

Growth and Development: Regulation of Adipogenesis and Adipose Tissue Development

822 Adipogenic differentiation state-specific gene expression as related to bovine carcass adiposity. C. L. Pickworth^{*1}, S. C. Loerch¹, F. L. Fluharty¹, D. H. Poole², S. G. Velleman¹, and J. L. Pate², ¹The Ohio State University, Wooster; ²The Pennsylvania State University, State College.

The regulation of site and rate of fat deposition in cattle is not well defined. The study objective was to investigate adipogenic differentiation state-specific gene expression in feedlot cattle (>75% Angus; <25% Simmental parentage) of varying adipose accretion patterns. Four groups of 4 steers were selected from a group of 80 steers via ultrasound to have low backfat-low intramuscular fat (LBF-LIM), low backfat-high intramuscular fat (LBF-HIM), high backfat-low intramuscular fat (HBF-LIM), or high backfat-high intramuscular fat (HBF-HIM). Adipose tissue from the subcutaneous (SQ) and intramuscular (IM) depots were collected at harvest. The relative expression of adipogenic differentiation state-specific genes were evaluated using quantitative PCR. Data were analyzed using the mixed model of SAS and gene expression data were analyzed using covariate analysis with ribosomal protein L19 as the covariate. No interactions were observed ($P > 0.10$) between IM and SQ adipose accretion for any of the variables measured. Therefore, only the main effects of high and low accretion within a depot and the effects of depot are reported. No differences ($P > 0.10$) in mRNA between high and low adipose accretion within a depot were detected for any of the genes in spite of differences ($P < 0.001$) in cell density, diameter, and carcass measures. Preadipogenic delta like kinase 1 mRNA was higher ($P < 0.001$) in the IM than the SQ adipose tissue; conversely, differentiating and adipogenic genes (lipoprotein lipase, peroxisome proliferator activated receptor, fatty acid synthetase, and fatty acid binding protein 4) were higher ($P < 0.001$) in the SQ than the IM depot. Intramuscular adipocytes were smaller ($P < 0.001$) than SQ adipocytes and had greater ($P < 0.001$) expression of preadipogenic markers, indicating that more hyperplasia was occurring in the IM while the SQ was undergoing differentiation and hypertrophy. Adipogenic differentiation state-specific gene expression was not different in cattle with various phenotypes but adipogenesis in the SQ and IM adipose tissues appears to occur independently.

Key Words: gene expression, adipose, differentiation

823 Palmitoleic acid (C16:1) changes fatty acid profiles and alters gene expression in bovine adipocyte cultures. T. A. Burns^{*}, S. K. Duckett, and S. L. Pratt, *Clemson University, Clemson, SC.*

Our objective was to determine if differences in fatty acid profiles or gene expression exist when adipocytes are exposed to increasing levels of C16:1. Three primary preadipocyte cell lines were isolated, propagated, and frozen for use in this study. Thawed cells were passaged 4 additional times and plated at a density of 1×10^5 cells/cm². Cells were allowed to reach confluence and held for 2 d. On D0, primary differentiation media [Dulbecco's modified eagles medium (DMEM) containing 10% fetal calf serum (FCS), and 2X antibiotic/antimycotic (AB/AM), insulin at 2.5 μ g/mL, 0.25 μ M dexamethasone, 20 μ M troglitazone (TRO), 0.5 mM isobutylmethylxanthine, and 10 mM acetate] was applied for 2 d and replaced with secondary differentiation media [DMEM, 10% FCS, 2X AB/AM, insulin at 2.5 μ g/mL, 5 μ M TRO, 10 mM acetate, containing 1 of 4 levels of C16:1(0, 50, 150, or 300 μ M)] from D2 to D12. Cells were harvested on D6 and D12 for fatty acid analysis using gas chromatography and gene expression by RT-qPCR.

At 0 μ M C16:1, there was no effect of harvest day on levels of C16:1, C18:1c11, or total mg fatty acids. However, these fatty acids and total fatty acids increased ($P < 0.05$) linearly in response to increasing C16:1 supplement. Additionally, they were elevated ($P < 0.05$) in 50, 150, and 300 μ M C16:1-supplemented cells harvested on D12 compared with D6. In contrast, C16:0, C18:0, C18:1c9, decreased ($P < 0.05$) in response to increasing C16:1 in the media and were not affected by harvest day. The ratio of C18:1c9/C18:0 decreased ($P < 0.05$) in response to increasing C16:1 supplementation. Stearoyl-CoA desaturase (SCD) and fatty acid synthase mRNA expression was reduced ($P < 0.05$) on D6 in C16:1-supplemented groups for each cell line. Therefore, C16:1 may have a regulatory role in the transcription of SCD and fatty acid synthesis. In conclusion, supplementing cells with C16:1 produced changes in fatty acid composition of bovine adipocytes by D6 and influenced mRNA expression.

Key Words: adipocyte, palmitoleic acid, gene expression

824 Effect of fatty acids on adipocyte differentiation specific genes expression. P. Cheguru^{*}, M. E. Doumit, G. Murdoch, and R. A. Hill, *University of Idaho, Moscow.*

Adipocyte differentiation has been extensively studied in vitro by using a standard hormonal cocktail induction treatment that includes dexamethasone, insulin and cAMP inducers like IBMX (isobutyl methyl xanthine). Pioneer work by Amri et al. (J. Lipid Res. 1991, 32; 1449–1456) provided evidence that fatty acids can also induce adipocyte-specific gene expression. We report here that long-chain fatty acids in the presence of insulin were able to induce lipid accumulation in mouse 3T3-L1 preadipocyte cell lines. 3T3-L1 cells were treated with either oleic acid (C18:1) or linoleic acid (C18:2) in the presence or absence of insulin. Confluent cells grown in complete growth medium were incubated with fatty acids in the presence or absence of insulin. Medium was changed every 2 d. Cells were stained with Oil Red O and RNA was collected on d 0, 2, 4, 6 and 8. Control cells were grown in complete growth medium only. Simultaneously, cells treated with hormonal cocktail containing dexamethasone, insulin and IBMX were observed at each time point. Oil Red O staining results showed that cells treated with fatty acids and insulin accumulated more lipid than cells treated with fatty acids alone or control cells. Cells treated with hormonal cocktail accumulated more lipid than other treatments. Adipocyte differentiation involves upregulation of PPAR γ (peroxisome proliferator activated receptor γ), C/EBP α (CCAAT enhancer binding protein α), FABP4 (fatty acid binding protein 4) and SREBP-1c (sterol regulatory element binding protein-1c). Other genes like Pref-1 (preadipocyte factor-1) and GATA2 (GATA binding protein 2) will be downregulated. We hypothesize that long chain fatty acids can upregulate PPAR γ , C/EBP α and FABP4 and downregulate Pref-1 gene expression. Lipid staining results confirm that cells treated with fatty acids and insulin had different metabolic changes than cells treated with fatty acids alone or control. We expect significant changes in adipocyte specific gene expression in cells treated with fatty acids and insulin at each time point.

Key Words: adipocyte differentiation, long-chain fatty acids, gene expression

825 Expression of genes associated with adipocyte differentiation differs with age and adipose tissue depot during growth. G. Go^{*}, D.

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We hypothesized that major subcutaneous (s.c.) adipose tissue depots would exhibit different developmental patterns of gene expression over time. Four Angus steers at each 9, 12, and 14 mo were harvested and s.c. adipose tissue samples were collected from over the chuck, rib, loin, sirloin, round, brisket, flank, and plate. AMPK α , CPT-1 β , SCD, GPR43, PPAR γ , and C/EBP β were analyzed with qRT-PCR. Data were analyzed using the general linear model of SAS statistical analysis program. When significant, means were separated by Fishers protected least significant difference method. Over time, the major backfat depots, loin, rib, and sirloin s.c. adipose tissues, had the highest SCD ($\Delta 9$ desaturase) gene expression, whereas, anterior depots, brisket, plate, and flank s.c. adipose tissues, expressed less SCD RNA ($P < 0.001$). AMPK α gene expression was the greatest in round and the least in loin and rib s.c. adipose tissues. There were few significant differences in gene expression over time. However, across tissues, AMPK α and GPR43 (short chain fatty acid receptor) decreased over time, and CPT-1 β , PPAR γ , and SCD gene expression was greatest at 14 mo of age (all $P < 0.07$). We conclude that there were few differences across adipose depots in adipogenic expression, although age of steer had major effects on gene expression.

Key Words: adipogenic genes, subcutaneous adipose tissue

826 Hedgehog signaling mediates adipogenesis in C3H10T1/2 cells via down-regulation of COUP-TFII. W. F. Yue*, J. X. Zhao, M. J. Zhu, and M. Du, *Department of Animal Science, University of Wyoming, Laramie.*

Effective manipulation of depot specific adipose accumulation will dramatically enhance animal production efficiency. In human, adipogenesis plays a central role in the pathogenesis and pathophysiology of metabolic syndromes. Chicken Ovalbumin Upstream Promoter Transcription Factor II (COUP-TFII) is expressed during the early stages of adipocyte differentiation. Hedgehog signaling is known to inhibit adipogenesis. We hypothesized that hedgehog signaling inhibits adipogenesis through regulating COUP-TFII expression. C3H10T1/2 multipotent cells were used. Addition of cyclopamine (3.6 μ M), an inhibitor of the hedgehog signaling, promoted the mRNA expression of COUP-TFII, while addition of sonic hedgehog (20 nM) which stimulates hedgehog signaling decreased COUP-TFII expression by $38.1 \pm 2.61\%$ ($P < 0.05$). The mRNA expression of COUP-TFII was correlated with the expression of peroxisome proliferator-activated receptor (PPAR) γ , with cyclopamine treatment increasing PPAR γ protein content by $73.4 \pm 14.56\%$ ($P < 0.05$). In addition, both CCAAT/enhancer binding protein (C/EBP) α and β , 2 transcription factors known to induce adipogenesis, were induced by $109.6 \pm 18.20\%$ and $137.0 \pm 44.05\%$ ($P < 0.05$) respectively when hedgehog signaling was inhibited by cyclopamine. In conclusion, hedgehog signaling decreases COUP-TFII expression and adipogenesis in C3H10T1/2 cells. COUP-TFII expression may stimulate early adipogenesis. Because hedgehog signaling is central to the morphogenesis and pattern formation during early animal development, hedgehog may regulate depot specific adipogenesis through COUP-TFII associated signaling pathways.

Key Words: COUP-TFII, adipogenesis, hedgehog

827 Characterization of fat mass and obesity associated gene (FTO) expression in the broiler chicken. A. Tiwari*, S. M. Krzysik-Walker, G. L. Hendricks III, and R. Ramachandran, *The Pennsylvania State University, University Park.*

Fat mass and obesity associated gene (FTO), also known as *Fatso* is a member of the Fe-II and 2-oxoglutarate-dependent dioxygenase superfamily. The FTO gene product is highly conserved across vertebrate species and is ubiquitously expressed in multiple tissues. Studies in humans and rodents suggest that FTO is involved in regulation of food intake and lipolytic activity in adipose tissue. Mutations in a FTO intron have been associated with obesity and related metabolic disorders in humans. While the physiological role of FTO is beginning to be understood in mammals, the chicken homolog of FTO has not been described. Objectives of the present study were to clone full length chicken FTO cDNA and to quantify FTO expression in primary metabolic tissues, adipose, liver and skeletal muscle to determine if age or feeding status affect FTO expression in male broilers. Using rapid amplification of cDNA ends (RACE), we cloned the full length chicken FTO cDNA. Chicken FTO cDNA and its deduced protein sequences were found to be 68% and 69% homologous to that of human FTO, respectively. Using Western blotting, we detected FTO protein in hypothalamus, adipose tissue, liver, skeletal muscle, spleen, kidney, heart and testes of broiler chickens. Quantification of FTO protein by Western blotting revealed significantly greater expression in liver (ANOVA, $P < 0.05$; $n = 5$ /treatment) of 8 week old broilers fasted for 48 h compared with *ad libitum* fed chickens maintained on standard commercial broiler feed. No significant difference in FTO protein expression, however, was found in 4-week-old versus 8-week-old broiler tissues (ANOVA, $P > 0.05$; $n = 5$ /age group). We conclude that FTO is expressed in multiple chicken tissues and that FTO expression is influenced by feeding status in the broiler chicken. We are currently investigating the physiological role of FTO in broiler chicken metabolism.

Key Words: metabolism, liver, energy balance

828 Effect of nutrition and chronic infusion of leptin on intake and body composition of *Bos indicus* heifers at puberty. M. V. Carvalho, J. D. Magalhães, L. U. Gimenes, G. P. Rodrigues, and L. F. P. Silva*, *Universidade de São Paulo, Pirassununga, SP, Brazil.*

The amount of fat in the carcass has been proposed as a regulator of initiation of puberty in cattle. To test if changes in energy intake and in leptin concentration is capable of altering body composition at puberty, 36 prepubertal Nelore heifers, 18 to 20 mo-old, 275.8 ± 17.2 kg BW and BCS of 5 ± 0.5 (1 to 9 scale) were randomly assigned to each of 3 treatments ($n = 12$): H (high energy diet), L (low energy diet), and LL (low energy diet + oLeptin). Diets were formulated to promote weight gain of 0.4kg/day (groups L and LL) or 1.2kg/day (H group). After 21 d of adjustment, heifers in LL group received subcutaneous injections of oLeptin at 4.8 μ g/kg BW twice a day, for 56 d. Groups H and L received similar injections of 2mL saline solution. Age at puberty was considered to be the age on first detection of a corpus luteum, confirmed by plasma concentrations of progesterone of > 1 ng/ml. Heifers were slaughtered during the luteal phase after the first estrus cycle. Individual intake was estimated using the markers chromic oxide and indigestible NDF for estimate total fecal excretion and digestibility, respectively. Leptin administration had no effect ($P > 0.05$) on dry matter intake, body weight gain, body composition and efficiency of energy use. High energy intake accelerated puberty and modified body composition at puberty. Heifers from the H group had heavier carcass weight (222 vs. 181.5 kg, $P < 0.01$), higher BCS (6 vs. 4.5, $P < 0.01$), larger longissimus muscle area (59 vs. 53 cm², $P < 0.01$), and greater subcutaneous fat thickness (5.4 vs. 3.4 mm, $P < 0.01$), than heifers from the L and LL groups together. Carcasses from the H group also had higher ether extract content (24.2 vs. 21.3, $P < 0.01$) than carcasses from the L and LL groups together. Animals receiving a high energy diet also had better efficiency of energy

use (0.25 vs. 0.16, $P < 0.01$). High energy intake during the prepubertal phase increased BW and carcass fat content at puberty, and improved the efficiency of dietary energy usage. These results do not support the hypothesis that puberty occurs at a constant percentage of body fat.

Key Words: carcass fat, cattle, maturity