Muscle Development and Growth

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Paracrine Effects of Porcine Muscle-Derived Fibroblasts on Satellite Cells in Culture

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Satellite cell proliferation and differentiation are essential for muscle growth to occur. The paracrine effects of non-myogenic cells which reside in muscle on satellite cell activity are not well understood. The objectives of this study were to 1) identify differences in growth and fusion characteristics of primary porcine satellite cell cultures and satellite cell clones, and 2) determine the effects of conditioned media from muscle-derived fibroblast-like cells and satellite cells on proliferation and differentiation of cloned porcine satellite cells. Primary cultures of porcine satellite cells were isolated from the semimembranosus muscles of 6-week-old pigs. Satellite cell clones were isolated using a cloning ring technique and clones of fibroblast-like cells were similarly isolated or obtained by serial passage of post-fusion primary cultures. All cells were grown in Minimum Essential Medium (MEM) containing 10% fetal bovine serum (FBS) and differentiation (assessed microscopically by counting total and myotube nuclei) of satellite cells was induced by exposure to MEM containing 2% FBS or MEM containing 2% FBS and 10^{-6} M bovine insulin. No myotube formation was observed in cultures of fibroblast-like cells at any time. Cloned porcine satellite cells treated for 3 days with 10^{-6} M insulin had a 6-fold greater fusion percentage than cells exposed to MEM + 2% FBS without insulin. However, insulin addition did not improve fusion of primary satellite cell cultures. Exposure of MEM + 10% FBS or 2% FBS to proliferating satellite cells or fibroblasts for 48 hours resulted in "conditioned media" (CM). MEM + 2% FBS CM harvested from both satellite cells and fibroblasts stimulated fusion of confluent satellite cell clones ($P<.05$ and $P<.001$, respectively), with fibroblast CM consistently inducing a greater fusion response than satellite cell CM. Treatment of proliferating satellite cells and fibroblasts with MEM + 10% FBS CM from both satellite cells and fibroblasts reduced proliferation compared to control MEM + 10% FBS ($P<.05$). Exposure to CM harvested from high density cultures resulted in fewer total nuclei than CM prepared from lower density cultures of both satellite cells and fibroblasts ($P<.05$). These data indicate that proliferating muscle-derived fibroblast-like cells induce fusion of porcine satellite cells via a mechanism which appears to involve insulin-like growth factor activity. Proliferating satellite cells and fibroblasts compete for nutrients/mitogens and/or secrete factors which reduce proliferation of satellite cells in culture. Paracrine interactions between porcine muscle-derived fibroblasts and satellite cells are likely to modulate satellite cell-induced muscle growth.

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Response Time of Broiler Chickens to Cimaterol

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The objectives of this study were to determine the response time to Cimaterol (CIM), a beta-adrenergic agonist, by broiler chickens for carcass characteristics, muscle composition, muscle fiber size, catheptic enzyme activity and tenderness. The study consisted of 160 thirty-three d old birds; one half received a diet containing 0 ppm CIM and the rest a diet with 1 ppm CIM. Chickens were *ad libitum* fed with free access to water. Twenty birds were killed at time 0 to establish baseline values. At 2, 4, 6, 8, 10, 12 and 14 d, 10 CON and 10 CIM-fed chickens were killed. Breast muscle (pectoralis major; BM) and leg muscle (gastrocnemius and peroneous longus; LM) wt, BM and LM cathepsin B and L activities, DNA, RNA and protein concentration, and BM and thigh muscle (biceps femoris, semitendinosus, semimembranosus, tensor facia latae; TM) shear values (SV) were measured. Fiber size of BM was measured (5 birds per treatment) at d 2, 6, 10 and 14 of the study. Breast muscle SV was higher at d 8 ($p=.06$) and d 10 ($P<.05$) after initiation of the study in CIM-fed chickens. Thigh SV of CIM-fed chickens was higher at d 4, 8, 10, 12 and 14 ($P<.05$). The BM of CIM-fed chickens had higher protein/DNA ($P<.05$) at d 6 through 14 whereas LM of CIM-fed chickens had higher protein/DNA at d 8, 10 and 14 of the study. Fiber size of the BM in CIM-fed chickens tended to be larger at d 10 ($P<.13$) and d 14 ($P<.17$). Total BM wt and BM percentage was higher at d 10 and 14 ($P<.01$) in CIM-fed chickens. Leg muscle wt and percentage was higher at d 14 ($P<.01$) in CIM-fed chickens.

No differences (P>.05) were found in total or specific cathepsin B and L activities in the BM and LM. In the BM of CIM-fed chickens, protein/DNA increased by d 6, SV by d 8, muscle fiber size by d 10 and muscle wt and percentage by d 10. For TM or LM from CIM-fed chickens, SV increased by d 4, protein/DNA by d 8 and muscle wt and percentage by d 14. Response time to CIM by broiler chickens depends upon the response variable in question and the muscle type studied.

Temporal Expression of the Myogenic Regulatory Gene Family in the Mouse Embryo

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Using a quantitative reverse transcription/polymerase chain reaction method, the temporal pattern of expression of the myogenic regulatory genes (myf, myogenin, herculin, myo D) in developing mouse muscle was analyzed and correlated to expression of growth factors (TGF-β, FGF, IGF-I and IGF-II) which modulate their activity in-vitro. In the whole embryo, myf is the first myogenic regulatory factor to appear, with expression evident 8.5 days post coitum (d pc). A transient peak of herculin expression occurred by 10.5 d pc. Appearing by 8.5 and 10.5 d pc, respectively, myogenin and myo D expression steadily increased. In the front limb bud myf is the first myogenic regulatory gene to appear, with transcripts easily detected 9.5 d pc. By 11.5 d pc myf expression increased 100 fold to maximal levels. Slight expression of herculin was evident in 9.5 d pc front limb buds, increased 60 fold by 10.5 d pc, and then diminished 80% by 11.5 d pc. Significant concentrations of myogenin or myo D mRNA were not detectable until day 11.5 pc. TGF-β was expressed at high concentrations by 9.5 d pc and decreased approximately 25 and 50% by 10.5 and 11.5 d pc, respectively. FGF expression was not detectable until day 10.5 d pc, after which it increased steadily. Both IGFs were expressed in a similar fashion, with high levels of expression in 9.5 d pc limb buds increasing over 2 fold to maximal concentrations observed in 11.5 d pc limb buds. In the hind limb bud, by 11.5 d pc myf expression was 80% of maximal levels observed by 14.5 d pc. Myogenin was detectable at trace levels by 11.5 d pc and increased approximately 40 and 90 fold by 12.5 and 14.5 d pc, respectively. Significant levels of herculin or myo D were not detected until day 12.5 pc. TGF-β was expressed at low levels by 11.5 d pc and increased 80% by day 12.5 pc. Trace expression of FGF in 11.5 d pc hind limb buds increased 75 and 100 fold by 12.5 and 14.5 d pc, respectively. Both IGFs were expressed at high levels in the hind limb buds by 11.5 d pc, and continued to increase thereafter. The results indicate that there are temporal kinetics to the expression of the myogenic regulatory gene family in developing mouse muscle. This pattern of expression seems similar for the whole embryo and anterior and posterior limb buds. There also seems to be a correlation between TGF-β, IGF-I, and IGF-II expression and expression of the myogenic regulatory gene family in the developing limb bud.

Production of Insulin-Like Growth Factor Binding Proteins by Cultured Porcine Myogenic Cells

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Porcine myogenic cells isolated from 50-55 day porcine fetuses were frozen and stored in liquid nitrogen until needed to establish cultures. Approximately 75.8 ± .59% of the clonal cultures established from these frozen stocks produced myotubes and 60.8 ± 2.3% of the nuclei in differentiated mass cultures were in myotubes. Differentiated cultures contained higher levels of creatine phosphokinase activity than undifferentiated cultures. Additionally, differentiated cultures incorporated ^{35}S-methionine into putative myosin heavy chain, α-actinin, and actin more rapidly than did undifferentiated cultures. Insulin, IGF-1 and sera stimulated total protein synthesis rate and decreased total protein degradation rate in myotube cultures.

This porcine myogenic culture system has been used to examine the production of insulin-like growth factor binding proteins (IGFBPs) by porcine myogenic cells. Ligand blots show that porcine myoblasts produce IGFBPs that band at 38-43 KD and at 31 KD. Porcine myotubes appear to secrete the same binding proteins as do myoblasts; but the relative amounts are different, with myotubes secreting approximately twice as much of the 38-43 KD IGFBPs and half as much of the 31 KD binding protein as do myoblasts. Porcine muscle-derived fibroblasts produce IGFBPs banding at 31 and 21 KD. Consequently, the 38-43 KD IGFBP detected in the medium of cultured porcine myogenic cells is not likely to be the result of fibroblast contamination of the myogenic cultures. L6 myoblasts secrete a single 21-KD binding protein that appears to be identical in molecular weight to the 21-KD binding protein secreted by muscle-derived fibroblasts. Differentiated L6 myotubes secrete a 27-KD binding protein in addition to the 21-KD protein observed in proliferating myogenic cells.

Transforming growth factor beta-1 dramatically affects IGFBP production of both porcine and L6 myogenic cells; however, each cell type is affected differently. Binding protein production by porcine myogenic cells (myoblasts and myotubes) is increased while binding protein production by L6 myogenic cells (myoblasts and myotubes) is decreased.

The Effect of Weekly BST Pellet Implantation on Carcass Parameters of Finishing Beef Cattle

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The objectives of the present study were to determine if relatively low doses of continuously delivered BST altered the carcass characteristics and feedlot performance of finishing beef cattle. After a five-week adaptation period, 180 cross-

bred steers (380 KG) were randomly assigned to three treatment groups. Cattle were either sham implanted with 20 mg pellets of BST at doses of 40 or 80 mg per week for twelve weeks. Pellet doses of 40 and 80 mg per week elicited rates of gain in rats that were comparable to 5 and 10 mg BST/day delivered continuously from Alzet minipumps. Growth rate and feed efficiency in cattle implanted weekly with 80 mg BST was 10% and 12% better than controls, respectively. The 40 mg dose of BST did not statistically alter growth rate and feed efficiency. Final body weight in cattle implanted with 80 mg BST weekly was 2% greater than controls. Fat depth was not altered, but %KPH fat was reduced by 19% and rib eye area was enhanced by 5% in cattle implanted with 80 mg BST weekly. The calculated yield of closely trimmed steaks and roasts was increased by 4.5 KG (3%) in cattle implanted weekly with 80 mg BST. Marbling in 80 mg BST treated steers was $Small^{00}$ reduced from a control value of $Small^{36}$. In control carcasses, 52% graded Choice or better; whereas 40% of the carcasses from 80 mg BST treated steers graded Choice or better. In conclusion, commercially relevant changes in feedlot performance and lean yield can be obtained by continuous delivery of relatively low doses of BST (approximately 10 mg/day). Changes in marbling score and quality grade may or may not be acceptable under current marketing systems.

Expression of Myogenic Genes in Bovine Muscle

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Meat animals which substantially differ in muscle mass have been shown to exhibit differences in myofiber size and/or number. While myofiber size is directly related to the transcriptional and translational efficiency of myofibrillar genes, the number of muscle cells may be influenced by the processes of myogenic cell determination and differentiation. The sequential and temporal transition of mesodermal cells into myotubes or myogenesis involves the developmental and potentially stage-specific transcription of a family of regulatory factors which include MyoD, myogenin, myf5 and MRF4. These proteins may operate in a dominant, hierarchial fashion to trans-activate muscle specific genes during embryonic development and satellite cells postnatally. It is well established that expression of MyoD in several mesodermal and nonmesodermal cell types activates muscle-specific gene expression. Our objective was to begin examination of the expression of myogenic genes in fetal bovine muscle, in bovine myoblast cultures during development and in myoblasts after exposure of cells to growth factors (GF) as part of continuing effort of identifying some of the molecular mechanisms of muscle growth. Semitendinosus muscles were excised from bovine fetuses (90-130d). After liberation, cells were sequentially preplated on uncoated flasks and seeded on basement membrane Matrigel® (1:10). Cells were plated (160 cm^2 flasks for RNA harvesting and 24-well Nunclon® plates for proliferation assays) for 24 h in DMEM, supplemented with 15% FBS and then switched to treatment medium consisting of basal medium (BM; DMEM + 5% FBS) alone and with IGF-1 (100 ng/ml), rFGF (100 ng/ml), or with 10% FBS for 24, 48 and 72 h with medium changes every 12 h. These concentrations of GF stimulated proliferation by approximately 2.5-fold ($P<.01$) over 5% FBS and this concentration was based on data of previous experiments. Replicate cultures were switched to fusion-medium (FM; DMEM + 1.0% FBS + 1 ug/ml BSA-linoleic acid) after 24 h of TRT with the GF. In the developmental experiment, RNA was isolated from cells in 10% FBS after 24, 48 and 72 h. In the experiment involving GF, RNA was isolated after 24 h after GF TRT. Replicate cultures were used to assess cell numbers. Total RNA from the developmental and GF experiment was spotted onto nylon and allowed to hybridize to ^{22}P-labeled cDNA for a 1785 bp MyoD obtained from H. Weintraub and a 230 bp quail fast myosin heavy chain (MHC) cDNA from C. Emerson. Northern analysis of the RNA from the developmental experiment also showed expression of ski (based on a 1.1 kb human cDNA from N. Nomura) at 24 but not at 48 or 72 h. Expression of MyoD based on relative hybridization signal from dot blot analysis was lower at 24 compared to 48 and 72 h cultures. Compared to 5% or 10% FBS, MyoD was increased ($P<.05$) 18% by IGF-1 and decreased 33% by FGF. MHC expression decreased by 42% in cultures with 10% FBS and FGF compared to 5% FBS. Results support the concept of involvement of MyoD-like genes in the physiological and developmental regulation of growth and differentiation of cultured bovine muscle cells.

Effect of a Beta-Adrenergic Agonist on Muscle Growth and Endogenous Proteinase Activities in Lambs

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To examine the effect of feeding a β-adrenergic agonist (BAA) on muscle growth, proteinase activities and postmortem proteolysis, 16 wether lambs were randomly assigned to receive 0 or 4 ppm of $L_{644,\ 969}$ (Merck Sharp and Dohme Research Laboratories) in a completely mixed high-concentrate diet for 6 wk. The biceps femoris muscle was 18.6% heavier ($P<.05$) in treated lambs. At 0 h, treated lambs had higher ($P<.05$) cathepsin B (35.6%), cathepsin B+L (19.1%), calpastatin (62.8%) and m-calpain (24.6%) activities than the non-treated lambs but had similar μ-calpain activity. In the longissimus and biceps femoris muscles, treated lambs had higher ($P<.05$) protein and RNA concentrations, but lower DNA concentrations. However, total DNA was not affected, indicating that the increase in muscle mass is probably due to muscle hypertrophy rather than hyperplasia. In treated lambs, postmortem storage had no effect on myofibril fragmentation index or degradation of desmin and troponin-T. These results indicated that the

ability of the muscle to undergo postmortem proteolysis has been dramatically reduced with BAA-feeding. Since similar proteolytic systems are thought to be involved in antemortem and postmortem degradation of myofibrillar proteins, it suggested that BAA-mediated protein accretion is due, at least in part, to reduced protein degradation. To examine whether protein synthesis was altered with BAA-feeding, the level of skeletal muscle α-actin mRNA was quantified. Longissimus α-actin mRNA abundance was 30% greater ($P<.05$) in the treated lambs. Collectively, these results indicate that dietary administration of a BAA increases muscle mass through hypertrophy and that the increase in muscle protein accretion is due to increased synthesis and reduced degradation of muscle proteins.

The Relation of Phosphoinositide Turnover to Myoblast Proliferation

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The L_6 myoblast cell line was utilized in a series of experiments designed to investigate the possible regulatory role of the phosphoinositide signal transduction system in the proliferation of myoblasts in culture. Cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal bovine serum (FBS). In initial experiments, cell number, ^{3}H-thymidine incorporation and protein content of cultures were determined at 24-h intervals (24-96 h) following addition of medium containing either 0 (control), 5, 10 or 20 mM lithium chloride (LiCl), an inhibitor of phosphoinositide synthesis. Control cells exhibited a rapid rate of proliferation (7.8-fold increase in cell number by 96 h) and the addition of 5 mM LiCl did not affect ($P>.20$) the rate or extent of proliferation (8.07 and 7.61 $\times$ 10^5 cells/well at 96 h for control and 5 mM treatments, respectively). In contrast, the addition of 10 and 20 mM LiCl resulted in 38.5 and 96.8% decreases ($P<.01$) in proliferation by 96 h in culture (5.32 and 1.15 $\times$ 10^5 cells/well for the 10 and 20 mM treatments, respectively). These results were reflected by measurements of DNA synthesis, with only the 10 and 20 mM LiCl treatments causing decreases (13.0 and 40.7%, respectively; ($P<.05$) in ^{3}H-thymidine incorporation into DNA when added to cultures of proliferating myoblasts. The inhibition of myoblast proliferation by the addition of LiCl resulted in 26.4 and 79.9% increases ($P<.01$) in protein content (62.3, 64.2, 78.8 and 122 $\mu g/10^5$ cells for control, 5, 10 and 20 mM LiCl treatments, respectively), which coincided with the apparent increase in cell size observed by phase microscopy for these treatments. In quiescent myoblast cultures labeled to equilibrium with ^{3}H-inositol (1 μCi/ml), stimulation with 10% FBS resulted in a rapid and transient increase ($P<.01$) in label recovered as inositol trisphosphate (196.7% increase within 30 s, with a return to initial levels by 60 s). The decrease in inositol trisphosphate was followed closely by a rise ($P<.05$) in the label recovered as inositol bisphosphate (100.0% increase at 60 s) and subsequently as inositol monophosphate (29.2% at 300 s). There also was a dose-dependent increase in the inositol phosphates in response to LiCl. In myoblasts preincubated with ^{3}H-inositol, 5, 10 and 20 mM LiCl increased total labeled inositol phosphates after 2 h incubation by 2.12, 4.03 and 4.53-fold, respectively. The same concentrations of LiCl increased inositol monophosphates by 4.14, 7.66 and 8.57-fold, respectively. The inositol bisphosphates were unaffected by 5 mM LiCl, and 10 and 20 mM LiCl increased this phosphoinositol pool by only 73.3 and 121.6%, respectively. Additionally, LiCl had no effect on the inositol trisphosphate pool after 2 h incubation, indicating the LiCl was more effective in increasing the inositol monophosphates than any other phosphoinositol pool. Overall, these data are supportive of a role for phosphoinositide metabolism in mediating the proliferation process in L_6 myoblasts, presumably by the phosphoinositide signal transduction pathway.

The Turkey Myogenic Satellite Cell: A Cell Culture System for the Study of Skeletal Muscle Development

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Cellular and hormonal mechanisms of avian skeletal muscle development are currently being investigated with myogenic satellite cells derived from young growing tom turkeys. Satellite cells isolated from the pectoralis major muscle were cloned to obtained pure populations of myogenic cells and used in all studies. A serum-free medium which promotes proliferation of the satellite cell was developed to provide a hormonally-controlled environment for in vitro developmental studies. This medium has allowed us to examine the effects of insulin-like growth factors (IGFs) and fibroblast growth factors (FGFs) on avian satellite cell proliferation. The role(s) of IGFs was further investigated by examination of the interaction of these hormones with cellular receptors and the release of IGF binding proteins from cells in culture. Our results demonstrate that, in contrast with mammalian muscle cells, IGF-I and IGF-II both interact with a type I IGF receptor on avian muscle cells. The release of IGF binding proteins from satellite cells decreases during myogenesis, suggesting a possible role in the development of the differentiated state of avian skeletal muscle.

Tissue Distribution of Insulin Receptors in Pigs, Cattle and Rats

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Insulin is a primary anabolic hormone in mammalian species and promotes adipose and muscle accretion via regulation of a variety of steps in metabolic pathways. Tissue responses to insulin are mediated through coupling to the plasma membrane bound insulin receptor. Responses to insulin vary and may result from regulated changes in receptor density, affinity for ligand, or receptor activity as mea-

sured by tyrosine kinase activity. Such changes may establish different patterns of nutrient partitioning as observed with age, between genotypes and with administration of somatotropin and phenethanolamines. Initial experiments compared insulin receptor density and activity in pig adipose and muscle tissues. Subsequent species comparisons were made by conducting similar experiments using bovine and murine tissues. Insulin binding to pig plasma membranes was 50% greater in middle-layer backfat compared to outer-layer whether standardized to membrane protein or to activity of 5′-nucleotidase, a plasma membrane marker. Insulin binding to muscle membranes was 20% that of adipose membranes regardless of the basis of data expression. Insulin receptor mRNA abundance also was nearly 80% lower in muscle, indicating an association between tissue content of receptors and mRNA levels. Basal and insulin-stimulated tyrosine kinase activity of WGA-purified receptors was similar for both adipose depots and muscle, suggesting that receptor populations were similar across tissues.

Similar to data in the pig, insulin binding to cattle and rat adipose plasma membranes was also 5-fold greater than to muscle membranes, indicating that the pig is not unique in the distribution of receptors in these two insulin-responsive tissues. Regional (adipose depots) and tissue differences in insulin receptor density likely reflect tissue-specific differences in metabolic control. Increased receptor density in middle-layer backfat is consistent with increased metabolic activity and rate of accretion of this depot. The extent to which changes in insulin receptor density/activity modify rates of tissue accretion remains to be determined.

Cell Culture Analysis of Bovine Double-Muscling

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Double-muscling (DM) is an inherited skeletal muscle hyperplasia which occurs in many strains of beef cattle. Breeding studies have indicated that the phenotype may be caused by the inheritance of a single, autosomal gene. We have developed an in-vitro system to study the cellular and biochemical basis of the muscle hyperplasia and systemic connective tissue abnormalities which characterize the DM phenotype. In previous studies, we observed that in cell cultures derived from normal (NC) and DM 90-day fetal muscle tissue, DM myogenic cells displayed an increased rate of proliferation and a delay in the onset of terminal differentiation. This resulted in the production of greater numbers of terminally differentiated muscle nuclei in the DM cultures; this effect was markedly enhanced by the addition of basic fibroblast growth factor (bFGF) to the cultures. Conditioned media experiments suggested that DM fibroblasts might be producing increased amounts of one or more factors which control myoblast proliferation and differentiation.

Our recent experiments have been directed towards: 1) comparison of growth factor responsiveness of NC and DM muscle-derived fibroblasts; and 2) analysis of factors produced by fibroblasts which might regulate myoblast growth. Using a serum-free culture system, we observed that the proliferative response of DM fibroblasts to bFGF and to combinations of bFGF and insulin-like growth factor −1 (IGF-1) was much greater than that of NC fibroblasts.

We have also examined production of binding proteins for the IGFs (IGF-BPs) by NC and DM fetal muscle-derived fibroblasts. Using ligand blot analysis of serum-free conditioned media, we found that bovine fetal muscle-derived fibroblasts produce several species of IGF-BPs. These include a high molecular weight, glycosylated BP with mobility similar to bovine serum IGF-BP3, and three low molecular weight, non-glycosylated IGF-BPs which co-migrate with bovine serum IGF-BP 2, 1 and 4. Preliminary experiments using purified BPs indicated that the low molecular weight forms inhibit myoblast proliferation and differentiation, possibly by binding and sequestering IGF. Pilot experiments with purified serum IGF-BP3 have indicated that this species does not interfere with IGF binding to myoblasts. Ligand blots of serum-free medium conditioned by NC and DM fibroblasts suggest that both fetal skin and fetal muscle-derived NC fibroblasts secrete increased amounts of the lower molecular weight, inhibitory binding proteins, compared to DM fibroblasts. Our data and the work of others indicate that levels of IGF-BP production by fibroblasts can be modulated by growth factors.

These findings support the hypothesis that alterations in the physiology of DM fibroblasts may be the underlying cause of the skeletal muscle hyperplasia and connective tissue abnormalities characteristic of DM animals. These results contribute to the growing body of evidence that locally-produced autocrine or paracrine factors may be of considerable significance in the control of muscle growth.

Regulation of Beef Cattle Growth by Somatotropin

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Research with myogenic cell cultures and meat animals has implicated both the systemic and local endocrine systems in the control of growth and lean tissue deposition. Research into the underlying mechanism(s) of bovine double muscling reveals a trophic (paracrine) effect of muscle-associated fibroblasts on myoblasts during myogenesis. However, the role of the systemic endocrine hormone, somatotropin (ST), in controlling growth during post-natal stages of growth in cattle is largely unknown. Research from this laboratory has utilized two approaches to investigate systemic hormone control of growth in beef cattle: 1) hypothalamic regulation of ST production and growth; and 2) exogenous administration of ST to regulate growth. In the first experiment, Charolais crossbred steers were immunized against somatotropin-release inhibiting hormone (SRIH). The immunized cattle were inoculated (d 0, 21 and 42) with 1 mg SRIH conjugated to 4.8 mg human serum alpha globulin. Blood samples were collected from selected steers at d 84 to determine ST secretory profiles. All cattle were slaughtered at d 100. Binding of antisera to labeled SRIH was 3.7% (1:320 final dilution) at d 82. Immunization against SRIH improved

(P<.09) average daily gain by 21% (1.48 vs. 1.22 kg) but did not influence (P>.10) feed efficiency. Plasma somatotropin overall mean, baseline mean and peak frequency were not different (P>.10) but peak amplitude was increased (P<.05) by 111% in SRIH-immunized steers. Adjusted hot carcass weight tended to be greater (P>.10) in SRIH-immunized steers. Fat depth, ribeye area, KPH, quality grade and yield grade were not influenced by SRIH immunization. Results from this experiment indicate that while steers immunized against SRIH during the finishing phase gain weight more rapidly, they do not exhibit altered carcass lean and fat characteristics. In a second experiment, Hereford crossbred steers were administered four dosages (0, 40, 80, 160 mg/wk) of recombinant DNA-derived bovine somatotropin (rbST) implants over a 100 d finishing period. ST decreased (P<.10) feed intake by 7% and improved (P<.05) feed efficiency (feed/gain) by 12%. Fat depth at the 12th rib was decreased (P<.10) by 21% and ribeye area was numerically increased (P>.10) by 4%. Chemical composition of 9-10-11 rib section was influenced by rbST treatment: protein was increased (P<.01) by 9% and fat was decreased (P<.01) by 12%. Results from these experiments demonstrate that the ST status of heavy beef cattle may be modified to alter growth and carcass characteristics. However, considering the discordant growth and carcass responses to SRIH immunization and rbST administration, it is not clear whether immunization against somatotropin is functioning primarily through the hypothalamic-pituitary axis or through other endocrine mechanisms.

Dose Response Effects of Porcine Somatotropin on Yield, Distribution and Composition of Edible Carcass Tissues

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Somatotropin has been shown to increase rate of gain and improve feed efficiency in growing-finishing pigs, while increasing muscle mass and decreasing carcass lipid content. The objectives of this study were to quantify the effects of somatotropin administration on the yield and composition of edible tissues, by wholesale cut, and to assess changes in tissue distribution. In order to achieve these objectives, 50 barrows were given daily injections of 0, 50, 100, 150 or 200 μg recombinant somatotropin per kg of body weight per day ($\mu g \cdot kg^{-1} BW \cdot d^{-1}$). Treatment began when pigs weighed approximately 30 kg and continued until the pigs were slaughtered as they individually reached 90 kg BW. Dosages were adjusted biweekly to accommodate growth. Left sides were separated into wholesale cuts. Each wholesale cut was separated into muscle, adipose, bone and skin components. Each muscle and adipose sample was subsampled for proximate composition determination. The tissues from the minor wholesale cuts (jowl, neckbones, spareribs, feet and tail) were pooled for analysis and are referred to as "other cuts." Furthermore, trimmings removed from the carcass while preparing the trimmed wholesale cuts were also pooled for analysis and are referred to as "rough trimmings."

Somatotropin treatment increased trimmed wholesale cut weights for the ham, loin, butt and picnic (P<.05) and decreased weights of the fat cuts, the belly and jowl (P<.05). Changes in wholesale cut distribution, expressed as a percentage of carcass weight, exhibited the same trends as observed for their absolute weights since carcass weights were only decreased by about 3 kg at the 200 μg dose when compared to the excipients.

As examples of the changes occurring in separable tissue mass of lean and fat cuts, loin and belly data are presented. Separable muscle mass from the loin increased (P<.05) at all doses of somatotropin, with a maximal increase of 37% observed at the 200 μg dose relative to controls. Separable loin adipose tissue decreased in a dose-dependent manner by as much as 69% at the highest somatotropin dose. Bone separated from the loin did not change (P>.05). By comparison, a dramatic 50% increase in separable muscle mass from the belly was observed at the highest dose, while adipose tissue mass decreased 84% and skin mass increased (P<.05) 47%. On a weight per side basis, total muscle tissue increased (P<.05) from 15.7 kg for the controls to a maximum of 21.4 kg at the 200 μg dose. Total side separable adipose tissue decreased in a dose-dependent manner (P<.05) from 11.6 kg for the excipients to 3.0 kg for the 200 μg dose group. Bone also increased (P<.05) from 3.7 kg (controls) to 4.3 kg (200 μg dose), and skin increased (P<.05) from 1.8 kg to 2.5 kg per side with somatotropin administration.

Proximate analysis revealed an approximate one percentage point increase (P<.05) in protein concentration in the muscle tissues of the loin from treated pigs. Moisture concentration also increased (P<.05), while lipid concentration decreased in a dose-dependent manner. Ash concentration of the loin muscle tissues was unchanged by somatotropin treatment (P>.05). Muscle tissue from the belly was similarly affected by somatotropin. A maximal increase (P<.05) of 6.1 percentage points for moisture concentration (68.5% for controls and 74.6% for the 200 μg treatment) was observed, while lipid concentration was decreased (P<.05) in a dose-dependent manner by as much as 7.3 percentage points (11.0% and 3.7% lipid for the excipients and highest dose treatments, respectively). Somatotropin caused profound dose-dependent changes in adipose tissue proximate composition in all cuts. Protein concentration was increased (P<.05) from 5.6% to 13.5% in loin adipose from pigs receiving the 0 and 200 μg doses, respectively. Similar changes from 4.4% to 14.3% protein were observed. Moisture concentration was increased (P<.05) for both the loin and belly adipose (from 24.0% to 44.4% for the loin and from 18.9% to 49.0% for the belly). Lipid concentration from adipose tissue was greatly reduced (P<.05) (from 69.5% to 41.4% in the loin and from 76.2% to 36.2% in the belly). Ash concentration increased (P<.05) in loin adipose, but did not change in belly adipose.

Total protein content of the soft tissues in the carcass (muscle and adipose) was increased (P<.05) by approximately 1 kg per side regardless of somatotropin dose. Total soft tissue moisture and ash content were also increased (P<.05) by somatotropin treatment (from 13.6 kg to 17.4 kg [28%] moisture and from 0.23 kg to 0.37 kg [61%] ash at the 0 and 200 μg doses, respectively). Total side soft tissue lipid

content was reduced (P<.05) with increasing dose by as much as 81% (from 9.7 kg to 1.8 kg at 0 and 200 μg doses, respectively).

Cholesterol concentration in the uncooked longissimus was not altered (P>.05), however, fatty acid profile of the longissimus was affected by somatotropin treatment. Total monounsaturated fatty acids were decreased (P<.05) by somatotropin administration (from 50.2% to 46.6% of the lipid for the 0 and 100 μg doses, respectively). Total saturated fatty acids were unaffected (P>.05), while total polyunsaturated fatty acids were increased (P<.05) from 11.8% to 15.0% of total extractable lipid) in muscle from somatotropin treated pigs.

In summary, somatotropin administration in growing finishing pigs dramatically increased skeletal muscle mass without significantly altering distribution among wholesale cuts. Adipose tissue mass was reduced to an even greater extent, leading to decreased weights of fat cuts, while weights of lean cuts were increased. The major change observed in nutritional composition of edible tissues was the reduction in percentage lipid, but adipose protein and moisture concentrations were also markedly increased in a dose-dependent manner.

Role of Insulin-like Growth Factors and Their Binding Proteins in Muscle Development

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The Insulin-Like Growth Factors (IGFs) are potent regulators of muscle growth and development. In biological fluids, the IGFs are bound to specific binding proteins (IGFBPs) which are capable of modulating the actions of the IGFs on cell growth and metabolism. The IGFs, their binding proteins and receptors comprise the IGF Growth Regulatory System. Here we have summarized three different studies which investigate the roles of IGF regulatory system components in muscle growth using various model systems. The model systems include: 1) in-vitro myogenesis, 2) intrauterine runting in pigs and 3) experimental diabetes. The parameters measured in these systems include: 1) serum IGF-1 levels, 2) serum IGFBP profiles, 3) IGF-1, 4) IGF-2, 5) IGFBP-2 and 6) IGFBP-3 gene expression. Results from the in-vitro myogenesis model indicate that a 1.8 kilobase IGFBP-2 mRNA transcript was highest in proliferating cells and decreased to 10% of initial levels during differentiation. Ligand Blots of serum free conditioned media (CM) revealed three IGFBP species of approximately 24,000, 30,000 and 32,000 M_r. The intensity of the 32,000 M_r band decreased with differentiation. These same blots were probed with an antibody raised against IGFBP-2. This antibody specifically bound to the 32,000 M_r IGFBP. The level of antibody binding decreased by nearly 100% as differentiation progressed. Thus, IGFBP-2 is differentially expressed and secreted by these myoblasts, suggesting that IGFBP-2 may be involved in the process of myogenesis. The study investigating in-utero runting revealed that IGFBP-3 mRNA in runt liver was decreased 20-40% at 90d of gestation and 10-30% at birth when compared with controls. IGFBP-3 expression and secretion were developmentally regulated. At 90d gestation, IGFBP-3 mRNA was relatively high, whereas serum levels of IGFBP-3 (43 and 39 kd bands) were very low. Postnatally, both IGFBP-3 serum levels and mRNA levels were low at birth and slowly increased to 49d of age. These data indicate that IGFBP-3 is developmentally regulated and is affected by runting. The experimental diabetes study investigated the effects of cellular nutrient restriction versus whole animal restriction such as fasting, on the IGFs. In diabetic pigs, IGF-1 mRNA was decreased by 50% in muscle and liver tissues, and by 70% in heart. The imposition of fasting to diabetic animals tended to further decrease IGF-1 mRNA levels, and fasting alone also decreased IGF-1 mRNA abundance in the three tissues ($p<0.05$). Insulin therapy restored IGF-1 mRNA levels to normal in muscle and liver, but did not restore IGF-1 expression in the heart. Serum IGF-1 was decreased 70% below control levels ($p<0.01$) in diabetic pigs. Expression of IGF-2 mRNA in the three tissues was unaffected by diabetes or fasting. These results demonstrate that IGF-1 expression is altered by diabetes and fasting, whereas IGF-2 mRNAs do not change. The combined results of these studies indicate that components of the IGF Growth Regulatory System are involved in muscle development, whole body development, cellular metabolism and growth. Understanding the actions and interactions of these factors will lead to the increased ability to efficiently produce muscle tissue in meat animals.

Effect of Growth Promotants on Myosin Gene Expression in Bovine Muscle Cells

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The purpose of this project was to determine if bovine skeletal muscle cells in culture were responsive to the addition of insulin, isoproterenol and triiodothyronine, three agents that affect protein metabolism or gene expression in muscle tissue. Each compound was added at the onset of myoblast fusion, and myosin gene expression was analyzed because myosin is the largest protein component by weight in the myofibril. Approximately five days after the onset of fusion, the quantity of myosin heavy chain protein, the apparent synthesis rate of myosin heavy chain protein, and the quantity of myosin heavy chain mRNA were measured from the same muscle cell culture dish. Apparent synthesis rate and the quantity of myosin heavy chain were measured by pulse labeling muscle cells with (^{3}H)Leu, followed by electrophoresis of myofibrillar protein preparations on polyacrylamide gels in the presence of sodium dodecyl sulfate. The quantity of myosin heavy chain mRNA was estimated by removal of a small portion of the initial cell homogenate for RNA isolation, followed by analysis of RNA dot blots with a bovine myosin heavy chain genomic clone. Insulin increased the quantity and apparent synthesis rate of myosin heavy chain, but caused a decrease in the quantity of myosin

mRNA. Isoproterenol stimulated the apparent rate of synthesis of myosin heavy chain protein, but had only a slight effect on the quantity of myosin heavy chain protein and no effect on the quantity of myosin heavy chain mRNA. Triiodothyronine caused a slight increase in the apparent synthesis rate of myosin heavy chain and in the quantity of myosin heavy chain mRNA, but had no effect on the quantity of myosin heavy chain protein. In summary, these data illustrate that this approach can be utilized to study the mechanisms by which different agents affect skeletal muscle gene expression and muscle protein metabolism. (This work was supported by Lilly Research Laboratories, Greenfield, IN)