**Project/Activity Number:** Multistate Research Project NC1184
**Project/Activity Title:** Molecular Mechanisms Regulating Skeletal Muscle Growth and Differentiation
**Period Covered:** 10/2012 to 10/2013
**Date of This Report:** 11/12/13
**Annual Meeting Dates:** 10/25-26/2013

**Participants:**
Paul Mozdziak (NCSU; host) - pemozdzi@ncsu.edu
Joshua Selsby (ISU; secretary) – jselsby@iastate.edu
Duane Keisler (Missouri) - keislerd@missouri.edu
Sarah Reed (UConn) - sarah.reed@uconn.edu
Gary Housman (Georgia) - ghausman@uga.edu
Anna Dilger (Illinios) - adilger2@illinois.edu
Michael Zeece (Nebraska) - michael.zeece@unl.edu
Shihuan Kuang (Purdue) - skuang@purdue.edu
Samer El Kadi (Virginia Tech) - elkadi@vt.edu
Rob Rhoads (Virginia Tech) - rhoadsr@vt.edu
David Gerrard (Virginia Tech) - dgerrard@vt.edu
John Gonzalez (Kansas State) - johngonz@k-state.edu
Wei Gou (Wyoming) - wguo3@uwyo.edu
Penny Riggs (Texas A&M) - riggs@tamu.edu
Gale Strasburg (Michigan State) - Stragale@anr.msu.edu

**Summary of Minutes of Annual Meeting:**

The annual meeting of the NC1184 technical committee meeting was held at North Carolina State University on October 25-26, 2013, and was hosted by Dr. Paul Mozdziak from Poultry Science. On October 25$^{th}$ the group was welcomed by Becky Boston, the Interim Assistant Director of the NCARS. Our planned interaction with a USDA official was cancelled due to the federal government shutdown. Next year's meeting of the NC1184 committee will be hosted by Dr. Joshua Selsby at Iowa State University. The group decided that the 2015 location will be hosted by Duane Keisler at the University of Missouri. The remainder of the day was filled by oral station reports summarizing each station's contributions to the objectives of the NC1184 project. The meeting adjourned and the group met for a dinner and social at the Sheraton in conjunction with the NE1034 committee. The meeting reconvened on October 26$^{th}$ and the remaining stations reported their activities. Following these presentations the meeting adjourned for the year.

**Accomplishments:**

**Objective 1: Characterize the signal transduction pathways that regulate skeletal muscle growth and metabolism including the influence of endogenous growth factors and various production practices.**

The **Connecticut Station** reports that the triggers for satellite cell activation are many but include growth factors, stretch, nitric oxide, and cytokines. Interleukin (IL)-6 is necessary for satellite cell proliferation and myonuclear accretion during compensatory hypertrophy in the mouse. To begin to understand the mechanisms behind cytokine induced satellite cell dysfunction, C2C12 myoblasts were cultured with 0, 1, 10, or 100 ng/mL IL-6 during proliferation or fusion conditions. Proliferation was inhibited by exposure to 1, 10 and 100 ng/mL of IL-6 for 36 hours. Myoblast fusion was also inhibited by 10 and 100 ng/mL IL-6. Ongoing work is investigating the signaling mechanisms involved in the downstream signaling pathways that may be involved in these inhibitions, including activation of the Jak/STAT3 pathway.

The expression of many genes encoding secreted and non-secreted factors have been studied in human and rodent adipose tissue with cDNA microarrays, but few such studies in adipose tissue from growing pigs have been reported. The **Georgia Station** reports that Total RNA was collected at slaughter from outer subcutaneous adipose tissue (OSQ) and middle subcutaneous adipose tissue (MSQ) samples from gilts at 90, 150 and 210 days (n55/age). Dye-labeled cDNA probes were hybridized to custom microarrays (70-mer oligonucleotides) representing about 600 pig genes involved in growth and reproduction. Gene expression intensity ratios changed little with age for 100 transcription factors, nuclear receptors, enzymes and other regulatory proteins in OSQ and MSQ from pigs between 90 and 210 days of age. However, the relative expression of 13 genes distinguished OSQ and MSQ depots in growing pigs. The expression of several genes were influenced by age including an increase in CCND3, HSF1 and PTGR1 expression in MSQ and a decrease in UCP2 and REA (prohibitin-2) expression in OSQ. These studies demonstrate for the first time the expression of several key regulatory genes in pig adipose tissue. Simple linear regression analysis showed that leptin gene expression was associated with expression of some of these regulatory genes. Negative associations between expression of some regulatory factors and leptin gene expression indicated that local leptin may decrease or antagonize adipogenesis.

Ractopamine-HCl (RAC) is a beta-adrenergic agonist that is commonly used in the livestock industries to positively affect live performance measures (average daily gain, feed to gain ratio) by promoting muscle accretion over fat deposition. Research trials have demonstrated that the trace mineral, Zinc (Zn), can enhance the response that swine exhibit to RAC supplementation, but the results are variable. The **Kansas Station** reports that Over the past year, the laboratory has participated in a research trial examining the effects of two sources of Zn (ZnO and Availa-Zn) supplemented at 3 different levels (75, 150, and 225 ppm) on the swine RAC response. Pigs were subjected to the RAC/Zn supplementation period for 35 d and muscle biopsy samples were collected on d 0, 8, 18, and 32 to supplementation. Biopsies were utilized for mRNA expression of 9 genes associated with muscle hypertrophy (*IGF I/II and receptors, Pax7, MyoD, Myf5, Myogenin, and beta-adrenergic receptors 1 and 2*) and examination of the mTOR protein synthesis pathway. Data was analyzed using orthogonal contrasts for the comparison of the RAC and Zn treatment groups. When compared to the controls, RAC supplementation tended ($P < 0.07$) to increase hot carcass weight, while adding Zn to RAC containing diets did not affect ($P > 0.10$) hot carcass weight. RAC supplementation did tend to increase ($P = 0.10$) loin eye area when compared to controls, while Zn supplementation tended to stimulate increases in loin eye area in a quadratic fashion. Zn150 carcasses possessed the largest loin eye areas and this was

catalyzed by a tendency of type IIB fibers to increase ($P < 0.10$) in CSA in the same quadratic manner. The modest increases in muscle hypertrophy stimulated by the Zn75 and Zn150 treatments occurred independently of an increase ($P > 0.10$) in protein synthesis as indicated by the lack of differences in the phosphorylation rate of key proteins of the mTOR pathway ($P > 0.10$; $Akt^{ser473}$, mTOR, and $RPS6^{ser\ 240/244}$). Hypertrophy was also stimulated independently of satellite cell incorporation into the myofiber as indicated by the lack of increases in fiber associated nuclei and mRNA expression of *Pax7* and the *myogenic regulatory factors* ($P > 0.10$). Interestingly, Zn supplementation decreased muscle *IGF1* mRNA expression in a quadratic fashion on d 8 and 18 of the feeding period ($P < 0.05$), which could account for the inability for Zn to enhance the RAC response.

The **Illinois Station** reports that in swine, a QTL that explained 15-30% of variation in muscle mass and 10-20% of variation in back fat was first reported in Large White and Peitran breeds. The mutation was later identified as a single base pair substitution in intron 3 of IGF2 (g3072A). The substitution altered a conserved CpG island that is hypomethylated in skeletal muscle and is thought to be a binding site for ZBED6, a repressor. Interestingly, IGF2 is one of many maternally imprinted genes. The mutation (inherited paternal A) resulted in an increase in IGF2 expression in postnatal muscle though it did not alter IGF2 expression in fetal muscle. Increased postnatal IGF2 expression may be responsible for increased differentiation in satellite cells resulting in increased muscle hypertrophy. However, the phenotype of IGF2 intron 3 g3072A animals has not been fully characterized; therefore, the mechanism by which this mutation alters muscle growth remains unclear despite the A allele being largely fixed in commercial swine populations.

The Illinois Station goes on to report that to more fully characterize the phenotype of this mutation and determine the mechanism of increased muscle growth in pigs carrying a paternal A allele, IGF2 mutant and wild-type animals were produced with a common genetic background and analyzed from d60 of gestation through market weight. The mutation did not alter body weight at any time point measured. However, the mutation did alter carcass composition was found. Pigs with a mutant A allele had decreased backfat thickness ($P < 0.05$), and increased loin muscle area ($P < 0.05$) and longissimus weight ($P < 0.01$), thereby resulting in a 2.4-percentage unit increase in lean carcass cutting yield ($P < 0.0001$). In contrast, the mutation only resulted in minimal changes in fresh meat quality parameters. The A genotype did increase post-natal IGF2 expression, up to a 4-fold increase in expression at a market weight time point ($P < 0.0001$), without significantly altering IGF1 expression. However, IGF type 1 and type 2 receptors, myostatin, and ZBED6 expression do not appear to be altered by the mutation.

The **Indiana Station** reports that myostatin (Mstn) is predominantly expressed in skeletal muscles and plays important roles in regulating muscle growth and development, as well as fat deposition. Mstn-knockout (Mstn(-/-)) mice exhibit increased muscle mass due to both hypertrophy and hyperplasia, and leaner body composition due to reduced fat mass. Here, we show that white adipose tissue (WAT) of Mstn(-/-) develops characteristics of brown adipose tissue (BAT) with dramatically increased expression of BAT signature genes, including Ucp1 and Pgc1α, and beige adipocyte markers Tmem26 and CD137. Strikingly, the observed browning phenotype is non-cell autonomous and is instead driven by the newly defined myokine irisin (Fndc5) secreted from Mstn(-/-) skeletal muscle. Within the muscle, Mstn(-/-) leads to

increased expression of AMPK and its phosphorylation, which subsequently activates PGC1α and Fndc5. Together, our study defines a paradigm of muscle-fat crosstalk mediated by Fndc5, which is up-regulated and secreted from muscle to induce beige cell markers and the browning of WAT in Mstn(-/-) mice. These results suggest that targeting muscle Mstn and its downstream signaling represents a therapeutic approach to treat obesity and Type 2 diabetes.

For some time the **Iowa Station** has been interested in developing novel therapeutic approaches for Duchenne muscular dystrophy (DMD), a fatal, progressive muscle disease. We have previously found that viral gene transfer of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) prevented disease onset and rescued muscle with active decline. We have recently found that gene transfer of PGC-1α will also prevent acute eccentric muscle injury in dystrophic skeletal muscle. This is important because this sort of injury is widely believed to contribute to muscle injury in DMD.

The Iowa Station has also used oral administration of a PGC-1α activator, quercetin, to attenuate disease severity. Previously, we found that dietary quercetin enrichment decreased, but did not eliminate muscle injury caused by dystrophin deficiency. We have been working of late to determine the mechanism underlying this effect. We will begin by performing knockdown and over-expression studies in cell culture. As an essential first step we are working to optimize dose and duration of exposure leading to maximal pathway activation. To this end we are nearing completion of an extensive dose-response and duration study in C2C12 cells. We have also initiated an in vivo feeding study where we are measuring respiratory function over time using unrestrained whole-body plethysmography.

The **North Carolina Station** reports that loss of muscle mass, or sarcopenia, is nearly universal in cirrhosis and adversely affects patient outcome. The underlying cross-talk between the liver and skeletal muscle mediating sarcopenia is not well understood. Hyperammonemia is a consistent abnormality in cirrhosis due to impaired hepatic detoxification to urea. We observed elevated levels of ammonia in both plasma samples and skeletal muscle biopsies from cirrhotic patients compared with healthy controls. Furthermore, skeletal muscle from cirrhotics had increased expression of myostatin, a known inhibitor of skeletal muscle accretion and growth. In vivo studies in mice showed that hyperammonemia reduced muscle mass and strength and increased myostatin expression in wild-type compared with postdevelopmental myostatin knockout mice. We postulated that hyperammonemia is an underlying link between hepatic dysfunction in cirrhosis and skeletal muscle loss. Therefore, murine C2C12 myotubes were treated with ammonium acetate resulting in intracellular concentrations similar to those in cirrhotic muscle. In this system, we demonstrate that hyperammonemia stimulated myostatin expression in a NF-κB–dependent manner. This finding was also observed in primary murine muscle cell cultures. Hyperammonemia triggered activation of IκB kinase, NF-κB nuclear translocation, binding of the NF-κB p65 subunit to specific sites within the myostatin promoter, and stimulation of myostatin gene transcription. Pharmacologic inhibition or gene silencing of NF-κB abolished myostatin up-regulation under conditions of hyperammonemia. Our work provides unique insights into hyperammonemia- induced myostatin expression and suggests a mechanism by which sarcopenia develops in cirrhotic patients.

Fetal growth in mammals depends in its entirety on "adequate" nutrition. Substantial evidence shows that perturbed nutrient supplies in prenatal life have profound, lasting effects throughout adulthood. Intrauterine growth restriction (**IUGR**), which is defined as impaired fetal growth and development, occurs when nutrient delivery falls short of meeting maximal needs of the growing fetus. IUGR results in changes in body composition and meat quality, reduced growth performance, increased morbidity and mortality, and abbreviated muscle development. All of these effects undoubtedly can lead to reduced profitability. Therefore, it is essential to understand the mechanism by which IUGR affects skeletal muscle growth so that dietary interventions may be designed to mitigate such deleterious effects on production efficiency. The principal objective of our research program is to determine how metabolic cues regulate macronutrient partitioning to anabolic and catabolic pathways. Over the last year the **Virginia Station** initiated two pig projects. We are employing a multifaceted approach to studying IUGR in pigs. First, we are characterizing the expression of muscle transcriptome with RNA-Seq technologies, and determining the relationship between transcript changes and protein expression. Second, protein synthesis and degradation will be studied with targeted metabolite analysis using stable isotope tracers. Finally, transcriptome and protein expression analyses will provide a framework for the development of nutritional interventions that would reverse the negative effects of IUGR on protein deposition.

The **Virginia Station** further reports that chickens from lines selected for low (LWS) and high (HWS) body weight differ by 10---fold in body weight at 56 days---old with differences in appetite, glucose regulation, and body composition. To evaluate if there are differences in appetite---regulatory factor and glucose transporter (*GLUT*) mRNA that are accentuated by hypoglycemia, blood glucose was measured and hypothalamus, liver, *Pectoralis major* and abdominal fat collected at 90 days of age from female HWS and LWS chickens, and reciprocal crosses, HL and LH, at 60 minutes after IP---injection of insulin. Neuropeptide Y (*NPY*) and receptor (*NPYR*) sub---types 1 and 5 mRNA were greater in LWS compared to HWS hypothalamus ($P < 0.05$), but greater in HWS than LWS in fat ($P < 0.05$). Expression of *NPYR2* was greater in LWS than HWS in *Pectoralis major* ($P < 0.05$). There was greater expression in HWS than LWS for *GLUT1* in hypothalamus and liver ($P < 0.05$), *GLUT2* in fat and liver ($P < 0.05$) and *GLUT9* in liver ($P < 0.05$). Insulin was associated with reduced blood glucose in all populations ($P < 0.05$), and reduced mRNA of insulin receptor and *GLUT* 2 and 3 in liver ($P < 0.05$). There was heterosis for mRNA, most notably *NPYR1* (–78%) and *NPYR5* (---81%) in fat and *GLUT2* (--- 70%) in liver. Results suggest that NPY and GLUTs are associated with differences in energy homeostasis in LWS and HWS. Reduced *GLUT* and *IR* mRNA after insulin injection suggest a compensatory mechanism to prevent further hypoglycemia.

Regenerating (Reg) proteins are a class of C---type lectin proteins found in a variety of inflamed and regenerating tissues. Reg proteins regulate normal regeneration progression in damaged tissues, which was observed across various tissues and organs in mice with genetic Reg protein mutations. Skeletal muscle is a dynamic tissue subject to bouts of degeneration and regeneration, however Reg proteins and their receptors (EXTL) are uncharacterized during these processes. Therefore, the objective of the **Virginia Station** was to evaluate Reg proteins during skeletal muscle regeneration using a well---characterized bupivacaine--- induced skeletal muscle damage and regeneration model. While all members of the Reg gene family except Reg IIIβ and IIIδ were expressed in damaged muscle tissue, only Reg I, IIIα, IIIγ and EXTL3 mRNA were detectable in isolated satellite cells. Reg IIIα RNA expression increases at the onset of muscle

injury, and remains elevated until 14 days post--damage, while Reg IIIγ expression peaked at 12 hours and then declines to moderate levels. Expression of all Reg subclasses were detectable with immunohistochemistry (IHC) and mainly localized to regenerating myofibers between 0.5 and 4 days post-damage. In satellite cells, Reg I and IIIγ gene expression peaked at 48 hours post---isolation then decreased over the next 5 days, while EXTL3 expression significantly jumped between 3 and 4 days post---damage. While Reg IIIα expression patterns in satellite cells were unremarkable over time, Reg IIIα had the highest overall mRNA concentration, which implies that this subclass is localized to satellite cells. Next, we supplemented damaged muscles with a Reg IIIα protein solution to discern the role of exogenous Reg protein on myogenesis. Reg---treated muscle samples showed significantly downregulated EXTL3, Pax7 and Myogenin expression at 2 or 3 days, as well as significantly decreased fiber numbers and diameters compared to the PBS--- treated samples. Taken together, our results indicate that each Reg subclass may perform different functions throughout the regeneration process to promote myogenesis.

One of the major research focuses of the **Wyoming Station** is on molecular mechanisms of gene alternative splicing in skeletal muscle growth and differentiation. RNA binding motif 20 (Rbm20) is muscle specific splicing factor that regulates alternative splicing of around 31 genes including myofibril protein-Titin. Titin undergoes alternative splicing in different skeletal muscle, for example, titin expresses larger isoform in Soleus, but smaller isoform in Tibialis anterior (TA). However, in Rbm20 deficient rat model, Titin appears as largest titin isoform in all different skeletal muscle, even soleus and TA express same size of titin. Interestingly, Thyroid hormone or growth factors change titin isoforms in cardiac muscle, but whether these hormones or growth factors alter titin isoforms in skeletal muscle remains unknown. In addition, whether Rbm20-regulated titin isoform transition connects thyroid hormone or growth factors is unclear as well. Therefore, our goal is to address these questions by performing in-vivo and in-vitro studies with molecular and cellular biology methods. Ultimately, the mechanisms will be used to test in livestock animals for improving quality and quantity of animal products.

**Objective 2: Characterize the cellular and molecular basis of myogenesis**.

The **Connecticut Station** reports that an important issue in maintaining muscle mass is the prevention of atrophy during periods of disease or disuse. IL-6 has multiple effects on satellite cell activity, depending on the context and environment in which expression is increased. C26 colon carcinoma induces IL-6 dependent muscle atrophy. To determine if this environment inhibits satellite cell function, the tibialis anterior (TA) of tumor bearing animals was injured via cardiotoxin injection two weeks after inoculation with C26 cells. Animals were injected with bromodeoxyuridine (BrdU) every other day until sacrifice. The cross sectional area (CSA) of uninjured TA muscles in tumor bearing was smaller than uninjured control muscles by 21 days after C26 inoculation ($P < 0.05$). Further, both seven and ten days following injury the CSA of injured muscles from TB animals was decreased compared to that of injured muscle from healthy animals, indicating a deficit in muscle regeneration ($P < 0.05$). Satellite cell proliferation in regenerating muscle (measured by the number of BrdU+ cells associated with muscle fibers) was decreased in TB animals compared with healthy animals 7, 10, and 14 days post injury ($P < 0.05$). To determine if the satellite cell pool itself was affected by the presence of cancer, the number of Pax7+ cells was quantified in muscles from control and TB animals in the presence or absence of muscle regeneration. There was no difference in the number of Pax7+ cells in non-

regenerating muscle from control or tumor bearing animals. However, in regenerating muscle, there were fewer Pax7+ cells in tumor bearing animals at both 3 and 7 days after injury ($P < 0.05$). Similarly, there were also fewer MyoD+ cells in regenerating muscle from tumor bearing animals compared with healthy animals ($P < 0.05$). These data suggest that muscle regeneration is inhibited and that satellite cells fail to function in a normal manner in response to regeneration in tumor bearing animals. Ongoing work is investigating the effect of an IL-6 receptor antagonist on muscle regeneration during tumor bearing conditions and will investigate the ability of satellite cells to contribute to regeneration.

The **Illinois Station** reports that alterations in muscle development due to prenatal insults can have life-long implications for both animal production and human health. We have recently began a series of projects to determine the fetal programming that results from nutritional alterations or from maternal infection. Choline, an essential nutrient, plays a key role in regulating growth and brain development. It is unclear, however, whether prenatal or postnatal deficiency in choline can alter skeletal muscle growth. Treatments were arranged in a 2x2 factorial with factors of choline status (deficient vs sufficient) and timing of treatment (prenatal vs postnatal). Sows were fed diets either deficient or sufficient in choline, and piglets were raised artificially on milk replacer deficient or sufficient in choline. Gene expression was analyzed in the longissimus dorsi muscle while cell size was measured in the semitendinosus muscle. Expression of IGF1 , IGF2, and myogenin were unaffected by treatment, while postnatal choline deficiency increased the expression of MyoD. Both prenatal and postnatal choline deficiency resulted in an increase in myostatin expression. Furthermore, MHC1 gene expression was increased and MHC2b expression was decreased in postnatal choline deficient animals. Expression of MHC2a and MHC2x were unaltered by treatment. Additionally, choline deficiency did not alter average muscle cell size.

To determine the influence of maternal infection on prenatal muscle development, sows were infected with porcine respiratory and reproductive syndrome (PRRS) virus at day 76 of gestation, and muscle samples were collected at birth and 28 days of age. As expected, maternal PRRS virus infection decreased crown to rump length, birth weight and the weight of the longissimus dorsi and psoas major muscles. Semitendinosus muscle has been collected to determine muscle cell size and number, while the longissimus dorsi and psoas major was collected for muscle gene expression. To contrast prenatal exposure to PRRS with a postnatal infection, we also collected tissue samples from pigs inoculated with PRRS virus at 5 weeks of age and sacrificed at 7 weeks of age. Inoculation with PRRS virus resulted in decreased body weight and crown to rump length and decreased longissimus dorsi and semitendinosus weights. However, PRRS virus did not alter the weight of the psoas major.

The **Indiana Station** reports that brown adipose tissues (BAT) are derived from a myogenic factor 5 (Myf5)-expressing cell lineage and white adipose tissues (WAT) predominantly arise from non-Myf5 lineages, although a subpopulation of adipocytes in some WAT depots can be derived from the Myf5 lineage. However, the functional implication of the Myf5- and non-Myf5-lineage cells in WAT is unclear. We found that the Myf5-lineage constitution in subcutaneous WAT depots is negatively correlated to the expression of classical BAT and newly defined beige/brite adipocyte-specific genes. Consistently, fluorescent-activated cell sorting (FACS)-purified Myf5-lineage adipo-progenitors give rise to adipocytes expressing lower levels of BAT-

specific Ucp1, Prdm16, Cidea, and Ppargc1a genes and beige adipocyte-specific CD137, Tmem26, and Tbx1 genes compared with the non-Myf5-lineage adipocytes from the same depots. Ablation of the Myf5-lineage progenitors in WAT stromal vascular cell (SVC) cultures leads to increased expression of BAT and beige cell signature genes. Strikingly, the Myf5-lineage cells in WAT are heterogeneous and contain distinct adipogenic [stem cell antigen 1(Sca1)-positive] and myogenic (Sca1-negative) progenitors. The latter differentiate robustly into myofibers in vitro and in vivo, and they restore dystrophin expression after transplantation into mdx mouse, a model for Duchenne muscular dystrophy. These results demonstrate the heterogeneity and functional differences of the Myf5- and non-Myf5-lineage cells in the white adipose tissue.

The **Indiana Station** goes on to report that Wnts are secreted proteins that play important roles in skeletal myogenesis, muscle fiber type diversification, neuromuscular junction formation and muscle stem cell function. Canonical Wnt signaling stabilizes β-catenin, which subsequently translocate to the nucleus to activate the transcription of TCF/LEF family genes. How Wnt proteins orchestrate such diverse activities remains poorly understood. Using TCF-reporter mice and conditional activation of Wnt signaling in muscle, we found that canonical Wnt signaling is strongly activated during fetal myogenesis and weakly activated in adult muscles limited to the slow myofibers. Muscle-specific transgenic expression of a stabilized β-catenin protein led to increased oxidative myofibers and reduced muscle mass, suggesting that canonical Wnt signaling promotes slow fiber types and inhibits myogenesis. By TCF-luciferase reporter assay, we identified Wnt-1 and Wnt-3a as potent activators of canonical Wnt signaling in myogenic progenitors. Consistent with in vivo data, constitutive overexpression of Wnt-1 or Wnt-3a inhibited the proliferation of both C2C12 and primary myoblasts. Surprisingly, Wnt-1 and Wnt-3a overexpression up-regulated BMP-4, and inhibition of BMP-4 by shRNA or recombinant Noggin protein rescued the myogenic inhibitory effect of Wnt-1 and Wnt-3a. Importantly, Wnt-3a or BMP-4 recombinant proteins promoted slow myosin heavy chain expression during myogenic differentiation of fetal myoblasts. These results demonstrate a novel interaction between canonical Wnt and BMP signaling that induces myogenic differentiation towards slow muscle phenotype.

The **Michigan Station** previously identified death-associated protein (DAP) as a candidate gene of interest for its potential role in turkey skeletal muscle development. Knockdown of *DAP* expression in cultured turkey satellite cells profoundly affected proliferation and differentiation. To investigate the mechanisms affected by *DAP* knockdown in proliferating and differentiating turkey satellite cells, small interfering RNA was used to knock down expression of *DAP*. Differential gene expression was determined using a turkey skeletal muscle long oligonucleotide microarray. Microarray data were validated using quantitative real-time PCR. In proliferating cells, a total of 397 genes showed differential expression (false discovery rate; FDR<0.05). Pathway analysis supports *DAP* involvement in mammalian target of rapamycin signaling. *AKT1* and several eukaryotic translational initiation factors showed altered gene expression, suggesting *DAP* involvement in the regulation of protein synthesis. In differentiating cells, 175 genes showed differential expression (FDR<0.05). Pathway analysis at this stage identified *DAP* involvement in regulation of calcium signaling. Down-regulation of proteins involved in sarcoplasmic reticulum calcium flux and a majority of myofibrillar proteins suggests *DAP* may

affect regulation of calcium homeostasis and muscle mass accumulation. This study provides the first evidence of global gene expression changes upon the knockdown of the *DAP* gene.

In subsequent experiments, chicken satellite cells were transfected with DAP1 cloned into the pCMS-enhanced green fluorescent protein vector or pcDNA3.1 vector, or a small interference RNA against the endogenous DAP1 gene. The cells were assayed for proliferation, differentiation, and apoptosis. The overexpression of DAP1 increased proliferation, differentiation, and myotube diameter, but it had no effect on satellite cell apoptosis. In contrast, knockdown of DAP1 in chicken satellite cells significantly decreased proliferation, differentiation, and number of nuclei per myotube, and it increased apoptosis of the cells. These results suggest that DAP1 is required for regulating myogenesis and apoptosis of satellite cells, which may affect muscle mass accretion and regeneration, and ameliorate muscle sarcopenia.

We have continued studies that test the hypothesis that differential gene expression is associated with the development of PSE turkey meat. Using a turkey muscle-specific oligonucleotide microarray, we analysed differential gene expression between normal and PSE turkey meat. Breast meat samples were collected from Randombred Control Line 2 turkeys at 22 wk of age, and classified as normal or PSE primarily based on marinade uptake (high = normal, low = PSE). Total RNA was isolated from meat samples with the highest (normal, n = 6) and the lowest (PSE, n = 6) marinade uptake. Microarray data confirmation was conducted using quantitative real-time PCR. Selection of differentially expressed genes for pathway analysis was performed using a combination of fold change (FC) ranking (FC < -1.66, FC >1.66) and false discovery rate (<0.35) as criteria. The calcium signaling pathway was highlighted as the top canonical pathway associated with differential gene expression between normal and PSE turkey. Dramatic downregulation of fast-twitch myosin heavy chain coupled with upregulation of slow-twitch myosin and troponin C suggested a switch of skeletal muscle isoforms, which may alter muscle fiber arrangement and formation of actin-myosin complexes. Changes in expression of genes in the actin cytoskeleton signaling pathway also suggest altered structures of actin filaments that may affect cell motility as well as strength and flexibility of muscle cells. Substantial downregulation of pyruvate dehydrogenase kinase, isozyme 4 was observed in PSE samples, suggesting altered regulation of the aerobic metabolic pathway in the birds that developed PSE meat defect.

We subsequently utilized deep transcriptome RNA sequence analysis (RNA-Seq) to identify differentially expressed genes and the associated molecular mechanisms between normal and PSE turkey breasts. Turkey breasts (n = 43) were previously classified as normal or PSE based on marinade uptake (high = normal; low = PSE). Total RNA from breast muscle samples with the highest (n = 4) and lowest (n = 4) marinade uptake were isolated and sequenced using the Illumina $GA_{IIX}$ platform. Differential expression of 494 loci was identified (false discovery rate < 0.05). Changes in gene expression were confirmed using quantitative real-time PCR. Pathway analysis of differentially expressed genes suggested abnormalities of calcium homeostasis and signaling pathways regulating actin cytoskeleton structure as well as carbohydrate metabolism and energy production in PSE samples. Dysregulation of postmortem glucose oxidation in PSE turkey was suggested by dramatic down-regulation of pyruvate dehydrogenase kinase, isozyme 4 (PDK4) mRNA, the most down-regulated gene, and decrease in protein (P = 0.0007) as

determined using immunoblot. These results support the hypothesis that differential expression of several genes, and their protein products, contribute to development of PSE turkey.

The **Texas Station** utilizes a unique cattle population to investigate differential gene expression and signal transduction in skeletal muscle resulting from inheritance of *Bos taurus* or *Bos indicus* alleles of genes associated with skeletal muscle growth, development and proteolysis. One objective of this project is to identify networks of genes in cattle that are critical for production of consistently tender and highly palatable beef, and for the effectiveness of electrical stimulation of carcasses. Our second objective is to integrate gene expression phenotype (microarray data) with SNP array data to refine QTL, and identify distinct genetic contributions that distinguish *Bos indicus* from *Bos taurus* cattle to influence tenderness and other meat quality traits. We are also interested in identifying genetic factors that are important for growth efficiency.

Considerable emphasis has been placed on identifying regions of the genome that harbor genes for traits that directly impact the consumer, such as marbling and tenderness. For mapping genes associated with production efficiency and nutrient utilization, a resource population of multiple F2 families of Nellore-Angus, Brahman-Angus, and Brahman-Hereford was generated at the Research Station in McGregor, TX. DNA has been collected for all animals, and phenotype has been scored for multiple characteristics including disposition, feed intake, age at puberty, and carcass and meat traits of steers.

Outputs: Network gene expression analyses combined with genotype and other expression analyses resulted in the identification of additional genes that affect shear force in beef.

ZBED6 was identified as a transcription factor that affects muscle mass and fat deposition in pigs. Mechanisms mediating effects on fat mass are unclear. The **Virginia Station's** objective was to determine the effect of *ZBED6* mRNA knockdown on 3T3---L1 preadipocyte differentiation and gene expression. Differentiation was associated with increased mRNA abundance of *CEBP/α* ($P < 0.05$), *CEBP/β* ($P < 0.05$), *CEBP/δ* ($P < 0.05$), *FASN* ($P < 0.05$), *PPARγ* ($P < 0.05$), and *SREBP---1* ($P < 0.05$), and decreased abundance of *PREF---1* ($P < 0.05$). Knockdown of *ZBED6* was not associated with changes in mRNA abundance of selected genes, lipid accumulation, lipid droplet size, or cell number. These results suggest that ZBED6 does not play a major role in preadipocyte differentiation.

The **Virginia Station** further reports that the STAC3 (Sh3 and cysteine-rich domain 3) gene is a functionally undefined gene predicted to encode a protein containing two SH3 domains and one cysteine-rich domain. We have found that this gene is predominantly expressed in skeletal muscle, and that STAC3 knockout mice died perinatally and had morphologically abnormal skeletal muscle with central located nuclei in myofibers, fewer and smaller myofibrils, and fewer but larger myofibers. In this study, we determined the potential role of the STAC3 gene in proliferation and differentiation of bovine satellite cells. We isolated satellite cells from skeletal muscle of adult cattle and transfected them with STAC3 small interfering RNA (siRNA) or scrambled siRNA. Cell proliferation assays revealed that STAC3 knockdown had no effect on the proliferation rate of bovine satellite cells. We assessed the differentiation status of bovine satellite cells by quantifying the expression levels of myogenin and myosin heavy chain genes, and by quantifying fusion index. STAC3 knockdown stimulated mRNA and protein expression of myogenin, and myosin heavy chain 3 and 7, and increased fusion index of bovine satellite

cells. These data together suggest that STAC3 inhibits the differentiation of bovine satellite cells into myotubes.

Myotome formation in the early mouse embryo is controlled by the coordinated expression of the myogenic regulatory factors (MRFs). Subsequently, muscle progenitors migrate from the dermomyotome structure and populate the trunk and limb regions. These cells are denoted by expression of Pax3 and Pax7. The **Virginia Station** examined the process of muscle formation in the bovine fetal forelimb with an emphasis on maternal nutrition. Multiparous beef cows were fed to meet their NRC requirements (CCC) or nutrient restricted (60% of NRC) through the first trimester (85 d; RCC) or second trimester (145 d; RRC) followed by realimentation to recommended levels. At d85, d140 and d254, subsets of the cows were slaughtered and the fetal infraspinatus was removed. The numbers of Pax7 immunopositive muscle precursors and the fiber cross---sectional area was measured for all fetuses. Results demonstrate that nutrient restriction for as little as 85 d causes a reduction in the numbers of Pax7 expressing cells. Presumably, these cells undergo precocious differentiation as the size of the primary fibers was larger in the nutrient restricted fetus at this time point. Realimentation of the cows at both the d 85 and d 145 endpoints allowed for catch---up hypertrophy of the fetal muscle fibers as no differences in fiber number or size was apparent between the groups at d 254. These results suggest that low caloric intake of pregnant beef cows through midgestation does not negatively impact the numbers of fibers or Pax7---expressing myogenic cells near birth in the offspring, if the cows are fed to meet their nutrient requirements during the final trimester.

The **Virginia Station** reports various species that experience serious heat stress---related health disorders enlist a number of heat stress---induced physiological metabolic alterations to cope with additional heat load, possibly in a similar fashion to humans with heat---related illness. A series of studies have been conducted aimed at understanding how hyperthermia influences the set points of several metabolic pathways within skeletal muscle. It appears that during heat stress skeletal muscle experiences mitochondrial dysfunction leading to impaired cellular energy status. This may have broad implications for the reduced growth and heat intolerance seen during heat stress especially if skeletal muscle is not able to make necessary contributions to whole---body energy homeostasis. Taken together, heat stress alters skeletal muscle metabolism and function, which may be important for whole---body metabolism and overall physiological adaptation to hyperthermia.

The major research focus of the **Washington Station** is to elucidate mechanisms linking AMP-activated protein kinase to myogenic differentiation of progenitor cells, which remains poorly defined. AMPK has two catalytic α subunits, α1 and α2. We postulated that AMPK promotes myogenesis in an isoform specific manner. Primary myoblasts were prepared from AMPK knockout (KO) mice and AMPK conditional KO mice, and knockout of the α1 but not the α2 subunit resulted in down-regulation of myogenin and reduced myogenesis. Myogenin expression and myogenesis were nearly abolished in the absence of both AMPKα1 and AMPKα2, while enhanced AMPK activity promoted myogenesis and myotube formation. The AMPKα1 specific effect on myogenesis was likely due to the dominant expression of α1 in myoblasts. These results were confirmed in C2C12 cells. To further evaluate the necessity of AMPKα1 subunit for myogenesis *in vivo*, we prepared both DsRed AMPKα1 knockout myoblasts and EGFP wild-type myoblasts, which were co-transplanted into Tibialis anterior muscle. A number of green fluorescent muscle fibers were observed, showing the fusion of engrafted wild-type myoblasts

with muscle fibers; on the other hand, very few or no red muscle fibers were observed, indicating the absence of myogenic capacity of AMPKα1 knockout myoblasts. In summary, these results indicate that AMPK activity promotes myogenesis through a mechanism mediated by AMPKα1.

The **Washington Station** has also spent time reflecting about muscle biology in order to 1) identify holes in the research being reported, so that I might capitalize on such to write grants in the near future. Moreover, 2) I have become convinced that (considering the present climate) something scientists need to do a better job of is to make/document impacts of work being conducted, and (could then be) of use by others. It is my thought that by informing others about the practical end-result of their research would go far in terms of opening-up new areas of study, recruiting others into the field, and (perhaps) be something granting agencies might use to establish/reestablish RFP's. Certainly, a younger scientist could not direct their focus in the manner I have done, but (I think) it is somewhat the responsibility of elderly statespersons/scientists to do so. Collaboration has been with numerous members of this group to "get papers out," and resultant papers from such have (already) received citations. This feedback [citation metrics] shows that others (at college campuses around the world) are experiencing/thinking similarly about the value of the conduct of research.

**Objective 3. Mechanisms of protein assembly and degradation in skeletal muscle.**

The **Nebraska Station** reports that bovine skeletal muscle (stearnomandibularis) was used to investigate the fraction of myofibrils termed easily releasable myofilaments (ERM). The method for isolating ERM was essentially that described by Neti et al (1) with a few modifications. Isolated ERM were characterized by electron microscopy, and a proteomic approach combining 2-dimentional electrophoresis and mass spectrometry. Considerable differences were observed in the 2-dimensional electrophoretic separation patterns of proteins in the myofibrillar and ERM fractions. Actin isoforms exhibited the most striking differences between these two samples. Several unique alpha and beta actin forms (with more alkaline pIs) were observed in the ERM fraction only. Mass spectroscopy confirmed the identity of these polypeptides. Additionally, analysis of the MS data of unique actin polypeptides showed several PTM changes including phosphorylation, glycosylation, biotinylation, ubitiquinylation and arginylation. Polyubiquitin tagged spots corresponding to tropomyosin and troponin (and other myofibrillar proteins) were also identified in ERM 2-DE separations. These results are consistent with changes in actin that are associated with thin filaments that are less stable and more prone to disassembly. Additionally, the presence of arignylation and Gly-Gly adducts (remnants of ubiquitin tagging) supports the hypothesis that ERM are selectively targeted for protein turnover.

**Impact Statements**

- An expression network and gene variants have been identified that are associated with shear force variability that remains in beef after electrical stimulation of carcasses. These findings can be applied to improve selection strategies for the beef cattle industry.
- Training of undergraduate and graduate students is being accomplished through this project.
- Work from R. Vaughn dissertation was selected as one of 8 late-breaking abstract for oral presentation at the ADSA-ASAS Joint Annual Meeting in Indianapolis, July 2013.

- Disclosure was filed with the office of technology transfer regarding potential DNA tests for tenderness in beef cattle.
- Information about project was presented to animal science students from the Universidad Autonoma Chapinga, Mexico. July 30. Texas A&M University, College Station, TX.
- Research efforts from this project were included in public Genomics Exhibition at the George Bush Presidential Library & Museum Aug 27, 2012-July 5, 2013.
- ERM were found to contain changes in actin that suggest these filaments are less stable, prone to disassembly, and selectively targeted for protein turnover.

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**Evidence of Future Productivity**

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**Other Publications**

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2. Brannan, J. L. Transcriptional profiling of immune responses in cattle experimentally-infested with amblyomma americanum ticks. Doctoral Dissertation, Texas A&M University, August, 2013.
3. Dodson, M.V. 2013. Professor Ugo Carraro and BAM: Friends for life. *European Journal of Translational Biology/Basic and Applied Myology* 23(4):
4. Dodson, M.V. 2013. It is only about the science. NACTA Journal 57(3):72
5. Dodson, M.V. 2013. A hint of things to come. NACTA Journal 57(3):75-76
6. Dodson, M.V. and S. Wei. 2013. What are we doing right? NACTA Journal 57(1):96-97
7. Elizabeth Harris 2013. Chemoprevention strategies for Spontaneus Ovarian Cancer in the Hen. MS Thesis
8. Herring A.D., D.G. Riley, J.O. Sanders, P.K. Riggs and C.A. Gill. 2013. Beef Cattle Genomics: Promises from the Past, Looking to the Future. Proceedings, Florida Beef Cattle Short Course.
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10. Lewis, W.C. and M.V. Dodson. submitted. The frustrations of learning how to write a scientific paper. *NACTA Journal*
11. Malila, Y. 2013. Differential gene expression and molecular mechanisms associated with development of pale, soft and exudative (PSE) turkey meat. Doctoral Dissertation, Michigan State University. August 2013.
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13. Stafuzza N.B., K.J. Kochan, P.K. Riggs, and M.E.J. Amaral. 2013. Single nucleotide variations in the buffalo kappa-casein gene (CSN3). Buffalo Bulletin. in press
14. Vaughn, R. N. Genetic mechanisms that contribute to differences in beef tenderness following electrical stimulation. Doctoral Dissertation, Texas A&M University, May, 2013.