

## Lactation Biology 2

**958 Regulation of mammary epithelial cell proliferation and gene expression by *Semen Vaccariae* active monomer.** Z. Y. WAN, H. L. TONG, Q. Z. LI, and X. J. GAO\*, *Key Laboratory of Dairy Science of Education Ministry, Northeast Agricultural University, Harbin, China.*

*Semen Vaccariae* is a traditional Chinese herb that is widely used to increase lactation. However, the underlying changes in gene expression that drive the increased milk production by *Semen Vaccariae*, especially the specific active monomer, remain an open question. Our lab has successfully separated active monomer dibutyl phthalate (DBP) from *Semen Vaccariae* to study its role in lactation. A model DT CASY Cell Counter was used to study the effect of increasing concentrations of DBP on proliferation and viability of primary cultured dairy cow mammary epithelial cells (DCMEC) harvested from a lactating cow at d 140 (n = 3). QRT-PCR and Western blot were used to study changes in mRNA and protein of *prlr*, *era*, *akt1*, *socs2*, *ppary* and *elf5* at 6, 12, 24, 36, 48, and 72 h. miRNAs (21, 125b, 143 and 195) and secretion of  $\beta$ -casein and lactose were detected by qRT-PCR analysis and RP-HPLC. Each assay was performed in 5 independent experiments using 3 different treatments and the data were analyzed with SPSS by ANOVA. The results showed that DBP (0.5 mg/ml) increased proliferation and viability of DCMEC significantly ( $P < 0.05$ ). DBP acted similarly to prolactin (PRL). It increased the expression of *prlr*, *era*, *akt1* and *elf5*, but repressed the expression of *ppary*. DBP promoted the expression of *socs2* mRNA, but inhibited the expression of *socs2* protein. Both DBP and PRL repressed the expression of miRNA-125b, miRNA-143 and miRNA-195 in DCMEC. DBP repressed the expression of miRNA-21, while the influence of PRL on miRNA-21 was uncertain. Both DBP and PRL enhanced the expression of  $\beta$ -casein ( $P < 0.05$ ) and the secretion of lactose ( $P < 0.05$ ) significantly. In conclusion, *Semen Vaccariae* active isomer increased proliferation and secretion of milk components by DCMEC. This is the first demonstration that miRNA expression can be changed by DBP and PRL. Further studies to uncover the lactogenic targets of DBP will help to shed light on the genetic mechanisms of mammary gland development and lactation.

*This work was supported by the Innovation Team of the Northeast Agricultural University (Grant No. LXT005-1-2).*

**Key Words:** *Semen Vaccariae*, dibutyl phthalate, lactation

**959 Deletion of thyroid hormone responsive spot 14 exacerbates the anti-lipogenic affect of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) in the mammary gland.** K. J. Harvatine\*<sup>1</sup>, Y. R. Boisclair<sup>2</sup>, and D. E. Bauman<sup>2</sup>, <sup>1</sup>*Penn State University, University Park*, <sup>2</sup>*Cornell University, Ithaca, NY.*

Inhibition of milk fat synthesis by fatty acid (FA) intermediates originating from ruminal biohydrogenation has been extensively studied in the cow and more recently in the mouse. In both species, *trans*-10, *cis*-12 conjugated linoleic acid (CLA) reduces milk fat concentration and markedly reduces milk fat concentration of de novo synthesized FA. During CLA treatment mammary lipogenic capacity is decreased by a coordinated downregulation of genes involved in milk fat synthesis. We also identified downregulation of thyroid hormone responsive spot 14 (S14) in mammary tissue of both the cow and mouse during CLA treatment. The functional role of S14 in CLA-induced inhibition of fat synthesis was tested using wild-type (WT) and S14 null mice in a randomized block design with a 2x2 factorial arrangement of treatments (genotype x CLA). Starting at 6–8 d of lactation, S14 null and WT dams

nursing 6–8 pups received oral doses of water (control) or 20 mg/d of CLA for 5 d. Pups and dams were weighed daily. On the last day of treatment dams were milked and killed. The effect of genotype, CLA, and genotype by CLA interaction was tested. Milk fat of S14 null mice was 25% lower than that of WT dams ( $P < 0.001$ ) and CLA treatment reduced milk fat concentration in both genotypes ( $P = 0.03$ ). However, there was a marked interaction of genotype and CLA treatment for milk concentration of de novo synthesized FA ( $P < 0.001$ ), where the WT dams reduced milk concentration of FA less than 16 carbons in length by 27% while S14 null mice reduced FA less than 16 carbons by 72%. In agreement, mammary lipogenic capacity measured as <sup>14</sup>C glucose incorporation into lipids by mammary tissue explants was decreased 23% in WT dams and 82% in S14 null dams. Mammary lipogenesis of S14 null dams is hyper-responsive to CLA treatment demonstrating a possible indirect effect of S14 on regulation of lipogenesis. Therefore, S14 may modify the activity of a second CLA responsive mechanism.

**Key Words:** milk fat, conjugated linoleic acid, lipogenesis

**960 The role of SREBP-1 in lipogenesis in bovine mammary epithelial cells.** L. Ma\* and B. A. Corl, *Virginia Polytechnic Institute and State University, Blacksburg.*

Sterol regulatory element binding proteins (SREBPs) are a family of transcription factors that regulate lipid metabolism. There are 3 isoforms, SREBP-1a, SREBP-1c and SREBP-2, among which SREBP-1a and SREBP-1c regulate fatty acid synthesis. The objective of this study was to determine the role of SREBP-1 in lipogenesis in bovine mammary epithelial cells. Bovine mammary epithelial cells (MACT) were used in this study. After reaching 80% confluence in a flask, cells were trypsinized and reseeded to plates at a density of  $2 \times 10^4$  cells/cm<sup>2</sup>. After incubation in base medium (DMEM+10% FBS) overnight, cells were transfected using small interfering RNAs (siRNA), against SREBP-1 (SSI), Cyclophilin B as positive control (POS), a non-targeting sequence as negative control (NEG), and no siRNA as untreated control (UNT), according to protocol (Dharmacon Inc.). Cells were harvested for mRNA measurement after 24 h, for measurement of protein and acetate incorporation after 72 h. After treatment with SSI at concentrations of 5, 25, 50, 75, and 100 nM, the expression of SREBP-1 mRNA was reduced 76%, 84%, 90%, 91%, and 92%, respectively. In POS, the expression of Cyclophilin B mRNA decreased 74%, 90%, 95%, 94%, and 96%, respectively. SSI reduced precursor SREBP-1 protein (10, 586, 705, and  $505 \pm 241$  for SSI, POS, NEG, and UNT, respectively;  $P < 0.07$ ) and mature form of SREBP-1 (0, 376, 453, and  $260 \pm 85$  for SSI, POS, NEG, and UNT, respectively;  $P < 0.05$ ) compared with controls. Acetate incorporation also decreased to 0.31 nmol/4 h with SSI at 5 nM, compared with 0.41, 0.56, and  $0.43 \pm 0.02$  nmol/4 h, for POS and NEG, and UNT, respectively ( $P < 0.01$ ). SSI at 5 nM reduced SREBP-1 mRNA and protein by 76% and 98%. When SREBP-1 decreased, there was a significant decrease in acetate incorporation, thus SREBP-1 might regulate milk fat through the de novo fatty acid synthesis pathway.

*This project was supported by National Research Initiative Competitive Grant no. 2009-35204-05358 from the USDA National Institute of Food and Agriculture.*

**Key Words:** SREBP, small interfering RNA, bovine

**961 Effects of t10,c12 CLA dose on mammary gland development, adiposity, and inflammation in mice.** M. R. Foote\*, S. L. Giesy, G.

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The t10,c12 CLA isomer has been shown to have a wide range of potential health benefits including anticarcinogenic and antiadipogenic effects in animal models. Despite evidence of beneficial effects in the rat, dietary t10,c12 CLA was recently shown to impair mammary gland development and to accelerate mammary tumorigenesis in the mouse. The level of dietary t10,c12 CLA (0.5% of the diet) in this study was typical of levels shown to result in white adipose tissue inflammation and insulin resistance, 2 factors known to promote mammary cancer. To evaluate the possibility that lower t10,c12 CLA doses might inhibit lipogenesis without detrimental effects on mammary development, FVB wild-type mice received a diet containing 0%, 0.1%, 0.2%, or 0.5% t10,c12 CLA (n = 8 to 10 per treatment) from 24 d to 49 d of age. As expected, the 0.5% CLA dose resulted in increased hepatic triglyceride content and weight and increased plasma insulin ( $P < 0.05$ ). In addition, the 0.5% dose caused abnormal mammary gland development (i.e., reduced ductal elongation combined with ductal hyperplasia) in parallel with dramatic mammary gland inflammation characterized by increased expression of monocyte chemoattractant protein 1 (MCP-1), egf-like module containing, mucin-like, hormone receptor-like 1 (EMR-1), tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), and interleukin 6 (IL-6) ( $P < 0.05$  for all). The 0.1% CLA dose did not affect any of these metabolic and inflammatory end-points or any indices of mammary gland development ( $P > 0.05$  for all). The 0.1% CLA dose, however, was as effective as the 0.5% dose in decreasing gonadal fat weights and fatty acid synthase expression in adipose tissue ( $P < 0.05$ ). Our results establish that a low dose of t10,c12 CLA decreases adiposity without impeding mammary gland development or causing inflammatory and metabolic complications. These results show, for the first time in the mouse, that the positive effects of t10,c12 CLA on adiposity can be dissociated from negative effects on mammary development, metabolism, and inflammation.

**Key Words:** CLA, mammary gland

**962 Impact of time of milk storage in the udder on fat.** M. Dutreuil<sup>1,2</sup>, C. Cébo<sup>3</sup>, J. Guinard-Flament<sup>2,1</sup>, and C. Hurtaud<sup>\*1,2</sup>, <sup>1</sup>INRA UMR1080 Production du lait, Saint-Gilles, France, <sup>2</sup>AGROCAMPUS OUEST UMR1080 Production du lait, Rennes, France, <sup>3</sup>INRA Unité GABI, Jouy-en-Josas, France.

Our objective was to study the effect of duration of milk storage on milk fat globule (MFG) secretion to better understand relationships between milk yield, milk fat and MFG secretion. Four milking frequencies were studied in 6 dairy cows averaging  $118 \pm 22$  dim: 2 milkings/d separated by 11- and 13-h intervals (2M11–13) or by 4- and 20-h (2M4–20) and 1 milking/d (1M24). The experimental trial was a double Latin square  $3 \times 3$  with 2 wk periods. In post-experiment, milking frequency of 36-h (1M36) was repeated twice. Compared with 2M11–13, 1M24 reduced milk and milk fat yields and increased fat content, without any effect on the size of MFG which agrees with previous research. 2M4–20 had no significant effect on milk fat yield and content but tended to increase the size of the MFG. Lipolysis, measured on morning milk, was weaker with 1M24. Milk fatty acid composition was not modified by milking frequency. When data were analyzed according to kinetics of milk storage duration (from 4 to 36-h), the highest fat content and the largest diameters of MFG were obtained on milks from 4- and 36-h milking (respectively 62.8 g/kg and 4.15  $\mu\text{m}$  and 57.7 g/kg and 4.09  $\mu\text{m}$ ). Such observations could have 2 origins: the richness in residual milk of the 4-h milk and the coalescence of MFG related to the long storage duration in the 36-h milk. Independently, for each duration of milk storage, there was a relationship between MFG size and fat yield

( $R^2$  from 0.31 to 0.81). Conversely, the relationship between MFG size and fat content was confirmed whatever duration of milk storage ( $R^2 = 0.55$ ). Speed of secretion of milk fat (storage of 4 h excluded) was also well correlated with MFG size ( $R^2 = 0.62$ ). For the 36-h milk, this relationship was also observed but with a significantly different slope suggesting a phenomena of MFG coalescence in response to the increased intra-mammary pressure. Duration of milk storage induces changes in MFG size under factors which interact.

**Key Words:** milk fat, milk storage, milk fat globule

**963 IGF-I regulates the expression of GLUT12 in bovine mammary epithelial cells.** Y. Shao\* and F.-Q. Zhao, *Department of Animal Science, University of Vermont, Burlington.*

Insulin-like growth factor-I (IGF-I) is a potent mitogen for mammary epithelial cells and plays an important role in mammary development. Glucose is an energy source for mammary epithelial cell proliferation and glucose uptake is mediated by facilitative glucose transporters in mammary epithelial cells. The objective of this study was to investigate the role of IGF-I in regulating the expression of the main glucose transporters GLUT1, GLUT8 and GLUT12 in bovine mammary epithelial cells. In the first experiment, Mac-T cells were treated for 12 h with increasing concentrations of IGF-I (20, 50, 100, 200 and 400 ng/mL). mRNA levels of GLUT1, GLUT8, GLUT12 and IGFBP3 were determined by real-time PCR. IGFBP3 mRNA increased 6- to 13-fold in all groups treated with 50 ng/mL or higher concentrations of IGF-I compared with non-treatment group ( $P < 0.001$ ), indicating that Mac-T cells are responsive to IGF-I. There were no treatment effects on GLUT1 and GLUT8 mRNA, but mRNA levels of GLUT12 decreased by 75% in all groups treated with 50 ng/mL or more IGF-I relative to control group ( $P < 0.001$ ). In the second experiment, Mac-T cells were treated with 100 ng/mL of IGF-I for 3, 6, 12, 18, 24 and 48 h. Interestingly, mRNA of GLUT12 decreased to 21–35% after 3 to 12 h treatment but returned to the same levels as in the non-treatment group after 48 h. In summary, these data indicate that IGF-I may regulate the expression of GLUT12, but not GLUT1 and 8 in bovine mammary epithelial cells.

**Key Words:** glucose transporter, IGF-I, mammary epithelial cells

**964 Mammary mitochondrial function is associated with lactation Performance in inbred mice.** J. Wei\*, S. Kiser, J. George, D. Anderson, and D. Hadsell, *Baylor College of Medicine, Houston, TX.*

Milk production in dairy cattle is influenced by genetics. In rodent models of lactation, oxidative metabolism and mitochondrial number/activity are increased dramatically with secretory activation. In addition, different inbred mice have different abilities to support litter gain through lactation implying that genetic background regulates milk synthesis capacity. However, comparison of mammary mitochondrial function among these inbred mice has not been studied. We hypothesized that variation in lactation capacity of different mouse strains is due to difference in mammary mitochondrial function. The FVB and C57BL/6 (C57) mice representing higher and lower lactation performance were used to assess mammary mitochondrial function and biogenesis. Lactation performance was assessed by crossfoster litter weight gain for the first 8 d of lactation. Mammary tissue was collected at Day 10 postpartum. mRNA levels of mitochondrial associated genes and mitochondrial DNA (mtDNA) copy number were measured using quantitative PCR (qPCR). Litter gain was higher with the FVB dams ( $26.81 \pm 0.64\text{g}$ ) than with the C57 ( $22.01 \pm 1.82\text{g}$ ) ( $P = 0.026$ ). Mammary mitochondrial ATP synthesis activity was 68% higher in FVB mice compared with C57 ( $P < 0.001$ ). Mammary mtDNA copy number per cell was

70% higher in the FVB compared with the C57 ( $P < 0.05$ ). Mammary mRNA levels of Ppargc1a (131.73%), Nrf1 (49.63%), Gabpa (51.34%), Tfb1m (71.65%), Tfb2m (92.37%), Nnt (5052.74%), Sirt1 (79.83%), and Sod2 (34.63%), in the lactating mammary gland were all higher ( $P < 0.05$ ) in FVB mice compared with C57. Mammary mRNAs for Tfam (65.61%) and Ucp3 (61.07%) were lower ( $P < 0.05$ ) in the FVB mice. Significant difference in the expression levels of these transcriptional factors regulating mammary mitochondrial biogenesis and ATP synthesis activity implicates their important roles in lactation performance. The lower expression of the mammary Ucp3 and Tfam in the FVB mice may also suggest novel regulatory mechanisms of mammary mitochondrial functions in lactation.

*Supported by USDA/ARS Cooperative Agreement # 6250-51000-048.*

**Key Words:** lactation, mitochondria, mammary

**965 Temporal changes in the mammary mitochondrial proteome of the mouse suggest that increases in a limited number of proteins are necessary to support increased ATP synthesis during early lactation.** D. Hadsell\*<sup>1</sup>, W. Olea<sup>1</sup>, R. Matsunami<sup>2</sup>, and D. Engler<sup>2</sup>, <sup>1</sup>*Baylor College of Medicine, Houston, TX*, <sup>2</sup>*The Methodist Hospital Research Institute, Houston, TX*.

The regulation of mammary mitochondrial biogenesis and function across the lactation cycle is not well understood. This study employed differential in-gel electrophoresis coupled with MALDI-tof/tof mass spectrometry to relate changes in mammary cell mitochondrial function during lactation to changes in the proteins that comprise this organelle.

Our hypothesis was that changes in mammary cell mitochondrial biogenesis and function during lactation would directly correlate with coordinated changes in the proteins that make up the oxidative phosphorylation (OXPHOS) pathway and that some of these proteins might also be linked to PPARGC1 $\alpha$  and AMP kinase. Markers of mammary mitochondrial biogenesis and function were measured in mammary tissue and mitochondria collected from lactating mice at d 2, 8, 14, 21, 28, and 35 postpartum. Mitochondrial ATP synthesis activity increased ( $P < 0.05$ ) during early lactation and then declined with prolonged lactation. Staining of mammary tissue sections for succinate dehydrogenase activity indicated that mitochondrial number increased ( $P < 0.05$ ) 5-fold during early lactation. Western blotting for the transcriptional co-activator PPARGC-1 $\alpha$  and immunofluorescent staining for phospho AMP kinase demonstrated that these proteins were most abundant on d 2 postpartum. Analysis of the proteome identified 154 proteins that changed ( $P < 0.05$ ) throughout the lactation cycle. Of these, only 3 members (NDUFAF3, UQCRB, and ATP6V1B2) of the OXPHOS pathway were increased during early lactation. In contrast, 23 OXPHOS proteins increased ( $P < 0.05$ ) during mid lactation, while most of these same proteins decreased ( $P < 0.05$ ) during late lactation. There were 6 proteins within the data set that could be directly linked to PPARGC1 $\alpha$  through analysis for interacting networks. The results suggest that the increased ATP synthesis activity of the mammary mitochondria during early lactation results from changes in only a limited number of rate limiting proteins.

*Supported by USDA/ARS Cooperative Agreement #6250-51000-048.*

**Key Words:** mammary, mitochondria, proteome