The plasma IGF-I concentration was measured by radioimmunossay after acidification and chromatography to remove binding proteins (25). All samples were measured in one assay, and the intra-assay coefficient of variation was 7.2%. Analysis of IGFBP was performed with a modified Western ligand blot procedure (6, 46). Blots were washed, allowed to dry at room temperature, and then exposed to X-ray film in the presence of intensifying screens at −70°C for 7–8 days. The band intensities on the autoradiographs were quantified with laser densitometry (Pharmacia LKB Biotechnology, Piscataway, NJ). Apparent molecular mass of IGFBP was estimated by comparison to Coomassie blue-stained protein standards run under identical conditions.

IGF-I receptor immunoblotting. Samples (~1 g) of frozen stomach, jejenum, and ileum were pulverized in liquid nitrogen and homogenized in a buffer (buffer A) containing 50 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM Na-EDTA, 2 mg/ml bacitracin, 348 mg/ml phenylmethylsulfonyl fluoride, 25 µg/ml leupeptin, and 25 µg/ml aprotinin. The homogenate was centrifuged at 10,000 g for 10 min at 4°C, and the resulting supernatant was then centrifuged at 100,000 × g for 1 h at 4°C. The 100,000 g pellet was solubilized in 3–5 ml of buffer A containing 1% Triton X-100 overnight at 4°C. The protein concentration of the solubilized membrane-containing fraction was determined with the BCA assay (Pierce).

For immunoblotting, equivalent amounts of membrane protein were mixed with an equal volume of 2× Laemmli sample buffer, pH 6.8, containing 10% β-mercaptoethanol, 0.06 M Tris–HCl, 2% SDS, and 10% glycerol and were placed in a boiling water bath for 5 min. Membrane proteins were separated on a 7.5% SDS-polyacrylamide gel run at 200 V for 1–2 h at 4°C. Proteins were electrotransferred from the gel to a nitrocellulose membrane (Bio-Rad, Hercules, CA) at 100 V for 1–2 h at 4°C. Nitrocellulose membranes were incubated in blocking buffer (TBS containing 30 g/l nonfat dry milk, pH 7.4) for 1 h; then the IGF-I receptor α-subunit antibody (0.25–0.50 µg/ml final concentration; Santa Cruz Biotechnology, Santa Cruz, CA) was added, and the blots were incubated overnight at 4°C. Membranes were rinsed at room temperature in wash buffer (TBS containing 1 ml/l Tween 20) and then incubated for 1 h at room temperature in TBS containing goat anti-rabbit IgG (Dako, Carpinteria, CA) diluted in blocking buffer. Membranes were rinsed at room temperature in wash buffer and then incubated for 1 h at room temperature with neutravidin-conjugated horseradish peroxidase (Pierce) diluted in wash buffer. After rinsing, the membranes were incubated with a chemiluminescent substrate as described by the manufacturer (SuperSignal). Immunoreactive proteins were visualized and detected by autoradiography. The band intensities on the autoradiographs were quantified with laser densitometry (Pharmacia LKB Biotechnology, Piscataway, NJ). The apparent molecular mass of the IGF-I receptor α-subunit was estimated by comparison to Coomassie blue-stained protein standards run on the same gel.
Calculations. Protein synthesis was calculated as a fractional rate (FSR, %/day) from the equations described by Burrin et al. (5)

\[
FSR = \frac{S_s}{S_a} \times \left( \frac{1.440}{t} \right) \times 100
\]

where \( S_s \) is the specific activity of the perchloric acid (PCA)-insoluble or protein-bound phenylalanine pool (Bq/µmol), \( S_a \) is the specific activity of the PCA-soluble or tissue free phenylalanine pool (Bq/µmol), \( t \) is time of labeling (in min), and 1.440 is the number of minutes in 1 day.

The absolute protein accretion rate (AAR) of the stomach, jejunum, and ileum was calculated from the difference in tissue protein mass measured at the end of the 7-day treatment period (TPm) and that predicted at day 0 (TPp):

\[
AAR (\text{mg/day}) = \frac{(TP_m - TP_p)}{7}
\]

The TPp was predicted by multiplying their respective body weight (measured on day 0) by the tissue protein mass per unit of body weight measured directly in the day 0 group of pigs. The absolute rates of DNA accretion were calculated similarly. The absolute protein synthesis rate (ASR) was calculated as the FSR measured at the end of the 7-day treatment period times the average tissue protein mass (TPavg):

\[
ASR (\text{mg/day}) = FSR \times TP_{avg}
\]

TPavg in control and Dex pigs during the 7-day treatment period was calculated from the TPp and TPm:

\[
TP_{avg} (\text{mg}) = \frac{(TP_p + TP_m)}{2}
\]

The absolute protein degradation rate (ADR) was calculated as the difference between the absolute protein synthesis and accretion rate:

\[
ADR (\text{mg/day}) = ASR - AAR
\]

The ADR includes secreted proteins because much of the protein synthesized in the stomach, jejunum, and ileum is first secreted into the gastrointestinal lumen and then degraded. The fractional protein degradation rate (FDR) was calculated as the ADR divided by the average tissue protein mass:

\[
FDR (\%/day) = \frac{ADR}{TP_{avg}} \times 100
\]

Statistical analysis. Data consisting of single observations in time, e.g., tissue weights and FSR, were analyzed by one-way analysis of variance (ANOVA) with treatment as the main effect. Observations taken across time, e.g., daily IGF-I concentrations, were analyzed by repeated-measures ANOVA (BMDFPV; 11), with treatment and sampling time as main effects. Data are expressed as means ± SD. Differences with \( P < 0.05 \) were considered significant.

RESULTS

The initial body weights were similar in control and Dex pigs (1.76 ± 0.23 and 1.74 ± 0.24 kg, respectively). Control pigs grew at more than twice the rate of Dex pigs (20.7 ± 3.6 vs. 9.24 ± 3.6 g/day, respectively; \( P < 0.05 \)) and were significantly heavier by the end of the study (2.24 ± 0.36 vs. 2.01 ± 0.36 kg, respectively; \( P < 0.05 \)).

The values in Table 1 represent the response to Dex treatment and are not confounded by a decrease in voluntary food intake typically associated with Dex treatment, because pigs were fed an equal amount of formula per unit of body weight. In both control and Dex pigs, the weight and protein, but not DNA, content of the stomach was greater at day 7 than at the initial day. Jejunum weight, protein, and DNA content in control pigs were greater at day 7 than at the initial day. Jejunum weight and DNA content in Dex pigs were lower than in control pigs at day 7. Similarly, ileum weight, protein, and DNA content in control pigs were greater at day 7 than the initial day and also were significantly greater than in Dex pigs at day 7.

The absolute rates of protein and DNA accretion in the stomach during the 7-day period were similar in control and Dex pigs (Table 2). In control pigs, there was significant net accretion of protein and DNA in the jejunum and ileum during the 7-day period. However, the absolute rate of protein accretion was nearly sixfold greater in the ileum compared with the jejunum, whereas the rate of DNA accretion was only 38% of the rate in the stomach.
control value. ANOVA and are for comparisons of Dex with corresponding saline administration to the jejunum, and ileum of neonatal pigs after either saline or Dex administration.

Table 3. Fractional and absolute rates of protein synthesis and degradation or secretion in stomach, jejunum, and ileum of neonatal pigs after either saline or Dex administration

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Dex</th>
<th>Pooled SD</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 6)</td>
<td>Dex (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fractional synthesis rate, %/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>31.8</td>
<td>44.1</td>
<td>6.0</td>
<td>0.006</td>
</tr>
<tr>
<td>Jejunum</td>
<td>79.2</td>
<td>75.1</td>
<td>9.0</td>
<td>0.452</td>
</tr>
<tr>
<td>Ileum</td>
<td>53.2</td>
<td>58.9</td>
<td>8.8</td>
<td>0.296</td>
</tr>
<tr>
<td>Absolute synthesis rate, mg/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>276</td>
<td>359</td>
<td>86</td>
<td>0.132</td>
</tr>
<tr>
<td>Jejunum</td>
<td>3,025</td>
<td>2,502</td>
<td>312</td>
<td>0.017</td>
</tr>
<tr>
<td>Ileum</td>
<td>2,529</td>
<td>2,007</td>
<td>441</td>
<td>0.071</td>
</tr>
<tr>
<td>Fractional degradation or secretion rate, %/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>25.2</td>
<td>38.2</td>
<td>5.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Jejunum</td>
<td>77.1</td>
<td>75.4</td>
<td>9.4</td>
<td>0.763</td>
</tr>
<tr>
<td>Ileum</td>
<td>45.3</td>
<td>57.9</td>
<td>8.7</td>
<td>0.032</td>
</tr>
<tr>
<td>Absolute degradation or secretion rate, mg/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>218</td>
<td>309</td>
<td>68</td>
<td>0.046</td>
</tr>
<tr>
<td>Jejunum</td>
<td>2,943</td>
<td>2,509</td>
<td>309</td>
<td>0.037</td>
</tr>
<tr>
<td>Ileum</td>
<td>2,154</td>
<td>1,971</td>
<td>417</td>
<td>0.471</td>
</tr>
</tbody>
</table>

Control and Dex values are means. P values are based on 1-way ANOVA and are for comparisons of Dex with corresponding saline control value.

The fractional rates of both protein synthesis and degradation in the stomach were significantly greater in Dex pigs than in control pigs (Table 3). The absolute rate of protein degradation-secretion in the stomach was greater in Dex than in control pigs, whereas the absolute rates of protein synthesis were similar. In control pigs, the fractional rates of both protein synthesis and degradation were generally higher in the jejunum than in the ileum. However, the difference between the fractional rates of both protein synthesis and degradation (i.e., an index of net protein balance or accretion) was higher in the ileum (7.9%/day) than in the jejunum (2.1%), which is consistent with the observed higher protein accretion rate in the ileum vs. jejunum.

It is important to note that, despite the lack of statistical significance compared with the control pigs, the fractional rates of protein synthesis and degradation in the jejunum were numerically lower in the Dex pigs. These small differences in the fractional rates of both protein synthesis and degradation in the jejunum translated into significantly lower absolute rates of protein synthesis and degradation in Dex compared with control pigs (Table 3). In contrast, Dex treatment increased the fractional protein degradation rate in the ileum ~28%. Despite the numerical increase in the fractional protein synthesis rates in the ileum, the overall decrease in protein mass resulted in a tendency (P = 0.07) for the absolute rates of protein synthesis to decrease (~21%), with no change in the absolute degradation rate.

In the jejunum, the villus height was similar in control and Dex pigs; however, the villus height in the ileum was significantly lower in Dex pigs than in control pigs (Table 4). In control pigs, the villus height was nearly twofold greater in the ileum than in the jejunum. There were no differences in either crypt depth or muscularis thickness in either intestinal segment between control and Dex pigs. The protein-to-DNA ratios in the jejunum and ileum on the initial day were 22 ± 0.04 and 21 ± 0.04, respectively. At day 7, the protein-to-DNA ratio in the ileum was significantly lower than in the jejunum, which was greater than in control pigs. The absolute accretion (i.e., an index of net protein balance or accretion) was higher in the ileum (7.9%/day) than in the jejunum. However, the difference between the fractional rates of both protein synthesis and degradation were generally higher in the ileum than in the jejunum.

Both the lactase specific activity and total lactase activity in the jejunum and ileum were lower in Dex pigs than in control pigs (Table 5). In control pigs, the specific and total activity of sucrase in the jejunum and ileum was greater in Dex pigs than in control pigs. In general, the effects of Dex on disaccharidase activity were proportionally greater in the ileum than in the jejunum.

The circulating IGF-I concentration in the control pigs did not change during the 7-day treatment period (Fig. 1). In the Dex pigs, the circulating IGF-I concentration was similar to that in the control group between days 1 and 3 but then decreased such that the IGF-I concentration at days 4, 5, 6, and 7 was significantly (P < 0.05) lower than in the control group.

In ligand blots of plasma samples collected after 7 days of treatment, we identified bands of IGF binding that corresponded to apparent molecular masses of 43, 39, 34, 29, and 24 kDa (Fig 2). By comparison with published reports that used immunological methods (6, 25, 31, 40), these bands were putatively identified as differentially glycosylated forms of IGFBP-3 for the 43- and 39-kDa bands, IGFBP-2 for the 34-kDa band, IGFBP-1 for the 29-kDa band, IGFBP-4 for the 24-kDa band. Moreover, we have previously found that anti-human IGFBP-3 antisera recognized only the 24-kDa band.
43- and 39-kDa proteins, and anti-bovine IGFBP-2 antiserum recognized only a 34-kDa protein (46); neither antibody recognized the 29- and 24-kDa bands. There appeared to be two bands between 29 and 31 kDa that were not resolved by densitometry. Although we have identified the 29-kDa band as IGFBP-1, it is conceivable that IGFBP-5 was present and, if so, also was decreased by Dex. Preliminary studies have confirmed the presence of two IGFBP with apparent molecular masses of 29–31 kDa in neonatal pig plasma that were immunologically identified as IGFBP-1 and IGFBP-5 (S. Donovan, personal communication). On the basis of densitometric analysis of the autoradiogram, the relative plasma abundance of IGFBP-1, IGFBP-2, and IGFBP-3, but not IGFBP-4, was significantly lower in Dex pigs than in control pigs (Fig. 2).

Table 5. Disaccharidase activities in jejunum and ileum of neonatal pigs administered either saline or Dex for 7 days

<table>
<thead>
<tr>
<th></th>
<th>Saline (n = 6)</th>
<th>Dex (n = 7)</th>
<th>Pooled SD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactase specific activity, µmol·min⁻¹·g protein⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>170</td>
<td>132</td>
<td>28</td>
<td>0.034</td>
</tr>
<tr>
<td>Ileum</td>
<td>179</td>
<td>115</td>
<td>51</td>
<td>0.045</td>
</tr>
<tr>
<td>Total lactase activity, µmol·min⁻¹·kg body wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>277</td>
<td>223</td>
<td>53</td>
<td>0.096</td>
</tr>
<tr>
<td>Ileum</td>
<td>487</td>
<td>213</td>
<td>195</td>
<td>0.028</td>
</tr>
<tr>
<td>Sucrase specific activity, µmol·min⁻¹·g protein⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>119</td>
<td>145</td>
<td>19</td>
<td>0.046</td>
</tr>
<tr>
<td>Ileum</td>
<td>15.4</td>
<td>60.3</td>
<td>21</td>
<td>0.002</td>
</tr>
<tr>
<td>Total sucrase activity, µmol·min⁻¹·kg body wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>196</td>
<td>241</td>
<td>42</td>
<td>0.090</td>
</tr>
<tr>
<td>Ileum</td>
<td>40</td>
<td>104</td>
<td>32</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Control and Dex values are means. P values are based on 1-way ANOVA and are for comparisons of Dex with corresponding saline control.

Results from immunoblots of solubilized membranes from each tissue indicated a prominent band at 125–130 kDa, which is consistent with the molecular mass range (120–140 kDa) reported previously for the α-subunit of the type I IGF receptor in humans and rodents (26). The relative receptor abundance in the stomach and ileum was significantly (2- to 3-fold) greater in the Dex pigs than in control pigs (Fig. 3). In the jejunum, the relative receptor abundance was much lower than in the stomach and ileum, and there was no effect of Dex treatment.

DISCUSSION

Glucocorticoids have been shown to cause a generalized catabolic effect on whole body protein metabolism in human neonates (4, 43, 47). However, the relative impact of glucocorticoids on protein metabolism in specific tissues has not been established in neonates. We examined the effect of Dex, a synthetic glucocorticoid commonly given to human neonates, on intestinal protein metabolism because compromised gastrointestinal function contributes to the morbidity of many of these infants. A recent study with neonatal rats indicated that tissues vary in their sensitivity to glucocorticoids and that the gastrointestinal tract is more sensitive than skeletal muscle to the protein catabolic effects of corticosterone treatment (3). Our present study conducted with neonatal pigs indicates that small intestinal protein metabolism is highly sensitive to
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Dex. Moreover, we found that both the relative response and the mechanism by which Dex affects protein metabolism varied among the different gastrointestinal tissues. Specifically, the catabolic response was confined to the small intestine and not the stomach and was mediated by decreased protein synthesis and increased protein degradation. The catabolic effect of Dex was proportionally greater in the ileum than the jejunum, consistent with a previous report in weanling rats (32); however, this result was largely due to the inherently higher rate of protein accretion in the ileum.

The differential growth rates among the three gastrointestinal tissues and their responses to Dex were evident in the relative rates of protein turnover. The respective rates of protein accretion and synthesis can be measured in individual tissues in vivo by quantifying the difference in protein mass and the rate of isotopic amino acid incorporation into tissue protein. However, for a variety of kinetic and analytical reasons, few isotopic methods are available to directly measure protein degradation in a given tissue in vivo. Thus, in this study, we used a combination of direct measurements of protein synthesis and accretion to indirectly estimate protein degradation. The fractional rate of protein synthesis or degradation is a measure of the inherent rate at which these processes are occurring in the cell at a given time point and is independent of changes in tissue protein mass. The difference between the fractional synthesis and degradation rates determines whether there is net gain or loss of tissue protein. In control pigs, the markedly higher rate of protein accretion in the ileum compared with the jejunum was due to a larger difference between the fractional rate of protein synthesis and degradation; this was despite the generally lower fractional rates of synthesis and degradation in the ileum. On the other hand, the absolute rates of protein synthesis and degradation are a function of both the fractional rate and the average protein mass of the tissue over a specified time period. Thus the absolute rates are influenced by the preceding rates of tissue protein gain or loss.

Dex significantly increased the fractional rates of protein synthesis and degradation in the stomach, consistent with evidence that glucocorticoids induce gastric acid, protease, and gastrin secretion in fetal and neonatal pigs (36–38). However, because the increases in the stomach fractional rates of both protein synthesis and degradation were similar, there was no difference in rate of protein accretion between control and Dex pigs. In contrast, Dex completely blocked protein accretion in both the jejunum and ileum, although the mechanism differed between the two. In the jejunum, Dex resulted in relatively small, statistically nonsignificant decreases in the fractional rates of both protein synthesis and degradation that, over the course of 7 days, translated into significantly lower absolute rates of protein synthesis and degradation. Because Dex reduced the absolute synthesis and degradation rate to a similar level (~2,500 mg/day), there was zero net protein balance during the 7 days. Thus, despite the subtle changes, the results could only be explained if Dex inhibited protein accretion in the jejunum by a proportionally greater decrease in rate of synthesis than of degradation.

In contrast, in the ileum, Dex increased the fractional protein degradation rate (~28%) without significantly affecting the fractional protein synthesis rate. As a result, Dex reduced the difference between the fractional synthesis and degradation rates (i.e., fractional accretion rate) nearly eightfold from 7.9%/day in the control pigs to 1.0%/day. As in the jejunum, the net effect was to reduce the absolute synthesis and degradation rate to a similar level (~2,000 mg/day), resulting in zero net protein balance after 7 days. More importantly, in the Dex-treated pigs, the absolute daily rate of protein degradation was similar to that of the control pigs despite having ~40% less protein mass. Thus the inhibition of protein accretion in the ileum was largely due to the proportionally greater increase in the fractional rate of protein degradation and not protein synthesis. In the only previous report of intestinal protein metabolism in young adult glucocorticoid-treated rats, the catabolic effect of Dex on intestinal growth also was ascribed to enhanced protein degrada-
tion by inference, because protein synthesis was unchanged (35).

The growth inhibitory effect of Dex on the ileum also was evident in decreased villus height and DNA accretion, suggesting that perhaps cell proliferation was reduced. However, most reports have indicated increased (18, 41) rather than decreased (7) intestinal mitotic activity in fetal and neonatal animals treated with glucocorticoids. Dex also might be expected to have protein catabolic effects on intestinal smooth muscle, yet we found no statistically significant differences in the muscularis thickness between the groups, suggesting that the response was confined to the mucosal epithelium.

Dex significantly decreased the specific activity of lactase and increased that of sucrase, consistent with the typical neonatal response to glucocorticoids (17). This contrasts with the stimulatory effect of glucocorticoids on intestinal lactase activity reported in late-gestation fetal pigs (39). The substantial reduction in ileal lactase activity may have resulted from either increased protein degradation or a shorter enterocyte life span secondary to the decreased villus height, both of which could decrease the amount of lactase processed into the mature form (42). However, we have no immediate explanation for the reduction in jejunal lactase activity. Nevertheless, in the total small intestine, the combined reduction in maximal specific activity and protein mass resulted in a >40% decrease in total lactase activity. Some preterm infants are born before the normal ontogenic increase in lactase activity during the third trimester and presumably have less than normal lactose digestive capacity (34). Thus the possible decrease in lactose digestive capacity resulting from Dex therapy may be of particular concern for this population of infants.

Previous studies have indicated that the protein catabolic response to glucocorticoids is associated with a decrease in plasma IGF-I concentration (40) and can be significantly ameliorated by simultaneous treatment with either IGF-I or growth hormone (20). Furthermore, increasing the plasma IGF-I concentration by systemic IGF-I administration specifically increased intestinal protein synthesis (27) and prevented intestinal atrophy associated with total parenteral nutrition (48). Therefore, we hypothesized that Dex could stimulate intestinal protein catabolism in part by suppressing the plasma IGF-I concentration. Indeed, we observed a decrease in plasma IGF-I concentration. Moreover, Dex significantly altered plasma IGFBP concentrations by decreasing IGFBP-3 and increasing IGFBP-1 and -2, which may have contributed to the decrease in circulating IGF-I by shortening the half-life in plasma and/or perhaps inhibited binding to cellular membrane IGF receptors (22, 28, 29, 44). However, we cannot exclude the possibility that Dex also decreased local intestinal and hepatic IGF-I production, thereby resulting in a local inhibition of growth and/or reduced hepatic release into circulation (1, 33).

Regardless of either the local concentration or source of IGF-I, the tissue responsiveness to IGF-I also can be modulated by the receptor abundance on the target cell. Although results from in vitro studies have shown an upregulation of receptor abundance by Dex (14), we are unaware of any previous reports documenting this response to Dex in vivo. In contrast to the reduction in plasma IGF-I, Dex significantly increased the abundance of the type I IGF receptor in the stomach and ileum. This increase in receptor abundance may be attributable to a direct effect of Dex on receptor expression and/or a secondary effect of reduced receptor turnover consequent to the reduction in plasma IGF-I concentration (26). Irrespective of the mechanism, however, there was tissue specificity in the response, in that increased receptor expression was observed in the stomach and ileum but not the jejunum. This pattern of response paralleled the protein synthetic response in these tissues despite the lower plasma IGF-I concentrations. Whether the protein catabolic effects of Dex are direct or are mediated indirectly via decreased IGF-I concentration cannot be reconciled from our results. Nevertheless, these observations suggest that both the stomach and ileum are more responsive to the effects of Dex than the jejunum and that, even within a tissue, there may be heterogeneity in the relative sensitivity of different cellular processes to Dex.

Perspectives. The results indicate that chronic treatment of neonatal pigs with Dex inhibits growth of the small intestine. The inhibition of growth was most pronounced in the ileum, the most rapidly growing region of the neonatal intestine, and the growth of the stomach was only minimally impacted. The inhibition of growth was mediated by increased protein degradation and by decreased protein synthesis and DNA accretion. The protein catabolic effects of Dex also were associated with a significant decrease in villus length and total intestinal lactase activity. This suggests that in those populations of low-birth-weight infants that receive Dex therapy, the protein catabolic effects on the small intestine may become manifest in reduced intestinal lactose digestive capacity.

It is important to contrast the protein catabolic effects on the intestine that we observed with a pharmacological dose of Dex, a more potent glucocorticoid than cortisol, with the physiological effects of cortisol in pigs during the perinatal period. During the perinatal period, pigs exhibit a marked increase in circulating cortisol levels. This perinatal hypercortisolism is associated with a significant increase in lactase activity and gastric function (36–38) and is concurrent with rapid somatic growth, including the intestine. Although this perinatal cortisol surge is a clear maturational signal in the intestine and stomach, it is less clear how this affects intestinal protein metabolism and cellular turnover in neonates. The previous report of Beckett et al. (3) demonstrated that in suckling rats, intestinal protein anabolism occurs at physiological glucocorticoid concentrations but becomes catabolic with higher levels. Thus whether glucocorticoids stimulate anabolism and maturation or catabolism likely depends on the circulating level and potency of the glucocorticoid, as well as the sensitivity of individual tissues. Given the
catabolic response we observed with Dex, further studies may be warranted to examine the intestinal response to more clinically relevant doses and tapered administration regimens.

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