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EFFECTS OF EXOGENOUS BOVINE SOMATOTROPIN ON LACTATION

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CONTENTS

INTRODUCTION

Over 60 years ago scientists first demonstrated the presence of a substance from the anterior pituitary that increased growth of rats (54, 55). Using pair-fed rats treated with extract from bovine pituitaries, Lee & Schaffer (86) further documented that effects of treatment included a shift in composition of the gain so that muscle was increased and fat was reduced. This extract factor was named "somatotropin" from the Greek derivation meaning "tissue

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growth." Somatotropin (ST) is also referred to as "growth hormone" in scientific and lay articles. About the same time, other scientists demonstrated that administration of an extract from the anterior pituitary also affected lactation in laboratory animals (133) and increased milk yield of lactating goats (6). In 1937, Russian scientists treated over 500 lactating dairy cows with subcutaneous injections of a crude extract from ox anterior pituitaries and observed a substantial increase in milk yield (7).

Major advances in our understanding of somatotropin occurred during World War II when food shortages caused British scientists to examine the possibility of using ST to increase milk supply. They established that ST was the galactopoietic factor in crude pituitary extracts and evaluated several dimensions of the milk response in dairy cows. However, results indicated that the amount of bovine somatotropin (bST) that would be available from pituitary tissue of slaughtered cattle would not be sufficient to have a substantial impact on their nation's milk supply [see review by Young (156)].

Over the next 40 years there was continued interest in bST. Particularly noteworthy were studies by Brumby & Hancock (29) and Machlin (92) that noted over 40% increases in milk yield of dairy cows over a 10- to 12-week treatment period. More recently, breakthroughs in biotechnology have made possible the production of proteins by recombinant DNA technology. The first study treating lactating cows with recombinantly derived bST was reported in 1982 (12), and the first long-term study in 1985 (16). Because treatment with bST results in unprecedented gains in productive efficiency, several companies have produced recombinant bST and this has led to an exponential increase in investigations to explore the potential for commercial use and to examine the role of ST in the biology of lactation. In some areas the research has been extensive and has led to a clear consensus. This is particularly true for many aspects of the production responses, and in these instances we will predominately cite reviews. However, in other aspects investigations have been less extensive. This is particularly true for the mechanisms of action where understanding is sometimes confused by apparently conflicting results and ideas. Finally, in the last section, we develop integrative concepts.

BACKGROUND

Somatotropin is a protein hormone synthesized by the anterior pituitary gland. Secretion from the pituitary gland is regulated by two peptides: growth hormone-releasing factor, which stimulates release, and somatostatin, which inhibits release. The amino acid sequence for somatotropin is known for many species (146). Bovine ST produced by the pituitary can have either a 191 or 190 amino acid sequence with either a leucine or valine at position 127 (153;

numbering based on the 191 amino acid variant). These represent the four major variants of bST that are produced naturally. Differences in the cleavage of the signal peptide cause the N-terminus to be an alanine (191 amino acid sequence) or a phenylalanine (190 amino acid sequence). Variation between valine or leucine at position 127 is due to differences in gene alleles, and the frequency of these alleles varies for the major dairy breeds (89). Recombinantly derived forms of bST that have been used experimentally can differ slightly from the bST produced by the pituitary gland. Depending on the manufacturing process, from 0 to 8 extra amino acids are attached to the N-terminus of the bST molecule (72). However, when the same purification techniques are used, recombinantly derived and pituitary-derived bST have similar potencies in various biological test systems (82, 153).

The discovery in the 1950s that some types of human dwarfism were due to inadequate pituitary production of ST stimulated interest in utilizing bST to treat this malady. However, clinical studies uniformly demonstrated that bST, as well as ST from other nonprimates, was not biologically active in humans. This led to the concept that ST was "species specific." Subsequent work demonstrated that the homology between hST and bST was only about 65% and that bST was not able to effectively bind to the ST receptor from human tissues (see reviews 72, 81). In contrast, the amino acid sequence for bovine and ovine ST only differs at a single position, and bST is biologically active in sheep (71).

The ST receptor has been isolated and characterized from several species and is a single peptide of about 620 amino acids consisting of an extracellular domain (about 250 amino acids, which is very similar to the ST-binding protein of plasma), a short trans-membrane domain (about 25 amino acids), and an intracellular domain (about 350 amino acids) (149). The receptor appears to belong to a novel family of receptors that includes the prolactin receptor (which has many similarities to the ST receptor) and a number of interleukin (cytokine) receptors (73, 94). A single ST molecule can bind to two receptor molecules, each binding to a different region of ST (46). The ST receptor may be internalized following binding of the ligand, but the role of internalization in signal transduction, if any, is unknown (126).

PRODUCTION RESPONSES

Milk and Milk Components

Milk-yield responses to bST have been reported in all dairy breeds. Milk yield gradually increases over the first few days of bST treatment and reaches a maximum during the first week. If treatment is terminated, milk yield gradually returns to pretreatment levels over a similar time period. However,

when treatment is continued, the increased milk yield is maintained. Thus, bST results in a greater peak milk yield and an increased persistency in yield over the lactation cycle (see reviews 71, 111, 114).

Milk-yield increases after bST treatment are observed in cows of all parities, but the magnitude of the increase in milk yield varies according to stage of lactation (31, 95, 111, 114). In general, response has been small or negligible when bST is administered in early lactation prior to peak yield. Therefore, possible commercial use would probably be over the last two thirds or three fourths of the lactation cycle.

The gross composition of milk (fat, protein, and lactose) is not altered by treatment with bST (9, 21, 30, 84, 91, 95, 114, 135). A variety of factors affect the fat and protein content of milk, including breed, stage of lactation, diet composition, nutritional status, environment, and season; these factors have the same effects on the milk composition of bST-treated cows. For example, certain breeds have a higher milk fat content, and an increase in milk fat typically occurs in late lactation for all breeds; treatment with bST does not alter these relationships. Likewise, the increase in milk fat content that occurs when the cow is in negative energy balance and the decrease in milk protein content that occurs when the cow has an inadequate protein intake are also observed in bST-treated cows. Overall, results demonstrate that the same factors that typically affect milk composition also affect the milk composition of bST-treated cows, and the variation in milk content of fat and protein is not altered (9, 11, 30, 91).

Milk from bST-treated cows does not differ in vitamin content or in concentrations of nutritionally important mineral elements (11, 21, 135). In addition, proportions of total milk protein represented by whey proteins and the different casein fractions are not substantially altered, and factors that affect the fatty acid composition of milk fat have the same effects in bST-treated cows. In addition to a lack of effects on milk composition, bST has no impact on the manufacturing characteristics of milk (9, 21, 84, 91, 135).

Lactational response to exogenous bST is a function of the daily dose represented by a hyperbolic dose-response curve with a pattern of diminishing marginal returns to increasing bST dose (17, 101, 111). The daily dose needed to optimize milk yield response results in blood concentrations of ST that are within the range typically observed during episodic release of endogenous hormone, but average daily concentrations are several-fold higher than before treatment. As in the case of other species, endogenous release of ST in dairy cows normally occurs as irregular, episodic bursts with a half-life of about 15–30 min. Studies have demonstrated that a similar milk response occurs regardless of whether the daily dose of bST is administered as a single bolus, a constant infusion, or as equal episodic pulses at 4-hr intervals (see review

17). Typically, bST has been administered by daily injection. However, several prolonged-release formulations have been recently developed in which a small volume is injected at intervals ranging from 2 to 4 weeks (31, 66, 101, 111).

Bioenergetics, Nutrition, and Animal Well-Being

Milk production responses to bST are not dependent on special diets or unique feed ingredients. Substantial increases in milk yield have been observed on diets ranging from pasture only to typical concentrate:forage mixtures (11, 30, 31, 41). Treatment does not alter digestibilities of organic components of the diet. Thus, the biological effects of bST are predominantly associated with the use of absorbed nutrients. Bioenergetic studies have demonstrated that bST treatment does not alter the energy expenditure for maintenance or the partial efficiency of milk synthesis, so that nutrient requirements for maintenance and per unit of milk are not altered (77, 127, 134). Overall, daily nutrient requirements are increased by an amount equal to the increase in milk yield, and productive efficiency (milk per unit of feed) is improved because a greater proportion of the nutrient intake is used for milk synthesis.

Most studies have involved bST treatment for a few weeks or for a single lactation. However, several dozen multilactational studies have been conducted and treatment has been continued for as long as eight successive lactations (see review 111). Responses in multilactational studies are of interest because cows typically utilize body fat reserves during the early phase of the lactation cycle and then replenish these reserves during latter phases of the lactation cycle. Over the course of the first few weeks of bST treatment, cows adjust their voluntary intake in a predictable manner related to the extra nutrients required for the increased production of milk (11, 30, 31, 41). In general, similar lactational responses have been observed when bST has been administered for two or more consecutive lactations, provided that management practices allow for an adequate replenishment of body energy reserves over the latter portion of the lactation cycle. However, in cases where cows were not adequately fed to allow for an adjustment of voluntary intake and replenishment of body energy reserves, milk yield response to bST was reduced or even absent in the next lactation cycle (11, 41).

The impact of bST treatment on animal health and well-being has also been of interest. Some investigators anticipated that administration of bST to modern high-producing dairy cows might result in metabolic disorders such as ketosis, fatty liver, and chronic wasting. These "postulated" catastrophic effects were based on the nutrient needs associated with a rapid, substantial increase in milk yield and on ideas, which originated in the 1940s, that ST had acute lipolytic and hyperglycemic (diabetogenic) effects. Such effects would likely occur during the first few days of bST treatment (milk yield

would have increased but voluntary intake would not yet have increased). Suffice it to say, these catastrophic effects have never been observed with bST treatment, even in animals with exceptionally large increases in milk yield (>10 kg/day) or animals that received exceptionally large doses of bST (dose equivalent to 4 yr of treatment given over a 2-week period), and the perceived mechanisms that were postulated to lead to such catastrophic effects are now known to be erroneous (11, 21, 30, 41, 85, 110, 118, 144).

Quality of management is the major factor affecting the magnitude of milk response to bST (11, 41, 113), and this comprises the nutritional program, milking procedures, herd health program, and environmental conditions. Several long-term studies have involved inadequate management conditions, and milk yield response to bST treatment was essentially zero. Adverse effects were not observed in any of these studies; cows simply had negligible milk yield response to bST (see reviews 11, 41, 111). Several reports have also summarized studies that encompass a range of environmental and management conditions in an effort to evaluate subtle health effects (1, 21, 38, 40, 56, 96, 109, 110, 118, 151). Variables have included physical examinations, blood chemistry, metabolic disorders, incidence of disease, mastitis and mammary health, and reproduction-related parameters of the treated cows, as well as the health and growth of their offspring. Results demonstrate that values for bST-treated cows are similar to controls and consistent with literature values for cows of comparable milk production. As a result of differences in management practices, substantial herd effects were apparent for many of these variables (e.g. mastitis or reproduction-related variables), but herd effects were the same for control and bST treatment groups. Analyses for subtle effects will be even more extensive as data accumulates and is used by regulatory agencies in their evaluation.

MECHANISMS OF ACTION

Somatotropin is a homeorhetic control that regulates utilization of absorbed nutrients. The dramatic increase in milk production that occurs in bST-treated cows requires the orchestration of diverse physiological processes in a number of tissues and must involve the metabolism of all nutrient classes. These adaptations involve both direct effects on some tissues and indirect effects that are probably mediated by somatotropin-dependent somatomedins (insulin-like growth factors, IGF-I and IGF-II) for other tissues. Two cell types that are well-established as major direct targets of ST are the adipocyte and the hepatocyte. In contrast, effects on mammary tissue are thought to be indirect. In this section we discuss the state of knowledge for whole body metabolism and these particular tissues.

Whole Body Metabolism

Physiological processes that are altered with bST treatment are summarized in Table 1. Adaptations in metabolism are major and of critical importance during the initial period of bST treatment, when milk yield has increased but intake has not. Overall, mammary uptake of all milk precursors increases

Table 1 Effect of bovine somatotropin on specific tissues and physiological processes in lactating cows[a]

Tissue	Process affected during first few days and weeks of treatment
Mammary	↑ Synthesis of milk with normal composition
	↑ Uptake of all nutrients used for milk synthesis
	↑ Activity per secretory cell
	↑ Number and/or maintenance of secretory cells
	↑ Blood flow consistent with increase in milk yield
Liver	↑ Basal rates of gluconeogenesis
	↓ Ability of insulin to inhibit gluconeogenesis
	ϕ Glucagon effects on gluconeogenesis and/or or glycogenolysis
Adipose	↓ Basal lipogenesis if in positive energy balance
	↑ Basal lipolysis if in negative energy balance
	↓ Ability of insulin to stimulate lipogenesis
	↓ Ability of adenosine to inhibit lipolysis
	↑ Ability of catecholamines to stimulate lipolysis
Muscle	↓ Uptake of glucose
Pancreas	ϕ Basal or glucose-stimulated secretion of insulin
	ϕ Basal or insulin/glucose-stimulated secretion of glucagon
Kidney[b]	↑ Production of 1,25-vitamin D_3
Intestine[b]	↑ Absorption of Ca, P, and other minerals required for milk
	↑ Ability of 1,25-vitamin D_3 to stimulate Ca-binding protein
	↑ Ca-binding protein
Whole body	↓ Oxidation of glucose
	↑ NEFA oxidation if in negative energy balance
	ϕ Insulin and glucagon clearance rates
	ϕ Energy expenditure for maintenance
	↑ Energy expenditure consistent with increase in milk yield (i.e. heat per unit of milk not changed)
	↑ Cardiac output consistent with increases in milk yield
	↑ Productive efficiency (milk per unit of energy intake)

[a] Adapted from Bauman et al (13). Changes (↑ = increased, ↓ = decreased, ϕ = no change) that occur in initial period of bovine somatotropin supplement when metabolic adjustments match the increased use of nutrients for milk synthesis. With longer-term treatment, voluntary intake increases to match nutrient requirements.

[b] Demonstrated in nonlactating animals and consistent with observed performance in lactating cows.

while metabolism of other body tissues is altered simultaneously so that a greater proportion of nutrients are used for milk synthesis.

In a high-producing dairy cow, glucose is derived predominately via hepatic gluconeogenesis. Glucose turnover is over 3 kg/day with 60–85% used for milk synthesis (15). Thus, adaptations in glucose metabolism are of particular importance. When bST treatment is initiated, glucose turnover increases and oxidation decreases (18); accordingly, hepatic production of glucose increases (37) and hindlimb use of glucose is reduced (98) (Table 1). Therefore, adaptations in glucose oxidation and production occur in bST-treated cows before the increase in voluntary feed intake, and the adjustments are quantitatively equal to the extra glucose required to support the increased milk synthesis (18).

Changes in lipid metabolism play an integral role in the response to ST treatment and vary according to the animal's energy balance (Table 1). When cows are near zero or in negative energy balance, bST treatment increases mobilization of body fat reserves as evidenced by chronic elevation in circulating concentrations of nonesterified fatty acids (NEFA), decreased body fat content, and an increased milk fat content with the pattern of these extra fatty acids reflecting body fat stores (22, 28, 31, 51, 128). Under such conditions, an increase in NEFA irreversible loss rate (ILR) is observed and the magnitude of the increase is related to the extent of the negative energy balance and quantitatively equal to the increase in whole body oxidation of NEFA and the increased secretion of milk fat (18). Rates of lipid synthesis in adipose tissue would already be low and relatively impervious to further attenuation by ST. This situation is most likely to occur when bST treatment is initiated in early- to mid-lactation and the increased reliance on NEFA as metabolic fuel facilitates the previously discussed reduction in glucose oxidation.

In contrast, when animals are in positive energy balance at the time bST-treatment is initiated (i.e. when some lipid synthesis and storage is occurring in adipose tissue), the major effect of ST is to inhibit lipid synthesis with little or no change in lipolysis or milk fat percent and fatty acid composition (51, 83, 114, 127). This situation is most likely to occur when bST is initiated in mid- or late-lactation and the decrease in nutrient utilization for body fat stores enables nutrients to be redirected to other tissues to support the increased milk synthesis. With prolonged ST treatment, voluntary food intake increases and animals can eventually return to a positive energy balance allowing the replenishment of body reserves (Table 1) despite continuing high circulating concentrations of ST.

The kinetics of amino acid metabolism have not been examined in bST-treated dairy cows. Abomasal infusions of casein or amino acids gave no increase in milk protein yield over that observed for the basal diet in

bST-treated cows (3, 90, 115). However, the characteristic reduction in milk protein content that occurs when dietary protein is inadequate is also observed in bST-treated cows. Therefore, standard NRC protein requirements and dietary recommendations are also applicable to bST-treated cows (30, 41, 102).

Partitioning of minerals is altered by bST as indicated by the fact that the increased secretion of milk has a normal composition of nutritionally important minerals. Mechanisms have not been investigated in lactating cows, but changes in flux are coordinated with the increased milk secretion because blood concentrations of these minerals are not altered (13, 21, 51, 135). Recent studies with nonlactating animals (26) have demonstrated an altered tissue response to signals that maintain mineral homeostasis and, in the long term, increased absorption as shown for Ca and P (Table 1).

Adipose Tissue

The adipocyte is a major target of ST action. The hormone acts chronically to facilitate lipolysis and decrease lipid synthesis, in part by altering the ability of the tissue to respond to acute endocrine and other signals, but in addition ST may have some seemingly conflicting actions. In laboratory species and under rather unusual conditions (lack of prior exposure to ST for several hours, acute surgical stress), ST can exert an acute, transient, "insulin-like" effect (61, 141). The physiological significance of this is unclear, as the conditions required are unlikely to occur in vivo except perhaps in the young male rat with its highly erratic ST secretion (70). ST also promotes differentiation in several cell-lines (e.g. 3T3 F-442 cells) that can develop into adipocyte-like cells (26, 141); neither this, nor the acute "insulin-like" effect are thought to have any role in the chronic effect of ST on milk production. Some reports suggest ST can have an acute lipolytic effect; in many of the early studies the ST preparations were most probably contaminated with other peptides, but even the availability of pure recombinant ST has not completely resolved the controversy. For ruminants and pigs, ST apparently has no acute lipolytic effect (see reviews 26, 53, 141). Thus, much of the literature on effects of ST on adipocyte function has little relevance to the lactating animal treated chronically with ST.

Chronic treatment of lactating cows (97, 129) or growing steers (117) with ST dramatically increases the lipolytic response to in vivo challenges with catecholamines (Table 1). Sechen et al (128) further demonstrated that maximum response, but not sensitivity, was altered. In contrast, when responses to catecholamines are measured with in vitro incubations of subcutaneous adipose tissue obtained from cattle receiving ST, the differences between these responses and those of controls are much smaller or absent (83, 117). The reason for this apparent discrepancy between in vivo and in vitro

measurements is not clear. The subcutaneous adipose tissue depot sampled for in vitro studies may not be representative of other depots. For example, in humans exercise has a greater effect on lipolysis in abdominal than in gluteal subcutaneous adipose tissue (5), whereas in sheep lactation has a greater effect on β-receptor number in omental adipose tissues than in carcass adipose tissues (25, 74). Alternatively, the adaptation may not be sufficiently robust to survive in vitro manipulations or ST may exert its effects by limiting inhibition by an antilipolytic factor. Adenosine is a possible candidate; an autocrine/paracrine factor, adenosine exerts an acute antilipolytic effect via its own receptor, which couples via Gi (an inhibitory GTP-binding protein) to adenylate cyclase (140). Chronic treatment with ST decreased response to adenosine in lactating rats (139) and cows (83) (Table 1). Diminished response to adenosine is also found after chronic exposure to ST in vitro (140). The mechanism has not been resolved but does not appear to involve changes in adenosine receptor number (150), which suggests that ST may be altering either the amount or activity of Gi. A decrease in the ability of adenosine to inhibit lipolysis would allow for an increased response to catecholamines and thereby provide a possible explanation for the enhanced in vivo response to catecholamines. Curiously, in rats and sheep, response to adenosine is increased during lactation (140) and perhaps acts as a brake to check lipolysis; treatment with ST may thus reduce the effectiveness of this putative brake.

Other mechanisms may also be operating. Culture of sheep subcutaneous adipose tissue with ST increased both responsiveness and sensitivity to catecholamines and also increased ligand binding to the β-adrenergic receptor (150). Curtailing lactation in rats either by litter removal (139) or by endocrine manipulation (10) causes a marked decrease in lipolytic response to catecholamines, which is prevented by treatment with ST. In these animals ST altered several components of the adrenergic signal transduction system, thereby increasing the number of β-receptors and hormone-sensitive lipase activity and decreasing cyclic AMP phosphodiesterase activity. The greatest effect of ST, however, was on the association of hormone-sensitive lipase with the lipid droplet following catecholamine stimulation (142).

In vivo treatment with ST decreases the rates of lipogenesis and activities of key enzymes involved in lipid synthesis (see reviews 26, 53, 141). Similar adaptations occur in untreated animals during the initial stages of lactation when concentrations of endogenous ST are high (15, 136). Evidence that effects are due to ST acting directly on adipose tissue comes from in vitro studies in which chronic exposure to ST decreases the rate of lipogenesis (see reviews 53, 141). Indeed it is possible by varying the concentration of insulin and ST in tissue culture to mimic the changes in lipogenesis seen in adipose tissue during the lactation cycle (138).

Most studies have focused on the control of lipogenesis and the key lipogenic enzyme acetyl CoA carboxylase (ACC), which exists in both active and inactive states within the cell. In ruminant animals in which acetate rather than glucose is the major precursor for fatty acid synthesis, ACC is a major control of flux and "total activity" is thought to reflect the amount of this enzyme (136). Tissue culture studies show that effects of ST on lipogenesis over the first 48 hr are due to a decrease in ACC in the active state with no change in total activity (137), which is consistent with the relatively long half-life (about 48 hr) for this enzyme (145). Exposure to ST for six days or more results in a decrease in total ACC activity; this has been observed with sheep adipose tissue in culture (137) or with in vivo ST treatment of lactating cows (83) and goats (R. G. Vernon, unpublished observation), or growing pigs (65, 88). The changes in activity were probably due to a decrease in enzyme synthesis, as Liu et al (88) demonstrated a decrease in ACC protein and message. The close relationship between total ACC activity and the rate of lipogenesis suggests that with prolonged ST treatment, a fall in the amount of ACC is responsible for the decreased rate of lipogenesis.

While ST reduces the effects of insulin on lipogenesis, it does not prevent an acute stimulation of lipogenesis by insulin in pigs in vivo (49) or in vitro (147). However, in vivo treatment of growing pigs with ST decreases sensitivity to insulin as measured by in vitro rates of glucose incorporation into adipose tissue lipid (147) or by in vivo rates of whole body glucose utilization (154). Similarly, treatment of lactating cows reduces glucose response to an insulin challenge (128). Thus, physiological concentrations of insulin would be less effective in increasing the rate of lipogenesis (Table 1). That ST does not completely abrogate the effect of insulin on lipogenesis in vivo can also be inferred from the recovery of adipose tissue reserves during the latter stages of lactation in bST-treated animals, despite high levels of ST.

The mechanism whereby ST inhibits the effects of insulin on lipogenesis is not known. ST treatment has no apparent effect either on the ability of adipocytes to bind insulin in pigs (93) or sheep (148), or on the ability of insulin to stimulate insulin-receptor tyrosine kinase activity (93) or down-regulate its receptor (148). Therefore, the site of action is at a postreceptor level, which is not surprising as ST does not inhibit all effects of insulin. For example, the antilipolytic effect of insulin (128) and insulin stimulation of protein synthesis (137) in cow and sheep adipose tissue, respectively, are not altered by ST. ST inhibits the activation of a phosphatidylinositol-specific phospholipase C by insulin in mouse adipocytes (32), possibly by interfering with the action of a putative Gi-like protein (125). This observation is of interest because lactation in rats results in a decreased ability of insulin to stimulate lipogenesis and activate lipogenic enzymes of adipocytes because

of a postreceptor impairment at the level of the plasma membrane (76). In sheep, onset of lactation also results in the loss of ability by insulin to increase the rate of lipogenesis in vitro (138), apparently owing to the loss of a putative protein required for activation of acetyl CoA carboxylase by insulin (137). Production of this mediating protein can be restored by prolonged incubation with insulin in vitro and is prevented by ST (137). While most work has focused on ST as an insulin antagonist, ST can also act chronically to decrease the rate of lipogenesis in the absence of insulin (24, 131).

Effects of ST on adipocytes are thought to be mediated by ST itself, for although ST stimulates IGF-I mRNA production (39, 155) and IGF-I secretion (J. Beattie and R. G. Vernon, unpublished observations) by adipocytes, the adipocytes themselves lack IGF-I receptors (141). The function of this locally produced IGF-I in the tissue is uncertain, but may have a role in angiogenesis. IGF-I also failed to mimic chronic effects of ST on lipogenesis and lipolysis during lactation (10; D. P. D. Lanna and D. E. Bauman, unpublished observations). IGF-I can mimic effects of insulin on adipose tissue but concentrations required were high, which suggests that they were mediated via the insulin receptor (52, 138, 147). One very provocative study suggested that IGF-I and IGF-II mediated acute lipolytic effects of ST on adipocytes in sheep (87), but attempts to confirm this have proved unsuccessful (68; R. G. Vernon, unpublished observations).

Adipocytes have been a major target of studies on the ST signal transduction system, but the paradoxical actions of ST have slowed progress in this area. The structure of the ST receptor suggests that it is unlikely to be associated with GTP-binding proteins (a common mediator of signal transduction) and that it is unlikely to be a protein kinase. However, ST binding apparently causes phosphorylation of its own receptor by sequestration of a cytosolic protein kinase (132). Studies with differentiating pre-adipocyte cell-lines suggest that ST, presumably through the sequestered kinase, causes tyrosine phosphorylation of a number of proteins including mitogen-activated protein kinase (MAP kinase) (4). Some effects of ST in these cell lines also appear to be mediated by protein kinase C (48), thus suggesting that the hormone activates a protein kinase cascade. Less is known about mechanisms involved in the chronic effect of ST on mature adipocytes. Protein kinase C may have a minor role (142), and it is not known if MAP kinase is involved. The chronic inhibitory effects of ST on lipogenesis are blocked by actinomycin D and appear to involve some relatively short-lived (half-life less than 3 hr) product of gene transcription (24); ornithine decarboxylase is an obvious candidate for this product because its half-life is less than 30 min and its activity is enhanced by ST in the liver (69). However, although polyamines are required for inhibition of lipogenesis by ST, they probably have a permissive rather than a mediatory role (R. G. Vernon, unpublished observations).

Hepatocytes

Hepatic rates of gluconeogenesis are increased with ST treatment of dairy cows as demonstrated by in vivo (37) and in vitro studies (78, 119) (Table 1). Evidence that this is a direct effect comes from studies with sheep hepatocytes maintained in culture (50). Mechanisms have not been resolved but include a decreased ability of insulin to inhibit gluconeogenesis (23, 62) (Table 1). In contrast, ST treatment had no effect on liver glycogen concentration in lactating cattle in positive energy balance (119), although such treatment did induce a small decrease in cows in negative energy balance (78). This lack of an effect of ST is not surprising, as hepatic glycogen reserves are not sufficient to sustain increased glucose output by the liver for long.

Effects of ST on hepatic lipid metabolism appear to be slight. In vivo treatment of lactating cows with ST increased fatty acid oxidation to CO_2 in liver slices (119), which was consistent with the increased rates of gluconeogenesis. Chronic treatment of cows with ST in vivo (119) and culture of sheep hepatocytes with ST (50) had no effect on the ketogenic capacity (i.e. rate in vitro with a saturating concentration of fatty acid). Consequently, any effects of ST on ketogenesis are indirect, via changes in plasma NEFA concentrations. Treatment with ST, in vivo (112) and in vitro (50), decreased rates of fatty acid esterification and lipoprotein secretion by sheep liver. If such changes occur in lactating cows, they must be highly coupled because hepatic concentration of triacylglycerol is not altered (119). The overall effect of ST on hepatic lipid metabolism thus appears to be a small increase in fatty acid oxidation (to support gluconeogenesis) at the expense of esterification.

Mammary Gland

The dramatic increase in milk yield is a clear demonstration that mammary uptake and utilization of nutrients is increased in bST-treated cows (Table 1). The change in the lactation curve suggests an increased rate of milk synthesis per cell and, in the long-term, an increased number of mammary epithelial cells. Clarification of these postulates and of the mechanisms responsible for them has proved difficult. This is not unexpected as biochemical changes in vivo are likely to be relatively small and mammary tissue from lactating animals is difficult to maintain in vitro because of its high metabolic rate. In addition, assessing the role of somatomedins is complicated by the presence of specific binding proteins.

Baldwin (8) demonstrated that bST-treated cows had increased RNA per gland, and therefore increased protein synthetic capacity; he also reported increased activities of several enzymes but the key enzymes controlling metabolic flux were not measured. Other studies of lactating cows (80) and goats (79) observed similar trends in enzyme activity after ST treatment, but

effects were not significant possibly owing to smaller increases in the milk yield response. Clear-cut evidence for an effect on activity and message level of mammary enzymes comes from studies of rats in which investigators treated animals with ST after blocking prolactin secretion with bromocriptine and neutralizing endogenous ST with an antiserum (10). The mammary gland is not metabolically homogeneous, so changes in synthetic capacity could result from an increased synthetic activity of active cells and/or the activation of resting differentiated cells (108). Treatment of cows with ST for a period during mid-lactation had no effect on total mammary DNA content (8), but in a longer-term study in which goats were treated with ST for 22 weeks, the decline in mammary cell number that normally occurs during lactation was prevented (79). No effect was observed on DNA synthesis (79), but the increase might have been too small to detect. The low milk levels of plasmin, a serine-protease associated with mammary gland involution, that are maintained during bST treatment (120) are also consistent with the proposed changes in maintenance and/or number of mammary cells.

Changes in mammary synthetic activity in response to ST are complemented by increased nutrient availability induced by the homeorhetic effect of ST and also by an increase in mammary blood flow (42, 57). However, merely increasing nutrient availability by itself does not mimic the effect of ST on milk yield (see review 114). Mepham et al (107) suggested that ST affects the mammary gland largely through an increase in blood flow, but it is now thought that the increased blood flow is the result rather than the cause of the increased mammary metabolism.

The mechanism whereby ST increases mammary gland function is still uncertain but appears to be indirect. Addition of bST to bovine mammary cells in culture had no effect on rates of synthesis of casein, fat, or α-lactalbumin (59). An attempt to demonstrate a direct effect of ST on the mammary gland using a close arterial infusion technique was unsuccessful (99); however, because of the half-life of ST and mammary blood flow rates, this approach would not allow for an adequate evaluation. Attempts to detect ST receptors in mammary tissue have been unsuccessful (2, 58, 75). Furthermore, concentrations of ST in milk are very low and not appreciably altered by bST treatment (72). Recent studies have reported the presence of mRNA for ST receptor in mammary tissue from pregnant (150 d), nonlactating heifers (67) and lactating cows (60), but in both cases the message level in mammary tissue was only a small fraction of that in liver. Therefore, although the message for bST receptors is present, either it is not translated or the number of receptors produced is too low to be detected by conventional techniques. As a result, the current view is that ST does not act directly on mammary epithelial cells, and efforts have focused on the role of the somatomedins as possible mediators.

Administration of exogenous bST to lactating cows causes an increase in concentrations of IGF-I in blood (36, 43, 124) and milk (72). Another candidate is IGF-II, although effects of ST on IGF-II are not consistent (106). Receptors for somatomedins are present in ruminant mammary tissue, and the number of available receptors increases during lactogenesis (45, 47, 63). When lactating cows are treated with bST, circulating concentrations of IGF-I begin to increase about 6–12 hr after the initial bST injection and reach maximum concentrations in approximately 48 hr (36). The response in milk yield is apparent about 24 hr after the first bST injection, and maximum production response occurs four to six days after start of treatment. In addition, IGF-I is present in milk, and concentrations increase with bST treatment (72). Therefore, the temporal pattern of changes in IGF-I is consistent with its possible role in mediating the effects of ST on milk production. Likewise, IGF-I stimulated casein synthesis in cultured mammary cells from lactating cows (64) and increased both casein synthesis and glucose transport in mammary explants from mid-pregnant mice (122). IGF-I also increased protein synthesis in mammary explants from pregnant rats (A. M. Gilhespy, C. J. Wilde, and R. G. Vernon, unpublished observations). On the other hand, IGF-I had no effect on fatty acid synthesis or α-lactalbumin secretion in mammary explants from lactating cows (130), but the medium also contained insulin (50 ng/mL), which may have masked effects of IGF-I. IGF-I also stimulates DNA synthesis in mammary tissue cultures (19, 116, 130, 152) and thus may play a role in maintaining cell number during long-term ST treatment.

Attempts to demonstrate an effect of IGF-I on milk secretion in vivo have had mixed success. While ST treatment increased milk secretion in goats, a three-day jugular infusion of IGF-I had no effect on milk yield even though blood concentrations of IGF-I were elevated to levels comparable to those of the ST-group (44). IGF-I injections also failed to mimic the effect of ST on mammary metabolism in rats treated with an antiserum to endogenous ST (10). In contrast, infusion of IGF-I into the pudendal artery of lactating goats for 6 hr increased milk production by about 30% (121). Differences in response to IGF-I in vivo could arise from problems relating to IGF-binding proteins.

The majority of somatomedins in physiological fluids are bound to soluble, high affinity binding proteins. There are six specific IGF-binding proteins (IGFBP) and their functions are not well established. Their postulated roles include serving as circulatory transport vehicles, retarding somatomedin degradation, facilitating transvascular movement, providing an extravascular pool, and/or modulating directly the actions of somatomedins at specific target cells either by enhancing or blocking their activity (20, 33, 123). The in vivo regulation of the two major IGFBP in bovine serum has been described more

fully (35). As in the case of humans (33), ST treatment of lactating cows results in a threefold elevation of circulating IGFBP-3 and a decrease of about two thirds in circulating concentrations of IGFBP-2 (35, 105, 143), so it is not surprising that IGF-I infusions or injections have not mimicked the effects of ST. Somatomedins themselves stimulate mammary cells to produce both IGFBP-2 and IGFBP-3 (100). Thus, local production of somatomedins and their binding proteins may also play a role in control of mammary tissue.

INTEGRATION

Although many details have yet to be clarified, we now know that exogenous ST enhances milk production in dairy cows by coordinating a complex series of adaptations within the body. In essence, bST both increases the rate of milk production within the mammary gland and provides the necessary nutrients in support of this enhanced rate of milk synthesis (Figure 1). Direct actions of ST appear to be primarily concerned with nutrient availability as illustrated by the aforementioned alterations in the metabolism of adipose tissue and liver. On the other hand, the indirect effects of ST appear to be primarily associated with the mammary gland and the actions of the IGF complex. We do not have a clear understanding of how the IGF complex is

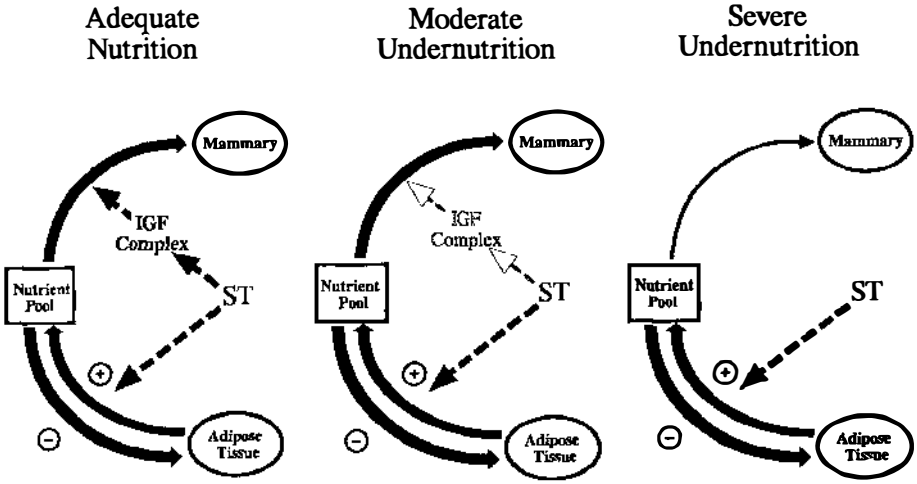

Figure 1 Conceptual model of the effects of somatotropin (ST). Direct effects include alterations in activities of key enzymes and tissue response to homeostatic signals as represented by plus and minus symbols on adipose tissue rates of lipolysis and lipogenesis, respectively. Indirect effects apparently involve the IGF complex (insulin-like growth factors and their binding proteins), and these are modulated by nutritional status as indicated. Model (developed by M. A. McGuire and D. E. Bauman, unpublished information) adapted from Bauman et al (14).

able to mediate mammary function, nor do we fully understand the interplay between the endocrine, autocrine, and/or paracrine aspects of the IGF complex. It is apparent, however, that changes in circulating concentrations of IGF-I and some of the IGFBP are closely tracking the biological events and the magnitude of milk responses that occur with bST treatment of dairy cows, indicating that the IGF complex has an important role in ST biology.

Nutritional status plays a key role in the regulation of somatomedins and their binding proteins (34, 106). In the lactating dairy cow, moderate undernutrition has no effect on basal concentrations of circulating IGF-I, but administration of bST results in a less dramatic increase in circulating IGF-I than when animals have an adequate nutritional status (104). When nutritional status is severely compromised by a short-term fast, basal concentrations of IGF-I are lower and the ability of bST to increase IGF-I is abolished (103; Figure 1). A similar impact of nutritional status on the somatotropin/somatomedin axis is observed in growing cattle (27) and other species including humans (34). Although not as extensively investigated, basal and bST-stimulated levels of IGFBP also appear to be modulated by nutritional status (103, 106).

The relationship between nutritional status and the somatotropin/somatomedin axis also provides a framework to consider variations in milk response to bST, which were discussed in the section on production responses. Moderate undernutrition attenuates both the increase in circulating IGF-I and milk yield response to bST (104). In addition, the small increases in milk yield that occur with bST treatment in the early portion of lactation are consistent with the representation in Figure 1. Cows in early lactation are typically in substantial negative energy balance, and the use of body fat reserves over the first 30 days of lactation can be energetically equivalent to one third of the milk produced (15). During this period, animals have high circulating levels of endogenous ST but low basal levels of IGF-I. Vicini et al (143) demonstrated that short-term bST treatment during early lactation resulted in lower responses in circulating IGF-I and milk yield than were found in cows during later lactation. Thus, the direct actions of ST on tissues such as adipose occur in early lactation to maximize nutrient supply to the mammary gland, but the somatotropin/somatomedin axis is attenuated by nutritional status.

Long-term studies with bST treatment have demonstrated that the magnitude and maintenance of the milk response is related to the quality of management (see section on production responses). As a major component of the management program, this would largely reflect the impact of nutritional status on the somatotropin/somatomedin axis. Thus, production studies in which bST was administered to cows with inadequate nutrient supply and/or to cows that had inadequate body reserves observed no adverse effects.

However, as would be predicted from the preceding discussion, the milk response to bST was negligible (see section on production responses). The situation in fasted or chronically underfed animals is an interesting comparison in other regards. At first it seems paradoxical that exogenous ST can increase milk production while one of the most dramatic ways to increase endogenous levels of circulating ST and decrease milk yield is to fast or severely underfeed an animal. In this case, the direct effects of ST are to partition nutrients away from storage toward utilization in an inadequately nourished cow, but effects on the IGF complex are uncoupled so that use by the mammary gland is not stimulated (Figure 1). Therefore, these adaptations provide nutrients for the animal's survival and minimize any use of nutrients for milk production.

CONCLUSIONS

Somatotropin treatment of dairy cows results in a remarkable increase in milk yield and an unprecedented gain in productive efficiency (milk per unit of feed). Aspects of the production responses including effects on milk components, bioenergetics, and animal well-being have been extensively examined with consistent results over a wide range of management and environmental conditions. Overall, somatotropin is a homeorhetic control that increases rates of milk synthesis by the mammary gland and coordinates a series of physiological adaptations in a variety of tissues to support nutrient needs for milk synthesis. These tissue adaptations include changes in activities of key enzymes and alterations in tissue response to homeostatic signals. In addition, nutritional status of the animal plays a major role in determining the extent to which milk yield is altered. As a result of the nutritional effects on the somatotropin/somatomedin axis, somatomedins and their binding proteins appear to be key links between nutritional state and cellular growth and developmental processes.

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