

# Lactation Biology Symposium: Novel Mechanisms Regulating Milk Secretion and Mammary Involution

**315 High fat diet suppresses de novo fatty acid synthesis in mammary epithelial cells independent of SREBP regulated gene expression.** S. M. Anderson\*, M. C. Rudolph, E. A. Wellberg, and M. C. Neville, *University of Colorado School of Medicine, Aurora.*

The lipid component of milk is an important energy source and a critical nutrient for proper development of the newborn. The lactating mouse secretes her entire body weight in fat during a normal lactation period. Fatty acids in milk triglyceride are blended from preformed sources (dietary and adipose stores), and de novo synthesized fatty acids. Factors that control lipogenic differentiation of mammary epithelial cells have not been identified. Gene expression profiling has identified potential controller genes including SREBP1c, Src, Spot14 (THRSP), Akt1, and the long form of the prolactin receptor. Metabolic genes that increase at parturition include the glucose transporter GLUT1, citrate synthase (CS), malic enzyme 1 (ME1), citrate transporter (SLC25a1), ATP citrate lyase (ACLY), acetyl-CoA carboxylase 1 (ACC1), fatty acid synthase (FASN), and stearoyl-CoA desaturase 2 (SCD2). SREBP1c regulates transcription of several of these genes in the liver; however, SREBP1c knockout mice do not display a lactation defect. Mice deficient in SCAP (SREBP Cleavage Activation Protein) have a significant lactation defect characterized by a 25% decrease in de novo synthesized fatty acids in milk and a 50% decrease in pup growth. Expression of FASN, Insig1, SLC25a1, and SCD2 in mammary epithelial cells is reduced, but there is no change in ACC1 and ACLY. This suggests SREBP-dependent and -independent regulation of lipid biosynthetic enzymes in mouse mammary epithelium. To compensate for loss of de novo fatty acid biosynthesis in SCAP null dams, we fed them a high fat diet (45% kCal). Although pup growth improved, lactation competency was not restored completely. Interestingly, the mRNA levels of ACC1, ACLY, FASN, SCD2, and SLC25a1 did not change, but the protein levels of ACC1, ACLY, and FASN were significantly reduced. This implies post-transcriptional regulation of fatty acid biosynthetic enzymes in mammary epithelial cells in response to dietary fat, rather than at the transcriptional level. Current efforts are focused upon understanding aspects of this post-transcriptional regulation in mammary epithelial cells.

**Key Words:** lactation, triglyceride, SREBP

**316 Serotonin: A homeostatic regulator of bovine lactation.** N. Horseman\*, *University of Cincinnati, Cincinnati, OH.*

Serotonin, a central neurotransmitter and peripheral hormone, was discovered in the mammary glands of mice using molecular genetic and gene expression profiling approaches. Physiological studies demonstrated that serotonin functions as a homeostatic regulator in the mouse mammary gland. Studies in human and bovine mammary gland experimental systems permitted us to generalize that serotonin is a conserved homeostatic regulator of lactation among mammals. Based on a foundation of basic science that has elucidated fundamental physiological, cellular, and molecular aspects of serotonin signaling in lactation, we have embarked on a variety of studies to understand the practical implications of serotonin signaling in human and bovine lactation, and in breast cancer. An extraordinary variety of drug targets and non-drug interventions can selectively impact serotonin signaling, providing a rich resource for modifying mammary gland cell functions.

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**Key Words:** serotonin

**317 Stanniocalcin-1 and local control of mammary involution.** P. Lacasse\*, *AAFC-Dairy and Swine R&D Centre, Sherbrooke, QC, Canada.*

Stanniocalcin-1 (STC-1) is a hormone that was first identified in fish and recently in mammals. In euryhaline teleost fishes, gill calcium uptake is decreased by STC-1, an action opposed by prolactin. In mice, some evidence suggests that STC-1 is implicated in lactation. Indeed, STC-1 is only detectable in blood during gestation and lactation, wild-type pups suffered growth retardation when fed by transgenic mothers overexpressing human STC-1, and passive immunization against STC-1 induced an alteration of milk composition and caused growth retardation of pups. Therefore, we have initiated a research program on the role of STC-1 in the regulation of bovine lactation. We have first demonstrated that injections of estradiol to lactating cows, while reducing milk production, increased the expression of STC-1 by the mammary cells and increased by several fold the concentration of STC-1 in milk. In a second experiment, we have measured STC-1 levels in blood and milk during lactation. We found that, as lactation progressed, STC-1 concentrations increased in milk but not in blood. In another experiment, cows were milked differentially for 8 wk, with half of the gland milked 1X and the other half milked 3X daily. Milk production and lactation persistency were greater in the udder half milked 3X while indicators of mammary involution, such as milk BSA and proteinase activity, increased in the udder half milked 1X. Milking 1X caused an increase in milk concentration of STC-1, an effect that persisted beyond the treatment period. Using unilateral milking, we have investigated the role of STC-1 during involution. Milk STC-1 concentration increased in the unmilked quarters and was correlated with milk proteinase activity and BSA. Mammary epithelial cells cultured in the presence of milk from the involuting quarters had more apoptotic cells and a reduced metabolic rate as compared with those cultured in milk from the milked quarters. Interestingly, the metabolic rate was negatively correlated with the STC-1 concentration in milk. These results suggest that STC-1 is implicated in the progression of involution. Nevertheless, more research will be needed to determine the target and function of this hormone.

**318 The role of Ca<sup>2+</sup>-ATPases in milk secretion and involution.** T. A. Reinhardt\*, *National Animal Disease Center, ARS/USDA, Ames, IA.*

The means by which calcium is transported into the milk is a poorly understood process. An older hypothesis is that calcium arrives in milk via exocytosis of secretory products from the Golgi pathway. This is consistent with more recent data showing that the secretory pathway Ca<sup>2+</sup>-ATPases (SPCA1 and 2), are induced in lactating mammary tissue. However, greater expression of the plasma membrane Ca<sup>2+</sup>-ATPase isoform 2bw (PMCA2bw) occurs during lactation. PMCA2bw expression is more strongly correlated with increases in milk calcium secretion and PMCA2bw's apical location suggested that calcium might be secreted directly into milk via this pump. This hypothesis was confirmed by examining calcium secretion in PMCA2 gene knockout mice compared with wild type controls. Milk from PMCA2-null mice have 60% less calcium than milk from wild-type mice. Total milk protein concentration was lower, and an indirect measure of milk production (litter weights) suggested that the PMCA2-null mice produce significantly less milk. These data demonstrated that PMCA2bw is required for maximal milk production and secretion of much of the calcium in milk. This major secretory function represents a novel biological role for the PMCA's, which were previously regarded as premier regulators of intracellular

Ca<sup>2+</sup> for cell signaling and general fine control of cell calcium homeostasis. The data available to date suggest that calcium transport into milk involves both the Golgi secretory pathway via the activities of SPCA1 and 2 as well as a major role for the apical pathway via PMCA2. It follows that lactating mammary cell calcium homeostasis is maintained by the high expression of PMCA2, SPCA1 and 2. Within 24 h after abrupt

cessation of lactation, PMCA2 and SPCA1 and 2 expression decreased 80–95%. The abrupt loss of Ca<sup>2+</sup>-ATPases, required by the mammary gland to maintain cell calcium homeostasis, could lead to accumulation of cell calcium, mitochondria Ca<sup>2+</sup> overload, calcium mediated cell death and thus may play a part in early signaling of mammary involution.

**Key Words:** mammary calcium transport, Ca<sup>2+</sup>-ATPase, involution