Effects of flax supplementation and a combined trenbolone acetate and estradiol implant on circulating insulin-like growth factor-I and muscle insulin-like growth factor-I messenger RNA levels in beef cattle[1,2]

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ABSTRACT: We evaluated effects of a 5% (dry matter basis) ground flaxseed supplement (flax) and a trenbolone acetate and estradiol-17β implant, Revalor-S, on circulating IGF-I and muscle IGF-I messenger RNA (mRNA). Sixteen crossbred yearling steers (initial BW = 397 kg) were assigned randomly to one of four treatments: 1) flax/implant; 2) nonflax/implant; 3) flax/nonimplant; and 4) nonflax/nonimplant. Serum was harvested from blood collected on d 0 (before implant or flax addition), 14, and 28, and used in subsequent analyses of circulating IGF-I. Biopsy samples (0.5 g) were obtained from the longissimus muscle on d 0, 14, and 28. Total RNA was isolated from the muscle samples, and real-time quantitative-PCR was used to assess relative differences in IGF-I mRNA. Flax supplementation had no effect ($P > 0.10$) on circulating IGF-I concentrations. Following implantation, sera from implanted steers had 52 and 84% greater ($P < 0.05$) IGF-I concentrations than sera from nonimplanted steers on d 14 and 28, respectively. On d 28, local muscle IGF-I mRNA levels increased 2.4-fold ($P < 0.01$) in biopsy samples obtained from implanted compared with nonimplanted steers. Muscle biopsy samples from nonflax cattle had 4.4-fold higher ($P < 0.01$) levels of IGF-I mRNA than those from flax cattle on d 28. To determine whether a component of flax, α-linolenic acid (αLA), was directly responsible for IGF-I mRNA down-regulation, we incubated primary cultures of bovine satellite cells, from implanted and nonimplanted steers, in two concentrations of αLA (10 n*M* and 1 μ*M*). An implant $\times$ dose interaction ($P < 0.05$) was observed for IGF-I mRNA concentrations in bovine satellite cells cultured for 72 h with αLA. Satellite cells from nonimplanted steers had similar ($P > 0.10$) IGF-I mRNA concentration regardless of the level of αLA exposure; however, satellite cells from implanted steers exposed to 10 n*M* and 1 μ*M* αLA had 2.5- and 2.0-fold greater IGF-I mRNA levels, respectively, than cells from implanted steers that were not exposed to αLA ($P < 0.05$). Administration of a Revalor-S implant increased circulating IGF-I and local muscle IGF-I mRNA concentrations in finishing cattle. However, muscle IGF-I mRNA levels were decreased by flax supplementation. Muscle cell culture experiments suggested that αLA was not responsible for the IGF-I mRNA down-regulation.

Key Words: Estradiol-17β, Flaxseed, Insulin-Like Growth Factor-I, α-Linolenic Acid, Steers, Trenbolone Acetate

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Introduction

Revalor-S is a combined trenbolone acetate (**TBA**) and estradiol-17β (E_2) implant. Administering Revalor-S improved both ADG and feed efficiency in feedlot cattle (Bartle et al., 1992; Johnson et al., 1996a). Additionally, serial harvest studies have reported that carcass protein and longissimus muscle area are increased in TBA/E_2 implanted steers relative to nonimplanted steers. Thus, it seems that a combined TBA/E_2 implant enhances muscle growth in feedlot cattle. Revalor-S-implanted cattle have higher serum IGF-I concentrations compared to nonimplanted cattle as soon as 6 d following implantation (Johnson et al., 1996b). In addition, longissimus muscle samples obtained from implanted steers had greater abundance of IGF-I messenger RNA (mRNA) than nonimplanted cattle 32 to 40 d after implantation (Johnson et al., 1998b; White et al., 2003). These changes in IGF-I could be partially

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responsible for the enhanced lean tissue accretion observed in feedlot cattle implanted with Revalor-S.

Dietary additions of omega-3 PUFA, in the form of flaxseed, to beef cattle diets have resulted in incorporation of these PUFA into the neutral and phospholipid fractions of muscle tissue (Scollan et al., 2001; LaBrune, 2000). When PUFA are incorporated into membranes, the fluidity of the membrane (Ginsberg et al., 1981) and IGF-I binding are significantly increased (Liu et al., 1994). It is possible that the addition of dietary omega-3 PUFA could enhance the binding of the increased levels of IGF-I produced as a result of implantation and result in enhanced lean tissue accretion superior to what has been observed in implanted cattle. Consequently, the objectives of the current study were to evaluate the changes in muscle IGF-I mRNA levels over the first 28 d of the finishing phase and to evaluate potential interactive effects of a 5% ground flaxseed supplement (flax) and a combined TBA (120 mg) and E_2 (24 mg) implant, Revalor-S, on circulating IGF-I and muscle IGF-I mRNA levels.

Table 1. Composition of experimental diets

Ingredient	Treatments: Flax	No Flax
	— Percentage of DM —	
Steam-flaked corn	77.54	82.54
Ground alfalfa hay	8.00	8.00
Corn steep liquor	5.90	6.00
Ground flaxseed	5.10	—
Dehulled soybean meal	0.49	0.49
Urea, 46% N	0.67	0.67
Limestone	1.71	1.71
Vitamin/mineral premix[a]	0.59	0.59
Rumensin/Tylan premix, kg/(steer·d)[b]	0.23	0.23
Dry matter, %	80.35	79.96
Crude protein, %	14.60	13.91

[a]Vitamin/trace mineral premix formulated to provide (total diet dry matter): 0.36% salt, 3,175 IU/kg vitamin A, 175 IU/kg vitamin E, 0.2 mg/kg cobalt, 13mg/kg copper, 72mg/kg manganese, 0.30 mg/kg selenium, 72 mg/kg zinc, and 75 mg/kg iodine.

[b]Elanco Animal Health, Indianapolis, IN. Fed to supply 330 mg of monensin and 90 mg of tylosin per steer daily in a ground corn carrier.

Materials and Methods

Animals. All experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee. Sixteen yearling crossbred steers with an average initial BW of 397 kg were stratified by weight and randomly and equally assigned, in a 2 × 2 factorial arrangement, to one of four treatments: 1) flax/implant; 2) nonflax/implant; 3) flax/nonimplant; and 4) nonflax/nonimplant. Steers assigned to the implant treatments were administered a Revalor-S implant in their right ear following the bleeding and biopsy procedures on d 0. Beginning 27 d before the initiation of the study, steers were stepped up to a 92% concentrate diet offered for ad libitum intake throughout the study (Table 1). On d 0, 14, and 28, venous blood samples were collected and sera were harvested for use in analyses of circulating IGF-I. Biopsies of the longissimus muscle, between the ninth and last rib, were taken from all steers on d 0 (before implant and flax addition), 14, and 28, as described below. The first and third biopsies were on the right side of the animal, but the third biopsy sample was obtained 5 cm anterior to the first.

Longissimus Muscle Biopsy. Steers were restrained in a hydraulic squeeze chute, hair was removed from the biopsy site, and a local anesthetic (lidocaine HCl; 20 mg/mL; 8 mL per biopsy site) was administered. A sterile cloth drape was placed over the biopsy site and a 1-cm incision was made with a scalpel. A sterile Bergstrom biopsy needle (6 mm) was used to obtain the tissue (0.5 g) from the longissimus muscle. The incision was closed with veterinary tissue glue and sprayed with a topical antibiotic followed immediately by application of a spray-on aluminum bandage. All steers were monitored for swelling 24 h after the biopsy.

Analysis of Circulating IGF-I. Blood collected from each steer on d 0, 14, and 28 was allowed to clot for 48 h at 4°C. Following centrifugation, sera were harvested and stored at –20°C for subsequent analyses of circulating IGF-I. Sera were analyzed for IGF-I using a commercially available two-site immunoradiometric assay (Diagnostic Systems Laboratories, Inc., Webster, TX) previously validated for use in bovine serum (Greenwood et al., 2001). Insulin-like growth factor binding protein interference was eliminated by acid-ethanol extraction. All samples were run in a single assay and had an intraassay coefficient of variation of 8.3%.

Sample Preparation and RNA Isolation. Muscle biopsy samples (0.5 g) from each steer were stored suspended in 5 mL of RNALater (Ambion, Austin, TX) in polypropylene tubes at –20°C. Samples were subsequently homogenized in 10 mL of a 5 *M* guanidine thiocyanate, 50 m*M* Tris-HCl, 25 m*M* EDTA, 0.5% lauryl sarcosine, and 1% β-mercaptoethanol solution (**Solution D**), followed by rapid freezing in liquid nitrogen and storage at –80°C for later RNA isolation. Total RNA was isolated according to Chomczynski and Sacchi (1987). Briefly, sodium acetate (2 *M*; pH 4.0), phenol, and chloroform/isoamyl alcohol (24:1, vol/vol) were added to a 2-mL aliquot of homogenized muscle sample. Samples were vortexed, chilled on ice for 15 min, and centrifuged at 10,000 × g for 20 min at 4°C. The aqueous layer was transferred to a new tube and reextracted with phenol and choloroform/isolamyl alcohol (24:1, vol/vol). Again, samples were vortexed, chilled on ice for 15 min, and centrifuged at 10,000 × *g* for 20 min at 4°C. After the second extraction, the aqueous layer was transferred to a new tube, mixed with cold isopropanol, chilled on ice for 15 min, and centrifuged at 10,000 × *g* for 20 min at 4°C. The resulting pellets were dissolved in Solution D and precipitated with 75% ethanol and dissolved in diethyl pyrocarbonate-treated water. The concentra-

tion of RNA was determined by absorbance at 260 nm. Electrophoresis of total RNA through a 1% agarose-formaldehyde gel followed by ethidium bromide staining to allow visualization of 28S and 18S ribosomal RNA (rRNA) was used to assess the integrity of RNA. After RNA integrity was assessed, samples were DNased to remove any contaminating genomic DNA using a commercially available kit (DNA-free; Ambion). One microgram of total RNA was then reverse-transcribed to produce the first-strand complementary DNA (cDNA) using TaqMan reverse transcription reagents and MultiScribe reverse transcriptase (Applied Biosystems, Foster City, CA) and following the protocol recommended by the manufacturer. Random hexamers were used as primers in cDNA synthesis.

Real-Time Quantitative PCR. Real-time quantitative-PCR was used to measure the quantity of IGF-I mRNA relative to the quantity of 18S rRNA in total RNA isolated from longissimus muscle tissue of steers. Measurement of the relative quantity of cDNA was carried out using TaqMan Universal PCR Master Mix (Applied Biosystems), 900 n*M* concentration of the appropriate forward and reverse primers, 200 n*M* concentration of appropriate TaqMan detection probe and 1 μL of the cDNA mixture. Bovine specific IGF-I forward and reverse primer and TaqMan detection probe were synthesized using published GenBank sequences (Genbank Accession #X15726). The sequences are as follows: forward primer; TGTGATTTCTTGAAGCAGGTGAA, reverse primer; AGCACAGGGCCAGATAGAAGAG, and TaqMan probe; 6-FAM-TGCCCATCACATCCTCCTC-GCA-TAMRA. Commercially available eukaryotic 18S rRNA primers and probe were used as an endogenous control (Applied Biosystems; Genbank Accession no. X03205). Assays were performed in an ABI Prism 7000 sequence detection system, (Applied Biosystems) using thermal cycling parameters recommended by the manufacturer (50 cycles of 15 s at 95°C and 1 min at 60°C). Relative expression of IGF-I was normalized with the 18S rRNA endogenous control and expressed in arbitrary units. Titration of 18S and IGF-I primer against increasing amounts of cDNA gave linear responses with slopes of –3.6 to –3.9.

*Alpha-Linolenic Acid (**αLA**) Exposure and Bovine Muscle Cell Culture IGF-I mRNA Concentrations.* Satellite cells were isolated from the semimembranosus muscle of nonimplanted and Revalor-S-implanted steers (n = 4) as described previously (Johnson et al., 1998a). The implanted steers received Revalor-S implants 56 d prior to slaughter. The nonimplanted steers had not received implants within the last 150 d and had never received TBA implants. Primary cultures of satellite cells were plated on tissue culture plates (9.62 cm^2/well) precoated with reduced growth factor-Matrigel (Becton Dickinson Labware, Franklin Lakes, NJ) diluted 1:9 (vol/vol) with DMEM. Cells were plated in 10% FBS/DMEM and stock solutions of αLA ([linolenic acid (9, 12, 15-octadecatrienoic acid], L-2376; Sigma Chemical Co., St. Louis, MO) dissolved in ethanol were added to each well immediately after plating to yield concentrations of 0.2% ethanol and either 10 n*M* or 1 μ*M* αLA. Control cultures were also exposed to 0.2% ethanol. All cultures were incubated at 37°C, 5% CO_2, in a water-saturated environment. At 48 h, cells were rinsed three times with DMEM, fed with fresh 10% FBS/DMEM, and the same concentrations of αLA were restored. Following 72 h of αLA exposure, total RNA was isolated from cells using the Absolutely RNA Microprep Kit (Stratagene, La Jolla, CA). The concentration of RNA was determined by absorbance at 260 nm. Electrophoresis of total RNA through a 1% agarose-formaldehyde gel followed by ethidium bromide staining to allow visualization of 28S and 18S rRNA was used to assess the integrity of RNA. One microgram of RNA was then reverse-transcribed to produce the first-strand cDNA using TaqMan Reverse Transcriptase Reagents (Applied Biosystems) following the protocol recommended by the manufacturer. Random hexamers were used as primers in cDNA synthesis. Real-time quantitative-PCR was used to measure the abundance of IGF-I mRNA relative to the abundance of 18S rRNA in total RNA isolated from primary cultures of muscle satellite cells as described above.

Statistical Analysis. Data were analyzed as a 2 × 2 factorial in a completely randomized design with four replicates and repeated measures over time. A split-plot analysis was employed to account for the repeated measures using the MIXED model procedure of PC SAS Release 8.1 (SAS Inst., Inc., Cary, NC), with steer serving as the whole-plot experimental unit. When interactions were detected ($P < 0.05$, unless otherwise noted), least squares means were separated ($P < 0.05$). Bovine satellite cell IGF-I mRNA data were analyzed as a 2 × 3 factorial using the Mixed Model of PC SAS Release 8.1. All main effect and interaction means were separated ($P < 0.05$) using the Least Significant Difference procedure when the respective F-tests were significant ($P < 0.05$).

Results

Performance. Average daily gains were calculated using individual steer weights obtained on d 0, 14, and 28. A flax implant interaction ($P < 0.05$) was observed for ADG from d 0 to 28 (Figure 1). The interaction is significant because the nonflax/implant steers had numerically higher gains than the flax/implant steers (2.27 kg/d vs. 2.08 kg/d), whereas the flax/nonimplant steers had numerically higher gains than the nonflax/nonimplant steers (1.56 kg/d vs. 1.30 kg/d).

Serum IGF-I Concentrations. Serum IGF-I concentrations were similar among all treatments on d 0 ($P > 0.10$). On d 14 and 28 following implantation, sera from implanted steers had 53% (560 ng/mL vs. 367 ng/mL) and 85%, respectively (708 ng/mL vs. 383 ng/mL) greater IGF-I concentrations ($P < 0.05$), relative to sera from nonimplanted steers (Figure 2A). Serum IGF-I concentrations increased 26.3% ($P < 0.05$) in sera obtained from implanted steers from d 14 to 28 (Figure

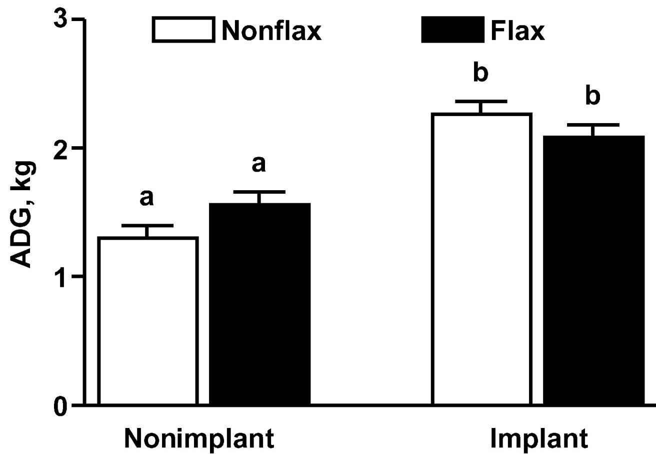

Figure 1. Average daily gains of steers from d 0 to 28. Bars with different letters differ, $P < 0.05$. Pooled SEM = 0.10 kg/d, n = 4.

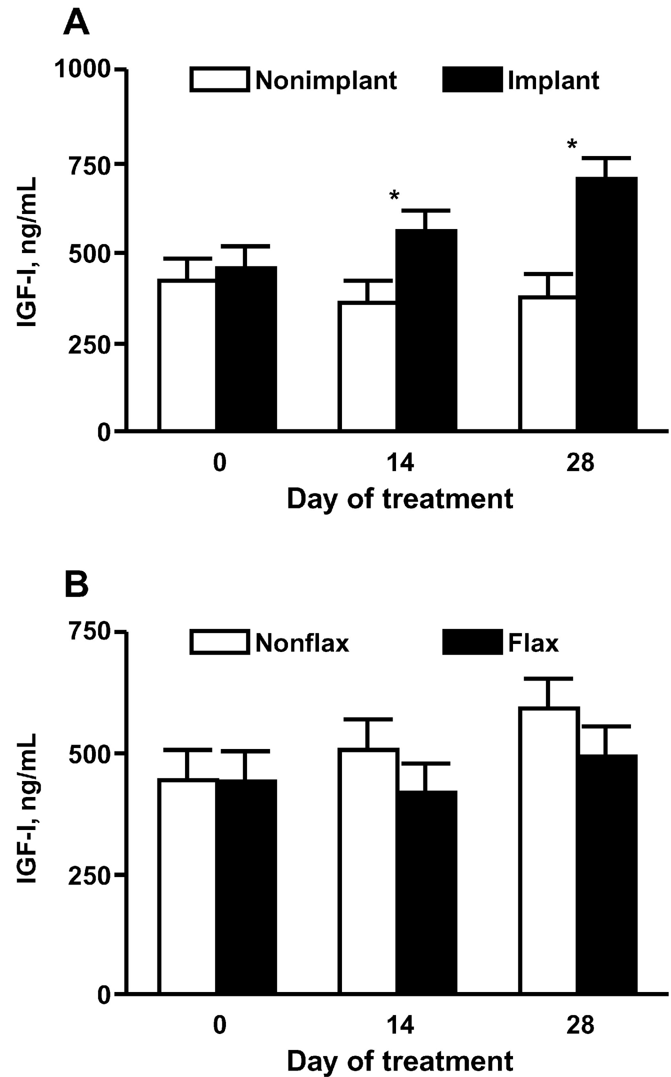

Figure 2. Insulin-like growth factor-I concentrations in sera obtained from Revalor-S-implanted and nonimplanted steers (Panel A) and flax-supplemented and nonflax-supplemented steers on d 0, 14, and 28 (Panel B). The asterisk indicates circulating IGF-I concentrations in the implanted and nonimplanted groups, on a given day, are different, $P < 0.05$. Pooled SEM = 60.2 ng/mL, n = 8.

2A). On d 28 subsequent to implantation, serum IGF-I levels in implanted steers increased 54% ($P < 0.001$) relative to concentrations measured in the same animals on d 0 (Figure 2A). However, serum IGF-I concentrations in nonimplanted steers did not differ ($P < 0.10$) on d 14 and 28 from concentrations of samples obtained from those same steers on d 0 (Figure 2A). Finally, flax did not alter ($P > 0.10$) serum IGF-I concentrations compared to serum collected from nonflax cattle (Figure 2B).

Longissimus Muscle IGF-I mRNA Concentrations. Longissimus muscle IGF-I mRNA concentrations did not differ ($P > 0.10$) among biopsy samples collected from implanted and nonimplanted steers on either d 0 or 14 (Figure 3A). However, on d 28, samples from implanted steers had 2.4-fold greater ($P < 0.001$) muscle IGF-I mRNA levels than samples from nonimplanted steers (Figure 3A). Muscle IGF-I mRNA concentrations did not differ among biopsy samples collected from flax and nonflax steers on either d 0 or 14 (Figure 3B). Nonflax steers had 4.4-fold greater ($P < 0.001$) muscle IGF-I mRNA levels than flax steers on d 28 (Figure 3B). There was a strong tendency ($P = 0.06$) for a flax × implant × day interaction in muscle IGF-I mRNA levels in biopsy samples obtained from steers on d 28 (Figure 4). Muscle biopsy samples collected from nonflax/implanted steers had 5.1-fold greater ($P < 0.01$) muscle IGF-I mRNA levels than flax/implanted steers whereas muscle samples from nonflax/nonimplanted steers had 3.3-fold greater ($P < 0.05$) muscle IGF-I mRNA levels than flax/nonimplanted steers (Figure 4).

Bovine Satellite Cell IGF-I mRNA Concentrations. To determine whether a component of flax, αLA, was directly responsible for IGF-I mRNA down-regulation, we incubated primary cultures of bovine satellite cells, from implanted and nonimplanted steers, in two concentrations of αLA (10 nM and 1 μM). An implant × dose interaction ($P < 0.05$) was observed for IGF-I mRNA concentrations in bovine satellite cells cultured for 72 h with αLA. Satellite cells from nonimplanted steers had similar ($P > 0.10$) IGF-I mRNA relative abundance regardless of the level of αLA exposure (Figure 5). However, satellite cells from implanted steers exposed to 10 nM and 1 μM αLA had 2.5- and 2.0-fold greater IGF-I mRNA levels, respectively, than cells from implanted steers that were not exposed to αLA ($P < 0.05$). Satellite cells from implanted and nonimplanted steers that were not exposed to αLA had similar ($P > 0.10$) IGF-I mRNA concentrations (Figure 5). Satellite cells from implanted steers that were exposed to 10 nM αLA had 7.7-fold higher ($P < 0.05$) IGF-I mRNA concentrations than cells from nonimplanted steers (Figure 5).

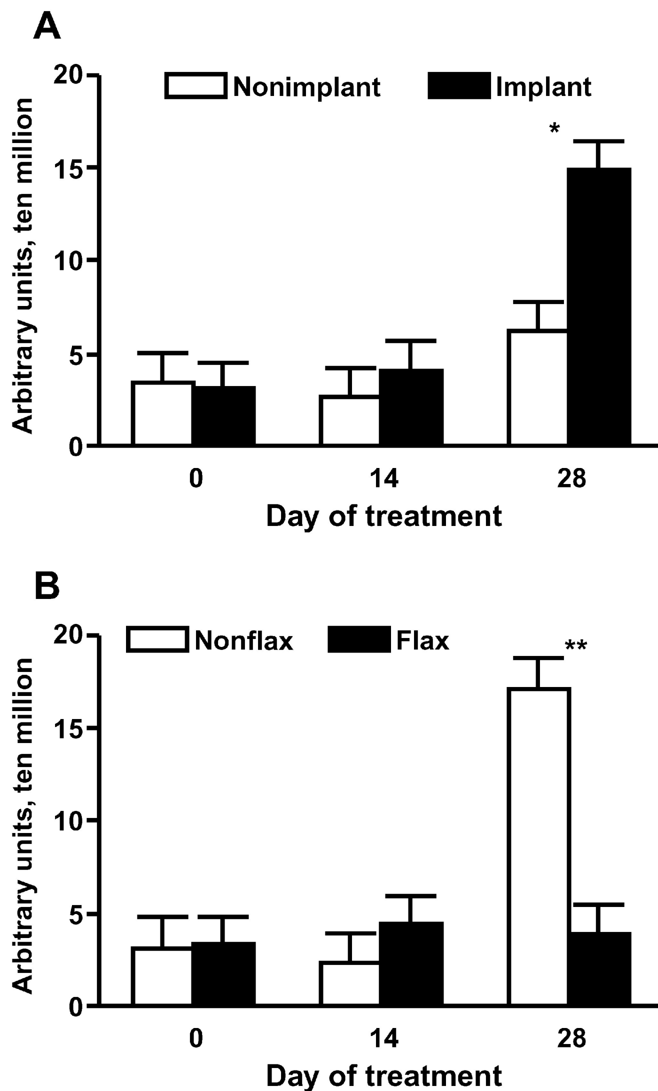

Figure 3. Muscle IGF-I mRNA levels in longissimus muscle biopsy samples obtained from implanted and nonimplanted steers (Panel A) and flax-supplemented and nonsupplemented steers (Panel B) on d 0, 14, and 28. The asterisk indicates IGF-I mRNA levels in the implanted and nonimplanted groups, on a given day, differ, $P < 0.001$. Two asterisks indicate IGF-I mRNA levels in the flax-supplemented and nonsupplemented groups, on a given day, differ, $P < 0.001$. Pooled SEM = 1.6 arbitrary units, n = 8.

Discussion

Previous research demonstrated that administration of a combined TBA/E_2 implant resulted in increased circulating IGF-I and IGF-I mRNA levels in the longissimus muscles of implanted steers as compared to nonimplanted steers (Johnson et al., 1996b; 1998b; White et al., 2003). In the current study, administration of a single combined TBA/E_2 implant resulted in substantial increases in circulating IGF-I levels in sera collected from implanted steers relative to nonimplanted steers by d 14 after implantation. Circulating IGF-I concentrations in sera from implanted steers further increased from d 14 to 28. Our data suggest that the implant was effective in increasing circulating IGF-I similar to previous experiments (Johnson et al., 1996b; 1998a).

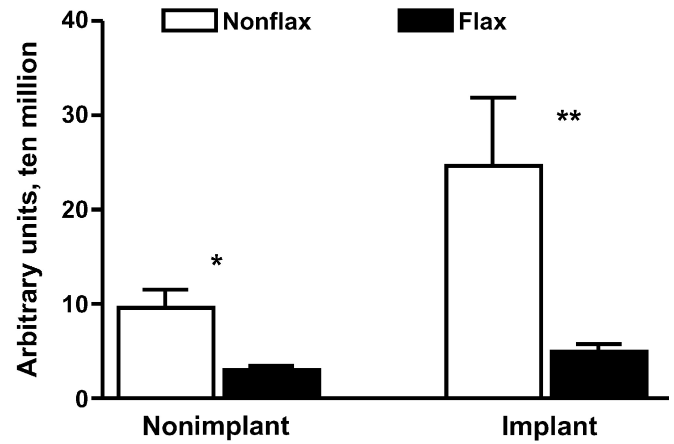

Figure 4. Muscle IGF-I mRNA levels in longissimus biopsy samples obtained from steers on d 28. A single asterisk indicates means differ, $P < 0.05$. Two asterisks indicate means differ, $P < 0.01$. Bars are mean values ± SE, n = 4.

It is generally recognized that the majority of the IGF-I found in the general circulation is synthesized in and secreted by the liver (Florini et al., 1996). Previous researchers have reported increased IGF-I mRNA levels in the longissimus muscle of implanted steers as compared to those from nonimplanted steers. Because

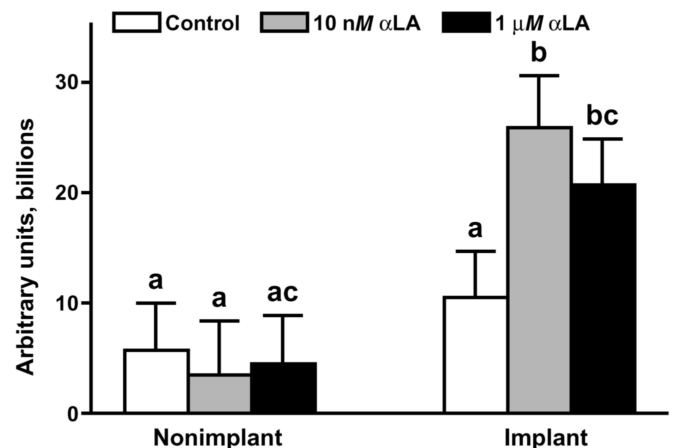

Figure 5. IGF-I mRNA concentrations in primary cultures of bovine satellite cells isolated from implanted and nonimplanted steers, n = 4, and incubated in 0, 10 n*M*, or 1 μ*M* α-linolenic acid (αLA). Following 72 h of αLA exposure, total RNA was isolated from cells and relative IGF-I mRNA abundance was determined using real-time quantitative-PCR. An implant × dose interaction was observed ($P < 0.05$). Bars with different letters differ, $P < 0.05$.

IGF-I is known to be a potent stimulator of both proliferation and differentiation of satellite cells (Florini et al., 1996), the locally produced IGF-I could act through autocrine and/or paracrine mechanisms to promote the proliferation and differentiation of muscle satellite cells, thus enhancing skeletal muscle hypertrophy. Insulin-like growth factor I is a critical mediator of skeletal muscle hypertrophy (Barton-Davis et al., 1999). Virally induced overexpression of IGF-I in skeletal muscle resulted in a 15% increase in overall muscle mass in young adult mice (Barton-Davis et al., 1998) and extended the proliferative lifespan of satellite cells (Chakravarthy et al., 2000). In the current study, IGF-I mRNA levels in muscle biopsy samples obtained from implanted steers were greater than those from nonimplanted steers on d 28. However, the IGF-I mRNA levels in muscle samples from implanted and nonimplanted steers did not differ on d 14. This is in contrast to the work of Pampusch et al. (2003) where IGF-I mRNA levels in longissimus muscle biopsy samples from implanted steers were greater than those of nonimplanted steers 12 d after implantation.

Flaxseed, also called flax, is an excellent source of the essential omega-3 polyunsaturated fatty acid, αLA. Scollan et al. (2001) demonstrated that steers fed whole linseed (flaxseed) had higher levels of αLA in both the neutral and phospholipid fractions of longissimus muscle tissue. In addition, LaBrune et al. (2000) reported that the addition of 10% ground flaxseed to beef cattle finishing diets resulted in significantly higher levels of αLA in plasma and in ribeye steaks. These data indicate that at least a portion of the dietary omega-3 PUFA can escape microbial biohydrogenation in the rumen. This is in contrast to earlier data demonstrating that dietary omega-3 PUFA are readily hydrogenated by microorganisms present in the rumen (Harfoot, 1978). Liu et al. (1994) demonstrated that skeletal muscle sarcolemma membranes obtained from rats fed a high-omega-3 fatty acid diet had significantly higher levels of omega-3 fatty acids and bound more insulin and IGF-I than rats fed a low omega-3 fatty acid diet.

In our study, supplementation with 5% ground flaxseed had no effect on circulating IGF-I concentrations throughout the 28 d. This contrasts the findings of Rickard et al. (2000), who recently reported rats supplemented with 5% flaxseed and an equivalent dose (1.5 mg/d) of the lignan precursor in flaxseed, secoisolariciresinol diglycoside, had significantly lower plasma IGF-I concentrations than nonsupplemented rats. These data suggest that the lignan precursor component of flaxseed may reduce circulating IGF-I levels in rats. It is possible that there are species differences that prevent the effects of flax supplementation on hepatic production of IGF-I from being measurably different in the circulation of steers after only 28 d.

Muscle IGF-I mRNA levels in longissimus biopsy samples obtained from nonflax steers after 28 d of supplementation were 4.4-fold greater compared to those from flax steers. On d 28, regardless of whether the steers were implanted or not, the concentrations of IGF-I mRNA in the longissimus muscle samples from nonflax steers were significantly greater than those from flax steers. These data are supported by a recent report that mice with established MDA-MB-435 human breast cancer tumors supplemented with 10% flaxseed had reduced IGF-I levels in tumors isolated upon necropsy compared to nonsupplemented mice (Chen et al., 2002). Insulin-like growth factor I is an important growth factor in the development of mammary cancers. Taken together, these data suggest that flax supplementation reduces the production of both IGF-I mRNA and the translated peptide. The fact that 28 d of flax supplementation reduced muscle IGF-I mRNA, yet had no effect on circulating IGF-I, is intriguing. It is possible that the PUFA in the flax supplement escaped ruminal biohydrogenation and were preferentially incorporated into the sarcolemma phospholipid bilayer (Scollan et al., 2001). Incorporation of these PUFA into the plasma membrane of the muscle fiber would result in increased membrane fluidity. This increased membrane fluidity may have resulted in increased binding of circulating IGF-I to the membrane-bound IGF-I receptors as was observed in the work of Liu et al. (1994). The increased sensitivity of the muscle tissue to circulating IGF-I could be responsible for the down-regulation of IGF-I mRNA production in the longissimus muscle that we observed in steers supplemented with flax; however, to our knowledge, no one has evaluated the effect of increased IGF sensitivity on subsequent IGF gene expression of the same cell. Furthermore, IGF-I expression may be affected differently in hepatic and skeletal muscle with inclusion of flax in beef cattle diets.

To increase our understanding of potential direct effects of αLA on IGF-I mRNA expression, we utilized primary cultures of bovine satellite cells isolated from nonimplanted and implanted steers. Muscle satellite cells are critical to postnatal skeletal muscle growth (Moss and Leblond, 1971; Allen et al., 1979; Campion, 1984), and IGF-I is known to be a potent stimulator of both proliferation and differentiation of satellite cells (Florini et al., 1996). To determine whether αLA was directly responsible for IGF-I mRNA down-regulation, we incubated primary cultures of bovine satellite cells, from implanted and nonimplanted steers, in two concentrations of αLA (10 nM and 1 μM). Here we report satellite cells obtained from implanted steers had higher levels of IGF-I mRNA when exposed to 10 nM αLA than cells from nonimplanted steers. It is unclear why satellite cell cultures from steroid-implanted steers had greater IGF-I mRNA levels in response to the αLA exposure than those from nonimplanted steers. In fact, it is somewhat surprising, since flax supplementation significantly reduced IGF-I mRNA levels in muscle biopsy samples. Satellite cell proliferation and differentiation was not assessed during this 72-h period. However, it is likely that satellite cell cultures established from implanted steers would have a shorter lag phase following plating as compared to primary cultures es-

tablished from nonimplanted steers. Thus, the activation state of the satellite cell may affect the response of αLA addition on IGF-I mRNA expression. Based on the findings above, it appears that the αLA component of flax is not responsible for the down-regulation of IGF-I mRNA levels in muscle. Other components, such as the lignan precursors, which are abundant in flax, may be causing the down-regulation of IGF-I mRNA in muscle.

Implications

Our results suggest that the administration of a combined trenbolone acetate and estradiol implant, Revalor-S, increased both circulating insulin-like growth factor-I and longissimus muscle insulin-like growth factor-I messenger RNA concentrations in finishing cattle. The increase in muscle insulin-like growth factor-I messenger RNA suggests that muscle tissue of Revalor-S-implanted steers may produce more insulin-like growth factor-I than that of nonimplanted steers. Supplementation with 5% ground flaxseed for 28 d had no effect on circulating insulin-like growth factor-I concentrations but decreased insulin-like growth factor-I messenger RNA in the longissimus muscle, regardless of implant status. Components other than α-linolenic acid seem to be responsible for the insulin-like growth factor-I messenger RNA down-regulation. These decreases in local insulin-like growth factor-I messenger RNA may ultimately affect skeletal muscle growth in beef cattle.

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