Temporal Change in Skeletal Muscle IGF-I mRNA Abundance and Nitrogen Metabolism Responses to Abomasal Casein Infusion in Steers¹

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ABSTRACT: Skeletal muscle IGF-I and α-actin mRNA responses to increased amino acid availability were investigated in young, rapidly growing steers. Four Holstein steers (208 kg BW) were surgically implanted with an abomasal cannula and jugular catheters and allowed 2 wk to recover. Steers were offered hourly a 43:57 forage:concentrate diet at 95% of ad libitum intake supplemented with continuous abomasal infusion of glucose (to replace 12.5% of metabolizable ad libitum energy intake) for 13 d before the start of abomasal infusion of 67 g of casein N/d. Biopsies of the liver and both semimembranosus muscles were removed and frozen in liquid N, and casein infusion was begun. Muscle biopsies were collected at 8, 16, 24, and 48 h, and on d 7 and 14. Nitrogen balance increased from 23.6 to 71.5 g/d (P < .001) within 24 h and remained elevated (mean = 58.4 g/d) during the 14 d of casein infusion. Plasma urea N increased from 4 to 9.5 mg/dL at 24 h and remained unchanged to d 14. Muscle IGF-I mRNA abundance increased to 215% of basal values at 16 h (P < .01), 244% of basal values at 24 h, and 222% of basal values at 48 h after initiation of casein infusion. Values reached a maximum of 274% of basal values on d 7 and then declined to near preinfusion levels on d 14. The IGF-I mRNA abundance was approximately 100 times higher in liver than in skeletal muscle and was not different on d 0 and 14. Although plasma IGF-1 concentrations were numerically higher during the first 24 h of abomasal casein infusion, they were not significantly higher during the chronic phase of treatment. Plasma IGF binding protein (BP)-2 concentrations were higher at 16, 24, and 48 h after casein infusion was begun, but IGFBP-3 concentrations were not altered at these sampling times. Neither acute (first 24 h) nor chronic (daily) plasma insulin concentrations were altered by abomasal casein infusion. Plasma somatotropin concentrations were lower (P = .008) at 24 h of casein infusion and beyond. Results suggest that enhanced amino acid availability may modulate skeletal muscle protein synthesis and accretion through an autocrine or paracrine IGF-I influence.

Key Words: Cattle, Skeletal Muscle, Growth, Nitrogen Metabolism, Casein


Introduction

Insulin-like growth factor I is involved in the regulation of postnatal skeletal muscle growth, but whether IGF-I exerts its effects in an endocrine, autocrine, or paracrine manner is not known. Myoblast and satellite cell proliferation and differentiation are stimulated by IGF-I in vitro (Allen and Rankin, 1990; Florini et al., 1991; Florini and Ewton, 1992), suggesting that it may exert an endocrine influence. Local IGF-I influence occurs in work-induced hypertrophy where muscle IGF-I mRNA abundance increases within 2 d of tenotomy in normal and hypophysectomized rats (DeVol et al., 1990). Although local IGF-I is considered an integral part of somatotropin-induced skeletal muscle growth (Isaksson et al., 1985), increased IGF-I mRNA abundance was not observed in enlarged skeletal muscles of pigs administered porcine somatotropin for 7 or 14 d (Grant et al., 1991; Coleman et al., 1994). Ramsay and coworkers (1995), however, observed that IGF-I mRNA abundance in liver, adipose tissue, and skeletal muscle increased to the greatest extent between 12 and 16 h after a single pST injection and exhibited differential rates of decline to 24 h postinjection. Their

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somatotropin, IGF-I, and IGF binding proteins changes in circulating concentrations of insulin, effect. We also sought to determine the associated acids causes an increase in IGF-I mRNA abundance in whether an increase postruminal supply of amino mechanism of action of pST (Ramsay et al., 1995). abundance occurs during the change, analogous to the deposition are also increased are not known. We hypothesized that an autocrine or paracrine influence of IGF-I is involved with the increased nitrogen response to increasing amino acid availability, and that a temporal change in IGF-I mRNA mechanism of action of pST (Ramsay et al., 1995).

Objectives of this experiment were to determine whether an increase in postruminal supply of amino acids causes an increase in IGF-I mRNA abundance in skeletal muscle and to define the time course of this effect. We also sought to determine the associated changes in circulating concentrations of insulin, somatotropin, IGF-I, and IGF binding proteins (IGFBP) during this treatment period.

Materials and Methods

The Cornell University Institutional Animal Care and Use Committee approved all procedures involving animals used in this experiment. Four Holstein steers approximately 7 mo of age were surgically implanted with an abomasal cannula and jugular catheters. Two additional steers were left unimplanted to serve as controls. Animals were allowed 2 wk to recover from surgery, during which ad libitum intake of the experimental diet (Table 1) was recorded. Mean intake during the final 7 d of this period was used to set the total metabolizable energy (ME) intake for the remainder of the experiment (95%). Abomasally infused glucose replaced 12.5% of ME intake on an individual animal basis for 14 d before the initiation of abomasal casein infusion. This was estimated to be equivalent to the energy contained in the casein infused. Casein was infused at a rate to achieve elevation of total N supply to 186% of daily dietary N intake on an individual animal basis. Glucose infusion was terminated when casein infusion was begun. The amount of feed offered daily was kept constant throughout the experiment and was provided in 24 equal amounts per day via automatic feeders to achieve steady state. Animals had continuous access to water. Daily N balance collections were conducted for 6 d before and for 14 d after initiation of abomasal casein infusion. Mean body weight before beginning baseline collections was 208 ± (SD) 7.8 kg. Animals were weighed before commencement of casein infusion and before slaughter on d 21 of the experiment.

Both hind legs were shaved, disinfected with Betadine, and rinsed with 70% ethanol before 5 to 10 mL of 2% lidocaine was administered s.c. to achieve local anesthesia. Subsequently, muscle biopsies (1 cm³) weighing approximately 500 mg were surgically removed from the semimembranous muscles with a #15 scalpel and trimmed of all adhering adipose and connective tissue. Right and left leg semimembranosus biopsies were removed in this manner and frozen in liquid N just before casein infusions were begun. Biopsies were also taken at 8, 16, 24, and 48 h after the initiation of casein infusion and on d 7 and 14. The incision was sutured, and a topical bactericidal preparation was applied. The initial biopsies were taken from the distal, lateral portion of the muscle. Subsequent biopsies were removed proximal and medial to the preceding biopsy to avoid muscle fibers previously sampled and to avoid wound drainage into succeeding biopsy sites.

Liver biopsies were obtained before initiating casein infusion and 16 d later. Following s.c. injection of lidocaine to achieve local anesthesia of the skin, samples were obtained before casein infusion with the use of an aspiration trocar inserted through an incision made just caudal to the last rib (Erwin et al., 1956), frozen in liquid N, and stored at −80°C. Postmortem liver samples were collected immediately after humane slaughter of each animal, frozen in liquid N, and stored at −80°C. Blood samples were collected via the jugular catheters daily for the 6-d “baseline” period, hourly for 8 h before, and hourly for 24 h after the initiation of casein infusion. Samples were also collected every 6 h for the next 24 h and daily for the remainder of the experiment.

Assay Protocols

Northern Analyses

Total RNA was extracted using density gradient CsCl cushion ultracentrifugation (Sambrook et al., 1989). Total RNA pellets were resuspended in buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) and

<table>
<thead>
<tr>
<th>Ingredient (air dry basis)</th>
<th>%</th>
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<tbody>
<tr>
<td>Ground shell corn</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Urea</td>
<td>1</td>
</tr>
<tr>
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<td>1</td>
</tr>
<tr>
<td>Vitamin and mineral mixb</td>
<td>1</td>
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<tr>
<td>Rumensin™</td>
<td>0.2</td>
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aProvided a minimum 2.9 g NaCl, 7.2 mg Mn, 8.5 mg Fe, 1.8 mg Mg, 1.1 mg Cu, 3 mg Co, .2 mg I, and .2 mg Zn per kg of diet DM. 

bProvided 2,200 IU Vitamin A, 1,100 IU Vitamin D, and 310 IU Vitamin E per kilogram of diet DM.
stored at -80°C. Aliquots of total RNA (20 μg) were denatured and electrophoretically separated. The RNA was transferred to Hybond N membranes (Amersham Corp., Arlington Hts., IL) with 10× SSC (1.5 M sodium chloride, 150 mM sodium citrate) and baked. The size of hybridized RNA was determined by including RNA markers in adjacent lanes. Marker lanes were cut from the gels before transfer to nylon membranes and stained with ethidium bromide. Blots were prehybridized in 50% formamide, 5× SSPE (150 mM sodium chloride, 10 mM sodium phosphate, 1 mM EDTA), 5× Denhardt’s solution (1× Denhardt’s: 0.02% BSA [fraction V, fatty acid free], 0.02% polyvinylpyrrolidone 40 (Sigma Chemical Co., St. Louis, MO), and .02% Ficoll 400 [Sigma]), 50 mM sodium phosphate buffer (pH 6.5), 1 mM EDTA, and .5 mg/mL tRNA (Boehringer Mannheim Biochemicals, Indianapolis, IN) for 2 h at 42°C. Hybridization solution was the same as prehybridization solution with the addition of 2 × 10^6 cpm/mL of a random-primed (Amersham Corp.) α-actin (L. Kedes, University of California, Los Angeles) cDNA probe. After an overnight hybridization, nylon membranes were washed for 10 min in 2× SSPE at room temperature and three times with .1× SSPE at 65°C for 45 min. Hybridized membranes were subjected to autoradiography with intensifying screens at 80°C. Blots were stripped and rehybridized under the aforementioned conditions using a random-primed (Amersham Corp.) 28S ribosomal RNA cDNA (provided by Lilly Research Laboratories, Greenfield, IN). Resulting autoradiographs were scanned using a laser densitometer to reveal relative abundance of each transcript. Muscle and liver IGF-I and α-actin expression were normalized to constitutively expressed 28S ribosomal mRNA.

Ribonuclease Protection Assay

Relative amounts of muscle IGF-I mRNA were quantified with a RNase protection assay similar to that described by Grant et al. (1991). A 710-bp cDNA fragment of the coding sequence for bovine IGF-I cloned into the Smal site of pBluescript was generously provided by Russ Anthony (Colorado State Univ., Ft. Collins). The IGF-I plasmid was linearized with HindIII (Promega, Madison, WI), and high specific activity cRNA probes were synthesized using T3 RNA polymerase (Promega) and [32P]CTP (400 Ci/mmol). Samples (50 μg total RNA) were annealed with 5 × 10^5 cpm of labeled probe for 18 h at 45°C in hybridization solution (80% formamide, 400 mM NaCl, 40 mM PIPES, 1 mM EDTA; pH 6.4). As a control, an internal standard was added to each reaction to ensure accurate normalization of the expression data. After hybridization, samples were treated with RNase A and T1 for 30 min at 37°C (Ambion, Austin, TX). The RNA was precipitated, reconstituted in loading buffer, denatured at 90°C for 4 min, and separated by electrophoresis in 8 M urea/5% acrylamide gels. Following electrophoresis, gels were dried and subjected to autoradiography. Relative IGF-I expression data were normalized to 28S ribosomal mRNA.

Metabolite Assays

Plasma glucose concentrations were measured using the Sigma kit (procedure no. 510, Sigma Diagnostics, St. Louis, MO) for colorimetric determination. Intraassay and interassay CV were 2.3 and 3.3%, respectively. Plasma urea nitrogen (PUN) was measured using the method of Chaney and Marback (1962) for colorimetric determination. Intraassay and interassay CV were 2.3 and 2.8%, respectively. Plasma NEFA concentrations were determined using a modification of an enzymatic colorimetric kit, (NEFA-C, Wako Pure Chemical Industries, Dallas, TX). Reagents A and B were diluted further to increase the number of samples assayed per kit. Sample volume was reduced to 25 μL instead of the recommended 50 μL. Intraassay and interassay CV were 2.2 and 2.8%, respectively. Urinary creatinine concentrations were measured using the sigma kit (procedure no. 555, Sigma Diagnostics) for colorimetric determination. The intraassay and interassay CV were 1.8 and 3.4%, respectively.

[125I]IGF-I Ligand Blot Assay

Ligand blotting with [125I]IGF-I was used to detect differences in plasma IGFBP concentrations in samples collected at the same time intervals as the muscle biopsies. Plasma samples were diluted 1:10 with 2× Scleicher and Schell sample buffer and subjected to SDS PAGE on gels consisting of 3.75% stacking and 10% separating gel. Separated bands were electrophoretically transferred onto nitrocellulose paper at 4°C. The blots were rinsed with saline (.1 M NaCl, .01 M Tris-HCl; pH 7.4) plus 1% Triton X-100 for 30 min, incubated with saline plus 1% BSA for 2 h, and then incubated with saline plus .1% Tween 20 for 10 min. Following these rinses, the blots were incubated overnight at 4°C with saline containing 1% BSA, .1% Tween 20, and 133,333 cpm [125I]IGF-I/mL buffer. The blots were then rinsed twice in saline plus .1% Tween 20 (15 min each) and then rinsed three times in saline (15 min each). The blots were allowed to dry and placed in a cassette with the Imager Plate for 19.5 h. Molecular size was estimated from the relative mobility of prestained protein standards (Bio-Rad, Hercules, CA). Identification of IGFBP-3 and IGFBP-2 bands was based on specific antisera immunoblotting molecular weight (M_r) estimates of 43,000 and 39,000 for IGFBP-3 and 34,000 for IGFBP-2, respectively, in bovine serum (Cohick et al., 1992). Band intensities on autoradiograms were evaluated by densitometry (FUJIX BAS, Japan).
Hormone Assays

Plasma insulin concentrations were determined using a Linco double-antibody RIA kit (Linco Research, St. Louis, MO). The procedure is based on the competition of circulating insulin and bovine \(^{[125I]}\)insulin for binding to guinea-pig anti-porcine insulin antibody (lot # 122-84SP) during incubation. The bound antibody-antigen complex was precipitated through the use of carrier (guinea pig IgG carrier, lot # NGP020) and a second antibody (goat anti-guinea pig IgG serum, lot # GP2016). Bovine insulin was obtained from Lilly Research Laboratories (Indianapolis, IN; lot # 615-70N-80; used for standards and tracer). Intraassay and interassay CV were 12.0 and 14.2%, respectively.

Plasma IGF-I concentrations were determined with a double antibody RIA (O'Connor et al., 1991; Plaut et al., 1991). The IGF-I binding proteins were dissociated and inactivated by acid, and plasma was incubated with 1 M glycyglycine HCl, pH 2.1, for 48 h at 38°C in polystyrene tubes. Acidified plasma was applied to Sephadex G-100 columns (.70 cm × 30 cm) and eluted with 1.0 M acetic acid to separate free IGF-I from the binding proteins. The column fraction containing free IGF-I was neutralized and resuspended in 1 mL of assay buffer. Recombinant n-methionyl human IGF-I was obtained from Gro-Pep (Adelaide, Australia; lot # GJA-C01) and used for iodination. Recombinant n-methionyl human IGF-I used for standards was provided by Dan Burleigh at Pitman-Moore, Inc. (Northbrook, IL; lot # 742-42). First antibody (murine monoclonal anti-human IGF-I) was obtained from NIADDK-NIH (batch #2) was diluted in non-immune murine serum obtained from Cambridge Medical Technology (Billerica, MA; lot # 019310). The second antibody (goat anti-mouse gamma-globulin) was obtained from Pel-Freez (Rogers, AR; lot # 13410). Intraassay CV was 7.8%. Only one assay was necessary.

Plasma ST concentrations were determined with a double antibody RIA (Beermann et al., 1991) using bST from Miles Laboratories (lot 12, 1.3 IU/mg protein; Naperville, IL) for iodination and standards and rabbit anti-ovine ST antiserum (NIADDK-anti-oGH-2). Intraassay and interassay CV were 9 and 14%, respectively. Somatotropin data were subjected to analysis by PC-Pulsar (Merrian and Wachter, 1982) to estimate baseline and peak amplitude concentrations, peak frequency, and mean concentrations.

Statistical Analysis

Nitrogen balance, hormone, and metabolite data were analyzed by analysis of variance using SAS GLM procedures (SAS, 1988) with animal and time main effects in a split-plot design. Skeletal muscle IGF-I mRNA and \(\alpha\)-actin mRNA abundance data were first analyzed by analysis of variance with animal, leg, and time main effects in a split-plot design. Because no significant differences between legs were observed, means for both legs were used in the final analysis. Individual means were compared by Duncan's Multiple Range Test when time main effects were significant.

Results and Discussion

Nitrogen balance averaged 24 g/d for the 6 d preceding initiation of the abomasal infusion of casein and increased to 71.5 g/d (\(P < .001\)) within 24 h of starting the continuous infusion. Nitrogen balance declined to 60 g/d within 3 d but remained elevated at a mean of 58.4 g/d during the 14 d of casein infusion (Figure 1). The rate of casein infusion was chosen based on results from earlier studies in our laboratory in which near-maximum rates of N balance were achieved in similar animals that received abomasal infusion of 90 g of casein N per day (Robinson et al., 1995). The objective was to create a large increase in amino acid absorption into the circulation and to stimulate skeletal muscle protein synthesis and deposition rates to the greatest extent possible without suppressing feed intake. Daily DM intake averaged 4.33 and 4.17 kg/animal (\(P = .05\)) during the glucose and casein infusion phases, respectively. This difference in intake reflects differences in the DM concentration of the diets because refusals were rare and small in both phases of the experiment. The corresponding daily N intake was 81.9 and 147.6 g (\(P < .001\)). The increase in N supply by casein infusion (80%) was close to the target of 86%.

The increase in N retention caused by casein infusion was reflected in an increase (\(P < .05\)) in body weight gain (764 g/d and 1,402 g/d during the glucose and casein phases, respectively). This difference in intake reflects differences in the DM concentration of the diets because refusals were rare and small in both phases of the experiment. The corresponding daily N intake was 81.9 and 147.6 g (\(P < .001\)). The increase in N supply by casein infusion (80%) was close to the target of 86%.

Plasma urea N concentrations were increased from 4 to 9.5 mg/dL during the first 24 h of casein infusion (\(P < .01\)) and remained unchanged throughout the treatment period (Figure 2). Preinfusion values agree with results reported for well-nourished cattle of similar genotype and body weight to which similar diets formulated to accommodate daily body weight gains of 1 kg/d (NRC, 1984) were fed (Houseknecht et al., 1992). Plasma urea N concentrations observed during
Figure 1. Daily N balance means for the four steers before and throughout the 20 d of continuous abomasal infusion. SEM = 1.8. Glucose solution was infused into the abomasum for the 6 d before starting the casein infusion to provide similar levels of metabolizable energy intake throughout the experimental period. The first 24 h after substituting casein for glucose is represented as d 1.

The abomasal casein infusion are similar to those observed in animals of similar genotype used in our earlier studies (Robinson et al., 1995).

Muscle IGF-I mRNA abundance was not different between the left and right legs at any time interval. Figure 3 is an autoradiogram that shows representative results of the ribonuclease protection assay. Values observed at d 0 and at 8 h (95% of basal) were also not different. Higher levels (P < .01) were observed at 16 h (215% of basal), 24 h (244% of basal), and 48 h (222% of basal) after initiating casein infusion (Figure 4). The IGF-I mRNA abundance increased to the greatest extent (274% of basal) on d 7 and then declined to near pre-infusion levels (131% of basal) on d 14. Results observed in control steers demonstrate the lack of measurable change in semimembranosus IGF-I mRNA abundance in response to the biopsy procedure at any time during the 14-d treatment period. We believe, however, that the anatomical pattern of skeletal muscle biopsy is critical for avoiding possible confounding effects of wound healing on subsequent biopsies.

The time course of the initial IGF-I mRNA abundance response to casein infusion seems to be similar to that observed in latissimus dorsi, semitendinosus, and vastus lateralis muscles of growing pigs administered a single s.c. injection of pST, although the time at which maximum levels were observed ranged from 8 to 16 h in these three muscles (Ramsay et al., 1995). Overload-induced skeletal muscle hypertrophy also exhibits a similar time course of IGF-I mRNA expression. Adams and Haddad (1996) observed a five- to sixfold increase (maximum) at 3 to 7 d following surgical removal of synergistic muscles, and a subsequent progressive decline in IGF-I mRNA at 14 and 28 d to near levels observed in control muscles. Results of our study and others provide strong evidence that sampling time is as critical as muscle selection in facilitating the detection of treatment effects on muscle growth.

The magnitude of response to abomasal casein infusion was twice as large as the response to the single pST injection but was similar to the twofold increase in IGF-I gene expression observed with low to high levels of dietary crude protein intake in pigs. Brameld et al. (1996) observed 228 and 184% greater (P < .10) expression of IGF-I mRNA abundance in semitendinosus muscles of pigs fed diets containing 16.2 and 19.4% crude protein compared to those of pigs fed 9.9 or 13.1% crude protein diets. Similar responses were not observed in the longissimus muscle, however, and the level of expression was greater in the longissimus than in the semitendinosus muscles at all levels of dietary percentage of crude protein.
Lack of diet and pST administration effect on IGF-I mRNA abundance response in the longissimus muscle was observed previously (Grant et al., 1991) and confirmed by Brameld et al. (1996). The basis for this difference is not known but was attributed to possible interactions of fiber type and differential pST response among muscles. The presence of differential IGF-I mRNA abundance response between individual muscles in an animal observed by Brameld et al. (1996) needs further investigation. We chose the semimembranosus muscle because we have observed significant increases in protein mass and weight of this muscle in steers administered close arterial infusion of .5 g/min of the β-agonist cimaterol (Byrem et al., 1998). We have also observed significant increases in net flux of essential amino acids across the hind limb of similar steers treated with cimaterol (Byrem et al., 1998) or administered abomasal casein infusion (Robinson et al., 1995). Samples were taken from only one muscle because serial biopsies were required in our experimental design, and the semimembranosus provided the largest surface area available for surgical surface biopsies with convenient fiber length orientation.

α-Actin mRNA abundance was also increased in a time-dependent manner after initiation of abomasal casein infusion (Figure 5). Significant increases (175%) in α-actin mRNA abundance were not observed until 48 h after starting the casein infusion. In contrast to the IGF-I mRNA response, α-actin mRNA abundance reached a maximum after 2 d, declined between 2 and 7 d of infusion, and then remained constant at 130% of basal levels of expression between 7 and 14 d. These data provide evidence that genes expressing myofibrillar proteins respond in association with the 100% increase in whole-body N balance over the course of the casein infusion. The later increase in α-actin mRNA abundance, when compared to the initial increase in IGF-I mRNA abundance, fits with the expected temporal pattern of growth factor gene activation preceding activation of the genes responsible for increasing rates of myofibrillar protein synthesis. Muscle IGF-I concentrations increase fourfold to sixfold between 3 and 7 d after surgically induced
Figure 4. Changes in IGF-I mRNA abundance in semimembranosus muscle biopsies taken from both legs of the four steers that received abomasal casein infusion and taken from only one leg of the two control steers. Values are expressed as percentage difference from basal (preinfusion sample) values normalized against the control steer samples that were assigned a value of 1. Asterisks denote values that were different from basal levels of abundance ($P < .05$). SEM = 57. No difference was observed between the contralateral hind legs of the infused steers, and no change in IGF-I mRNA abundance was observed in control steers, demonstrating the lack of measurable effect of the biopsy procedure on IGF-I mRNA abundance in serial muscle biopsies.

Table 2. Plasma somatotropin concentrations and secretion pattern responses to abomasal infusion of casein in steers

<table>
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<tr>
<th>Day</th>
<th>Mean concentration</th>
<th>Baseline concentration</th>
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<th>Peak amplitude</th>
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<tr>
<td>0</td>
<td>6.5</td>
<td>4.7</td>
<td>1.75</td>
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</tr>
<tr>
<td>1</td>
<td>5.2</td>
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<td>1.25</td>
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Somatotropin concentration values are expressed in units of nanograms/milliliter. Data represent samples taken at 15-min intervals for 6 h from four steers. Data were analyzed by PC-Pulsar.

Figure 5. Change in α-actin mRNA abundance in semimembranosus muscle biopsies taken from both legs of the four steers that received abomasal casein infusion. Values are expressed as percentage difference from basal (preinfusion sample) values. Asterisks denote values that were different from basal levels of abundance ($P < .005$). SEM = 6.
Figure 6. Plasma IGF-I concentrations before and after initiation of abomasal casein infusion. Values shown are means for four steers. Blood samples were collected via jugular catheters inserted at the beginning of the experiment. SEM = 38.

14 of casein infusion. Plasma IGFBP-2 concentrations increased (P < .05) to levels 1.3, 1.2, 1.3, and 1.6 of those present before initiating casein infusion at 16, 24, and 48 h and 14 d of casein infusion, respectively.

Mean plasma ST concentrations were lower (P < .01) during abomasal infusion of casein at d 2, 7, and 14 of the experiment (Table 2). A similar trend (P = .16) was observed for baseline ST concentrations. Number of episodic secretions, expressed as peaks/6 h, was not affected, but peak amplitude was higher after 24 h of casein infusion than at any other sampling time.

Neither somatotropin nor IGF-I concentrations were elevated in similar steers infused with 20 g of casein N/d (Houseknecht et al., 1992) or higher rates (Robinson et al., 1995) via abomasal infusion. Abomasal infusion of casein and glutamate to increase total N intake to 150% of dietary intake increased insulin and IGF-I concentrations in beef steers of similar weight, but dietary crude protein level was low (8.5%) and no glucose infusion was used during the pretreatment period (Reecy et al., 1996). Plasma IGF-I concentrations were high in the steers used in this study, and the early increases were relatively small and not significant. The cause for the decline in ST mean concentrations is not clear but suggests that increased concentrations of amino acids in the circulation may mediate a decreased rate of ST secretion or an increased clearance rate.

In summary, abomasal casein infusion was used to study the acute and chronic effects of increased amino acid availability on skeletal muscle IGF-I mRNA and α-actin mRNA abundance in growing steers. A transient increase in both was observed, but neither circulating insulin nor IGF-I concentrations were increased during the treatment period. Results suggest, therefore, that enhanced amino acid availability, achieved through abomasal casein infusion in this study, may modulate increased muscle growth through an autocrine or paracrine IGF-I influence.

Implications

Results demonstrate that increased postruminal protein intake alone, at a constant level of energy
intake, is capable of inducing insulin-like growth factor I (IGF-I) gene expression in skeletal muscle in young, rapidly growing steers. Results provide evidence that enhanced amino acid availability alone may modulate skeletal muscle protein synthesis and accretion through an autocrine or paracrine IGF-I influence, although involvement of endocrine influences by circulating IGF-I or IGF binding proteins cannot be ruled out.

Literature Cited


