**Accomplishments Report**

**Project/Activity Number: NC1184**

**Project/Activity Title:** **Molecular Mechanisms Regulating Skeletal Muscle Growth and Differentiation**

**Period Covered: October 1st, 2022 to September 20th, 2023**

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**Annual Meeting Date(s): October 6th – 7th, 2023**

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**Brief summary of minutes of annual meeting**:

The NC1184 annual meeting was held on October 6th-7th at Washington State University (WSU) in a hybrid format, with both participants attending in-person and those attending remotely. Dr. Min Du, WSU Department of Animal Sciences was the host of this meeting. On October 6th, the group was welcomed by Dr. Gordon Murdock, Chair of Department of Animal Sciences, and Holly Neibergs, Assistant Director of Agricultural Experimental Station at WSU. The group then began with oral station reports. A conference call was made with Dr. Mark Mirando, USDA/NIFA, who outlined current funding opportunities, the USDA NIFA budget, and statistics on the number of proposals submitted annually and funding rates. After the call with Dr. Mirando, the groups continued with station reports. On October 7th, the group finished the remaining station reports.

The group voted to hold the 2025 meeting at the University of Hawaii, to be hosted by Dr. Caleb Reichhardt.

The 2024 meeting will be held at the Louisiana State University, to be hosted by Dr. Xing Fu.

**Accomplishments:**

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

**Kansas Station**

1. Abundance of several oxidative enzymes that play a role in metabolite entry and exit from the TCA cycle change in skeletal muscle during finishing phase of swine production. In addition, pyruvate- and glutamate- derived metabolite flux through the TCA cycle undergo changes during this time.
2. The protein kinase NUAK (mammalian NUAK1/ARK5) coordinates with the insulin signaling pathway to control the serial addition of sarcomeres.

**Iowa Station**

1. In collaboration with Dr. Rhoads (Virginia Tech) and Dr. White (TAMU), the Iowa Station has been working to better understand metabolic dysregulation and muscle injury caused by heat stress. They have expanded our investigations to explore the role of heat stress on the myocardium and kidney as we are considering more holistically the impact of heat stress on an organism with the underlying premise that total health needs to be optimized to maximize animal production efficiency.
2. In conjunction with Dr. Rhoads (Virginia Tech) and Dr. White (TAMU), they have made further inroads into the role of biological sex on the muscular response to heat stress. They are continuing to work through analysis of phenotypical outcomes and biochemical and histological measures.
3. They have expanded a collaboration with an expert in proteomics technologies to more holistically capture changes to the proteome caused by muscle injury to include investigation of sex effects of heat stress. They are also working with an expert in transcriptomics and have completed an early/preliminary analysis of the muscle transcriptome following 1 and 7 d of HS in barrows and gilts.

**Utah Station**

1. Learned more about the mechanism through which anabolic implants improve growth of skeletal muscle in beef cattle.

**Wisconsin Station**

1. A collaboration among Dr. Wei Guo at University of Wisconsin, Dr. Gale Strasburg at Michigan State University, and Dr. Sandra Velleman at the Ohio State University initiated a research topic in the journal of Frontier in Physiology to collect the recent research update on poultry muscle pathogenesis and the consequent effects on muscle physiology, growth and meat quality.

**Connecticut Station**

1. PCR arrays testing the effects of F0 maternal diet on inflammatory factors on F2 offspring have been completed, demonstrating sex by treatment and treatment effects on gene expression.
2. LPS challenge completed in 7-month-old F2 offspring of over-, restricted-, and control fed F0 ewes. Cytokine assays in progress as of fall 2023.
3. LD samples from 10-month-old F2 male offspring of over-, restricted- and control-fed F0 ewes are collected, undergoing histological analysis for muscle fiber cross-sectional area is underway.
4. Antioxidant status and oxidative markers have been measured in LD and plasma samples from F1 and F2 offspring of control- over-, restricted-fed F0 ewes. Analysis of additional antioxidants and markers of oxidative stress is in progress.

**New Jersey Station**

The research at the New Jersey Station shows that

1. Different modes of exercise affect the metabolome during recovery more similarly than previously predicted or appreciated.
2. Liver is a testing platform to examine the kinetic proteome during sulfur amino acid restriction with future application to skeletal muscle.
3. Amino acid transport is upregulated in skeletal muscle and brown fat during acute cold to support thermogenesis.
4. How the integrated stress response is activated and executed across organs and tissues including skeletal muscle.

**Louisiana Station**

1. Single-nucleus RNA sequencing analysis identified multiple cell types in bovine skeletal muscle.
2. Multiple transcription factors possibly regulating fibro/adipogenic progenitor diversity and differentiation, as well as those possibly responsible for the differences in fibro/adipogenic progenitors between Wagyu and Brahman were identified.

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

**Ohio Station**

Effect of Thermal Stress on Pectoralis Major Satellite Cells (with Michigan Station)

1. Poultry selected for growth have an inefficient thermoregulatory system and are more sensitive to temperature extremes.
2. Cold temperatures inhibited rates of proliferation and differentiation of pectoralis major (p. major) muscle satellite cells.
3. If the hot temperature was applied during p. major muscle satellite cell proliferation, it resulted in greater stimulatory effects on differentiation than if the hot temperature was administered only during differentiation. Similarly, cold temperature administered during proliferation tended to have more suppressive effects on differentiation than if the cold temperature was applied only during differentiation.
4. Growth selection has resulted in the p. major muscle satellite cells from the faster-growing commercial turkeys to be more sensitive to hot temperature during both proliferation and differentiation. The increased rates of proliferation and differentiation in vivo may result in a greater potential to accrete more satellite cells to drive myofiber hypertrophy, which could impact post-hatch breast muscle growth and structure.
5. Adipogenic gene expression is more responsive to thermal challenge in proliferating satellite cells than in differentiating satellite cells, and that growth-selection has increased temperature sensitivity of satellite cells.
6. Thermal stress can affect breast muscle hypertrophic potential by changing satellite cell proliferation and differentiation, in part, through the mTOR/S6K pathway in a growth-dependent manner.
7. Thermal stress affects poultry breast muscle growth potential and protein to fat ratio by altering function and fate of SCs through the Fzd7-mediated Wnt/PCP pathway in a growth-dependent manner.

**Kansas Station**

1. NUAK kinase activity is required for final muscle size and viability, although sarcomerogenesis proceeds normally.
2. NUAK physically interacts with and is required for phosphorylation of the CryAB chaperone protein.

**Washington Station**

1. The Washington Station continued to study the roles of bone morphogenetic protein (BMP) signaling in myogenesis of early embryos. They found that maternal obesity enhances BMP signaling and suppresses myogenesis; on the other hand, fibro-adipogenesis is increased due to elevated BMP signaling. On the other hand, maternal exercise improves fetal muscle development.

**Indiana Station**

1. Used cell and molecular biology techniques, animal models and production animals to study molecular regulation of muscle growth and metabolism.
2. Developed new research techniques and methods; used state-of-art single cell RNA sequencing to illustrate cell dynamics and reveal novel subpopulations in murine and porcine skeletal muscles.

**Utah Station**

1. Gained insight into which genes are actively being transcribed during the process of myogenesis in growing satellite cells.

**Objective 3: Characterize mechanism of protein assembly and degradation in skeletal muscle**

**Kansas Station**

1. The conserved Striatin-interacting phosphatase and kinase (STRIPAK) complex regulates autophagic protein turnover, primarily at the autophagosome-lysosome fusion step.

**Utah Station**

1. Identified new information relating to how mitochondria contribute to postmortem proteolysis and tenderization of meat.

**Impacts:**

1. The Ohio Station, in collaboration with the Michigan Station, reported impacts including, 1) Thermal stress may have long-term implication on breast meat quality through changes in muscle development and growth potential; 2) Growth selection plays a role in the response of the breast muscle to thermal stress; and 3) Immediate post hatch thermal stress both cold and hot effects both the proliferation and differentiation of satellite cells required for muscle growth and muscle mass accretion.
2. Research related to projects performed in the Indiana Station will lead to fundamental understanding of the cell intrinsic molecules and extrinsic signals that regulate skeletal muscle development, growth, and regeneration. Such knowledge will serve as the foundation for translational approaches to increase meat production and improve meat quality in animal agriculture, and to improve health of the muscular system of humans.
3. Heat stress continues to negatively impact swine production and will do so at progressively greater magnitudes should environmental models be accurate and/or pigs continue to be selected for protein accretion. The Iowa Station’s discovery of a sex effect of heat stress could lead to renewed focus of sexed semen, influence the location of new barn construction, and barrow and gilt management strategies.
4. The effort at Wisconsin Station provides new knowledge of the effect of muscle pathogenesis on avian physiology, animal welfare and quality of muscle as a food for long-term outcomes in the field.
5. The research performed at the Connecticut Station provides new knowledge 1) F0 maternal diet impacts F1 and F2 offspring basal inflammatory profile in a sex and diet dependent manner. 2) F1 offspring of F0 over-fed ewes had altered cortisol and interleukin-10 responses to LPS challenge relative to offspring of control- and restricted-fed ewes. 3) F1 and F2 offspring of over- and restricted-fed ewes have diet and sex specific changes in antioxidants and oxidative markers relative to control offspring.
6. The study at the Louisiana Station suggested the molecular basis underlying quality differences between Wagyu and Brahman beef.

**Collaborative Grants:**

1. Strasburg, G., Velleman, S.G., Reed, K. NIFA AFRI, “Turkey Breast Muscle Development: The Biological Response to Thermal Challenge in Production Birds,” $500,000, 4/1/2020-3/31/24.
2. Selsby J. (PI), Baumgard, Rhoads, and White-Springer. USDA/AFRI Foundation Grant. Calcium regulation as a contributor to heat stress-mediated skeletal muscle dysfunction. $500,000. (1/1/21-12/31/23)
3. Selsby J. (PI), Baumgard, Rhoads, and White-Springer. USDA/AFRI IDEA Grant, Basic and applied consequences of heat stress in barrows and gilts. $1,000,000. (9/1/20-8/30/25)
4. Selsby J. (PI), and White-Springer. MDA. **Obesity as a modifier of disease progression caused by dystrophin deficiency.** $300,000. (9/2022-9/2025)
5. Thornton KJ (PI), B.M. Murdoch, and G. K. Murdoch. USDA-AG2PI: Impact of breed type on beef production and sustainability. $75,000. (01/22-03/23)

**Publications:**

**Collaborative Publications**

1. Xu J, Strasburg GM, Reed KM, Bello NM, Velleman SG. Differential effects of temperature and mTOR and Wnt-planar cell polarity pathways on syndecan-4 and CD44 expression in growth-selected turkey satellite cell populations. PLoS One. 2023;18(2):e0281350.
2. Reed KM, Mendoza KM, Xu J, Strasburg GM, Velleman SG. Transcriptome Response of Differentiating Muscle Satellite Cells to Thermal Challenge in Commercial Turkey. Genes (Basel). 2022;13(10). Epub 20221014. doi: 10.3390/genes13101857. PubMed PMID: 36292741; PMCID: PMC9601516.
3. Kim KH, Oprescu SN, Snyder MM, Kim A, Jia Z, Yue F, Kuang S. PRMT5 mediates FoxO1 methylation and subcellular localization to regulate lipophagy in myogenic progenitors. Cell Rep. 2023;42(11):113329. Epub 20231024. doi: 10.1016/j.celrep.2023.113329. PubMed PMID: 37883229.
4. Qiu J, Yue F, Zhu P, Chen J, Xu F, Zhang L, Kim KH, Snyder MM, Luo N, Xu HW, Huang F, Tao WA, Kuang S. FAM210A is essential for cold-induced mitochondrial remodeling in brown adipocytes. Nat Commun. 2023;14(1):6344. Epub 20231010. doi: 10.1038/s41467-023-41988-y. PubMed PMID: 37816711; PMCID: PMC10564795.
5. Oprescu SN, Baumann N, Chen X, Sun Q, Zhao Y, Yue F, Wang H, Kuang S. Sox11 is enriched in myogenic progenitors but dispensable for development and regeneration of the skeletal muscle. Skelet Muscle. 2023;13(1):15. Epub 20230913. doi: 10.1186/s13395-023-00324-0. PubMed PMID: 37705115; PMCID: PMC10498607.
6. Kim KH, Jia Z, Snyder M, Chen J, Qiu J, Oprescu SN, Chen X, Syed SA, Yue F, Roseguini BT, Imbalzano AN, Hu C, Kuang S. PRMT5 links lipid metabolism to contractile function of skeletal muscles. EMBO Rep. 2023;24(8):e57306. Epub 20230619. doi: 10.15252/embr.202357306. PubMed PMID: 37334900; PMCID: PMC10398672.
7. Wang L, Gao P, Li C, Liu Q, Yao Z, Li Y, Zhang X, Sun J, Simintiras C, Welborn M, McMillin K, Oprescu S, Kuang S, Fu X. A single-cell atlas of bovine skeletal muscle reveals mechanisms regulating intramuscular adipogenesis and fibrogenesis. J Cachexia Sarcopenia Muscle. 2023;14(5):2152-67. Epub 20230712. doi: 10.1002/jcsm.13292. PubMed PMID: 37439037; PMCID: PMC10570087.
8. Unsihuay D, Hu H, Qiu J, Latorre-Palomino A, Yang M, Yue F, Yin R, Kuang S, Laskin J. Multimodal high-resolution nano-DESI MSI and immunofluorescence imaging reveal molecular signatures of skeletal muscle fiber types. Chem Sci. 2023;14(15):4070-82. Epub 20230323. doi: 10.1039/d2sc06020e. PubMed PMID: 37063787; PMCID: PMC10094364.
9. Chen J, Yue F, Kuang S. Labeling and analyzing lipid droplets in mouse muscle stem cells. STAR Protoc. 2022;3(4):101849. Epub 20221119. doi: 10.1016/j.xpro.2022.101849. PubMed PMID: 36595920; PMCID: PMC9679676.
10. Wang Y, Troughton LD, Xu F, Chatterjee A, Ding C, Zhao H, Cifuentes LP, Wagner RB, Wang T, Tan S, Chen J, Li L, Umulis D, Kuang S, Suter DM, Yuan C, Chan D, Huang F, Oakes PW, Deng Q. Atypical peripheral actin band formation via overactivation of RhoA and nonmuscle myosin II in mitofusin 2-deficient cells. Elife. 2023;12. Epub 20230919. doi: 10.7554/eLife.88828. PubMed PMID: 37724949; PMCID: PMC10550287.
11. Roths M, Abeyta MA, Wilson B, Rudolph TE, Hudson MB, Rhoads RP, Baumgard LH, Selsby JT. Effects of heat stress on markers of skeletal muscle proteolysis in dairy cattle. J Dairy Sci. 2023;106(8):5825-34. Epub 20230620. doi: 10.3168/jds.2022-22678. PubMed PMID: 37349209.

**Other publications:**

1. Ma Z, Huang Z, Zhang C, Liu X, Zhang J, Shu H, Ma Y, Liu Z, Feng Y, Chen X, Kuang S, Zhang Y, Jia Z. Hepatic Acat2 overexpression promotes systemic cholesterol metabolism and adipose lipid metabolism in mice. Diabetologia. 2023;66(2):390-405. Epub 20221115. doi: 10.1007/s00125-022-05829-9. PubMed PMID: 36378328; PMCID: PMC9665029.
2. Kargl CK, Sullivan BP, Middleton D, York A, Burton LC, Brault JJ, Kuang S, Gavin TP. Peroxisome proliferator-activated receptor γ coactivator 1-α overexpression improves angiogenic signalling potential of skeletal muscle-derived extracellular vesicles. Exp Physiol. 2023;108(2):240-52. Epub 20221201. doi: 10.1113/ep090874. PubMed PMID: 36454193; PMCID: PMC9949767.
3. Chen X, Ferreira CR, Kuang S. Targeted Lipidomics Analysis of Adipose and Skeletal Muscle Tissues by Multiple Reaction Monitoring Profiling. Methods Mol Biol. 2023;2640:351-68. doi: 10.1007/978-1-0716-3036-5\_25. PubMed PMID: 36995607.
4. Tien PC, Chen X, Elzey BD, Pollock RE, Kuang S. Notch signaling regulates a metabolic switch through inhibiting PGC-1α and mitochondrial biogenesis in dedifferentiated liposarcoma. Oncogene. 2023;42(34):2521-35. Epub 20230711. doi: 10.1038/s41388-023-02768-6. PubMed PMID: 37433985; PMCID: PMC10575759.
5. D'Souza RF, Figueiredo VC, Markworth JF, Zeng N, Hedges CP, Roberts LA, Raastad T, Coombes JS, Peake JM, Mitchell CJ, Cameron-Smith D. Cold water immersion in recovery following a single bout resistance exercise suppresses mechanisms of miRNA nuclear export and maturation. Physiol Rep. 2023;11(15):e15784. doi: 10.14814/phy2.15784. PubMed PMID: 37549955; PMCID: PMC10406566.
6. Liu Q, Li C, Deng B, Gao P, Wang L, Li Y, Shiri M, Alkaifi F, Zhao J, Stephens JM, Simintiras CA, Francis J, Sun J, Fu X. Tcf21 marks visceral adipose mesenchymal progenitors and functions as a rate-limiting factor during visceral adipose tissue development. Cell Rep. 2023;42(3):112166. Epub 20230228. doi: 10.1016/j.celrep.2023.112166. PubMed PMID: 36857185; PMCID: PMC10208561.
7. Ford H, Liu Q, Fu X, Strieder-Barboza C. White Adipose Tissue Heterogeneity in the Single-Cell Era: From Mice and Humans to Cattle. Biology (Basel). 2023;12(10). Epub 20230927. doi: 10.3390/biology12101289. PubMed PMID: 37886999; PMCID: PMC10604679.
8. Liu Q, Li C, Li Y, Wang L, Zhang X, Deng B, Gao P, Shiri M, Alkaifi F, Zhao J, Stephens JM, Simintiras CA, Francis J, Sun J, Fu X. Progenitor cell isolation from mouse epididymal adipose tissue and sequencing library construction. STAR Protoc. 2023;4(4):102703. Epub 20231109. doi: 10.1016/j.xpro.2023.102703. PubMed PMID: 37948186.

**Abstracts, Posters, and Professional Presentations:**

1. Rudolph TE, Roths M, Freestone AD, Rhoads RP, Baumgard LH. and Selsby JT. Sex-specific alterations in mitochondrial dynamics following environment-induced heat stress. Advances in Skeletal Muscle Biology in Health and Disease. Gainesville, FL, March 14-17th, 2023.
2. Rudolph TE, Roths M, Freestone AD, Rhoads RP, Baumgard LH. and Selsby JT. The contribution of biological sex to heat stress-mediated production outcomes in growing pigs. IPIC/Iowa Pork Congress, Des Moines, IA, January 25-26, 2023.
3. Rudolph TE, Roths M, Freestone AD, Rhoads RP, Baumgard LH, and Selsby JT. The impact of biological sex on skeletal muscle metabolism following heat stress. Iowa Physiological Society, Iowa City, November, 2022.
4. Shira KA, Becker GM, Murdoch BM, Thornton KJ, Chibisa GE, Murdoch GK. Myokine expression by cultured bovine satellite cells from 3 and 11 month old steers. Annual Meeting of the American Society for Animal Science. July 16-20, 2023. Albuquerque, NM.
5. Kawaida MY, Gonzalez D, Gonzalez JM, Tillquist NM, Reiter AS, Smith BI, Zinn SA, Govoni KE, Reed SA. Poor maternal nutrition during gestation decreases shear force and alters the expression of genes involved in muscle growth. 2022 ASAS Annual Meeting, Oklahoma City, OK.
6. Anthony TG, Indiana University School of Medicine, Indianapolis, IN, April 24, 2023
7. Anthony TG, NIH-OFAS Workshop on Healthy Aging, Sept 6-7, 2023
8. Kuang S, Regenerative Medicine Lecture, West Michigan University School of Medicine. Kalamazoo, MI, October, 2022
9. Kuang S, Seminar for Neurotrauma and Diseases, Center for Paralysis Research, Purdue University, IN, November, 2022
10. Kuang S, Stem cells and regenerative medicine, President’s Council Back to Class. Naples, FL, February, 2023
11. Kuang S, Metabolic H-Talk (#178). Chinese Society for Metabolic Biology. Virtual, April, 2023
12. Kuang S, Gordon Research Conference on Myogenesis, June, 2023
13. Kuang S, Fralin Life Sciences seminars, Virginia Tech University, September, 2023
14. Kuang S, IBP seminar series, University of Minnesota, September, 2023
15. Kuang S, Big 10 Lipid Symposium, University of Iowa, October, 2023

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