Growth Research: Invited Posters

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Decreases in Local Hormone Biosynthesis Accompany Differentiation of Porcine Preadipocytes

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To better understand possible autocrine/paracrine mechanisms involved in adipose development, we have studied the biosynthesis of insulin-like growth factor I (IGF-I) and prostaglandin E_2 (PGE_2) by cultured porcine preadipocytes in response to factors known to modulate growth and differentiation. The expression of c-*fos* was monitored because of the potential role of that protooncogene in coordination of growth and differentiation. Preadipocytes were grown to confluence and then maintained in one of three media treatments: (i) standard medium supplemented with 10% fetal calf serum (FCS), (ii) FCS supplemented with dexamethasone (Dex), (iii) FCS supplemented with dbcAMP. Indirect measurements of growth indicated that cell proliferation did not differ due to media type. Histochemical and enzymatic measurements of adipocyte development revealed that differentiation occurred only in those cultures exposed to Dex. The increase in adipocyte differentiation in response to Dex was associated with a decrease in *c-fos*, actin and IGF-I RNA expression and decreased secretion of IGF-I and PGE_2. These data provide the first demonstration that biosynthesis of IGF-1 by preadipocytes can be modulated by a potent inducer of adipocyte differentiation. Immunocytochemical analysis indicated that induction of Fos protein was possible only in undifferentiated cells. The combined results indicate that glucocorticoids may stimulate adipocyte differentiation by suppressing intracellular and putative intercellular mitogenic signals.

Evidence for Non-Growth Hormone Mediated IGF Modulation in Adipose Tissue and Muscle

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Insulin-like growth factor I (IGF-I) is a 70 amino acid peptide sharing structural homology with proinsulin and has both insulin-like and growth-promoting properties. This somatomedin appears to be synthesized and secreted primarily by the liver, but has also been demonstrated to be produced by fibroblasts, myocytes and many other tissues. We have previously reported IGF-I and IGF-II mRNA production by muscle as well as adipose tissue. Both nutrition and GH have been shown to regulate hepatic IGF production. Since IGF-I is the product of a single copy gene, we hypothesized that control of gene expression in paracrine tissues producing IGF might be similar to control of circulating IGF (hepatic production) although time course and magnitudes of effect might vary. To test this hypothesis, we measured the effect on tissue levels of IGF mRNA of treatments known to decrease blood levels of IGF. The specific objectives were: 1) to examine the expression of IGF-I mRNA by rat adipose tissue, cardiac muscle and skeletal muscle in response to a short-term (2 day) and long-term (5 day) fast; and 2) to determine the role of insulin on the expression of IGF-I mRNA in rat adipose tissue, cardiac muscle and skeletal muscle. Relative expression of IGF-I mRNA was determined with a solution-hybridization nuclease-protection assay using total cellular RNA. In the current study, both nutritional status and insulin levels had pronounced effects on IGF-I expression in all tissues examined. IGF-I expression in white adipose tissue was 2.5 fold lower in the diabetic and 3.5 fold lower in the long-term fasted rats. Short-term fasting had no effect on white adipose tissue expression. Cardiac muscle IGF-I expression was decreased 2.5 fold with diabetes and approximately 1.5 fold in both fasted groups. Skeletal muscle IGF-I expression was the most dramatically affected, resulting in a 5 fold decrease in the diabetic rats and 4.5 and 6.5 fold decline with short-term and long-term fasting, respectively. This study demonstrates that the control of gene expression in paracrine tissues producing IGF-I appears to be similar to control of circulating IGF (hepatic production).

Fetal Somatomedin and Adipogenic Response to Maternal Diabetes in Swine

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Previous research has demonstrated that maternal diabetes in swine can result in an increase in lipid accumulation in the newborn pig. The potential effects of maternal diabetes upon systemic adipogenic factors and the regulation of preadipocyte proliferation and differentiation have not been examined in the fetal pig. Fourteen pregnant, crossbred gilts were utilized in this study. Seven gilts were injected with alloxan (50 mg/kg) at day 75 of gestation to induce diabetes.

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Gilts underwent caesarean section on day 105 of gestation. Maternal and fetal blood were collected for assay of glucose, insulin, IGF-1 and adipogenic activity as determined upon cell cultures of rat and porcine adipose tissue. Isolated cell cultures from the stromal-vascular fraction of the subcutaneous adipose tissue of fetal pigs or young rats were utilized for these experiments. Cells were exposed to medium 199 containing 2.5% test sera from day 5 to 15 (post-confluency) or from day 1 through 15 (pre- and post-confluency). Cell cultures were harvested on day 15 of culture for analysis of alpha-glycerol phosphate dehydrogenase (GPDH), citrate lyase (CL), lipoprotein lipase (LPL), lactate dehydrogenase (LDH), cell number and protein content. Maternal glucose levels were increased 491% while insulin levels were reduced to undetectable levels by diabetes. Fetal glucose levels were increased 166% by maternal diabetes. Fetal IGF-1 concentrations were increased from 13+/−1 to 18+/− 1 ng/ml by maternal diabetes. Adipogenic activity of the sera from the fetuses of the diabetic and control animals did not differ as assessed enzymatically on primary cultures of porcine or rat adipose tissue. Cells obtained from fetuses of diabetic pigs demonstrated a greater proliferative response (57%) and higher rates of differentiation as determined by GPDH (142%) and LPL (80%) activities than cells acquired from control fetuses. The present data indicate that the increased lipid accumulation in the fetuses of diabetic pigs may be a consequence of changes in the preadipocyte fraction of cells within the developing adipose tissue which result in the formation of more adipocytes being present to utilize the increased quantity of available substrates. (This work was supported by USDA Grant 86-CRCR-1-2126.)

Muscle IGF-I Expression and Serum Factors Affecting Myoblast Differentiation in Runt Pigs

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Before birth, runt pigs have undergone retarded fetal growth and abnormal muscle development resulting in small for date animals with fewer muscle fibers as compared to control littermates. The objectives of this study were to determine the effects of *in utero* runting on 1) neonatal skeletal muscle and liver gene expression of Insulin-like growth factor-I (IGF-I), 2) tissue content of IGF-I, 3) serum levels of IGF-I and 4) the ability of serum to regulate *in vitro* mouse myoblast (C_2C_{12}) differentiation. Blood and tissue samples were collected from runt and control crossbred littermates of the same sex (5-10 animals per group) at birth. Runts were defined as those animals weighing less than 67% of the average litter weight and less than 900 gm. Sera obtained from these animals were used to measure circulating IGF-I levels and to study the ability of these sera to promote *in vitro* myoblast differentiation, as measured by creatine phosphokinase (CPK) activity. Tissue samples collected at birth were immediately frozen in liquid nitrogen for processing of RNA or tissue extracts. Serum and tissue IGF-I levels were determined using a heterologous RIA (Nichol's Institute), after acid ethanol extraction of samples. RNA was extracted using the guanidine thiocyanate procedure. Gene expression of IGF-I was investigated using dot blotting techniques with autoradiography, followed by laser densitometry of autoradiographs. Differentiation of myoblasts incubated with serum from runt pigs did not differ significantly from those incubated with control serum. However, cells incubated with runt serum tended to differentiate to a lesser extent than cells incubated with control serum. Serum IGF-I levels in runt pigs were 63% higher than in sera from control littermates. Extractable tissue levels of IGF-I tended to be slightly higher in runt muscle and over two-fold higher in runt liver compared with control samples. Gene expression of IGF-I in *semitendinosus* was 49% lower in runts compared with controls, while runt liver was modestly lower in IGF-I expression compared with control. These data indicate that the molecular regulation of IGF-I in skeletal muscle may be altered in runt pigs. Since the IGF's are known to be potent regulators of *in vitro* muscle growth, these changes may be involved in the growth retardation observed in runts.

Biology of Insulin-like Growth Factor Binding Proteins in the Pig

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Insulin-like growth factors (IGFs) are transported in pig blood by specific IGF-binding proteins (IGF-BPs). One of our objectives is to purify these IGF-BPs and gain a better understanding of the role they play in modulating the biological effects of IGF-I and IGF-II. In the pig, there are two IGF-BP complexes that transport IGF-I and three IGF-BP complexes that transport IGF-II. IGF-I is bound to a large complex that has a M_r of 150,000 and is GH-dependent. This IGF-BP transports about 60% to 70% of the immunoreactive IGF-I found in blood. The remainder of the immunoreactive IGF-I is associated with an IGF-BP complex with a M_r of 40,000. IGF-II is also associated with these IGF-BP complexes. However, there is a larger IGF-BP ($M_r \approx 450{,}000$) that is specific for IGF-II. When postnatal pig serum is subjected to ligand blotting, there are five specific IGF-BPs with M_r of 43,000, 40,000, 34,000, 30,000 and 26,000. We have purified the two larger IGF-BPs and found that they are part of the GH-dependent 150 kDa IGF-BP complex. Although there are two bands on ligand blots, they have the same NH_2-terminus amino acid sequence, which suggests that there is one protein which has different quantities of carbohydrate bound. Antisera raised against the purified IGF-BP recognizes the 150 kDa IGF-BP complex in pig serum. Treatment of pigs with GH results in a dose-dependent increase in the 43 and 40 kDa IGF-BPs and a dose-dependent decrease in the 34 kDa IGF-BP. In summary, the IGF-BPs are heterogenous with respect to size, their specificity for IGF-I and IGF-II and hormonal regulation of production. Studies are underway to purify all the pig IGF-BPs and determine which tissues produce them.

RNA Transcription in Skeletal Muscle Nuclei: Developmentally and Physiologically Induced Alterations

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The objective of these studies was to assess the transcriptional activity (TA) of fetal bovine or porcine skeletal muscle nuclei in response to various physiological parameters and exogenous stimuli. TA of nuclei was assessed by measuring [^{3}H]UTP incorporation. The inclusion of actinomycin D (ACT-D) in the reaction buffer resulted in ablation of TA and the inclusion of 100 or 0.5 ug/ml of the toxin alpha amanitin (AMAN) decreased TA by 55% and 44%, respectively. Responses of nuclei to ACT-D and AMAN were characteristic of nuclei engaged in DNA-dependent RNA polymerase activity. In order to determine the effect of exogenous steroids on fetal muscle TA, muscle tissue was isolated from fetuses that were approximately 150 d of gestation, minced and incubated in the presence of .01, .1, or 1.0mM of either testosterone propionate (TEST), estradiol-17 beta (EST), or hydrocortisone (CORT). [^{3}H]UTP incorporation was enhanced at the two lower concentrations of test ($p<.10$), the two highest concentrations of CORT ($p<.10$), and the highest concentration of EST ($p<.10$) relative to controls. During a thirty-minute incubation in reaction buffer, nuclei isolated from muscle exposed to .01 mM EST exhibited increased levels of Myosin Heavy Chain (MHC) RNA production but no increase in overall nuclear RNA production. Nuclei isolated from tissue exposed to .01mM TEST increased overall RNA production but had reduced levels of MHC RNA. In another experiment, TA of nuclei isolated from bovine muscle tissue preincubated with serum from somatotropin-treated swine (pST) was examined. Including 5% serum in the preincubation mixture caused over a 600% increase ($p<.05$) in TA over controls. A similar response ($p<.05$) was observed when 10% serum was included in the preincubation buffer with TA declining to control values at the 20% serum level. MHC RNA production was examined in muscle nuclei isolated from neonatal pigs which had fasted for 96 h. MHC RNA production increased relative to nuclei isolated from fed controls. Developmental changes in TA of fetal bovine muscle were assessed by isolating nuclei from fetal bovine muscle at 80, 120, 180 and 210 d of gestation. TA declined from 80 to 180 d gestation and remained the same from 180 to 210 d. It can be concluded from these studies that isolated muscle nuclei can be used to assess the TA of muscle in response to exogenous hormone administration and developmental and physiological parameters.

Satellite Cell Proliferative Activity in Rats Genetically Selected for Fast and Slow Growth

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Muscle growth occurs as a result of a combination of proliferation and differentiation of embryonic myoblasts and postembryonic satellite cell (SC) myoblasts, and expression of genes encoding families of myofibrillar proteins which results in bulk protein accretion. Because SC are of a myogenic lineage, they may become activated by a variety of stimuli postnatally to engage in mitotic activity or differentiate more fully. Postnatal replication of SC nuclei and their intrusion into myofibers results in increased myonuclei numbers and DNA, which endows muscle with greater protein synthetic potential. The objective of this study was to begin characterization of the intrinsic differences in SC metabolic activity in rats that differ genetically in rate of growth with the long-range goal of identifying the mechanisms of SC activation. Eighteen male Charles River CD rat pups from eight litters resulting from the matings of rats that have been selected for rapid or slow rates of growth for 12 generations were injected twice with ^{3}H-dThd (20 uCi/g BW) at 1, 2 and 3 wk age between 0600 and 0900 h. Injections were interspaced by 1 h. One h after the second injection, rats were euthanized by chloroform affixation. Muscles were clamped *in situ* and fixed in Carnoy's solution. Muscle fiber segments were isolated after collagenase digestion. Myofibers isolated from the *triceps brachii* and *semitendinosus* were prepared for satellite cell enumeration by light microscope autoradiography. Body weight, *triceps* muscle weight, *semitendinosus* weight, nuclei/unit volume, nuclei/standard myofiber length, DNA/muscle were larger in the muscles of fast strain rats. At 7 and 14 d of age, percentage tagged nuclei was higher ($P<.05$) in *triceps* (4.4 & 3.6, 3.2 & 2.2, and 1.0 & .9% for fast and slow strains at 7, 14 and 21 d, respectively) and *semitendinosus* (2.6 & 2.0, 1.8 & 1.3, and .9 & .7% for fast and slow strains at 7, 14 and 21 d, respectively) muscle of fast compared to slow growing rats and declined with age in both strains ($P<.01$). Results indicate that growth of skeletal muscle during the neonatal period in the rat is associated with a developmental reduction in SC proliferative activity and that genetic selection for rapid growth enhanced proliferative activity in muscle relative to genetic selection for slow rates of growth and small body size.

Examination of Skeletal Muscle Protein Turnover by Stable Isotope Labelling of Embryonic Chick Muscle Cell Cultures

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Embryonic chick muscle cell cultures were used as an experimental model to determine the effects of added compounds on skeletal muscle proteolysis. Cell protein was labelled with a combination of stable-isotope and radioisotope labelled leucine and degradation was measured by determining the release of labelled leucine into the culture medium. Separate experiments were done to examine the effects of leupeptin and cycloheximide on proteolysis. Leupeptin, a protease inhibitor, has been shown to inhibit

degradation in isolated muscles and cell cultures, but the magnitude of this effect has not been clearly established. Cycloheximide, an inhibitor of protein synthesis, has been used to measure rates of proteolysis in the absence of synthesis in various model systems, but the effect of cycloheximide on proteolysis in muscle cells has not been examined. For this study, primary cell cultures derived from leg muscles of 12-d chick embryos were grown in medium supplemented to contain all 20 amino acids. Cultures were labelled on days 2-8 with [5,5,5-D_3]-leucine and [1,1-$^{18}0_2$]-leucine by replacement of the unlabelled leucine in the medium with an equimolar mixture of these two stable isotopes. On day 8, cultures were radio-labelled by addition of [1-^{14}C]-leucine. On day 9, medium was replaced with release medium containing 25% of the normal concentration of unlabelled leucine and the release of D_3-, $^{18}0_1$ amd ^{14}C-leucine into the medium was measured over a 24-h period. To determine the amount of leucine uptake during the measurement period, [4,5-^{3}H]-leucine was added to the release medium. Each experiment consisted of two trials with 3 control plates and 3 plates treated with either leupeptin (75 ug/ml) or cycloheximide (0.375mM) for each trial. Leupeptin treatment resulted in an 11% to 15% decrease in release of D_3-, $^{18}0_1$- and ^{14}C-leucine into the medium ($P<0.01$). This experiment demonstrated the validity of the labelling methods for comparing effects of added compounds on muscle cell proteolysis. Cycloheximide treatment resulted in an 8% increase ($P<.02$) in release of ^{14}C-leucine, but a >50% decrease ($P<.01$) in release of D_3-leucine. Uptake of ^{3}H-leucine was completely inhibited in cycloheximide-treated plates. The results demonstrated that cycloheximide treatment affects proteolysis in muscle cells, and suggest that this effect is quantitatively and qualitively different for proteins with long and short half-lives.

Quantitative Aspects of Gene Regulation

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Recombinant DNA technologies have been utilized recently for the quantitation of the effects of phenethanolamines and other compounds affecting growth on the processes of growth and development in livestock species. To address the possibility that compounds such as clenbuterol or ractopamine stimulate muscle protein synthesis, RNA typically is extracted from a suitable muscle frozen in liquid nitrogen immediately postmortem. After extraction by standard procedures, the RNA is separated by size on agarose gels and transferred to nitrocellulose (or nylon-supported) filters (Northern transfer), or is blotted directly onto filters (slot- or dot-blots). Hybridization to radiolabeled cDNA clones such as that specific for myosin light-chain-1/3 has revealed that treatment with ractopamine increased the muscle concentration of the myosin light chain-1/3 mRNA. This indicates that these compounds increase muscle mass in livestock species by increasing myofibrillar protein synthesis. This same technique has been used to demonstrate that zeranol, with or without bGRF, increases the concentration of growth hormone mRNA in cultured adenohypophyseal cells. This suggests that zeranol may increase the growth rate of cattle by stimulating growth hormone secretion. Recombinant DNA technologies also have been used to demonstrate restriction fragment length polymorphisms (RFLPs) in bovine DNA. Bovine DNA, prepared from blood samples, is digested with restriction endonucleases (e.g., *Pvu* II, *Bam* HI, or *Pst* I), and the size fragments are separated on agarose gels. Specific genomic or cDNA clones (such as for the adipose tissue fatty acid-binding protein), when hybridized to Southern transfers of the DNA fragments, can reveal DNA size polymorphisms. These RFLPs currently are being used in both plant and animal agriculture to select for individuals with specific phenotypic traits. Similar techniques have been used in DNA fingerprinting. DNA from parents and offspring, prepared as described above, can be used to verify (or disprove) parentage. These are a few of the methods by which the quantitative aspects of gene expression can be utilized in animal agriculture.

Myoblast Proliferation and Phosphatidylinositol Metabolism

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The myogenic cell line (L_6) was utilized in an *in vitro* study designed to investigate the proliferation and differentiation of myoblast cells in culture. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with the addition of 10% fetal bovine serum (FBS) and proliferation and differentiation monitored through changes in cell number, ^{3}H-thymidine uptake and changes in creatine kinase activity. In addition, the possible regulatory role of phosphoinositide metabolism in the proliferation process of these cells was investigated. The cells exhibited a rapid rate of proliferation with large increase ($P<.05$) in cell number from 24 through 96 h of culture and were confluent at 120 h. DNA synthesis, as monitored via ^{3}H-thymidine incorporation, paralleled the change in cell number with FBS stimulating large (approximately 3-fold) increases ($P<.01$) in ^{3}H-thymidine incorporation as compared to controls up to 96 h in culture, but did not increase incorporation after 120 h in culture. Creatine kinase activity reflected the proliferation data, with very little activity at 24 h, but increasing ($P<.01$) nearly 14-fold by 144 h in culture (10.6 vs 142.3 mU). These data suggest the decrease in proliferative capacity of these cells with time in culture is the result of the differentiation of the myoblast. Inhibition of inositol phosphate turnover with increasing levels of lithium chloride (LiCl) resulted in an increasing ($P<.05$) inhibition of DNA synthesis (^{3}H-thymidine incorporation) with 100 mM LiCl causing an 85.7% decrease in ^{3}H-thymidine incorporation and a near complete inhibition of proliferation (7.6% increase in cell number over initial cell number; $P>.30$). Stimulation of the cells with FBS resulted in a 196% increase ($P<.01$) in inositol trisphosphate (IP_3), within 30 s ($P<.01$) and subsequently was followed by increases ($P<.05$) in the dephosphorylation products of IP_3, inositol bisphosphate (IP_2) and inositol monophosphate (IP). Taken

together, these data are supportive of a role for phosphoinositide metabolism in mediating the proliferation process in myoblast.

Preliminary Results on the Effectiveness of the Destron PG-100 Electronic Probe as a Predictor of the Carcass Composition of Pigs Treated with an Analog of Human Growth Hormone-Releasing Factor

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Sixteen crossbred Yorkshire (75%) X Landrace (25%) barrows were used to evaluate the effect of an analog of human growth hormone-releasing factor (hGRF) on the relationship between lean yield prediction obtained by linear measurements and carcass composition. Eight animals were used as controls and eight were treated with hGRF. Pearson correlation coefficients between percent separable lean and percent predicted lean yield obtained by probe were 0.6789 ($P = .06$) and 0.6477 ($P = .08$) respectively for control and hGRF treatments, respectively. Also high negative correlation coefficients were found between crude fat and predicted lean yield ($P<0.05$) for the two groups. Regression analysis of backfat measurements with percent separable lean obtained by dissection yielded residual standard errors (RSE) of 1.51 and 2.05 for the hGRF group and the control group, respectively. No significant differences ($P>0.05$) were observed between regression lines. Clearly, a study with a larger number of animals is needed. However, these results indicate that hGRF treatment does not reduce the predictive value of the probe measurements. Revision of the regression equation is suggested.

Key words: Carcass composition, grading, hGRF, growth promoter, fat thickness.

The Level of Calcium-Dependent Proteases, Lysosomal Enzymes and Nucleic Acid Content in a Lean and Obese Strain of Pigs

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Lean and obese animals may provide a model for protein turnover to help understand the causes of obesity, and to better understand the mode(s) of skeletal muscle protein turnover. The objectives of the present study were to look at parameters that are implicated in these processes. Strains of lean and obese swine from similar genetic lineage (Duroc x Yorkshire, 50:50) have been well established and may prove ideal models for this purpose. This experiment was executed in 2 phases, involving 32 pigs. Phase 1 involved 8 lean and 8 obese pigs at 10-12 wks of age. Phase 2 involved 8 lean and 8 obese pigs, at 7 months of age. Immediately after slaughter, *longissimus* muscle samples were processed to measure activities of calcium-dependent protease-I (CDP-I), CDP-II and CDP inhibitor as well as lysosomal cathepsins B and B+L. Additional samples were frozen immediately in liquid nitrogen for DNA, RNA, mRNA of α-actin and total protein determinations. Phase 2 and the mRNA measurements have not been done at this time. No significant differences ($P>0.05$) were observed in total CDP-I, CDP-II and cathepsin B activities per gram of muscle between lean and obese pigs. Total CDP inhibitor and cathepsin B+L activities per gram muscle, as well as total protein per gram muscle, were significantly greater ($P<.05$) in obese than lean pigs. In contrast, total DNA and RNA per gm muscle were greater ($P<.05$) in the lean pigs. The data obtained to date suggest that these two strains of pigs may make them a good model for the study of skeletal muscle protein turnover.

Key words: Proteases, Cathepsins, DNA, RNA.

The Effect of a β-Adrenergic Agonist on the Activity of the Calcium-Activated Proteinase System and on Myofibrillar Protein Degradation in Ovine Muscle

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Dietary administration of 4 ppm of the β-agonist L-644,969 (Merck Sharpe and Dohme Research Laboratories) to finishing lambs induced a decrease in extractable calpain I activity in the *longissimus* (LD) muscle (10-14%, $p<.05$). At 4 days postmortem (d-4), extractable calpain I levels in the LD of both control and treated lambs were significantly reduced ($p<.001$) from those present at d-0; and the extractable activity in the LD from control lambs was reduced to a greater extent than in treated lambs. Calpain II activity was increased 34-42% ($p<.005$) in LD of treated lambs; however, no significant differences were observed between d-0 and d-4 calpain II activity within treated or control LD samples ($p>.1$). Calpastatin activity was higher in the LD of treated lambs than in the LD of control lambs (59-74% on d-0, $p<.001$ and 365-430% on d-4, $p<.001$). On days 2, 4 and 6 postmortem, degradation was decreased in myofibrils isolated from the LD of treated lambs as compared to myofibrils isolated from control lambs. Warner-Bratzler shear values for loin chops from treated lambs were higher on both days 3 (111%) and 6 (108%) postmortem than for chops from control lambs ($p<.001$). L-644,969-induced decreases in muscle proteolytic capacity may limit postmortem myofibril degradation and contribute to reduced tenderness observed in meat from L-644,969-treated lambs. Additionally, this decreased proteolytic capacity may contribute to increased protein accretion in muscles of L-644,969-treated lambs.

Influences of Beta-Agonist L-655,871 and Electrical Stimulation on Post-Mortem Muscle Metabolism and Tenderness in Lambs

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Inter-relationships between dietary administration of the beta-adrenergic agonist L-665,871, electrical stimulation, postmortem muscle metabolism and tenderness were studied in growing-finishing lambs. Eighty crossbred ewes and wethers were fed 0, 0.25, 1.0 or 4.0 ppm L-665,871 or 1.0 ppm L-644,969 for 6 weeks. One-half of all lambs received low-voltage electrical stimulation at slaughter. Growth performance and carcass characteristics were improved in a dose-dependent manner. Instron shear force of one-half inch cores of *longissimus* (LD) cooked to 70 C, was increased 4.1%, 26.8% ($P<.05$), and 36% ($P<.05$) with .25, 1 and 4 ppm L-665,871, respectively. Controls averaged 5.26 kg; shear force was 39.9% higher in lambs fed 1 ppm L-644,969. Stimulation reduced shear force 7% on average ($P<.05$); range between treatments was 5.3%-17%. Decreased tenderness was not caused by differences in sarcomere length, measured at 48 hours postmortem, or cooking loss. Stimulation accelerated rate of pH decline in the LD, but the beta agonists fed at 1 or 4 ppm increased pH .2 units at 0 and 2 hours, and .1 unit at 4, 8, 24 and 48 hours post-mortem (all $P<.05$). Calcium-dependent proteinase I and II and calpastatin activities were measured in 100 g of LD from lambs fed 0 or 4 ppm L-665,871. Treatment effects were different in ewes and wethers ($P<.05$). In wethers fed L-665,871 CDP-I activity was 36% lower, CDP-II activity was 22% lower and calpastatin activity was 21% lower than in control lambs. Ewes fed the beta agonist exhibited 52% higher CDP-II ($P<.05$) and 32% higher calpastatin ($P<.05$) activities, compared with controls, while CDP-I activities were not different. Results demonstrate that 1) the dose-dependent increase in shear force of cooked muscle from beta-agonist treated lambs is still in an acceptable range, 2) the reduction in tenderness is not caused by cold shortening or greater cooking loss, 3) that electrical stimulation can improve tenderness in lambs fed the beta-agonist, and 4) that changes in CDP or calpastatin activities may explain a portion of the tenderness reduction.

Interaction of β-Adrenergic Agonists and Insulin with Porcine Adipocytes and L6 Muscle Cells

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Certain β-adrenergic agonists have been shown to decrease insulin binding and sensitivity in rodent adipocytes. The opposite response has been observed in rodent skeletal muscle. The objectives of these experiments were to; 1) examine the interaction of β-adrenergic agonists and insulin on swine adipocyte metabolism, 2) determine the effectiveness of ractopamine and clenbuterol in interfering with insulin action of swine adipocytes, and 3) using L-6 muscle cells, determine the effects of β-adrenergic agonists on insulin binding kinetics. Adipocytes were prepared from backfat and incubated in Krebs-Ringer bicarbonate buffer (KRB; 3% BSA) for 2 h at 37°C (lipolysis and lipogenesis) or in KRB (1% BSA) for 1 h at 30°C (insulin binding). Myotubes from L-6 myoblast were cultured for 36 h in medium containing 10% horse serum and 10^{-4} M isoproterenol (ISO) followed by 24 h at 4°C in KRB (1% BAS) plus labelled insulin. Sensitivity of lipolysis to ISO was enhanced approximately 20 fold by the addition of adenosine deaminase (ASA). Insulin negated the lipolytic response to ISO in the absence of ADA and reduced ISO sensitivity approximately 10 fold in the presence of ADA. Epinephrine (EPI), ractopamine (RAC) and clenbuterol (CB) decreased ($P<.05$) basal lipogenic rate in the presence of ADA or theophylline. Insulin stimulated lipogenesis 25% and prevented some of the inhibitory effects of EPI and RAC and all of the antilipogenic response to CB. Adipocytes incubated with EPI or RAC (10^{-5} M) or dibutyryl-cAMP specifically bound less insulin (15-40%) than non-treated cells. L-6 myotubes exposed to ISP for 36 h specifically bound 36% more insulin than non-treated cells. Taken together, these data clearly show the counter regulatory actions of the β-adrenergics and insulin in modulating adipose tissue metabolism. In muscle, on the other hand, β-adrenergic agonist and insulin appear to be complementary. Differential regulation of insulin action by β-adrenergic agonists may be responsible, in part, for tissue growth patterns in pigs fed these agents.

Effect of Ractopamine Treatment and Subsequent Withdrawal on Porcine Skeletal Muscle Alpha-Actin mRNA Abundance

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A total of 60 crossbred market pigs were used to study the effect of ractopamine treatment and subsequent withdrawal on porcine skeletal muscle alpha actin mRNA abundance. Ractopamine was administered through the feed (20 ppm) for periods of 2, 4 and 6 weeks. Six pigs were allotted to each treatment and control group for these time periods. In addition, four control pigs and four pigs that were fed ractopamine for 6 weeks were slaughtered at 1, 3 and 7 days after withdrawal of ractopamine. At slaughter, a *longissimus* muscle sample was immediately removed and frozen in liquid nitrogen. Total RNA was isolated using lithium chloride – urea precipitation followed by a guanidinium thiocyanate – cesium chloride centrifugation. Northern blots and dot blots were used to quantify the relative abundance of skeletal muscle alpha actin. A full length human skeletal muscle

alpha actin probe was used for hybridization under stringent conditions. Alpha actin levels were 41% and 60% higher ($P<.05$) in treated animals than controls after feeding ractopamine for 2 and 4 weeks, respectively. No significant difference between control and treated animals was detected at 6 weeks or during the withdrawal period. These results indicate that the ractopamine enhanced muscle growth and elevated protein synthesis rates may result from increased gene transcription or other pretranslational events that increase alpha actin mRNA levels.

Effect of Ractopamine Treatment and Subsequent Withdrawal on Growth and Carcass Composition of Pigs

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Thirty crossbred barrows weighing 72.4 kg were allotted to a control group and another 30 were allotted to a group fed 20 ppm ractopamine. Water and the 18% crude protein corn-soybean meal diet were provided ad libitum. Six control and six barrows were slaughtered at 2, 4 and 6 wk of the trial. At 6 wk of the trial, all pigs received the control diet. Four control barrows and four barrows that had been fed ractopamine for 6 wk were slaughtered after 1, 3 and 7 d of withdrawal. At slaughter, the right *longissimus* muscle and perirenal fat from both sides were removed and weighed. After chilling for 24 h, tenth rib backfat thickness, *longissimus* muscle area were measured and carcass muscle weight calculated. Feeding ractopamine increased live weight and average daily gain at 2 and 4 wk but the slightly greater weight and gain of treated pigs were not different at 6 wk or during the withdrawal period. Tenth rib backfat tended ($P>.05$) to be less in treated pigs at all slaughter periods. Perirenal fat weight was not different between treatments at 2 and 4 wk but was decreased at 6 wk by ractopamine which persisted throughout the withdrawal period. *Longissimus* muscle area was increased by feeding ractopamine at 4 and 6 wk and after 1 d of withdrawal. While *longissimus* areas of ractopamine-fed pigs were larger after 3 and 7 d, they did not differ from controls. *Longissimus* muscle weight was significantly increased by ractopamine at 2, 4 and 6 wk as well as following 1, 3 and 7 d of withdrawal. Total carcass muscle weight also was increased by feeding ractopamine at 2, 4 and 6 wk; and although the increase in muscle of treated pigs was maintained following withdrawal, the differences were not different from controls. These data indicate that the advantages of feeding ractopamine on growth and carcass composition generally are maintained throughout 7 d of withdrawal.