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## Effects of an acute enteric disease challenge on IGF-1 and IGFBP-3 gene expression in porcine skeletal muscle

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## EFFECTS OF AN ACUTE ENTERIC DISEASE CHALLENGE ON IGF-1 AND IGFBP-3 GENE EXPRESSION IN PORCINE SKELETAL MUSCLE

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### Summary

Eighteen pigs (initial weight 25 lb and approximately 5 wk of age) were used in a 14-d trial to determine the effects of an acute *Salmonella enterica* serotype typhimurium (ST) disease challenge on both circulating insulin-like growth factor-1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) and steady-state IGF-1 and IGFBP-3 mRNA levels in skeletal muscle. Muscle biopsies and blood samples were obtained from all pigs on d 0, 3, 7, and 14 relative to ST-challenge. Results suggest that an acute ST-challenge decreased circulating IGF-1 levels on d 3 and 7 but did not affect circulating IGFBP-3 concentrations. Additionally, ST-challenge had no effect on steady-state IGF-1 and IGFBP-3 mRNA levels in skeletal muscle following the onset of disease. These data suggest that an acute enteric disease insult can lower circulating IGF-1 but more chronic conditions may be necessary to affect local IGF-1 levels in skeletal muscle. Additionally, the increased muscle IGF-1 mRNA without increased IGFBP-3 levels on d 14 most likely results in increased IGF-1 synthesis that contributes to circulating IGF-1 concentrations.

(Key Words: Pigs, *Salmonella*, Muscle, IGF-1, IGFBP-3.)

### Introduction

Our previous data with an acute enteric disease (*Salmonella enterica* serotype typhimurium, ST) challenge showed a precipitous decrease in circulating IGF-1 and IGFBP-3 concentrations 48 h after challenge. These data suggest that alterations in IGF/IGFBP levels during a diseased-state may contribute to the reduced skeletal muscle protein accretion. To our knowledge no one has ascertained the effect of an acute enteric disease challenge on local IGF-1 and IGFBP-3 synthesis in skeletal muscle tissue. A more thorough understanding of alterations in local IGF-1 and IGFBP-3 mRNA levels in skeletal muscles of pigs during an acute disease challenge will increase our understanding of the effect of disease on these important growth mediators and, ultimately, protein synthesis and degradation in skeletal muscle. The objective of the current study was to determine the effect of an infectious dose of ST on changes in steady-state IGF-1 and IGFBP-3 mRNA of porcine skeletal muscle.

### Procedures

The experimental protocol used in this study was approved by the KSU Institutional Animal Care and Use Committee. A total of 18 pigs (initial weight 25 lb and approxi-

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mately 5 wk of age) were randomly allotted to one of two treatments (n=9 pigs per treatment) with weight balanced across the treatments in a 14-d experiment. The two treatments were control or *Salmonella*-challenged (ST). Preceding the study fecal samples were obtained and cultured using standard microbiological direct plating and enrichment techniques at the Kansas State University Veterinary Diagnostic Laboratory to ensure that pigs were not shedding ST prior to challenge.

On d 0, nine pigs were challenged with ST using a challenge model described previously. Pigs were challenged orally with  $11 \times 10^9$  cfu of ST. The control pigs received sterile medium orally.

Pigs were weighed and feed disappearance was measured on d 0, 7, and 14. On d 7 after challenge, fecal samples were obtained from all pigs and cultured for ST at the Kansas State University Veterinary Diagnostic Laboratory. Rectal temperature was measured on pigs daily from d 0 to 7. Blood samples were obtained via venipuncture on d 0 (prior to challenge), 3, 7, and 14.

Muscle samples (0.5 g) were obtained from the gluteus medius of pigs on d 0, 3, 7, and 14, relative to disease challenge using a biopsy technique. Briefly, pigs were administered general anesthesia. Once pigs reached a surgical plane of anesthesia (approximately 10 min.) the biopsy site was scrubbed thoroughly and a 1-cm incision was performed. A Bergstrom biopsy needle was inserted to obtain approximately 0.5 g of muscle tissue. The incision site was closed with tissue adhesive. Pigs fully recovered within 1.5 hr of the procedure. Muscle samples were immediately homogenized in 10 mL of a preservative solution, followed by rapid freezing in liquid nitrogen and stored at -80°C for subsequent analysis.

Total RNA was isolated according to established procedures. RNA integrity was determined by electrophoresis of total RNA through a 1% agarose formaldehyde gel followed by ethidium bromide staining to visualize 28 and 18S ribosomal RNA (rRNA). Once RNA integrity was assessed, any contaminating DNA was removed. The RNA (1 ug) was reverse transcribed to synthesize the first-strand of cDNA.

All real-time quantitative (RTQ-PCR) reactions were performed on a ABI Prism 7000 sequence detection system, (Applied Biosystems, Foster City, CA). Specific cDNA sequence, forward and reverse primer sequences, and Taq Man detection probe sequences were:

IGF-1: Genbank Accession # M31175, forward primer (38)  
TCTTCTACTTGGCCCTGTGCTT, reverse primer (110) GCCCACAGAGGGTCTCA, and TaqMan probe (65) CCTTCAC-CAGCTCTGCCACGGC

IGFBP-3: Genbank Accession #AF085482, forward primer (692) AGCACG-GACACCCAGAACTT, reverse primer (753) CGGCAAGGCCCGTATTC, and TaqMan probe (713)  
TCCTCTGAGTCCAAGCGCGAGA

Relative expression of IGF-1 and IGFBP-3 were normalized to 18S rRNA with the eukaryotic 18S rRNA endogenous control (ABI Cat. # 4319413E; Genbank Accession # X03205) and were reported as arbitrary units.

Blood collected on d 0, 3, 7, and 14 was allowed to clot at 4°C for 48 h and serum harvested by centrifugation. Serum IGF-1 was determined via immunoradiometric assay as described previously for use in pigs. Circulating concentrations of IGFBP-3 were also determined with a commercially available im-

munoradiometric assay (Diagnostic Systems Laboratories, Inc., Webster, TX, Cat. # DSL 6600).

All data were analyzed with the PROC MIXED procedure of SAS as a completely randomized design with repeated measures over time. The model included terms for the fixed effects of treatment, day, and treatment  $\times$  day interaction. Unless noted on figures, comparisons of treatment or time were conducted only when a significant main effect or interaction was found.

## Results and Discussion

None of the pigs were shedding ST prior to challenge. On d 7 following challenge, 77.8 % (7/9) of pigs orally-challenged with ST were shedding ST in their feces as compared to 0 % (0/9) in the control group. Rectal temperature in ST-challenged pigs did not differ from control pigs during the 14-d trial (data not shown). However, daily feed intake was dramatically reduced in pigs challenged with ST the first week following challenge as compared to control pigs (0.84 lb/d  $\pm$  .15 vs. 1.61 lb/d  $\pm$  .11).

Circulating IGF-1 levels did not differ ( $P>0.10$ ) between control and ST-challenged pigs prior to challenge (Figure 1). Following the oral ST-challenge, infected pigs exhibited a precipitous drop in circulating IGF-1 levels on d 3 as compared to the d 0 value (33 vs. 97 ng/mL). Sera from pigs challenged with ST had reduced ( $P<0.05$ ) IGF-1 on d 3 and 7 as compared to sera from control pigs (Figure 1). However, by d 14 following challenge, sera from ST-infected pigs had similar circulating IGF-1 as compared to sera from control pigs, suggesting the pigs were recovering from the acute disease challenge (Figure 1). The circulating IGF-1 results need to be viewed with some caution. While the treatment  $\times$  day interaction tended to be significant ( $P=0.09$ ) in

this study, we believe the treatment comparisons within day are representative. First, the changes reported here mirror results published previously which were obtained from the same ST- disease challenge model. Furthermore, due to the precipitous drop in feed intake the first week following ST-challenge, we would fully expect circulating IGF-1 concentrations to be reduced during this period.

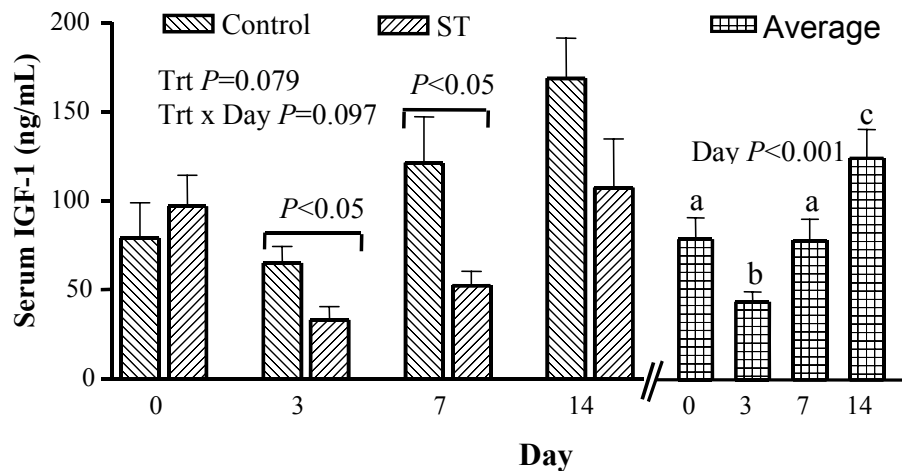
Serum concentrations of IGFBP-3 are illustrated in Figure 2. No significant ( $P>0.10$ ) treatment  $\times$  day interaction was observed for circulating IGFBP-3. We did observe a significant day effect ( $P<0.001$ ). Circulating IGFBP-3 in sera from pigs on d 7 and 14 were elevated ( $P<0.01$ ) as compared to samples obtained on d 0 and 3.

The effects of ST-challenge on steady-state IGF-1 mRNA levels in muscle are illustrated in Figure 3a. No significant treatment  $\times$  day interaction was observed for steady-state IGF-1 mRNA levels in muscle of pigs. However, we did detect a significant day effect. Steady-state IGF-1 mRNA in muscle samples obtained on d 14 were significantly greater ( $P<0.001$ ) than d 0, 3, and 7. In this study, skeletal muscle IGF-1 mRNA levels were unaffected by ST-challenge even though circulating IGF-1 levels were reduced following the acute ST-challenge. These data suggest that an acute disease challenge may not be sufficient to alter local IGF-1 levels in skeletal muscle. However, a more chronic disease challenge could lower IGF-1 levels in skeletal muscle which would affect protein synthesis and degradation rates.

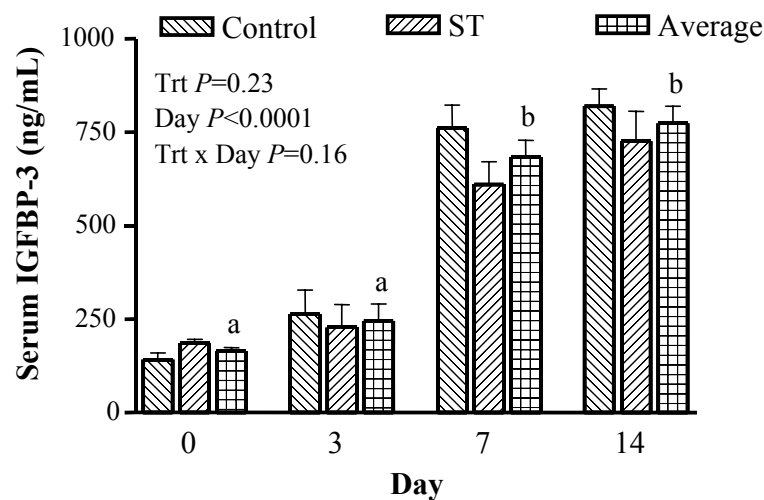
No treatment effect was observed for steady-state IGFBP-3 mRNA levels in muscle samples obtained by biopsy (Figure 3b). It is noteworthy that during the period of increasing muscle IGF-1 mRNA concentrations (d 14) local muscle IGFBP-3 mRNA levels were unaffected. This difference between responses

between muscle IGF-1 and IGFBP-3 gene expression on d 14 may contribute to increased skeletal muscle protein synthesis during periods of rapid muscle accretion in the growing pig. The increased muscle IGF-1 mRNA

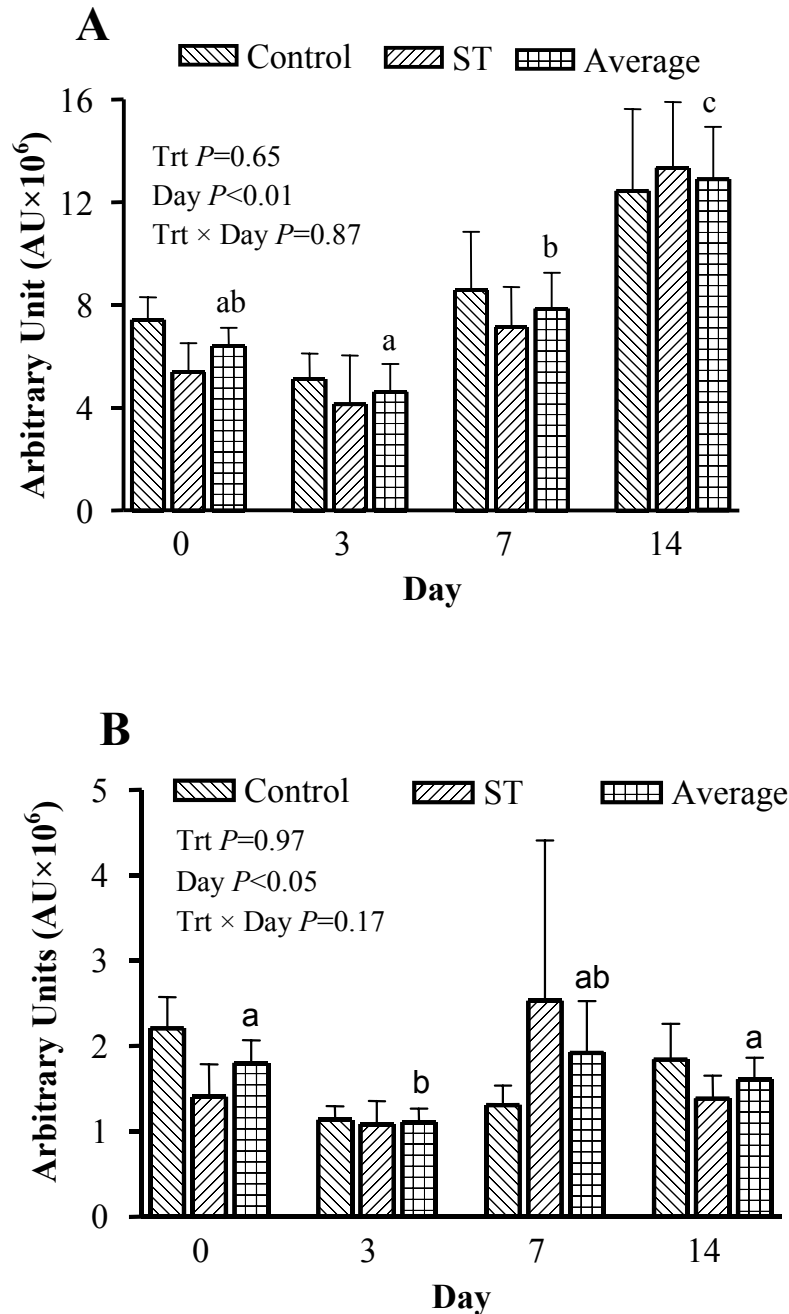
without increased IGFBP-3 levels on d 14 most likely results in increased IGF-1 synthesis. It is likely that this IGF-1 production exits the muscle and contributes to the increased circulating IGF-1 levels.



**Figure 1. Serum Insulin-Like Growth Factor 1 (IGF-1) Concentrations in ST-Challenged and Control Pigs (n=9).** Sera were obtained on d 0, 3, 7, and 14 following challenge.



**Figure 2. Serum Insulin-Like Growth Factor Binding Protein 3 (IGFBP-3) Concentrations in ST-Challenged and Control Pigs (n=9).** Sera were obtained on d 0, 3, 7, and 14 following challenge. Average (day) values with different superscripts differ  $P<0.05$ .



**Figure 3.** A) Steady-State Muscle IGF-1 mRNA Levels in Muscle Biopsy Samples Obtained from ST-Challenged and Control Pigs on D 0, 3, 7, and 14 Relative to Disease Challenge. IGF-1 mRNA levels were normalized to 18S rRNA and expressed as arbitrary units. B) Steady-state muscle IGFBP-3 mRNA levels in muscle biopsy samples obtained from ST-challenged and control pigs. IGFBP-3 mRNA levels were normalized to 18S rRNA and expressed as arbitrary units similar to IGF-1.