# Galactopoiesis, Effect of Treatment[1] with Bovine Somatotropin

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## Introduction

In the 1930s, injection of crude extracts from bovine anterior pituitary was shown to increase the milk yield of dairy goats and cows. Approximately 10 years later, the component of these extracts that was responsible for increased milk production was identified as growth hormone or somatotropin. Initial advances toward understanding the myriad of physiological effects of somatotropin were achieved using pituitary-derived somatotropin, culminating in the idea that somatotropin orchestrates coordinated metabolic responses of tissues throughout the body to regulate nutrient partitioning and enhance milk production. Two groups led by Dale Bauman and Ian Hart proposed this concept of 'homeorhetic' control by somatotropin. With the production of recombinantly derived bovine somatotropin (bST), it became feasible to utilize the hormone for increasing lactational performance of dairy cows. Subsequent investigations and commercial use expanded our knowledge of the physiological effects of bST and demonstrated its efficacy and safety as a stimulant of milk production. The efficacy of bST as a stimulant of milk production has also been demonstrated for goats, sheep, and buffaloes. Indeed, somatotropin appears to be the primary galactopoietic hormone (i.e., hormone that increases milk production) in mammals, except for rodents, in which prolactin appears to be the primary galactopoietic hormone. bST signaling pathways include direct signaling by bST and indirect signaling by insulin-like growth factors (IGFs). Homeorhetic control is exerted, in large part, by altering the response to homeostatic signals. While bST exerts homeorhetic regulation, homeostatic regulatory processes that ensure animal well-being, and other homeorhetic mechanisms such as those that support body growth and fetal development during pregnancy, are still operative. Thus, bST exerts an overarching control, but not an overriding control, on processes that support milk production.

[1] Mention of a trade name or proprietary product does not constitute a guarantee or warranty by the United States Department of Agriculture and does not imply approval to the exclusion of others not mentioned.

## Effects of bST on Milk Production

Administration of bST to lactating dairy cows increases the yield and efficiency of milk production. In response to injection of bST, milk secretion increases within a day and is maximized within a week. The increased milk yield is maintained as long as treatment is continued but quickly returns to control levels when bST is discontinued. The milk yield response is dose dependent and the response curve is hyperbolic in shape. At approximately 40 mg of bST per day, nearly maximal response is obtained. Milk yield achieved with near-maximal doses of bST is impressive, with increases reported as high as 30–40%. Typically, bST increases milk production by 4–6 kg $day^{-1}$, approximately a 10–15% increase in yield. The magnitude of response to a particular dose of bST depends upon biological variation, stage of lactation, and management parameters.

During midlactation, the pattern of bST administration does not affect the magnitude of the galactopoietic response. Similar increases in milk yield are obtained with the same daily dose of bST whether administered as once-daily injections, 4-h pulses, or constant infusion. The bST formulation currently approved for use in the United States is a prolonged-release *n*-methionyl-bST (Posilac, Monsanto Co.) that was approved by the US Food and Drug Administration in November 1993. It is administered at a dose of 500 mg per lactating cow every 2 weeks. Treatment should be initiated after peak lactation at >60 days postcalving, when cows are at or near positive energy balance. During early lactation, response to bST is minimal.

In addition to increasing milk yield, bST increases the efficiency of lactation. Cows treated with bST increase feed intake over the first few weeks to match increased nutrient demands for milk synthesis and thus cows remain in neutral or positive energy balance during the majority of lactation. However, because milk secretion increases more rapidly than voluntary intake, bST-treated cows initially experience a temporary period of negative energy balance. Respiration calorimetry studies demonstrated that the energy requirements for body maintenance and the partial efficiency of milk synthesis from absorbed nutrients were not changed in bST-treated cows. Milk production efficiency is therefore increased by bST treatment because the increased milk production is achieved without nutritional overhead. Assuming an 11% increase in milk production, 9 bST-treated cows can yield

the same amount of milk as 10 control cows, and the energy savings would be the maintenance requirements for 1 cow. However, it is important to note that bST is not unique in this regard. There are other methods of increasing milk production, such as increasing milking frequency, that increase the efficiency of lactation because production is increased without increasing energy requirements for maintenance. In contrast, thyroid hormone supplementation increases milk production, but it also increases body metabolism and maintenance requirements and there is no gain in efficiency. A recent analysis demonstrated that bST provides a means to increase dairy production efficiency while reducing environmental impact. Conventional herds supplemented with bST had lower acidification, eutrophication, and global warming potential than conventional herds without bST or herds adhering to organic production guidelines.

Administration of bST typically does not alter the gross composition of milk from cows in positive energy balance. Syntheses of milk proteins, fat, and lactose are all increased proportion in to milk volume and normal milk composition is maintained. Additionally, there is little change in the composition of milk protein or milk lipid when cows are treated with bST. Casein proteins are expressed in the same proportions in milks produced from control and bST-treated cows; whey proteins that have been evaluated appear similarly unaffected by treatment, and the ratio of whey protein to casein protein is unaltered. Lipid classes and fatty acid composition of milk fat are not altered or altered very slightly by bST treatment when cows are in positive or neutral energy balance. For example, there may be a small increase in the relative amount of long-chain fatty acids in the milk of bST-treated cows. For reasons discussed subsequently, the fat content of milk increases, and fatty acid composition may change, if cows are in negative energy balance when bST is administered. The mineral content of milk appears largely unaffected by bST treatment and, although vitamin content has been less thoroughly examined, vitamin concentration also seems to be unaltered.

When bST is provided as a sustained-release formulation, small cyclical effects on milk yield and composition have been noted. With the biweekly injection protocol, milk production peaks 7–9 days after injection and then declines until the next injection, seemingly as a function of bST concentrations in the blood. The concentration of milk lactose follows the same cyclical pattern as milk yield, although the reasons for this effect are unclear. Milk fat and protein cycle in a manner that is out of phase with milk yield, that is, the concentration of milk protein and fat is at a nadir when milk yield peaks. With the biweekly injection protocol, a steady state of the metabolic alterations coordinated by bST is seemingly never fully achieved. Thus, synthetic processes for the synthesis of milk components may not be fully coordinated, resulting in minor and temporary alterations in milk composition. Other changes, such as changes in nutrient balance or changes in mammary blood flow, may also occur in response to biweekly injections of bST. These may produce small changes in the availability of nutrients to the mammary glands and may partially explain the small fluctuations in milk volume and composition. However, it should be noted that these cyclical fluctuations in composition are not apparent in the bulk tank milk because cows within a herd typically calve asynchronously and are injected with bST asynchronously. Thus, this effect is of no importance to milk processors or consumers. Indeed, these variations in milk composition during bST treatment are minor compared with normal variation in milk composition that occurs between herds and within a herd. Milk composition is more strongly influenced by season, stage of lactation, genetics, nutritional management, and energy balance.

## Mode of Action of bST

The galactopoietic action of exogenous bST may be the result of a combination of direct and indirect effects: (1) direct stimulation of mammary tissue; (2) indirect stimulation of mammary tissue; (3) direct effects on other tissues to supply nutrients to support increased milk production; and (4) indirect effects on other tissues to supply nutrients to support milk production. There is a preponderance of evidence that suggests that bST enhances milk production largely by partitioning nutrients to support milk production, by both direct and indirect actions; however, bST does not alter the digestibility of nutrients. The effects on the mammary gland appear to be indirect, and whether the direct effects of bST are operative remains to be determined.

It is questionable whether bST has direct effects on the lactating mammary gland. Although bST is galactopoietic *in vivo*, the addition of bST to mammary culture systems has failed to increase the synthesis of milk components, and receptor-binding assays have failed to detect somatotropin receptor in mammary tissue. These early results argued against a direct effect of bST on the mammary gland, and it was presumed that if endocrine stimulation of the mammary gland occurred, it was via bST-induced increases in circulating IGFs. Because bovine mammary epithelial cells have receptors for IGF-I and IGF-II, they appear to be target cells for IGF signaling. Indeed, infusion of IGF-I or IGF-II into the local arterial supply to one of the mammary glands of a goat caused an increase in milk production and blood flow to the infused gland within 2–4 h. Although this increase in milk yield is consistent with a direct galactopoietic effect of IGFs on

mammary tissue, it may also have been an indirect outcome of increased blood flow and nutrient supply to the mammary gland. It is interesting to note that *in vitro* treatment with bST increased milk fat synthesis by mammary explants when cocultured with adipose and liver explants, but not in the absence of liver and adipose tissue. This is consistent with a nutrient- and hepatic IGF-mediated effect on milk component synthesis.

More recently, somatotropin receptor mRNA has been detected in lactating bovine mammary tissue by Northern blotting and by *in situ* hybridization. The mRNA for somatotropin receptor was localized in both epithelial and stromal (nonsecretory) elements of mammary tissue, but IGF-I mRNA was restricted to stroma. Assuming that the bST receptor transcripts are translated into protein (as they are in rabbit and rat mammary epithelium, where somatotropin receptor protein has been detected by immunohistochemistry), the results open the possibility that bST may have effects on the mammary gland that are mediated by local production of IGFs.

Several levels of complexity are involved in modulating IGF-regulated functions: the local concentration of IGF, expression of IGF receptors and their downstream signaling pathways, the types and quantities of IGF-binding proteins (IGFBPs), and abundance of, the acid-labile subunit (ALS) protein. The majority of IGF-I circulates in a ternary complex with one of the IGFBPs and ALS. Consequently, ALS has the capacity to regulate the availability of systemic IGF-I to tissues. To date, six high-affinity IGFBPs (IGFBP-1–6) and nine low-affinity IGFBPs, known as IGFBP-related proteins (IGFBP-rp1–9), have been identified. A number of IGFBPs are synthesized by mammary epithelial cells. Depending upon the specific IGFBP, the binding proteins may reduce IGF activity by competing with IGF receptors for ligand, increase IGF activity by serving as delivery vehicles to the target cell, or serve as a reservoir for IGFs, causing their slow release and reducing their turnover. Furthermore, the IGFBPs may have activities that are independent of their interaction with IGFs and they are subject to enzymatic modifications that may alter their various activities. The picture that emerges is one of a highly complex IGF system with multiple levels of regulation, making the specific actions of IGFs during lactation difficult to resolve.

Somatotropin directly or indirectly coordinates metabolic adaptations that promote increased milk production in the lactating dairy cow. These adaptations involve chronic alterations in carbohydrate, lipid, and protein metabolism in a number of tissues and serve to preferentially direct nutrients toward the mammary gland. This coordinated regulation to support the priorities of a physiological state has been termed homeorhetic regulation. The importance of integrated nutrient partitioning is illustrated by udder perfusion studies. Milk secretion by isolated goat udders is dependent on the presence of glucose in the perfusate. Removal of acetate or amino acids allows continued milk synthesis; however, the secretion of fat and protein is reduced. Normal milk composition is dependent upon balanced nutrient supply to the mammary gland.

Lipid metabolism is strongly influenced by bST administration. Although bST has no acute effects on lipogenesis or lipolysis, it has chronic effects on these processes in adipose tissue. When cows are in negative energy balance during bST administration, lipolysis is increased. This is manifested as increases in the level of nonesterified fatty acids (NEFAs) in blood, increased milk fat percentage, and an increase in the percentage of long-chain fatty acids in the milk fat. (In the mammary gland, the long-chain fatty acids incorporated into milk triacylglycerols originate from mobilized fat stores and from dietary sources, whereas short- and medium-chain fatty acids are synthesized within mammary tissue.) When cows are in positive energy balance during bST administration, lipogenesis is inhibited. These effects are achieved by altered responsiveness to key homeostatic signals and changes in the quantity of key enzymes (**Figure 1**).

Modulation of insulin responsiveness provides an important means to regulate lipid metabolism, and bST antagonizes some of the actions of insulin. Insulin is a key homeostatic regulator of nutrient metabolism. It promotes the facilitated transport of glucose into most cells of the body (the central nervous system and mammary glands are not insulin dependent) and inhibits many of the liver enzymes that catalyze gluconeogenesis. It also promotes the synthesis of glycogen and inhibits glycogenolysis. It stimulates the deposition of fat by enhancing the activity of key enzymes of fatty acid synthesis and by inhibiting lipolysis of triacylglycerol. It stimulates protein deposition by enhancing facilitated uptake of amino acids and increasing the activity of some ribosomal enzymes involved in protein synthesis. Only a few of insulin's actions are antagonized by bST treatment. Most importantly, bST inhibits the lipogenic activity of insulin, and the effect appears to be exerted on processes that are downstream in the signaling cascade from the insulin receptor, consistent with the targeted inhibition of a limited number of insulin actions. Lipoprotein lipase (LpL) is an enzyme that is partly regulated by insulin. bST treatment of lactating dairy cows causes a decrease in LpL in adipose tissue but no change in mammary tissue. LpL is an enzyme that hydrolyzes triacylglycerols of very-low-density lipoproteins and chylomicrons in the serum, permitting the uptake of NEFAs by surrounding cells. Reduced LpL in adipose tissue and normal LpL in mammary tissue, along with the inhibition of lipogenesis in adipose tissue, ensure the preferential delivery of NEFAs to the mammary gland for synthesis of milk fat. bST also

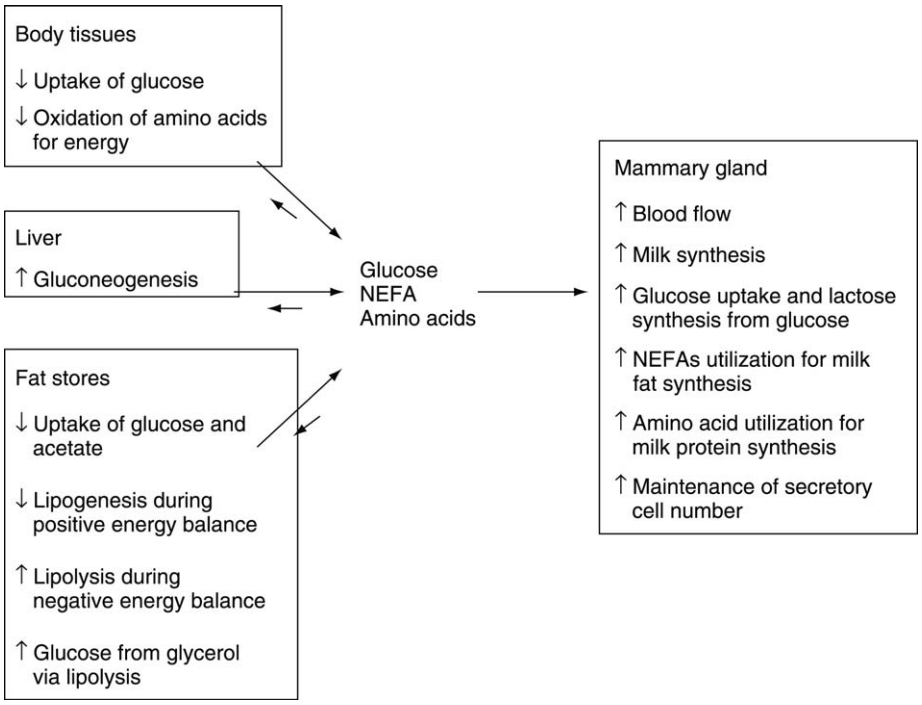


**Figure 1** Effect of bovine somatotropin administration on nutrient partitioning to support increased milk production. Metabolism is altered in an organ-specific fashion to establish nutrient flux toward the mammary gland. NEFAs, nonesterified fatty acids.

decreases expression of key enzymes involved in fatty acid synthesis, such as fatty acid synthase and acetyl-CoA carboxylase.

When nutrients are in limited supply, bST enhances lipolysis again by altering the response to homeostatic regulators. Dairy cows treated with bST mobilize considerably more NEFAs following epinephrine challenge than do control cows. However, there is little change in adrenergic receptor numbers, and no change in the stimulatory G proteins and other components of the cyclic-AMP lipolytic signaling pathway. Rather, it has been discovered that bST enhances lipolysis by antagonizing antilipolytic regulators. Treatment with bST decreases the activity of the inhibitory G proteins. Thus, bST promotes lipolysis by chronic inhibition of antilipolytic regulation. The ability to enhance lipolysis often comes into play when bST treatment is initiated. Because cows are typically near neutral energy balance when treatment is initiated and feed intake does not increase immediately, bST induces a period of negative energy balance that requires the mobilization of energy stores. When cows enter positive energy balance, for the majority of lactation, inhibition of lipogenesis is the hallmark of bST action on lipid metabolism.

Carbohydrate metabolism is altered by bST treatment to meet the increased glucose requirement for greater milk secretion. More glucose is made available for milk synthesis by increasing hepatic glucose production and decreasing oxidation by body tissues. In ruminants, the products of rumen fermentation are volatile fatty acids, and only a small percentage (15%) of blood glucose is derived from the diet. Body glucose supply is met by hepatic gluconeogenesis, which can amount to production of $3\,kg\,day^{-1}$ in a lactating cow. Administration of bST enhances hepatic gluconeogenesis at least in part by antagonizing the ability of insulin to inhibit gluconeogenesis. Glucose serves as the substrate for lactose synthesis in the mammary gland, and in high-producing lactating dairy cows, nearly 85% of total glucose turnover is used for milk synthesis. Treatment with bST increases net utilization of glucose in mammary tissue and decreases glucose utilization by nonmammary tissues. These metabolic adaptations are sufficient to provide the necessary glucose for milk synthesis; no glucose deficit is encountered and ketosis is not induced. Somatotropin treatment also decreases expression of glucose transporters in skeletal muscle and in fat stores, but has no effect on transporters in the mammary gland, thus increasing the proportional flux of glucose into the mammary gland. Other data suggest that the effects on glucose uptake are primarily secondary to nutrient gradients created by metabolic effects on the tissues.

Protein metabolism of bST-treated lactating dairy cows is altered to support the added amino acid requirements for increased milk protein synthesis. This appears to be largely the result of more efficient utilization of amino acids. Whole-body oxidation of amino acids is reduced in bST-treated dairy cows and there is a

resulting decrease in the concentration of urea and a decrease in urinary nitrogen loss. Increased milk protein synthesis appears to be supported primarily by increased availability of precursors to the mammary gland due to decreased oxidation of amino acids by other tissues.

bST exerts an overarching control, but not an overriding control, on metabolic processes that support milk production. While bST exerts homeorhetic regulation, homeostatic regulatory processes that ensure animal well-being, and other homeorhetic mechanisms such as those that support body growth or fetal development during pregnancy, are still operative. Increases in milk yield are greater in multiparous than in primiparous cows because the milk response to bST is reduced by an amount that is dictated by the nutrient requirements of continued body growth. Similarly, when bST-treated cows are simultaneously lactating and pregnant, milk production declines normally during the later months of pregnancy and thus minimizes conflict with nutrient demands for fetal growth. Use of bST does not ensure increased milk production to the detriment of a young lactating animal's continued body growth or of a lactating animal's ability to support pregnancy. Normal physiological processes that serve to ensure the well-being of a lactating animal and survival of her fetus are still operative during prolonged use of bST during lactation. Indeed, use of bST over multiple lactations has proven to be safe and effective.

Although much has been learned about the nature of the metabolic alterations induced by bST and the tissue-specific effects of bST, the means by which the hormone signal elicits the biological response is poorly understood. The effects of bST on adipose tissue can be demonstrated *in vitro*, suggesting that these effects are direct and are mediated by the somatotropin receptor. The effects on the liver are presumed to be direct effects, because these too can be mimicked *in vitro*. However, within these tissues, locally acting paracrine or autocrine effects cannot be ruled out. In contrast, the effects of bST on muscle and mammary tissue appear to be primarily mediated by the IGF system. Although the metabolic effects of bST on nonmammary tissues effectively spare nutrients to support milk synthesis, there also appear to be effects at the level of the mammary gland. Infusion of IGFs into the local arterial supply of the mammary gland stimulates milk production and argues for IGF-mediated effects on mammary gland synthetic ability. Associations between energy balance, bST, the IGF system, and milk production suggest the importance of IGF-mediated effects on the mammary gland. Moderate undernutrition causes a muted IGF response to administration of bST and a reduced galactopoietic effect. Thus, during early lactation, when cows are in negative energy balance, IGF response to bST administration is reduced and bST is a less effective stimulator of milk production. During severe undernutrition, there is dissociation between bST and the IGF system, and both IGF response and the milk production response to bST are abolished.

Other than bST, thyroxine is the only other hormone known to increase milk production in dairy cows, and there are numerous interactions between the somatotropic and thyroid hormone axes. Indeed, tissue-specific changes in thyroid hormone metabolism alter the local action of systemic thyroid hormones, which appear to be important for supporting milk production and for modulating the galactopoietic response to prolactin and somatotropin in mice and rats. The situation in cows is less clear and has received scant attention.

In addition to metabolic effects, bST appears to alter population kinetics within the mammary gland. Production data indicate that bST increases the persistency of lactation, and this may be achieved by decreasing the loss of secretory cells during lactation and by increasing cell proliferation. Data for goats indicate that bST administration results in maintenance of cell number as lactation progresses, due to decreased cell loss. Recent data suggest that cell proliferation is increased in the mammary tissue of bST-treated dairy cows and heifers during midlactation. These data are consistent with the *in vitro* mitogenic activity of IGF-I and IGF-II in bovine primary cell culture, mammary tissue slices, and an established line of bovine mammary epithelial cells (MAC-T cells), and suggest that the IGFs mediate this effect. However, decreased cell death and increased cell proliferation assist in the partial maintenance of the population of secretory cells, as previous studies have demonstrated that bST does not actually increase mammary cell number. These effects supplement the metabolic alterations induced by bST and lessen the decline in milk production with advancing lactation.

## Galactopoiesis in Other Species

Although dairy cows have been the subject of most investigations, bST has been shown to be an effective galactopoietic hormone in other dairy animals, including sheep, goats, and the Italian water buffaloes. As with dairy cows, substantial increases in milk production are obtained (14–30%) and the composition of milk remains unaffected. The processing attributes of milk are unimpaired. In fact, coagulation time is improved in milk from ewes during late lactation. Because sheep, goats, and buffaloes are seasonal breeders, the ability to increase persistency of lactation is particularly attractive to maintain milk production throughout the year.

## Effects on Udder Health

The effect of bST on mastitis has been studied extensively in the 1990s using more than 11 000 cows in 19 investigations. The conclusion of these experiments is that bST treatment does not significantly alter the incidence of mastitis and has negligible effects on milk somatic cell count. In a review of Dairy Herd Improvement records from dairy herds in the northeastern United States (8 years, >80 000 cows and >2 million test days), it was reported that there was no change in the productive life of cows from herds that used bST. Although bST caused a small but significant increase in the milk somatic cell count, the increase is of little biological significance. It is consistent with the small increase in somatic cell count that accompanies increased milk yield, and the increase is considerably lower than the effects of season, parity, breed, and age in control cows. bST helps to maintain milk production in mastitic quarters, and there is evidence that it enhances neutrophil function and quickens the recovery from (coliform) mastitis.

## Effects on Reproduction

Dairy cows undergo a period of reduced fertility during early lactation when they are in a state of negative energy balance. Administration of bST is approved for use after 63 days of lactation to minimize its use during this period of negative energy balance. Nonetheless, both heifers and multiparous cows treated with bST often experience a reduction in pregnancy rate and an increase in days open, which is partially due to increased frequency of undetected estrus. Despite these apparent effects, the reproductive performance of bST-treated cows typically does not differ from that of cows with equivalent milk production. However, administration of bST increases the incidence of twinning and follicular development. The influence of the bST/IGF axis on ovarian function is an area of active research that should provide information to improve fertility.

*See also*: **Lactation:** Galactopoiesis, Effects of Hormones and Growth Factors.

## Further Reading

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