Station Reports
NCCC210 Mtg., San Diego 2016

General Highlights

- NCCC210 member and *ad hoc* Attendees: Jim Kinder (remote), Voy Brynn with students Ronique Beckford and Sarah Howard, Jack Odle, Kola Ajuwon, Min Du with student Boo Wang, Stone Ding, Werner Bergen, Sean Adams, Ching Hu, Woo Kim, Brian Piccolo (co-chair), and Theo van Kempen (chair)

- The meeting started a bit hectic because of computer problems. This opportunity was used to start a discussion on the role of the NCCC210 group. The key activities that were not yet filled in were to set up two symposia. The action that was taken was to set up a small task force to explore the possibilities let by Kola Ajuwon with help of Voy Brynn and Stone Ding. Sean Adams and Jack Odle would serve as advisors. Although some directions were discussed no clear conclusions were drawn on this, except the suggestion to team up with other working groups and to formulate a proposal within the next month.

- The split of ASN with EB was also discussed. The majority of the attendants felt a better affiliation with EB than with ASN, and as such, the current thinking was to keep this meeting affiliated with EB.

- Jim Kinder indicated that a mid-term review would take place in 2017. The annual reports were due within 60 days of today. Jim will stay advisor to this group despite a sabbatical in Australia for one year.

- Subsequently, research updates were given by most of the attendees, accompanied by productive and pleasant discussions.

Action points:

- Provide station reports using the format mailed to Theo by 30 April, 2016
- Organize a symposium: Kola, Voy, and Stone to lead this effort
- For the 2017 meeting, Woo Kim was chosen as Incoming Chair, with Brian Piccolo as Chair. Dr. Kinder shall continue in the role of NCCC210 Administrative Lead.

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1. University of Arkansas

2. Sean Adams

3. Accomplishments:
Our laboratory focuses on metabolic physiology, which involves in part the cross-talk of nutrients and hormones across metabolically-important tissues such as fat and muscle. The research also focuses on sub-cellular events that are involved with fuel management including sites that control the fates of fatty acids. At the NCCC210 meeting, recent work was presented that relates to the generation of fatty acylcarnitines, metabolites generated by enzymes within and outside of mitochondria acting on acyl-CoA molecules. Acylcarnitines can accumulate in tissues when fat fuel delivery exceeds ATP turnover (metabolic driver of fuel combustion), and this has been shown to be exacerbated under conditions of diabetes, obesity, and some inborn errors of metabolism. The laboratory has published data supporting a bioactive role for acylcarnitines, in which they impinge on enzyme systems to elicit inflammation and other outcomes. At the NCCC210 meeting, new results showing the effects of weight loss and exercise in pre-diabetic women were presented; that study tested whether improved metabolic health leads to better-matched fatty acid oxidation (lower accumulation of acylcarnitines in blood). Preliminary evidence suggests that this is the case, since even with higher fat oxidation globally in the body, the degree of incomplete fat oxidation was not increased. Furthermore, novel data were presented showing an effect of acylcarnitines on muscle nerve signaling—this raises the possibility that metabolites in muscle (and perhaps other tissues such as adipose) signal to the brain to provide information on metabolic status.

4. Publications
   a) D.K. Layman, T.G. Anthony, B.B. Rasmussen, **S.H. Adams**, C.J. Lynch, G.D. Brinkworth, T.A. Davis. Defining meal requirements for protein to optimize metabolic roles of amino acids. *Am. J. Clin. Nutr.*, 101(Suppl):1330S–8S, 2015
   b) C. Aguer, C.S. McCoin, T.A. Knotts, R. McPherson, R. Dent, D. Hwang, **S.H. Adams***, M-E. Harper***.** Acylcarnitines: potential implications for skeletal muscle insulin resistance. *FASEB J*, 29(1):336-45, 2015 (2014 Oct 23. pii: fj.14-255901. [Epub ahead of print]) (*co-corresponding)
   c) T.N. Dunn, T. Akiyama, H-W. Lee, J-B. Kim, TA. Knotts, S.R. Smith, D.D. Sears, E. Carstens, **S.H. Adams***. Evaluation of the synuclein-γ (SNCG) gene as a PPARγ target in murine adipocytes, dorsal root ganglia somatosensory neurons, and human adipose tissue. *PLoS One*, 10(3): e0115830. doi:10.1371/journal.pone.0115830, 2015
   d) B.D. Piccolo, N.L. Keim, O. Fiehn, **S.H. Adams**, M.D. Van Loan, J.W. Newman. Habitual physical activity and plasma metabolomics patterns distinguish individuals with low vs. high weight loss during controlled energy restriction. *J. Nutrition*, 145(4): 681-690, 2015
   e) B.D. Piccolo, K.B. Comerford, S.E. Karakas, T.A. Knotts, O. Fiehn, **S.H. Adams***. Whey protein supplementation does not alter plasma branched-chained amino acid profiles but results in unique metabolomics patterns in obese women enrolled in an 8-week weight loss trial. *J. Nutrition*, 145(4): 691-700, 2015

f) D.H. Bedinger, I.D. Goldfine, J.A. Corbin, M.K. Roell, **S.H. Adams***. Differential pathway coupling of the activated insulin receptor drives signaling selectivity by XMetA, an allosteric partial agonist antibody. *J. Pharmacol. Exp. Ther.*, 353(1): 35-43, 2015

g) C.S. McCoin, T.A. Knotts, K.D. Ono-Moore, P.J. Oort, **S.H. Adams***. Long-chain acylcarnitines activate cell stress and myokine release in C2C12 myotubes: calcium-dependent and -independent effects. *Am. J. Physiol. Endocrinol. Metab.* 308(11): E990-E1000, 2015

h) D.H. Bedinger, D.A. Kieffer, I.D. Goldfine, M.K. Roell, **S.H. Adams***. Acute treatment with XMetA activates hepatic insulin receptors and lowers blood glucose in normal mice. *J. Cell Biochem.* 2015 Mar 23. doi: 10.1002/jcb.25168. [Epub ahead of print], 2015

i) S.V. Chintapalli, G. Bhardwaj, R. Patel, N. Shah, R.L. Patterson, D.B. van Rossum, A. Anishkin, **S.H. Adams***. Molecular Dynamic Simulations reveal the structural determinants of palmitic acid binding to oxy-myoglobin. *PLoS One*, Jun 1;10(6):e0128496, 2015

j) C.S. McCoin, T.A. Knotts, **S.H. Adams***. Acylcarnitines: old actors auditioning for new roles in metabolic physiology. *Nature Reviews Endocrinology* 11(10):617-25, 2015

k) D.H. Bedinger and **S.H. Adams***. Metabolic, anabolic, and mitogenic insulin responses: a tissue-specific perspective for insulin receptor activators. *Mol. Cell. Endocrinol.* 415:143-56, 2015

Station Reports
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1. University of Georgia

2. Woo Kyun Kim

3. Accomplishments

a) Isolation and differentiation of mesenchymal stem cells (MSC) from broiler compact bones.
Bone disorders and excessive fat accumulation have the potential to present major economic and welfare problems in the poultry industry. Calcium, phosphorus and vitamin D_3 have been the major ingredients and nutritional factors to maintain bone health in poultry. Developing chicken MSC culture model has potential to provide an opportunity for better understanding of adipogenic and osteogenic mechanisms of chicken MSC and identification of novel nutrients/bioactive molecules for promoting skeletal health and efficient feed utilization in poultry. MSC are multipotent progenitor cells that have the capacity of differentiating into other tissues like bone, fat, cartilage and muscles. The objective of our study was to develop a method to isolate the MSC from compact bones of broilers. After femur and tibia of 3 day old male broiler chicks were collected aseptically, bone marrow was flushed 6 times with PBS from the compact bones. The compact bones were chopped to small pieces and digested with digestion buffer containing 0.25% collagenase type II and 20% Fetal Bovine Serum at 37° C for 50 minutes. Digested cells containing media were filtered and then centrifuged. After removing the supernatant, the cell pellet was cultured in a specific growth medium Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum. Cells took 6-7 days to grow and define in tissue culture treated 100mm dish before they could be passaged further. Microscopically, the cultured cells showed morphology resembling fibroblasts and divided actively and remained as a monolayer. They were mainly spindle-shaped cells with both ends elongated and proliferated rapidly like whirlpool or flamboyance. The multilineage differentiation potential of chicken MSC was revealed by their ability to undergo adipogenic and osteogenic differentiation. Cells treated with osteogenic differentiation media containing 5% FBS, 50ug/ml ascorbate, and 3mM β-glycerophosphate were able to grow and change their general morphology. Cells treated with adipogenic cocktail and oleic acid were positive for Oil Red O stain as early as 48 hrs of treatment compared to control group. The results suggest that MSCs can be isolated from broiler compact bones that possess similar characteristics to those from other species and their capability to differentiate into other tissues. These MSCs can be used as *in vitro* model for studying effect of different compounds on adipogenesis and osteogenesis in chicken which could address many practical health and production issues in the poultry industry.

b) Effect of adipogenic cocktail and different levels of oleic acid on adipogenic differentiation of mesenchymal Stem cells derived from broiler compact bones
Mesenchymal stem cells (MSCs) are multipotent cells that are capable of differentiating to different lineages of tissues like bone, fat, muscles, cartilage and dermis. The objective of the

study was to evaluate the adipogenic effect of adipogenic cocktail and oleic acid (OA) in primary MSCs isolated from compact bones of broiler chicks. Primary MSCs isolated from broiler chicks were plated into 6 well plates using Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and treated with respective treatments at confluency. 1) Control (C); 2) 75 uM OA; 3) 150 uM OA; 4) 300 uM OA; 5) DMI alone (adipogenic cocktail (DMI) containing 500 nM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine and 20 mg/mL insulin); 6) DMI + 75 uM OA; 7) DMI+ 150 uM OA; and 8) DMI + 300 uM OA . At 48 hrs of treatment, cells were stained with Oil red O to observe the adipocyte formation in MSCs. Total area of Oil Red O stained cells were determined in each well. The non-induced cells (control) were negative for Oil red O stain. Cells treated with OA alone showed increased adipocyte formation in a dose response manner, whereas cells treated with DMI alone did not show any Oil red O stain at 48 hrs of treatment. At the same time point, cells treated with DMI and OA together showed positive for Oil red O stain similar to the OA alone treatments. Cells treated with 300uM of OA have the most distinct Oil red O stain among other 3 levels of OA. The study indicates that use of OA alone or OA with DMI can induce differentiation of MSCs derived from compact bones into adipocytes as early as 48 hours of treatments, but DMI alone was not sufficient to induce adipogenesis in MSC derived from broiler compact bones unlike the adipogenic differentiation of mammalian MSC which is mainly dependent on DMI. Current result provides rationale for further study about regulatory mechanism of OA and adipogenic cocktail in changing the fate of MSCs when treated alone and combined.

c) 20(S) oxysterol induces the in vitro expression of proinflammatory cytokines in chicken preadipocytes

20(S) oxysterol has the potential to reduce adipogenesis in mammalian cells through induction of the hedgehog signaling pathway. This study was conducted to examine the effect of 20(S) oxysterol on the expression of proinflammatory cytokines. Preadipocytes were isolated from 20-wk old laying hen (*Gallas gallus*, Lohman strain) and treated with an adipogenic cocktail (DMIOA) containing 500 nM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, 20 μg/mL insulin and 300 μM oleic acid, DMIOA + 5 μM 20(S) oxysterol, and 5 μM 20(S) alone in 1X Dulbecco's Modified Eagle's Medium (DMEM) for 96 h. Gene expression were measured using real-time quantitative polymerase chain reaction (qRT-PCR). Data were analyzed using GLM procedure of the Statistical Analysis System (SAS Institute version 9.2). The mRNA expression of TNFAIP3, IL-8, IL-10, IL-1β, and INFG were higher ($P < 0.05$) in cells treated with DMIOA +20(S) and 20(S) compared with cells treated with DMIOA. The highest expression of these cytokines ($P < 0.05$) were measured in cells treated with 20(S) alone compared with cells treated with a combination of DMIOA and 20(S), DMIOA alone, and non-treated control cells. In conclusion, although further investigation is required, the current data showed that 20(S) is may stimulate the humoral immune response during the in vitro maturation preadipocytes isolated from laying hen.

4. Published Papers
   a) Moseti* D., A. Regassa, and **W.K. Kim** (2016) Molecular regulation of adipogenesis and potential anti-adipogenic bioactive molecules. International Journal of Molecular Science, 17, 124; doi:10.3390/ijms17010124.

b) Regassa*, A. and **W.K. Kim** (2015) Global transcriptome analysis of laying hen preadipocytes treated with adipogenic cocktail and oleic acid with or without 20(S)-hydroxylcholesterol. BMC Gemonics 16:91-106.
c) Regassa*, A. , Adhikari R, C.M. Nyachoti and **W.K. Kim** (2015) Effects of 25-(OH)D3 on Ca and P utilization, Ca and P transporter mRNA expression, bone mineralization, and performance in growing female pigs. Journal of Environmental Science and Health, Part B 50:293-299.
d) Shang* Y., A. Regassa, J.H. Kim and **W.K. Kim** (2015) The effect of dietary fructooligosaccharide supplementation on growth performance, intestinal morphology and immune responses in broiler chickens challenged with Salmonella Enteritidis lipopolysaccharide. Poultry Science 94: 2887-2897

5. Impacts

We successfully isolate mesenchymal stem cells from chicken compact bones. These cells will be very useful to understand molecular mechanisms of fat and bone cell differentiation. We also found that 20S oxysterol is a novel activator of Hedgehog signaling to regulate mesencymal cell differentiation. Thus, this compound can be potentially used for intervention of obesity and osteoporosis in humans and animals.

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1. National Taiwan University

2. Shih-Torng Ding

3. Accomplishments

We have established a better methodology of getting adipose-derived stem cells from the stromal vascular fraction of subcutaneous white adipose tissues. In which, pig adipose-derived stem cells (pADSC) are isolated from 7- to 9-day old piglets. The dorsal white fat depot of porcine subcutaneous adipose tissues is sliced, minced and collagenase digested. These pADSC exhibit strong potential to differentiate into adipocytes. We have published the protocol in JOVE this year. These pADSC derived in this protocol provide an abundant and assessable source of adult mesenchymal stem cells with full multipotency for studying adipose development and application to tissue engineering of regenerative medicine.

We developed a dietary induced metabolic syndrome model using miniature pigs to study the involvement of AMPK and sirt1. The results were published in Eur. J. Clin. Invest. This model can be used to evaluate the long-term feeding of the Western diet to Lee-Sung miniature pigs to create obesity and used for other metabolic disease studies. For our study, we found that SIRT1/AMPK and their downstream pathways might be one of the possible regulators for pathological obesity in Lee-Sung pigs.

We have also used two fatty liver model animals to study the development of fatty liver and possible mechanism underlined the pathology and recovery of the disease by nutritional factors.

4. Publications for Ding, Shih-Torng 2015

a) Liu, I.P., Y.Y. Lin, **S.T. Ding**, and C.Y. Chen. 2015. Development of a dietary induced metabolic syndrome model using miniature pigs-involvement of AMPK and sirt1. Eur. J. Clin. Invest. 45: 70–80 (IF=2.834, ranking 27/156, MEDICINE, GENERAL & INTERNAL).

b) Li SJ, Liu CH, Chang CW, Chu HP, Chen KJ, Mersmann HJ, **Ding ST**, Chu CH, and **Chen CY**. 2015. Development of a dietary induced metabolic syndrome model using miniature pigs—Involvement of AMPK and SIRT1. Eur J Clin Invest 45:70-80. (corresponding author) (Impact factor: 2.734, ranking 30/153, **MEDICINE, GENERAL & INTERNAL**)

c) Babic, S., M. Pokusa, V. Danevova, **S.T. Ding**, and D. Jezova. 2015. Effects of atosiban on stress-related neuroendocrine factors. J. Endocrin. 225:9-17. (SCI, ENDOCRINOLOGY & METABOLISM, 44/128, IF=3.718)

d) Chang, C.C., W.C. Lin, L.M. Pai, H.S. Lee, S.C. Wu, **S.T. Ding**, J.L. Liu and L.Y. Sung. 2015. Cytoophidium assembly reflects upregulation of IMPDH activity. J. Cell Sci. 128:3550-3555. (SCI)

e) Wang, Y.W., T.W. Hong, S.H. Tsai, R. Chu, **S. T. Ding**, K. Irie, T.K. Li, S.S. Tzean, T.L. Shen, Y.L. Tai and Y.J. Wang. Evaluation of an epitypified Ophiocordyceps formosana (Cordyceps s.l.) for its pharmacological potential. Evidence-Based Comp. Alt. Med.. **DOI:** 10.1155/2015/189891. (SC I, IF=1.880)

f) Lin**,** H.J., S.H. Wang, Y.H. Pan, and **S.T. Ding (coresponding).** Primary endodermal epithelial

cell culture of the yolk sac membrane of Japanese quail embryos. JoVE (In press, SCI, IF=1.325)

g) Tsai, M.T., C.Y. Chen, Y.H. Pan, S.H. Wang, H.J. Mersmann and **S.T. Ding (corresponding author)**. 2015. Alleviation of carbon-tetrachloride-induced liver injury and fibrosis by betaine supplementation in chickens. Evidence-Based Comp. Alt. Med. In Press. **DOI:** 10.1155/2015/725379. (SCI, IF=1.880)

h) Wu, J.T., T.P. Sun, C.W. Huang, C.T. Su,C.Y.Wu, S.Y. Yeh, D.K. Yamg, L.C. Chen, **S.T. Ding**, and H.Y. Chen. Tunable coverage of immobilized biomolecules for biofunctional interface design. Biomat. Sci. 3:1266-1269. (SCI, MATERIALS SCIENCE, BIOMATERIALS 10/33, IF=3.831)

i) Chen, Y.J., H.Y. Liu, Y.T. Chang, HY.H. Cheng, H.J. Mersmann, and **S. T. Ding (coresponding).** 2015. Isolation and differentiation of adipose-derived stem cells from porcine subcutaneous adipose tissues. JoVE (In press, SCI, IF=1.325)

j) Li, S.J.,S.T. Ding, H.J. Mersmann, C.H. Chu, and C.Y. Chen. 2015. A Nutritional Non-Alcoholic Steatohepatitis Minipig Model. J. Nutr. Biochem. 28:51-60. **(SCI, NUTRITION & DIETETICS Top 15% IF: 3.891, 11/72)**

k) Huang, C.W. , Y.-T. Lin , **S.T. Ding**, L.L. Lo , P.H. Wang , E.C.g Lin , F.W. Liu and Y.W. Lu. 2015. Efficient SNP discovery by combining microarray and Lab-on-a-Chip data for animal breeding and selection. Microarrays *4*:570-595.

5. Impacts:
   We have established several animal and cell models for obesity related research. These models can be utilized to advance the discovery of the knowledge of this field.

Station Reports
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1. Auburn University/Animal Sciences

2. Werner G. Bergen (Alabama Agricultural Experiment Station –voting member), Terry D. Brandebourg.

3. Accomplishments

   The Bergen and Brandebourg laboratories in Animal Science at Auburn University have overlapping goals for their activities. The labs may pursue collaborative work or distinctly different projects. The Bergen Lab has worked on molecular factors regulating marbling in cattle in a time-longitudinal model. Other experiments were conducted to assess the potential of using expression of target genes a phenotypic marker and a sentinel for production efficiency. Sadly all the Bergen lab samples (Most in the process of analysis) from three trials were lost due to freezer failure. The lab continues to collaborate in work on by-product feeding on lipid gene expressions in grower and finishing pigs.

   The Brandebourg Lab has focused on molecular regulation of residual feed intake, molecular regulation of marbling in cattle, and the development of a swine model that will produce obesity without feeding high fat diets.

   Bergen involved in Objectives 1,2,4. Papers where published including a review of 50 years of meat animal growth research with contemporary focus on adipose and muscle stem cells.

   Work in the Brandebourg lab directly addresses Objectives 2 and 4 and could also enhance Objective 5, especially with the establishment of the Mangalica pig as a novel model of hyperphagic obesity that spontaneously develops indices of metabolic syndrome. During the past calendar year, six manuscripts have been submitted for publication that are currently in various stages of the review process or in press.

4. Publications:
   a) Tanco VM, Whitlock BK, Jones MA, Wilborn RR, **Brandebourg TD**, Foradori CD.
      Distribution and regulation of gonadotropin-releasing hormone, kisspeptin, RF-amide related peptide-3, and dynorphin in the bovine hypothalamus.
      Peer J. 2016 Mar 21;4:e1833. doi: 10.7717/peerj.1833. eCollection 2016
   b) Campos CF, Duarte MS, Guimarães SE, Verardo LL, Wei S, Du M, Jiang Z, **Bergen WG**, Hausman GJ, Fernyhough-Culver M, Albrecht E, Dodson MV.
      Review: Animal model and the current understanding of molecule dynamics of adipogenesis.
      Animal. 2016 Jan 18:1-6.
   c) Dodson MV, Allen RE, Du M, **Bergen WG**, Velleman SG, Poulos SP, Fernyhough-Culver M, Wheeler MB, Duckett SK, Young MR, Voy BH, Jiang Z, Hausman GJ.
      INVITED REVIEW: Evolution of meat animal growth research during the past 50 years: Adipose and muscle stem cells.
      J Anim Sci. 2015 93(2):457-81. Review.

5. Impact

   The role of NCCC210 (and its predecessors) in understanding of animal lipid metabolism has been enormous. This Multistate committee has been at the forefront in lipid metabolism topics

germane to animal production and development of muscle–foods products consistent with the nutritional needs of the consuming public since the 1940s. At Auburn, new-global laboratory techniques and bioinformatics have been utilized to delineate molecular regulation of marbling and also feed efficiency. Initial rounds of these studies (as elsewhere) will require extensive additional work to develop paradigms which consistently will result in high production efficiency and "healthy" animal foods products by the US livestock industry.

Of particular interest is data from the Brandebourg laboratory indicating the Mangalica pig exhibits superior meat quality and Red Mangalica could serve as a unique model for studying marbling. Also as Mangalica pigs become fatter, they spontaneously develop risk factors associated with metabolic syndrome: obesity, hyperglycemia, hyperinsulinemia, dyslipidemia, and low-grade chronic inflammation. Thus, Mangalica pigs now represents a novel pig model that develops metabolic syndrome simply by being allowed to eat to their voluntary intake. Also, these pigs represent a novel model to study feed intake as they eat 2-3 times more than is necessary to maximize bone and muscle growth unlike other swine breeds that essentially "eat to their energy". Furthermore, feeding high fat diets fails to suppress voluntary feed intake in these pigs indicating they are a novel model of hyperphagia. Thus we now have the necessary data to satisfy review panels and study sections for both NIH and USDA funding mechanisms which can now be targeted.

Station Reports
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1. Washington Station Publications [2015--2016]

2. Min Du

3. Accomplisments:
   During the past year, the Washington Station studied the impact of maternal Vitamin A intake on adipogenesis and metabolic health of offspring.

   Retinoic acid regulates various types of progenitor differentiation and tissue development including adipose development. However, the impact of maternal vitamin A supplementation on fetal adipose development and its long-term consequence in adulthood remain unclear. To address, we supplemented maternal mice with 0, 15 or 30 IU/mL retinyl palmitate in water during gestation and lactation. Adipose tissue and serum of weanling offspring, and E7.5, 12.5 and 18.5 fetuses were collected. We found that retinoic acid is the main retinoid in fetuses that was elevated by maternal vitamin A supplementation. The populations of platelet-derived growth factor receptor alpha positive ($PDGFR\alpha^{+}$) adipocyte progenitors in both fetuses and weaned offspring were increased due to vitamin A supplementation, which were correlated with heightened vascular density in adipose tissue at weaning. We further found that retinoic acid upregulated vascular endothelial growth factor α (VEGFα) which might explain the enhanced vasculogenesis in adipose tissue of fetuses supplemented with vitamin A. Using florescence tracing, we found that, under cold or retinoic acid stimulation, $PDGFR\alpha^{+}$ progenitor cells were located in neovascular system. In summary, our results reveal that maternal vitamin A supplementation increases offspring adipose progenitor populations by promoting vascular system development during the fetal stage, which is mediated by retinoic acid receptor signaling.

   Retinoic acid (RA) is known to maintain the preadipocyte phenotype and inhibit adipocyte differentiation and maturation. Although RA has been showed to induce epigenetic changes during embryonic stem cell differentiation, the effect of RA on epigenetic modifications of adipogenic genes are unknown. C3H10T1/2 mesenchymal progenitor cells were induced adipogenesis with/without 1mM all-trans retinoic acid (ATRA), and epigenetic changes in the *zfp423* promoter, a critical transcription factor initiating adipogenesis, were analyzed. We found that Growth arrest and DNA-damage-inducible protein GADD45 alpha (*gadd45α*) is involved in adipogenesis of C3H10T1/2 by inducing DNA demethylation in the *zfp423* promoter. During adipogenesis, both *gadd45α* and *zfp423* expression was upregulated. *Gadd45α* overexpression upregulated zfp423 expression and enhanced adipogenesis whereas *gadd45α* knock down had opposite effects. RA strongly inhibited the expression of *zfp423* and down-stream adipogenic genes in both control and *gadd45α* overexpressing cells and completely blocked lipid accumulation, which was associated with a reduction in *gadd45α* expression and binding to the *zfp423* promotor. Consistently, *in vivo*, we found that 30 IU/mL vitamin A supplementation (through water) prevented high fat diet (60 kcal% fat) induced obesity, which was correlated with attenuated *zfp423* and *pparg* expression in the adipose tissue of mice. After one month of high fat diet challenge, vitamin A supplemented mice had lower body weight gain, less white fat deposition, smaller adipocytes size and higher insulin sensitivity compared with control mice. These data demonstrate that GADD45α mediated *zfp423* promoter demethylation is required for adipogenesis. RA inhibits adipogenesis by preventing DNA demethylation of the *zfp423*

promoter and lipid accumulation. Thus, RA is beneficial for the prevention of high fat diet induced obesity.

4. Publications:

a) Wei, S. J., **M. Du**, Z. H. Jiang, G. J. Hausman, L. F. Zhang, and M. V. Dodson. (2016). Long noncoding RNAs in regulating adipogenesis: new RNAs shed lights on obesity. *Cellular and Molecular Life Sciences*, *In press*.
b) Wang, B., Q. Yang, C. L. Harris, M. L. Nelson, J. R. Busboom, M. J. Zhu, and **M. Du**. (2016). Nutrigenomic regulation of aidpose tissue development – role of retinoic acid. *Meat Science*, In press.
c) Liang, X., Q. Yang, X. Fu, C. J. Rogers, B. Wang, H. Pan, M. J. Zhu, P. W. Nathanielsz, and **M. Du**. (2016). Maternal obesity epigenetically alters visceral fat progenitor cell properties in male offspring mice. *Journal of Physiology*, In press.
d) Campos, C. F., M. S. Duarte, S. E. F. Guimaraes, L. L. Verardo, S. Wei, **M. Du**, Z. Jiang, W. G. Bergen, G. J. Hausman, M. Fernyhough-Culver, E. Albrecht, and M. V. Dodson. (2016). Review: Animal model and the current understanding of molecular dynamics of adipogenesis. *Animal*, In press.
e) Li, N., Q. Y. Yang, R. Walker, T.B. Thompson, **M. Du**, and B. R. Rodgers. (2016). Myostatin attenuation in vivo reduces adiposity, but activates adipogenesis. *Endocrinology*, 157: 282-291.
f) Miao, Z. G., L. P. Zhang, X. Fu, Q. Y. Yang, M. J. Zhu, M. V. Dodson, and **M. Du**. (2016). Invited review: Mesenchymal progenitor cells in intramuscular connective tissue development. *Animal*, 10: 75-81.
g) Fu, X., M. J. Zhu, S. Zhang, F. Marc, V. Benoit, and **M. Du**. (2016). Obesity impairs skeletal muscle regeneration via inhibition of AMP-activated protein kinase. *Diabetes*. 65: 188-200.
h) Martins TS, ML Chizzotti, W Silva, LN Renno, LMP Sanglard, NVL Serao, FF Silva, SEF Guimaraes, MM Ladeira, MV Dodson, **M Du**, and MS Duarte. (2015). Molecular factors underlying the deposition of intramuscular fat and collagen in skeletal muscle of Nellore and Angus cattle. *PLOS One*, 0139943.
i) Fu, X., M. J. Zhu, M. V. Dodson, and **M. Du**. (2015). AMP-activated protein kinase stimulates Warburg-like glycolysis and activation of satellite cells during muscle regeneration. *Journal of Biological Chemistry*. 290: 26445-26456.
j) Wang, S., M. J. Zhu, and **M. Du**. (2015). Prevention of obesity by dietary resveratrol: how strong is the evidence? *Expert Review of Endocrinology and Metabolism,* 10: 561-564.
k) Yang, G., Y. Xue, H. Zhang, **M. Du**, and M. J. Zhu. (2015). Favorable effects of GSE on intestinal epithelial differentiation and barrier function in IL10-deficient mice. *British Journal of Nutrition*, *2015:1-9*.
l) Chen, D., W. Li, **M. Du**, M. Wu, and B. Cao. (2015). Sequencing and characterization of divergent marbling levels in the beef cattle (Longissimus dorsi muscle) transcriptome. *Asian Australasian Journal of Animal Science,* 28: 158-165.
m) **Du, M.**, W. Bo, M.J. Zhu. (2015). Fetal programming in meat production. *Meat Science*, 109: 40-47.
n) Wei, S., X. Fu, X. Liang, M. Zhu, Z. Jiang, S. M. Parish, M. V. Dodson, L. Zan, and **M. Du**. (2015). Enhanced mitogenesis in stromal vascular cells derived from subcutaneous adipose tissue of Wagyu compared with those of Angus cattle. *Journal of Animal Science*, 93: 1015-

1024.

Station Reports
NCCC210 Mtg., San Diego 2016

1. North Carolina State University

2. Jack Odle

3. Accomplishments

Synergistic effects of dietary prebiotics and long-chain polyunsaturated fatty acids (LCPUFA) on intestinal health of the suckling neonate.

This project is underway and funded by a research grant from USDA-NIFA.

Aberrations to intestinal barrier function in early life can lead to chronic inflammation and may predispose adult-onset allergies and autoimmune disease. We suggest that such maladies are rooted in molecular signaling induced by microbial-associated molecular patterns (MAMP) and modulated by pro- and anti-inflammatory eicosanoids derived from LCPUFA. Our studies will be predicated on preliminary data showing independent effects of dietary prebiotics and LCPUFA on the microbiome, eicosanoid production and intestinal barrier function. Our experiments will interrogate the molecular convergence of signals derived from these nutrients on key transcription factors that drive enteric inflammation and immune development. We will employ in vivo and ex vivo methods in our well-developed piglet model to retain physiological relevance but also gain insight into underlying molecular mechanisms. The overall goal of this USDA-AFRI funded project is to determine whether formula supplemented with prebiotics and LCPUFA will enhance intestinal health by altering the microbiome, improving barrier function and resolution of inflammation to promote intestinal health in a neonatal piglet model. SPECIFIC AIMS are to assess the synergistic effects of dietary prebiotics and LCPUFA on: 1. Intestinal microbiome, mucosal eicosanoids and pattern recognition receptors. 2. Ex vivo inflammation induced by microbial-associated molecular patterns. 3. Resolution of intestinal inflammation following dextran-sodium-sulfate-induced colitis. These studies will provide molecular insights into mechanistic interactions between prebiotics and LCPUFA in the neonatal GI tract to optimize barrier function and support healthy modulation of inflammation.

4. Publications mid 2014-todate
   a) Eisemann, J., H. Lewis, A. Broome, K. Sullivan, D. Boyd, **J. Odle** and R. Harrell. 2014. Lysine requirement of 1.5 to 5.5 kg pigs fed liquid diets. *Anim. Prod. Sci.* 54:608-615.
   b) Pi, D., Y. Liu, H. Shi, S. Li, **J. Odle**, X. Lin, H. Zhu, F. Chen, Y. Hou, W. Leng. 2014. Dietary supplementation of aspartate enhances intestinal integrity and energy status in weanling piglets after lipopolysaccharide challenge. *J. Nutr. Biochem.* 25:456-462
   c) INVITED **Odle, J.**, S.H. Adams and G. Vockley. 2014. Carnitine. *Adv. Nutr.* 5:289-290.
   d) Xie, J., L. Tang, L. Lu, L. Zhang, L. Xi, H.C. Liu, **J. Odle**, and X. Luo. 2014. Differential expression of heat shock transcription factors and heat shock proteins after acute and chronic heat stress in laying chickens (Gallus gallus). *PLoS ONE* 9:e102204.
   e) Bai, X., X. Lin, J. Drayton, Y. Liu, C. Ji, and **J. Odle.** 2014. Clofibrate increases long-chain fatty acid oxidation by neonatal pigs. *J. Nutr.* 144:1-6.
   f) Xi, L., S. Jacobi and **J. Odle**. 2015. Transplacental induction of fatty acid oxidation in term fetal pigs by the PPARα agonist clofibrate. *J. Anim. Sci. Biotech*. 6:1-12.
   g) Zhu, Y.W., L. Lu, W.X. Li, L.Y. Zhang, C. Ji, X. Lin, H.C. Liu, **J. Odle** and X.G. Luo. 2015. Effect of dietary manganese on antioxidant status and expression levels of heat-shock proteins and

factors in tissues of laying broiler breeders under normal and high environmental temperatures. *Br. J. Nutr.* 114:1965-1974.

h) Rosero, D.S., **J. Odle**, A.J. Moeser, R.D. Boyd and E van Heugten. 2015. Peroxidised dietary lipids impair intestinal function and morphology of the small intestine villi of nursery pigs in a dose-dependent manner. *Br. J. Nutr.* 114:1985-1992.

i) Zhu, Y.W., L. Lu, W.X. Li, L. Y. Zhang, C. Ji, X. Lin, H.C. Liu, **J. Odle** and X.G. Luo. 2015. Effects of maternal dietary manganese and incubation temperature on hatchability, antioxidant status, and expression of heat shock proteins in chick embryos. *J. Anim. Sci.* 93:5725-5734.

j) Kuchibhatla, R., B.W. Petschow, **J. Odle** and E.M. Weaver. 2015. Nutritional impact of dietary plasma proteins in animals undergoing experimental challenge and implications for patients with inflammatory bowel disorders: A meta-analysis. *Adv. Nutr*. 6:541-551.

k) Rosero, D.S., **J. Odle**, S.M. Mendoza, R.D. Boyd, V. Fellner and E. van Heugten. 2015. Impact of dietary lipids on sow milk composition and balance of essential fatty acids during lactation in prolific sows. *J. Anim. Sci.* 93:2935-2947.

l) Xie, J., L. Tang, L. Lu, L. Zhang, X. Lin, H.C. Liu, **J. Odle** and X. Luo. 2015. Effects of acute and chronic heat stress on plasma metabolites, hormones and oxidant status in restrictedly fed broiler breeders. *Poultry Sci.* 94: 1635-1644.

m) Rosero, D.S., **J. Odle**, C. Arellano, R.D. Boyd and E. van Heugten. 2015. Development of prediction equations to estimate the apparent digestible energy content of lipids when fed to lactating sows. J. Anim. Sci. 93:1165-1176.

n) Zhu, Y.W., J. J. Xie, W.X. Li, L. Lu, L. Y. Zhang, X. Lin, H.C. Liu, **J. Odle**, C. Ji, and X.G. Luo. 2015. Effects of environmental temperature and dietary manganese on egg production performance, egg quality, and some plasma biochemical traits of broiler breeders. *J. Anim. Sci.* 93:3431-3440.

o) Bost,.K.L., K.J. Piller, **J. Odle** and C.H. Stahl. 2016. A sublethal swine model for defining in vivo superantigen-induced responses following exposure to staphylococcal enterotoxin B. *Methods Mol.Biol.* 1396:115-124.

p) Jacobi, S.K., T. Yatsunenko, D. Li, S. Dasgupta, R.K. Yu, B. Berg, M. Chichlowski and **J. Odle**. 2016. Dietary isomers of sialyllactose increase ganglioside sialic acid concentrations in the corpus callosum and cerebellum and modulate the colonic microbiota of formula-fed piglets. *J. Nutr.* 146:200-208. doi: 10.3945/jn.115.220152

q) Rosero, D.S., R.D. Boyd,, M. McCulley, **J. Odle**, and E. van Heugten. 2016. Essential fatty acid supplementation during lactation is required to maximize the subsequent reproductive performance of the modern sow. *Anim. Reprod. Sci.* 168:151-163.

5. Impact

This research is at an early stage so the ultimate impact(s) are yet to be determined. In the short term, impacts are measured in terms of procedures developed, implemented and refined. For example, we have determined the minimal effective dose of dextran sodium sulfate required to induce colitis in the suckling pig. This was a preliminary but important first step. Early impacts are also measured in student development and training. A postdoc, a Ph.D. student and 6 undergraduate students received mentoring on this project. Similarly 3 collaborative principal investigators are combining their expertise to achieve the goals of this project.

Station Reports
NCCC210 Mtg., San Diego 2016

1. University of Tennessee

2. Brynn H. Voy

3. Accomplishments
   Objective 1: Share and critique new techniques, experimental designs, and in progress-recent data. In 2015 I co-organized and participated in the annual NCCC210 workshop in Boston, MA. This workshop, once again, provided the opportunity to present results from ongoing, unpublished studies and to benefit from the lively discussions and perspectives of other group members. During 2015 my lab increased its use of 'omics-scale technologies by implementing LC-MS-based lipidomics (*Objective 2: increasingly implement newly emerging high through-put omics based technologies and experimental methodologies*). We modified and used methods to comprehensively profile phospholipids, acylcarnitines and bile acids in tissue extracts, including adipose tissue. We applied this methodology to profile acylcarnitine profiles and bile acids in adipose tissue across a period of progressive fasting. We also defined the impact of maternally-provided fish oil on tissue phospholipid species in offspring, using our boiler hen-chick model. A portion of this work was presented at EB 2015 in Boston, MA, and at Poultry Science 2015 in Louisville, KY. Our background in this area contributed to a review article published in 2015, in which we described progress in adipocyte biology gained from studies in agricultural animal models over the last 50 years (*Objective 3: Publish peer-reviewed scientific reviews*).

4. Publications.
   a) Evolution of meat animal growth research during the past 50 years: Adipose and muscle stem cells. Dodson MV, Allen RE, Du M, Bergen WG, Velleman SG, Poulos SP, Fernyhough-Culver M, Wheeler MB, Duckett SK, Young MR, Voy BH, Jiang Z, Hausman GJ. J Anim Sci. 2015 Feb;93(2):457-81. doi: 10.2527/jas.2014-8221.

5. Impacts.
   As in past years, the workshop organized in 2015 provided ample opportunity for our lab and others to exchange and discuss data, and to foster new interactions among group members. The lipidomics results we have obtained in 2015 have spawned new hypotheses about lipid mediators and their roles in adipocyte biology. Thus far, two new grant proposals have arisen from these 'omics efforts in 2015, and work in 2016 is in part built upon these data.

Station Reports
NCCC210 Mtg., San Diego 2016

1. North Carolina State University/Nutreco

2. Theo van Kempen

3. Accomplishments
   The bio-availability of vitamin E in swine was studies using a pharmacokinetics approach. Swine received a test meal with deuterated all-rac tocopheryl-acetate while control animals received an IV dose of deuterated RRR-tocopherol. Serial blood samples were obtained for analysis of tocopherol, and data were analyzed using various pharmacokinetics models. For the orally dosed animals, a model with a lag phase was most appropriate with the lag phase corresponding with gastric passage, while for the IV dosed animals a 2-phase model described the data best; IV dosed tocopherol was quickly eliminated from the blood stream (presumably by the liver) and subsequently released back slowly into the blood stream and then eliminated with a half-life of 5.9 h. Oral vitamin E had a bio-availability of 12.5%, a half-life of only 2.6 hours, and hence a bio-efficacy of only 5.4%. This value was in line with predictions based on published literature.

4. Publications
   a) Fouhse, J.M., M.G. Gänzle, T.A.T.G. van Kempen, and R.T. Zijlstra (2015) High amylose starch with low in vitro digestibility stimulates hindgut fermentation and has a bifidogenic effect in weaned pigs. J. Nutr. 145:2464-2470.

5. Impact
   Vitamin E is the primary cost component in premixes and as such an important factor in profitability of the livestock industry. Bio-availability information allows for comparison of alternatives to tocopheryl-acetate.

Station Reports
NCCC210 Mtg., San Diego 2016

1. Purdue University

2. Kolapo Ajuwon

3. Accomplishments

   Dr. Ajuwon was involved in various activities of NCCC210 in the past year. As a participant at the annual NCCC210 meeting in Boston in 2015, Dr. Ajuwon participated in the important discussions on critiquing new experimental techniques and designs in the area of adipocyte function and lipid metabolism. Dr. Ajuwon was also in involved in the review of multiple manuscripts for multiple journals. In addition, Dr. Ajuwon served as an external reviewer for grant applications from Brazil and Belgium as well as serve on an NIH review panel. In addition, in the past year. Dr. Ajuwon conducted research on the use of fecal water for metabolomics analysis in the pigs. The results of experiments in this area were presented at the Digestive physiology conference in Kliczcov, Poland in 2015. Dr. Ajuwon published many peer-reviewed publications in 2015. The list is given below. Dr. Ajuwon is conducting research in the area of heat stress effect on pig adipose tissue in order to understand the effect of climate change and an increasing ambient temperature on the growth and metabolism of pigs. Results from these studies were presented at the Experimental Biology conference in Boston in 2015.

4. Publications:
   a) Yan H, Ajuwon KM. Mechanism of Butyrate Stimulation of Triglyceride Storage and Adipokine Expression during Adipogenic Differentiation of Porcine Stromovascular Cells. PLoS One. 2015 10(12):e0145940. doi: 10.1371/journal.pone.0145940.
   b) Qu H, Donkin SS, Ajuwon KM. Heat stress enhances adipogenic differentiation of subcutaneous fat depot-derived porcine stromovascular cells. J. Anim. Sci. 2015. 93(8):3832-42. doi: 10.2527/jas.2015-9074.
   c) Shih CL, Ajuwon KM. Inhibition of MMP-13 prevents diet-induced obesity in mice and suppresses adipogenesis in 3T3-L1 preadipocytes. Mol Biol Rep. 2015 Jul;42(7):1225-32. doi: 10.1007/s11033-015-3861-2.
   d) Ajuwon, K. M. 2015. Toward a better understanding of mechanisms of probiotics and prebiotics action in poultry species. *J. Appl. Poult*. Res. 0:1–7. http://dx.doi.org/10.3382/japr/pfv074.

   Research abstracts:
   a) Ajuwon, K. and H. Zhao. 2015. Heat stress and LPS activate multiple signaling pathways to regulate cytokine expression in macrophages. FASEB J. 29: 756.9.
   b) Yan, H and Kolapo M. Ajuwon 2015. Effect of butyrate on inflammatory and oxidative gene markers in porcine IPEC-J2 intestinal epithelial cells. J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2.
   c) Horn, N.L. Guy Miller, C. R Little, F. Ruch, K. M. Ajuwon, and O.Adeola. 2015. Impact of acute water and feed deprivation at weaning and subsequent heat stress on serum stress markers and ileal mucosa gene expression in nursery pigs J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2.
   d) Xue, P.C., D. Ragland, K.M. Ajuwon, and O. Adeola. 2015. Dietary nitrogen level affects

ileal phosphorus digestion in growing pigs J. Anim. Sci. Vol. 93, Suppl. s3/J. Dairy Sci. Vol. 98, Suppl. 2.

5. Impacts
   a. Several graduate students were exposed to new techniques and knowledge of adipose biology. In addition, several undergraduate students were given training on techniques that stimulated their interest in pursuing research or academic careers related to adipose biology/obesity.
   b. There were positive interactions with several members of the NCCC210 and other colleagues from the government, academia and industry that may lead to joint research projects.