oxygen barrier. In addition, these coatings and films can serve as a carrier for a variety of edible antimicrobial agents. Technologies designed to pasteurize processed meats after final packaging have received copious attention. Post pasteurization of vacuum packaged cooked products consists of hot water or steam immersion followed by immediate chilling. Irradiation (gamma, electron beam or X-ray) of cooked vacuum or MAP packaged products can produce very acceptable product at the correct dose. High pressure processing involves submerging the packaged meat product in water increasing the pressure to 87,000 psi. The process has no effect on product color, texture, flavor, or package purge. Post pasteurization, irradiation and high pressure all increase product shelf life and significantly reduce pathogens.

**Key Words:** Packaging, Cooked meat, Food safety

## Molecular Manipulation to Influence Mammary Development and Function

**418 Physiological phenotypes of estrogen receptor knock-out mice.** K.S. Korach*[1], [1]*NIEHS/NIH, Research Triangle Park, NC.*.

Estrogen receptors (ER) play a crucial role in development and reproduction. Gene targeting allowed generation of mice homozygous for either the disrupted ER$\alpha$ ($\alpha$ERKO) or ER$\beta$ genes ($\beta$ERKO). $\alpha$ERKO mice were unresponsive to uterotropic assays with estradiol, hydroxy TAM, or DES. Estrogen, EGF or IGF-1 treatment to induce DNA synthesis in $\alpha$ERKO uteri, even though EGF and IGF-1 signaling was shown to be intact by stimulation of c-fos also failed. Progesterone receptor mRNA was detected in $\alpha$ERKO mice, but was not stimulated by estrogen in the uterus, mammary gland or ovary, indicating an estrogen dependent and independent gene regulation. $\alpha$ERKO females are infertile and have hypoplastic uteri and hyperemic ovaries. The $\alpha$ERKO ovarian phenotype occurs developmentally and can be reversed by a GnRH antagonist. Serum estrogen and LH are elevated compared to WT or $\beta$ERKO females. Analysis of the mammary glands of adult $\alpha$ERKO females showed a primitive ductal rudiment rather than the fully developed ductal tree seen in WT or $\beta$ERKO mice. $\alpha$ERKO males are also infertile, with atrophy of the testes and seminiferous tubule dysmorphogenesis resulting in decreased spermatogenesis and inactive sperm. Bone length is decreased in $\alpha$ERKO of both sexes, but not in $\beta$ERKO mice. $\alpha$ERKO males have reduced bone density and some alterations in cardiovascular function. Phenotypic differences were seen in sex and aggressive behavior in both $\alpha$ERKO males and females compared to the patterns in WT or $\beta$ERKO mice. In contrast to the $\alpha$ERKO, the $\beta$ERKO males are fertile with normal sexual behavior. Recent development of a viable double ER $\alpha/\beta$-knock out shows a unique ovarian phenotype of transdifferentiation of granulosa to sertoli cells. Further characterization of the mice and comparison of the individual and double ER gene KO phenotypes will be required to more fully understand the physiological consequences of ER mediated actions and the specific roles of the two different forms of ER in estrogen hormone responsiveness.

**Key Words:** estrogen knockout, mammary , reproduction

**419 Genetic manipulation of the IGF-I axis to regulate mammary development and function.** D.L. Hadsell*, S.G. Bonnette, and A.V. Lee, *Baylor College of Medicine, Houston, TX.*.

Insulin-like growth factor I is believed to regulate several processes within mammary epithelial cells during mammary gland development. Firstly, IGF-I stimulates cell cycle progression, in both normal mammary epithelial cells and in breast cancer cells. Secondly, IGF-I can stimulate milk protein gene expression and/or milk synthesis in a number of model systems. Lastly, IGF-I inhibits apoptosis in both normal mammary epithelial cells and breast cancer cells. Our laboratory has studied the IGF-I-dependent regulation of these processes by using transgenic and knockout mouse models that exhibit alterations in the IGF-I axis. Our studies on transgenic mice that overexpress IGF-I during pregnancy and lactation have demonstrated that this growth factor slows the apoptotic loss of mammary epithelial cells during the declining phase of lactation while having minimal effects during early lactation on milk composition or lactational capacity. In contrast, our analysis of early developmental processes in mammary tissue from mice which carry a targeted mutation in the IGF-I receptor gene suggests that IGF-dependent stimulation of cell cycle progression is more important to early mammary gland development than potential anti-apoptotic effects. With both models, the effects of perturbing the IGF-I axis are dependent on the physiological state of the animal. The diminished ductal development that occurs in response to loss of the IGF-I receptor is dramatically restored during pregnancy while the ability of overexpressed IGF-I to protect mammary cells from apoptosis does not occur if the mammary gland is induced to undergo forced involution. Data from our laboratory on the expression of IGF-signaling molecules in the mammary gland suggests that this effect of physiological context may be related to the expression of members of the IRS, or insulin receptor substrate, family.

**Key Words:** Transgenic mice, Mammary, Apoptosis

**420 Regulation of IGF signaling by IGF binding protein-3 in the mammary gland.** Wendie Cohick* and Constance Grill, *Rutgers, The State University of NJ, New Brunswick, NJ/USA.*

The insulin-like growth factors (IGF) mediate mammary epithelial cell (MEC) growth and thus play a critical role in mammary gland growth and development. The biological activity of IGF is modulated by IGF binding proteins, a family of six structurally related yet distinct proteins. The immortalized bovine MEC line MAC-T synthesizes four forms of IGFBP. Under basal serum-free conditions, minimal IGFBP-3 protein is secreted. However, IGF-I specifically upregulates the synthesis of IGFBP-3, while having no effect on other IGFBP forms. Stable cell lines genetically engineered to constitutively express IGFBP-3 exhibit enhanced responsiveness to IGF-I in terms of DNA synthesis relative to mock-transfected cells (controls), suggesting that IGF-I regulation of IGFBP-3 acts as a regulatory loop that functions to increase IGF bioactivity. DNA synthesis is also increased relative to controls by factors that activate the IGF receptor but do not bind IGFBP, hence the mechanism does not require a physical interaction between IGF and IGFBP-3. IGF-I receptor number and affinity are similar between IGFBP-3 transfected and control cells. Therefore IGFBP-3 may enhance IGF action by directly influencing intracellular signaling events downstream of the IGF-I receptor. To investigate this, the signaling molecules that mediate IGF action in bovine MEC were first determined. IGF-I does not activate ERK 1/2, suggesting that IGF-I does not stimulate DNA synthesis via this MAP kinase pathway. In contrast, the p85 regulatory subunit of PI3 kinase co-precipitates with IRS-1 following stimulation with IGF-I, indicating involvement of the PI3 kinase signaling pathway. Activation of the downstream effector Akt is observed by 1 min and maximal by 15 min following exposure to IGF-I. Akt phosphorylation is greater at 1 min in MAC-T cells expressing IGFBP-3, relative to controls, and this enhanced activation is maintained through 10 h. In vitro kinase assays confirm that Akt activity is 1.4- to 1.9-fold higher in IGFBP-3 transfected cells. Therefore, IGFBP-3 may potentiate IGF-I activity by enhancing the activation of the PI3 kinase signaling pathway via Akt. Studies are in progress to further define the signaling molecules responsible for this effect.

**Key Words:** Mammary gland, Insulin-like growth factor binding protein-3, Signaling

**421 Regulation of apoptosis during mammary involution by the p53 tumor suppressor gene.** D. Joseph Jerry*[1], Ellen S. Dickinson[1], and Amy L. Roberts[1], [1]*University of Massachusetts.*

Regulation and functions of the p53 tumor suppressor gene have been studied extensively with respect to its critical role in maintaining the stability of genomic DNA following genotoxic insults. However, p53 is also induced by physiologic stimuli resulting in cell cycle arrest and apoptosis. In other situations, the activity of p53 must be repressed to prevent inappropriate removal of cells. The mammary gland provides a valuable system in which to study the mechanisms by which the expression and biological responses to p53 can be regulated under a variety of physiological circumstances. The proapoptotic role of p53 during involution of the post-lactating mammary epithelium is especially relevant to animal agriculture. We have utilized p53-deficient mice to establish the molecular targets of p53 in the mammary gland and biological consequences when it is absent. We have demonstrated that induction of the p21/WAF1 gene (Cdkn1a) is p53-dependent in the involuting mammary epithelium. Abrogation of p53 resulted in delayed involution of

the mammary epithelium demonstrating the physiological role of p53 in this process. Stromal proteases were induced in the mammary gland by 5 days post-weaning providing a p53-independent mechanism that resulted in removal of the residual secretory epithelium. These processes can be interrupted by treatment with hydrocortisone. These data establish p53 as a physiological regulator of involution that acts to rapidly initiate apoptosis in the secretory epithelium in response to stress signals. Therefore, p53 activity may be used as a physiological indicator to select treatments or animals with increased persistency of lactation.

**Key Words:** Mammary, Involution, p53

**422 The Production and Regulation of Leptin in Bovine Mammary Epithelial Cells.** J.L. Smith* and L.G. Sheffield, *University of Wisconsin-Madison, Madison, WI, USA.*

Western blot analysis indicated the presence of leptin in bovine milk, while reverse-transcription polymerase chain reaction (RT-PCR) indicated the presence of leptin mRNA in mammary tissue. Leptin was found to be produced by cultured bovine mammary epithelial cells (MAC-T cell line) by western blot and RT-PCR analysis. A real time RT-PCR method was developed that allowed quantitative assessment of bovine leptin mRNA over approximately 3 orders of magnitude. Time course studies indicated a rapid increase in leptin mRNA in response to insulin or IGF-I. When normalized against bovine GAPDH as an endogenous control, 30 minute or 1hr treatment with 10 ng/ml insulin gave 39±4 and 64±2 fold increase in leptin mRNA compared with 0hr control. Leptin mRNA was increased 257±9 and 75±23 fold by 30 minute or 1hr treatment with 10 ng/ml IGF-I. Dose response studies indicated significant increases in leptin mRNA in response to as little as 1 ng/ml insulin or 0.1 ng/ml IGF-I. Maximum increase in leptin mRNA was observed in response to 10 ng/ml insulin and 10 ng/ml IGF-1. These results indicate that production of leptin by bovine mammary epithelial cells can be regulated by factors known to alter mammary function and nutrient partitioning. This suggests that leptin may be an autocrine/paracrine signal in the bovine mammary gland.

**Key Words:** Leptin, Mammary epithelial cells, IGF-I

**423 Mammogenic effects of estrogen and growth hormone are mediated by local changes in mammary IGF-1 and IGFBP-3.** S. D. Berry*[1,2], T. B. McFadden[1,3], R. E. Pearson[2], and R. M. Akers[2], [1]*AgResearch, Hamilton, New Zealand,* [2]*Virginia Polytechnic and State University, Blacksburg, VA,* [3]*University of Vermont, Burlington, VT.*

An epithelium-free mammary fat pad was surgically prepared in twenty-five one-month-old, Friesian heifers. At 18 months of age, the heifers were randomly assigned to one of four treatment groups. Treatments were: control (C), growth hormone (GH), estrogen (E) or growth hormone + estrogen (GE). Hormones were administered for 40 hours before the animals were sacrificed to provide mammary samples of parenchyma (PAR), intact fat pad (MFP), and epithelium-free or "cleared" fat pad (CFP). IGF-1 and IGF binding protein-3 (IGFBP-3) mRNA was higher in CFP and MFP than PAR ($P < 0.001$) whereas the protein products were higher in PAR ($P < 0.001$). IGFBP-2, a 28-kDa IGFBP and a 24-kDa IGFBP were more abundant in CFP and MFP. E ($P < 0.01$) and GH ($P < 0.05$) increased incorporation of $^3$H-thymidine into DNA of PAR by an average of 350% and 125% respectively. Incorporation of $^3$H-thymidine into DNA of MFP or CFP was minimal. Coincident with the changes observed in mammary epithelial proliferation, the overall effect of E was to increase IGF-1 protein content by 190%, 40% and 60% in MFP ($P < 001$), PAR ($P < 0.01$), and CFP ($P < 0.05$), respectively. E increased IGF-1 mRNA levels in MFP ($P < 0.08$), but not CFP, indicating that the regulation of IGF-1 expression is modulated by adjacent epithelium. GH and E reduced IGFBP-3 content in PAR to less than 40% of IGFBP-3 protein content in controls, whereas the 24-kDa IGFBP in CFP and MFP was increased to between 40% and 150% of controls. Increased proliferation of mammary parenchymal cells was associated with increased IGF-1 and reduced IGFBP-3 protein in mammary tissue. An increased ratio of local mammary IGF-1: IGFBP-3 likely mediates the stimulatory effects of GH and E in heifer mammogenesis.

**Key Words:** Estrogen, Growth Hormone, Mammary

**424 Influence of feeding level and bovine somatotropin (bST) on transforming growth factor-beta (TGF-$\beta$)and its receptor in mammary tissue of growing heifers.** K. Plaut*[1], R. Maple[1], X. Cui[1], and S. Purup[2], [1]*University of Vermont, Burlington, VT/USA,* [2]*Danish Institute of Agricultural Sciences,Foulum/DK.*

Transforming growth factor-$\beta$ is a potent inhibitor of mammary epithelial cell growth. The objective of this study was to determine whether TGF-$\beta$ or its receptors were altered in heifers when mammary growth was altered. Twenty-four heifers weighing 195 kg were fed to gain at approximately 550 or 1100 gm/day for low or high feeding level, respectively. They were then treated for 35 days with bst injections (0 or 15 mg/day)at either high or low feeding level. Therefore, the treatments (n=6 per treatment) were low feeding level, placebo (LC), low feeding level, bST treated (LST), high feeding level, placebo (HC) and high feeding level, bST treated (HST). The heifers were slaughtered at approximately 230 kg live weight and mammary tissues were removed. TGF-$\beta$ mRNA expression was analyzed using RNase protection assay and quantified by densitometric scanning. The type II TGF-$\beta$ receptor was analyzed by competitive binding assay, immunohistochemistry and western blot analysis. TGF-$\beta$ mRNA was normalized to 100 for HC and compared to all other treatments. The least squares mean for TGF-$\beta$ mRNA expression was 112 for LC, 86 for LST, 222 for HST ± SEM of 80. Binding was expressed as cpm/100 micrograms of protein and was 8338 for LC, 7590 for LST, 9310 for HC, 4978 for HST ± 1396. Even though mRNA expression was approximately two-fold greater and receptor binding was one-half in animals fed to gain 1200 gm/day and treated with bST compared to all other treatments, differences were not significant due to large variability. Western blot analysis was used to determine that all tissue expressed both the Type I and II receptors. Immunohistochemistry revealed that receptors were localized in ductal and myoepithelial cells consistent with a role for TGF-$\beta$ in regulating ductal morphogenesis. While TGF-$\beta$ may play a role in mammary development, it does not seem to be regulated by somatotropin or plane of nutrition during early development.

**Key Words:** Mammary, Transforming Growth Factor-beta, Bovine Somatotropin and Feed Intake

**425 The role of insulin in the modulation of milk fatty acid composition.** B. A. Corl*[1], S. T. Butler[1], W. R. Butler[1], and D. E. Bauman[1], [1]*Cornell University, Ithaca, NY.*

Milk fat fluidity is regulated by controlling the pattern of fatty acids that comprise the triglycerides. Specifically, shorter chain fatty acids and unsaturated fatty acids lower the melting point of the triglycerides. $\Delta^9$-desaturase plays an important role in regulating milk fat melting point by adding a *cis*-9 double bond to several medium and long chain saturated fatty acids. In several species, insulin regulates $\Delta^9$-desaturase gene expression. We utilized a hyperinsulinemic-euglycemic clamp to examine the role of insulin in regulating milk fatty acid composition and $\Delta^9$-desaturase in lactating cows. This involved continuous infusion of insulin to elevate circulating concentrations and maintenance of euglycemia by variable rates of glucose infusion. The treatment period was 4 days and commenced when cows were 10 DIM. Five cows received intravenous saline infusion (control) and four cows received the insulin clamp. Milk samples were collected two days prior to and during the treatment period. Plasma insulin concentrations were increased 8-fold during the insulin clamp as compared to cows infused with saline. Over the course of the experiment, milk fat yield and milk yield were reduced in cows receiving the insulin clamp. Milk fatty acid composition of cows during saline infusion did not change when compared to pre-treatment values. In contrast, cows receiving the insulin clamp had a decrease in milk fat content of long chain fatty acids as compared to pre-treatment values. Consequently, milk fat content of *de novo* fatty acids increased considerably during the insulin clamp. This effect was most obvious for $C_{14:0}$ and $C_{16:0}$ which increased 68% and 48%, respectively. The ratio of $\Delta^9$-desaturase fatty acid products to substrates serves as a proxy for $\Delta^9$-desaturase activity. Insulin infusion increased activity of $\Delta^9$-desaturase. Although milk fat content of $C_{18:0}$ and *cis*-9 $C_{18:1}$ was reduced 56% and 38%, respectively, the ratio of *cis*-9 $C_{18:1}$ to $C_{18:0}$ was increased 42%. Similar increases were observed for the ratio of *cis*-9 $C_{14:1}$ and $C_{14:0}$ (86%) and the ratio of *cis*-9, *trans*-11 CLA and *trans*-11 $C_{18:1}$ (96%). These data indicate that insulin directly or indirectly regulates $\Delta^9$-desaturase and also milk fat content of long chain fatty acids presumably via effects on rates of lipolysis.

**Key Words:** insulin, desaturase