MAMMARY DEVELOPMENT

Regulation of mammary development

Inactivation of Stat5 in mouse mammary epithelium during pregnancy reveals distinct functions in cell proliferation, survival, and differentiation.
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This study explored the functions of the signal transducers and activators of transcription 5a and 5b (referred to as Stat5 here) during different stages of mouse mammary gland development by using conditional gene inactivation. Mammary gland morphogenesis includes cell specification, proliferation and differentiation during pregnancy, cell survival and maintenance of differentiation throughout lactation, and cell death during involution. Stat5 is activated by prolactin, and its presence is mandatory for the proliferation and differentiation of mammary epithelium during pregnancy. To address the question of whether Stat5 is also necessary for the maintenance and survival of the differentiated epithelium, the two genes were deleted at different time points. The 110-kb Stat5 locus in the mouse was bracketed with loxP sites, and its deletion was accomplished by using two Cre-expressing transgenic lines. Loss of Stat5 prior to pregnancy prevented epithelial proliferation and differentiation. Deletion of Stat5 during pregnancy, after mammary epithelium had entered Stat5-mediated differentiation, resulted in premature cell death, indicating that at this stage epithelial cell proliferation, differentiation, and survival require Stat5.

Diet and Mammary Development/Function

Diet-induced obesity impairs mammary development and lactogenesis in murine mammary gland.
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We have developed a mouse model of diet-induced obesity that shows numerous abnormalities relating to mammary gland function. Animals ate approximately 40% more calories when offered a high-fat diet and gained weight at three times
the rate of controls. They exhibited reduced conception rates, increased peripartum pup mortality, and impaired lactogenesis. The impairment of lactogenesis involved lipid accumulation in the secretory epithelial cells indicative of an absence of copious milk secretion. Expression of mRNAs for beta-casein, whey acid protein, and alpha-lactalbumin were all decreased immediately postpartum but recovered as lactation was established over 2-3 days. Expression of acetyl-CoA carboxylase (ACC)-alpha mRNA was also decreased at parturition as was the total enzyme activity, although there was a compensatory increase in the proportion in the active state. By day 10 of lactation, the proportion of ACC in the active state was also decreased in obese animals, indicative of suppression of de novo fatty acid synthesis resulting from the supply of preformed fatty acids in the diet. Although obese animals consumed more calories in the nonpregnant and early pregnant states, they showed a marked depression in fat intake around day 9 of pregnancy before food intake recovered in later pregnancy. Food intake increased dramatically in both lean and obese animals during lactation although total calories consumed were identical in both groups. Thus, despite access to high-energy diets, the obese animals mobilized even more adipose tissue during lactation than their lean counterparts. Obese animals also exhibited marked abnormalities in alveolar development of the mammary gland, which may partially explain the delay in differentiation evident during lactogenesis.

MAMMARY CANCER


Prepubertal estradiol and genistein exposures up-regulate BRCA1 mRNA and reduce mammary tumorigenesis.

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Prepubertal exposure to soy or its biologically active component genistein reduces later breast cancer risk in both animal models and human populations. We investigated whether that might be due to reported estrogenic properties of genistein. Our study indicated that daily prepubertal exposures between postnatal days 7 and 20 to 10 microg 17beta-estradiol (E2) reduced later risk of developing 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumors. Assessment of mammary gland morphology revealed that both prepubertal E2 and genistein (50 microg daily) exposures reduced the size of mammary epithelial area and number of terminal end buds (TEBs) and increased the density of lobulo-alveolar structures, suggesting that these exposures induced elimination of targets for malignant transformation by differentiation. Next, the mechanisms mediating the protective effects of E2 and genistein were investigated. E2 is shown to up-
regulate BRCA1, a tumor suppressor gene that participates in DNA damage repair processes and cell differentiation and that down-regulates the activity of estrogen receptor (ER)-alpha. The expression of BRCA1 mRNA was up-regulated in the mammary glands of rats exposed to E2 or genistein during prepuberty, when determined at the ages of 3, 8 and 16 weeks. Prepubertal E2 exposure reduced ER-alpha levels in the mammary gland, while prepubertal genistein exposure had an opposite effect. Our results suggest that prepubertal estrogenic exposures may reduce later breast cancer risk by inducing a persistent up-regulation of BRCA1 in the mammary gland.

HORMONES AND MAMMARY FUNCTION

Role of prolactin, growth hormone and insulin-like growth factor 1 in mammary gland involution in the dairy cow.

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Bovine mammary involution, an important process for subsequent lactations, is characterized by loss of epithelial cells by apoptosis, but its hormonal regulation is still not well defined. Prolactin (PRL) and growth hormone (GH) play a specific role on rat mammary gland apoptosis, through insulin-like growth factor 1 (IGF-1) and the IGF binding protein (IGFBP) system. The purpose of our investigation was to determine the possible role of PRL, GH, and IGF-1 on cell survival and on IGFBP-5 expression in the bovine mammary gland. Mammary gland explants were cultured in the presence of cortisol, 17beta-estradiol, progesterone, insulin, PRL, GH, and IGF-1 and with the same treatment but without PRL, GH or IGF-1, respectively. After 24 h of culture, we determined the level of apoptosis through evaluation of DNA laddering in the oligonucleosomal fraction and examined IGFBP-5 messenger RNA (mRNA) expression. The results show a high level of DNA laddering and an increase in IGFBP-5 mRNA content in mammary explants cultured in the absence of PRL, GH, or IGF-1 with respect to explants treated with all hormones. Moreover, explants cultured in presence of PRL, GH, or IGF-1 show a low level of DNA laddering and IGFBP-5 expression with respect to explants cultured without any hormones. These data demonstrate a relationship between levels of apoptosis and IGFBP-5 mRNA expression in the bovine mammary gland and confirm the involvement of this binding protein programmed cell death and its relationship with the main lactogenic hormones.
Leptin affects prolactin action on milk protein and fat synthesis in the bovine mammary gland.

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Leptin, a protein hormone produced and secreted predominantly by white adipose tissue, has a critical role in the regulation and coordination of energy metabolism. Identification of leptin in the milk of several mammals, including humans, led us to investigate its presence and regulatory effect in the cow mammary gland. The expression of leptin receptor in tissue culture of lactating mammary gland was augmented approximately 25 times by prolactin, but had no effect on virgin calf mammary tissue. Expression of leptin in tissue culture from mammary glands of lactating cows was enhanced 2.2-fold by prolactin. No effect of prolactin on leptin and leptin receptor expression was found in mammary gland tissue culture from calves. Leptin-enhanced fatty acid synthesis in the presence of prolactin, but had no effect without presence of prolactin. A similar pattern was found in the expression of alpha-casein and beta-lactoglobulin in mammary gland explants from a lactating cow. Our findings indicate that leptin plays an important role in mammary gland lactogenesis, and that the expression of leptin requires the presence of prolactin.

**MAMMARY UPTAKE AND METABOLISM**

Dietary protein concentration affects plasma arteriovenous difference of amino acids across the porcine mammary gland.

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The objective of this study was to determine whether the porcine mammary gland responds to increasing dietary CP concentration through changes in AA arteriovenous difference (a-v). Sixteen Landrace x Yorkshire lactating sows were provided ad libitum access to one of four isocaloric diets varying in CP concentration (7.8, 13.0, 18.2, and 23.5 %; as-fed basis). Litters were adjusted to 11 pigs within 48 h of birth. Sows were fitted with catheters in the carotid artery
and main mammary vein on d 4. On d 10, 14, 18, and 22 of lactation, arterial and venous blood samples were obtained every 30 min over 6 h. Milk yield was estimated on d 11 and 21 using the D2O dilution technique. Final litter sizes on d 21 were 10.3, 11, 9.5, and 11 piglets for sows fed the 7.8, 13.0, 18.2, and 23.5% CP diets, respectively. Piglet ADG tended ($P = 0.088$) to increase with increasing dietary CP concentration and were 186, 221, 220, and 202 g for sows fed the 7.8, 13.0, 18.2, and 23.5% CP diet, respectively. Daily total milk yield on d 21 (kg milk/d) tended ($P = 0.099$) to increase, and average milk yield per nursed piglet (kg of milk-pig(-1)d(-1)) increased ($P < 0.05$) with increasing CP concentration and were, on a per-piglet basis, 0.95, 1.19, 1.14 and 1.13 kg of milk/d for the 7.8, 13.0, 18.2, and 23.5% CP diets, respectively. As dietary CP increased from 7.8 to 23.5%, isoleucine and leucine a-v increased linearly only (linear, $P < 0.01$); all other AA a-v increased, reached a maximum in sows fed 18.2% CP, and decreased thereafter in sows fed 23.5% CP (quadratic, from $P = 0.10$ to $P < 0.05$). Amino acid uptake by the entire udder and by each gland increased (linear, $P < 0.05$) with increasing dietary CP. Arteriovenous differences response to increasing day of lactation varied among AA, from no change for histidine, isoleucine, lysine, methionine, tryptophan, and valine, to a linear trend increase for arginine ($P = 0.055$), leucine ($P = 0.064$), phenylalanine ($P = 0.101$), and threonine ($P = 0.057$). In summary, for the majority of AA, a-v increased with increasing dietary CP concentration from 7.8 to 18.2%, but decreased when CP concentration exceeded 18.2%. In contrast, mammary AA uptake, piglet ADG and milk yield per pig increased linearly with increasing dietary CP, suggesting a coordinated regulation between AA delivery and transport to meet the demand for milk yield.

**Glucose Metabolism**  

**Changes in gene expression of glucose transporters in lactating and nonlactating cows.**  
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Glucose delivery and uptake by the mammary gland are a rate-limiting step in milk synthesis. It is thought that insulin-independent glucose uptake decreases in tissues, except for the mammary gland, and insulin resistance in the whole body increases following the onset of lactation. To study glucose metabolism in peak-, late-, and nonlactating cows, the expression of erythrocyte-type glucose transporter (GLUT1) and the insulin-responsive glucose transporter (GLUT4) in the mammary gland, adipose tissue, and muscle were assessed by Western blotting and real-time PCR. Our results demonstrated that the mammary gland of lactating cows expressed a large amount of GLUT1, whereas the mammary gland
of nonlactating cows did not (P < 0.05). On the other hand, adipose tissue of late and nonlactating cows expressed a large amount of GLUT1, whereas the adipose tissue of peak-lactating cows did not (P < 0.05). There were no significant differences in the abundance of GLUT4 mRNA in adipose tissue and muscle, whereas GLUT4 mRNA was not detected in the mammary gland. The plasma insulin concentration was greater (P < 0.05) in nonlactating cows than in peak- and late-lactating cows. The results of the present study indicate that in lactation, GLUT1 expression in the mammary gland and adipose tissue is a major factor for insulin-independent glucose metabolism, and the expression of GLUT4 in muscle and adipose tissue is not an important factor in insulin resistance in lactation; however, the plasma insulin concentration may play a role in insulin-dependent glucose metabolism. Factors other than GLUT4 may be involved in insulin resistance.

MODELING AND LACTATION


A mathematical model for mammary fatty acid synthesis and triglyceride assembly: the role of stearoyl CoA desaturase (SCD).

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An increase in the proportion of unsaturated fatty acids in milk is considered desirable for human health. A prerequisite for the manipulation of milk fat composition is a co-ordinated understanding of the complex interactions in its biosynthesis. It has been suggested that an increase in the expression of mammary stearoyl-CoA-desaturase (SCD) would enrich mono-unsaturated fatty acids in milk, and therefore improve its nutritional properties. To investigate the potential effects of changes in expression of mammary enzymes and substrate availability on milk fat composition, we constructed, parameterized and evaluated a mechanistic mathematical model of fatty acid biosynthesis and milk-fat triglyceride assembly. The objective was to describe changes in the amount and composition of milk fat produced by bovine mammary cells due to changes in nutrition. Using the model we found that a 50% up-regulation in SCD activity increased the molar fraction of milk triglyceride 18:1 from 0.30 to 0.33 and 16:1 from 0.04 to 0.06. Up-regulation of SCD therefore did not appear to be the optimal method for increasing the content of unsaturated fatty acids in milk fat. The model was also used to determine the likely rate-limiting processes for the incorporation of unsaturated fatty acids into milk fat. Halving the concentration of glycerol 3-phosphate increased the molar fraction of milk triglyceride 18:1 from
0.30 to 0.35 and decreased the molar fraction of milk triglyceride 16:0 from 0.30 to 0.22. This achieved the desirable outcome of producing more unsaturated low-fat milk. Our model also predicted that a K232A mutation in the bovine mammary DGAT1 gene that is linked with an increase in milk fat yield would be consistent with a 120% increase in the DGAT acylation rate and also would be associated with a decrease in milk mono-unsaturated fatty acids.

**MASTITIS**


**Treatment of persistent intramammary infections with Streptococcus uberis in dairy cows.**

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A survey was conducted of the prevalence of environmental pathogens, especially Streptococcus uberis, as causes of clinical mastitis in dairy cows. The response of intramammary infections with *S. uberis* to conventional treatment was monitored by taking milk samples for bacteriology and somatic cell counting seven, 14 and 21 days after the treatment. The results showed that 51 per cent of the infections failed to respond, and the odds of cases failing to respond was significantly increased when the individual quarter somatic cell count seven days after the treatment was greater than 201,000 cells/ml. Ninety-six per cent of the suspected *S. uberis* isolates identified by culture were confirmed as *S. uberis* by using the *api 20 Strep* system. Restriction endonuclease fingerprinting was used to type the strains of *S. uberis* isolated from 75 milk samples from 32 cows. Analysis showed that 96 per cent of the cases of *S. uberis* that failed to respond to conventional treatment were persistent infections with one strain rather than reinfections with different strains. The persistent cases of *S. uberis* were treated further with an extended course of intramammary preparations containing either procaine penicillin with dihydrostreptomycin or cefquinome. There was no significant difference between the cure rates achieved by the two preparations, and 55 per cent of the cases that had failed to respond to conventional treatment responded to the additional treatment.
Bovine mammary gene expression profiling using a cDNA microarray enhanced for mammary-specific transcripts.


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A cDNA microarray resource enhanced for transcripts specific to the bovine mammary gland (BMAM) has been developed and used in pilot studies to examine gene expression profiles in the mammary gland. One goal driving development of this resource was to shed some light on the pathways and mechanisms specifically related to bovine mammary gland growth and development. To accomplish this, gene expression patterns from bovine adipose, liver, adrenal, lymph, spleen, thymus, gut, and developing mammary tissue were compared using the BMAM microarray. We have thus identified a putative set of 16 genes being preferentially expressed in developing mammary gland. Another of our long-term goals is to elucidate the genes and pathways associated with bovine lactation and involution and to use these as a model for human mammary gland development as it relates to human breast cancer risks. To begin this process, we conducted a pilot study, comparing gene expression profiles of lactating bovine mammary tissue against nonlactating tissue on the BMAM microarray. Our results have yielded many novel and interesting genes exhibiting differential expression in lactating mammary tissue, including oncogenes (VAV3, C-myc), mediators of apoptosis (Caspase 8), and cell cycle regulators (LASP1).

LACTATION IN OTHER SPECIES

Characterisation of proteins in the milk of fur seals.

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Milk protein composition was investigated throughout the lactation periods of the Australian fur seal (Arctocephalus pusillus doriferus) and Antarctic fur seal.
(Arctocephalus gazella). The mean protein content of the milk was found to be 10.9% and 10.6% respectively. The concentration of total protein did not change during lactation, although a decline in casein content of the milk in late lactation was apparent. Milk protein concentration during a foraging/suckling cycle of the Antarctic fur seal analysed at the time of arrival on shore, and 24 h and 72 h after arrival was 12.8%, 11.4% and 12.5% respectively. Re-feeding animals at 72 h resulted in a significant increase in milk protein content to 14.9%. Characterisation of milk protein by SDS-PAGE analysis revealed 5 casein and 10 major whey protein bands. Amino-terminal sequencing indicated that the majority of the whey fraction of the milk is beta-lactoglobulin (beta-LG). The limited amino acid sequence indicated 3 different beta-LGs were secreted in the milk. Subsequently, RT-PCR was used to extend the sequence of one of the beta-LGs and translation of the 464 bp fragment indicated that it shared 79% sequence identity with feline beta-LG II.

BIOTECHNOLOGY


Genetically enhanced cows resist intramammary Staphylococcus aureus infection.
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Mastitis, the most consequential disease in dairy cattle, costs the US dairy industry billions of dollars annually. To test the feasibility of protecting animals through genetic engineering, transgenic cows secreting lysostaphin at concentrations ranging from 0.9 to 14 micrograms/ml [corrected] in their milk were produced. In vitro assays demonstrated the milk's ability to kill Staphylococcus aureus. Intramammary infusions of S. aureus were administered to three transgenic and ten nontransgenic cows. Increases in milk somatic cells, elevated body temperatures and induced acute phase proteins, each indicative of infection, were observed in all of the nontransgenic cows but in none of the transgenic animals. Protection against S. aureus mastitis appears to be achievable with as little as 3 micrograms/ml [corrected] of lysostaphin in milk. Our results indicate that genetic engineering can provide a viable tool for enhancing resistance to disease and improve the well-being of livestock.