Meeting attendees:
Kola Ajuwon, James Kinder, Jack Odle, Xing Li, Kim Barnes, Jan Novakofski, Donald Beitz, Shih-Torng Ding, Sean Adams, Min Du.

Accomplishments for NCCC210
Indiana Station (Ajuwon). The focus of our work in 2011 continues to center around the regulation of extracellular matrix composition in adipose tissue. In 2011, we investigated the impact of anti-inflammatory treatment with sodium salicylate on high fat diet induced upregulation of expression of regulation of extracellular matrix (ECM) proteins. Sodium salicylate was fed to C57BL6 mice on either a control (10% fat calories) or a high fat diet (HFD) (60% fat calories) for 8 weeks. Expression of adipose tissue ECM and inflammatory cytokine genes was then determined by RT-PCR. Results from this experiment shows that specific ECM genes such as biglycan and COL1A1 and inflammatory genes IL-6 and TNFα was significantly induced by high fat diet. However, supplementation with sodium salicylate resulted in significant downregulation of expression of BGN, COL1A1, and COL6A1 and a concurrent downregulation of TNFα and IL-6 and TLR4 expression. Additionally, correlation reveals significant positive correlation between biglycan, IL-6 and TNFα expression. In addition, lower expression of IL-6 and CD68 was found in the mesenteric adipose tissue of BGN knockout mice compared to the wild type. These studies provide evidence of a strong link between inflammatory state and expression of ECM genes in adipose tissue. It also suggests that anti-inflammatory treatments may prevent excessive deposition of ECM that is linked to adipose tissue dysfunction in obesity. Through collaborative work, we also showed that a synthethic estrogenic compound, (+)-Z-Bisdehydrodoisynolic acid, enhances fatty acid oxidation in female obese Zucker rats. Several studies were also conducted on the effect of maternal high fat diet on offspring adiposity and metabolic status in the pig model. These studies, published in abstract form at present, indicate that maternal overnutrition results in metabolic abnormalities in the offspring.

Washington Station (Dodson). Dr. M.V. Dodson's research continues with attempts to increase the efficiency of mature adipocyte cell isolation and propagation of progeny cells. Cells derived from all cell isolations are being put to use by personnel of Dr. Min Du in a variety of molecular experiments. Moreover, Dr. Zhihua Jiang is initiating studies with such isolated cells to attempt to define molecular switching among mature adipocytes and dedifferentiated progeny cells. Papers and grants have been/are being written to continue this line of collaborative research at the Washington station.
Washington Station (Du). Dr. Du joined Washington Station recently. His lab continues to define the mechanisms associated with intramuscular fat deposition, focusing on adipogenic differentiation. In mice, very recent studies show that adipocytes and fibroblasts share a common pool of progenitor cells, with Zinc finger protein 423 (Zfp423) as a key initiator of adipogenic differentiation, while fibrogenesis is promoted by transforming growth factor-β (TGF-β) signaling pathway. To evaluate the role of Zfp423 in intramuscular adipogenesis and marbling in beef cattle, we sampled beef samples for separation of stromal vascular cells. These cells were immortalized with pCI neo-hEST2 and individual clones were selected by G418. A total of 288 clones (3 x 96 well plates) were isolated and induced to adipogenesis. The presence of adipocytes was assessed by Oil-Red-O staining. Three clones with the high and low adipogenic potential were selected for further analyses. The expression of Zfp423 was much higher ($307.4 \pm 61.9\%$, $P < 0.05$) in high adipogenic cells, while TGF-β was higher ($156.1 \pm 48.7\%$, $P < 0.05$) in low adipogenic cells. Following adipogenic differentiation, the expression of peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/Enhancer Binding Protein α (C/EBPα) were higher ($239.4 \pm 84.1\%$ and $310.7 \pm 138.4\%$, respectively, $P < 0.05$) in high adipogenic cells. Over-expression of Zfp423 in

stromal vascular cells and cloned low adipogenic cells dramatically increased their adipogenic differentiation, accompanied with the inhibition of TGF-β expression. In conclusion, data show that Zfp423 is a critical regulator of adipogenesis in stromal vascular cells, and Zfp423 may provide a molecular target for enhancing intramuscular adipogenesis and marbling in beef cattle.

North Carolina Station (Odle, Li). To test the effect of clofibrate on the fatty acid (FA) oxidation in vivo, newborn pigs received 5 mL of vehicle (2% tween 80) with or without clofibrate (75 mg/kg bw) once daily via intragastric gavage for 3 days. Total FA oxidative capacity was measured in respiration chambers after gastric infusion of piglets (n=5 /treatment) with isoenergetic amounts of [1-^{14}C] labeled triglycerides (TG) -- either tri-C18:1 (3.02 mmol/kg bw$^{0.75}$) or tri-C22:1 (2.46 mmol/kg bw$^{0.75}$). Total expired $^{14}CO_2$ was collected and quantified at 20-min intervals over 24 h. Hepatic in vitro FA oxidation was determined simultaneously using [1-^{14}C] labeled C18:1 and C22:1. The average 24 h accumulative [1-^{14}C] TG oxidative utilization (% of energy intake/kg bw$^{0.75}$) tended to increase with clofibrate supplementation ($P<0.14$), but there was no difference in the peak utilization rate. The maximal extent of tri-C18:1 oxidation was 61 % greater than for tri-C22:1. Hepatic FA oxidation in vitro increased significantly with clofibrate. The relative abundance of mRNA increased 2-3 fold for hepatic PPARα and its target genes (fatty acyl-CoA oxidase (FAO), carnitine palmitoyltranserase (CPT) I and CPT II) in the pigs given clofibrate. The increase in mRNA enrichment by clofibrate was greater for CPT I than for ACO. In conclusion, clofibrate may improve in vivo FA oxidative utilization in neonatal pigs. Supported by CSREES, USDA NRI program award 2007-35206-17897.

Alabama Station (Bergen). His lab has continued to screen mRNA abundance in skeletal muscles and subcutaneous adipose tissues of key target genes involved with lipid accretion (PPARγ, fatty acid synthase, SREBP-1c, SCD), fat catabolism and the role of uncoupling proteins (PPARα, Acyl CoA dehydrogenase, beta oxidation, UCP) and protein metabolism (key genes in the proteasomal pathway) in cattle under varying production systems in the Southeast. We are also studying the relative pattern of PPARγ and DLK-1 (Pref-1) gene expression from serial samples (170-310days of age) of loin muscle containing marbling (intramuscular fat, imf) and in subcutaneous adipose tissue. It is still not clear whether DLK-1 has a role in muscle tissue imf-adipocyte differentiation and delays the onset (via upstream factors) of marbling compared to differentiation in subcutaneous depot fat. The putative effect of DLK-1 on PPARγ expression appears to be mediated via SOX 9 and other transcription/ regulatory factors and we are doing further work to dissect these relationships in growing and finishing steers.

Alabama Station (Brandebourg). Dr. Brandebourg since his hire into the Animal Sciences Department at Auburn University has been establishing a comprehensive program in lipid biology of pigs and important lipid metabolism related issues in Southeast beef cattle production.

Work with pigs: 1) The Mangalica pig is a suitable model for obesity induced metabolic syndrome and development of the diabetic heart in humans. As fat accretion progresses, these pigs exhibit perturbed glycemic control, liver dysfunction, markers of adipose tissue inflammation and the development of cardiac muscle-associated lipid deposition. These data validate the Mangalica as a translational model for obesity-associated metabolic disease. 2) The expression of neuronal genes in pig preadipocytes and

adipocytes suggest that suggest porcine adipose tissue is under the regulation of circadian control. The expression of neuronal genes, Synuc, Npas2, and Bmal1 is detectable in both porcine preadipocytes and adipocytes while the expression of Per2 mRNA is not. Quantitative real-time PCR indicates that ACRP, Npas2, Bmal1 and Synuc expression is induced 36, 11-, 8-, and 5-fold by day 16 of adipogenesis. These data point to a unique feedback mechanism between fat and the brain.

Work with beef cattle: 1) The role of PPARγ appears to regulate intramuscular adipose tissue development in heifers in response to age and diet. The timing of the development of marbling appears linked to the expression of a transcription factor, PPARγ, as this key regulator of fat cell differentiation is down regulated in IMF until the finishing stage in cattle and in response to ryegrass versus grain finishing diet. This study does not support a role for Pref-1 in this mechanism. 2) The role of neuropeptides in regulating feed efficiency in growing cattle during conditions of thermoneutrality and heat stress. Growth efficiency is linked to increased levels of POMC and lower levels of NP-Y, relaxin-3 and GnRH in the arcuate nucleus of growing steers. These data suggest neuropeptides in the feeding center of the brain play an important role in regulating feed efficiency in cattle. The overall aim of the work with beef cattle: Ultimately these efforts should enhance the sustainability and profitability of cattle production in our state by allowing Alabama cattlemen to overcome major limitations currently preventing wide scale adoption of grass-based finishing systems in the southeast and by improving heifer performance in cow-calf operations.

Illinois Station (Novakofski). The North American river otter (*Lutra canadensis*), once native to Illinois, was hunted nearly to extinction throughout the Midwest in the mid 1800s. They have recently been reintroduced in several states and populations have begun to recover. However, there are economically important and endangered species that may be preyed upon by otters including game fish, mussels and turtles. The goal of this project is to use fatty acid profiles from both otters and potential prey species to infer typical diets. This approach is based on the assumptions that 1) FA profiles, particularly unusual FA, are a proportional representation of the diet, and 2) different prey have unique FA profiles (The level of possible taxonomic distinction, family, genus, species, is unclear). One challenge in this study is similar to use of the method in marine mammals: primary feeding studies to determine depot partitioning and relative metabolism of specific FA are not possible. Other complications are unknown seasonal and geographical variation in energy balance and prey species. Use of PCA analysis in this case is analogous to maximum likelihood estimation to evaluate the proportion of each prey candidate in the diet that makes the otter profile most likely. A pilot study was done with principle-components analysis (PCA) on published FA profiles of several carnivores (otters, raccoon dogs, wolves, brown bears, and two subspecies of ringed seals-from the Artic and Baltic Seas). Two principle components (60.31% of the variation) separated species except for wolves and brown bears and the ringed seal subspecies, which consume different fish species, were identifiable. The FA composition of carnivores displays depot variation as in livestock with the interesting addition of foot pad depots. Analysis of FA from 9 incidentally killed otters to date indicates reasonable variation in FA composition between and within individuals (for example 10-16% 16:0, 17-26% 18:1 between depots). Variation between depots Tail adipose has the greatest and food pad adipose the lowest variation in FA. We are currently analyzing samples from 48 otters and prey including crayfish, mussels, frogs and fish.

West Virginia Station (Barnes). The primary goal of our laboratory is to understand the mechanism(s) of action of dietary conjugated linoleic acid-induced body fat loss. We have utilized a model of enhanced CLA responsiveness in mice raised from weaning, for 6 weeks prior to CLA supplementation, on diets deficient in polyunsaturated fatty acids (coconut oil). As well, we have established a cell culture (3T3-L1) model of CLA-induced lipolysis to study the oil x CLA interaction we have observed in mice. We have also investigated the effect of different sources of omega-3 fatty acids on body composition and serum lipids.

Major findings from this year's work include: 1). CLA feeding increased the mRNA expression of Malic Enzyme and SCD1 in coconut oil-fed mice; indicating that these mice may have greater fatty acid turnover, contributing to the enhanced loss of body fat. 2) 3T3-L1 adipocytes responded maximally to trans-10, cis-12 CLA with increased lipolysis when cells were treated with 50 μM CLA vs LA for 12 hours following overnight serum starving. Also, adipocytes that were exposed to coconut oil prior to CLA responded with a greater increase in lipolysis than adipocytes exposed to soy oil. Therefore, 3T3-L1 adipocytes under these conditions can be used as a model for the enhanced response to CLA observed in coconut oil-fed mice. 3). Vegetarian sources of EPA (yeast oil) and DHA (algal oil) can reduce serum cholesterol levels in mice but did not affect serum triglycerides or body fat.

Iowa Station (Beitz). Project 1: A Mitochondria-Targeted Vitamin E Derivative Decreases Hepatic Oxidative Stress and Inhibits Fat Deposition in Mice. Our objective in this study was to determine whether a mitochondria-targeted vitamin E derivative (MitoVit E) would decrease oxidative stress and associated obesity by preventing a previously proposed aconitase inhibition cascade. Sixty-four mice were fed a high-fat (HF) diet for 5 wk. They were then switched to either a low-fat (LF) or a medium-fat (MF) diet and gavaged with MitoVit E (40 mg MitoVit E • kg body weight^{-1}) or drug vehicle (10% ethanol in 0.9% NaCl solution) every other day for 5 wk. Epididymal fat weight, as well as liver lipid and remaining carcass lipid, were significantly lower in the MF group receiving MitoVit E (MF-E) than in the MF group receiving vehicle only (MF-C). Liver mitochondrial H_2O_2 production and the protein carbonyl level were also significantly lower in MF-E than in MF-C mice. In contrast, none of the biochemical variables (aconitase activity, ATP and H_2O_2 production, and protein carbonyl level) in the muscle mitochondria were modified by MitoVit E in either MF or LF groups. Expression of acetyl-CoA carboxylase and fatty acid synthase in both liver and adipose tissue of MF groups was not affected by MitoVit E. However, expression of carnitine palmitoyltransferase 1a in the liver and uncoupling protein 2 in adipose tissue were significantly enhanced by MitoVit E in both LF and MF groups. In conclusion, MitoVit E attenuates hepatic oxidative stress and inhibits fat deposition in mice but not through alleviation of the aconitase inhibition cascade.

Project 2: Effect of a mitochondria-targeted vitamin E derivative on mitochondrial alteration and systemic oxidative stress in mice. The objective of the present study was to determine whether a mitochondria-targeted vitamin E derivative (MitoVit E) would affect certain mitochondrial parameters, as well as systemic oxidative stress. A total of sixty-four mice were fed a high-fat (HF) diet for 5 weeks. They were then switched to either a low-fat (LF) or a medium-fat (MF) diet, and administered orally with MitoVit E (40 mg MitoVit E/kg body weight) or drug vehicle (10 % (v/v) ethanol in 0.9 % (w/v) NaCl solution), every other day for 5 weeks. Mitochondrial ATP and H_2O_2 production rates in both the liver

and the gastrocnemius were not affected by MitoVit E administration in either LF or MF diet-fed mice. However, the number and average size of the subsarcolemmal mitochondria, but not the intermyofibrillar mitochondria, from the soleus muscle were significantly higher in the MF group receiving MitoVit E (MF-E) than in the MF group receiving vehicle only (MF-C). After the mice were switched from the HF diet to the four dietary treatments (LF-C, LF-E, MF-C and MF-E), the decrease in urinary isoprostane concentration was significantly greater in the LF-E group than in the other three groups during the whole study (weeks 6–10). In addition, MitoVit E significantly increased plasma superoxide dismutase (SOD) activity in the MF diet-fed group without affecting plasma glutathione peroxidase activity or H_2O_2 levels. Overall, these data suggest that MitoVit E affects subsarcolemmal mitochondrial density and systemic oxidative stress parameters such as plasma SOD activity and urinary isoprostane concentration.

Impacts

1. Knowledge obtained deepens our understanding of nutritional, environmental and genetic regulation of adipogenesis, depot specific and whole body fat accumulation.
2. Advances are also made in our understanding of lipid metabolism in adipose tissue.

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