CHAPTER II

BACKGROUND INFORMATIONS

The structure of Growth hormone

Growth hormone or somatotropin is a protein hormone synthesized by the anterior pituitary gland. The secretion of growth hormone from the pituitary gland is regulated by two peptides: the growth hormone-releasing factor (GRF), which stimulates the release and the somatostatin, which inhibits the release. In addition to these two peptides, a third as yet unidentified hormone binds to the growth secretagogue receptor to stimulate growth hormone release using a signal transduction pathway distinct from that of GRF is reported (Etherton and Bauman, 1998).

Growth hormone contains 191 amino acids, and bovine somatotropin (bST) and porcine somatotropin (pST) share a high degree of amino acid sequence similarity (~ 90%) (Bauman and Vernon, 1993). In contrast, the amino acid sequence of both bST and pST is appreciably different from that of human somatotropin (hST) (~35% of the amino acids in hST differ from these of bST and pST). Because of this difference, bST and pST have no effect on human growth, which is consistent with their binding affinity to the hST receptor being several orders of magnitude lower than that of hST.

It is noted that there are variant forms of growth hormone. For example, bST is released from the pituitary as four variants. These variants have either a leucine or valine substitution at position 127 and an alanine (191amino acid sequence) or a phenylalanine (190 amino acid sequence) at the NH_2 terminus. The variation in the NH_2 terminus is due to differences in the cleavage of the signal peptide. The

frequency of these gene alleles differs between dairy breeds (Bauman and Vernon, 1993).

Recombinantly derived forms of bST (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. However, when the same purification techniques are used, recombinantly derived and pituitary-derived bST have similar potency in various biological test systems (Bauman and Vernon, 1993; Etherton and Bauman, 1998).

Exogenous growth hormone must be injected to be biologically active. The digestive tract secretes enzymes that break proteins down to amino acids to be absorbed. If exogenous growth hormone is given orally, it is broken down to amino acids in the digestive process just like other dietary proteins. These reason is that bST is cleared rapidly from the blood stream and is not stored in the body. Clearance of bST occures via is by normal body mechanisms and involves breaking the protein down to amino acids. Thus, to achieve a sustained increase in milk yield, one needs to give daily injections or use a prolonged-release formulation of bST. Several prolonged-release formulations have been developed that are small volumes to be administered by subcutaneous injection at time intervals ranging from 2 to 4 weeks (Bauman, 1992; Muller, 1992).

Production performances

For all species, milk yield follows the lactation curve, increasing to peak yield and then declining at weaning or the cessation of milking. For dairy cows, genetic selection has greatly increased two interrelated factor, peak yield and lactation persistency, defined as the change of yield with time in mid-lactation. Broster and Broster (1984) calculated that peak yield of dairy cow's accounts for 66 to 80% of the variance in total yield compared with 8 to 12% for persistency. Peak

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yield is in turn determined by secretory cell number and by secretory activity per cell. Studies in goats by Knight and Wilde (1993) show that parenchyma cells increase in number during pregnancy and early lactation. Between parturition and peak lactation secretory cells increase in size and become more fully differentiated. After peak, cell loss is largely responsible for decline in milk yield, but the activity per cell is maintained.

Milk yield increase after bST treatment is observed in cows of all parities, but the magnitude of the increase in milk yield varies according to stage of lactation (Peel and Bauman, 1987). In general, the response has been small or negligible when bST is administered in early lactation prior to peak yield. Therefore, the possible commercial use would probably be over the last two- third or three-fourth of the lactation cycle (Bauman and Vernon, 1993). Lactation response to bST is a function of the daily dose represented by a hyperbolic dose-response curve with a pattern of diminishing marginal returns to increasing doses (McGuffey and Wilkinson, 1991).

Exogenous growth hormone enhances milk production in dairy cows by coordinating a complex series of adaptations within the body. Treatment with bST increases the rate of milk production within the mammary gland and provides the necessary nutrients in support of this enhanced rate of milk synthesis. Voluntary feed intake does not increase until several weeks after increases in milk yield in bST-treated cows (Burton et al., 1994). The magnitude of the increase in feed intake is dependent upon the increase in milk yield, the degree of body condition change, and the nutrient density of the diets. The nutrient partitioning response to bST treatment that supports increased milk yield particularly concerns the preferential oxidation of fatty acids and the sparing of glucose by peripheral tissues. The increased substrate utilization by the mammary gland may in turn provide a stimulus for increasing feed intake (Bauman and Vernon, 1993).

The major blood precursors for the formation of milk are glucose, acetate, β -hydroxybutyrate, triglyceride, fatty acids and amino acid. Treatment of lactating cows with bST has been shown to augment insulin resistance in peripheral tissues (Sechen et al., 1990). bST had no effect on plasma glucose concentrations but increased the irreversible loss rate of glucose by 12% (Bauman et al., 1988). Milk lactose production represented a major use of lost glucose. McDowell et al. (1987) demonstrated that bST treatments reduced glucose uptake by the hind limb muscle and increased glucose uptake by the mammary gland. Peel et al. (1983) reported that bST treatments did not affect plasma concentrations of glucose, insulin, glucagon, prolactin, tri-iodothyronine, thyroxine or cortisol in either early or late lactation. In many mammalian cells, a major point of metabolic regulation of glucose utilization is the transport of glucose across cell membranes, which is mediated by a family of tissue-specific facilitative glucose transporter (Kahn, 1992). Mammary gland mainly expresses GLUT1 glucose transporter protein (Zhao et al., 1999 and Zhao et al., 1996) whereas muscle and fat primarily express GLUT 4 (Zhao et al., 1996).

Analysis of milk for intracellular constituents, such as glucose, has proved useful, changes in the concentration of glucose in milk have been found to correlate significantly with changes in milk production under a variety of situations such as feed restriction (Chaiyabutr et al., 1981) and suppression of secretion (Faulkner et al., 1981). These, together with direct data on mammary glucose concentrations, indicate that milk glucose concentrations reflect intracellular concentrations (Faulkner et al., 1981). Faulkner (1999) reported that there were increased in the availability of glucose within the mammary epithelial cell in response to growth hormone treatment.

Increased milk yield should reflect increased flow of blood carrying milk precursors to the mammary gland. Control of mammary blood flow (MBF) may be a way to control nutrient partitioning. Cardiac output was reported to be 10% higher and MBF increased by 35% in bST-treated cows studies by Davis et al. (1988).

Tannaer and Hauser (1989) also reported higher cardiac output in a study. Heart rates were monitored regularly in several studies. Heart rate was slighter higher in bST-treated cows at high dose rate but was still within normal range (Soderholm et al., 1988 and Eppard et al., 1987).

The daily outputs of major milk constituents (lactose, fat, protein, minerals and vitamins) are elevated by an amount comparable to milk volume in bST-treated cows (Bauman and Vernon, 1993 and Burton et al., 1994). The concentrations of fat and protein in milk normally vary as a result of factors such as genetics, breed, stage of lactation, season, diet and nutritional status. These similar factors also affect the composition of milk from bST-treated cows (Etherton and Bauman, 1998).

Mechanisms of action

Growth hormone is a homeorhetic control that affects numerous target tissues in ways that is highly coordinated to affect marked changes in nutrient partitioning among these tissues. Two cell types that are well established, as major direct targets of growth hormone are the adipocyte and the hepatocyte. In contrast, effects on mammary tissue are thought to be indirect.

Effects on lipogenesis and lipolysis

Propionate and acetate are the main energy sources in ruminant animals because of their availability and high rate of uptake by the lactating mammary gland, acetate and to a lesser extent, β-hydroxybutyric acid are considered the most important energy metabolites in mammary gland metabolism of ruminants. Two of the most significant functions of acetate are to supply carbon atom for *de novo* synthesis of fatty acids and to generate adenosine triphosphate through the tricarboxylic acid acid cycle and the electron transport system. Growth hormone has

dramatic effects on adipose tissue and lipid metabolism. Both lipogenesis and lipolysis are altered by growth hormone treatment, with effects on lipid synthesis being of major importance if animals are in positive energy balance, whereas effects on lipolysis predominate when animals are at an energy balance near zero or negative (Bauman and Vernon, 1993).

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). An decreased body fat content and an increased milk fat content with the pattern of these extra fatty acids reflecting body fat stores (Bitman et al., 1984; Eppard and Bauman, 1985 and Sechen et al., 1990). This situation is most likely occurring 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 tissues), the major effect of growth hormone is to inhibit lipid synthesis with little or no change in lipolysis or milk fat percent and fatty acid composition (Eppard and Bauman, 1985; Peel and Bauman, 1987 and Sechen et al., 1989). 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.

The regulation of lipolysis involves cAMP and a signal tranduction system that includes stimulatory G proteins (G_S) and inhibitory G protein (G_i). Catecholamines affect lipolysis through the G_S system, and growth hormone treatment dramatically increases the lipolytic response to catecholamines in lactating cows (McGuffey and Wilkinson, 1991 and Sechen et al.1990). This change in response to catecholamines is evident within 15 h after the initiation of growth

hormone treatment and is observed regardless of whether animals are in a positive or negative net energy balance (Etherton and Bauman, 1998).

Growth hormone treatment, *in vivo* or *in vitro*, result in only modest changes in β-and α_2-adrenergic receptor numbers. Furthermore, examination of the G_S proteins and other downstream components of the lipolytic signal transduction cascade demonstrated no differences in adipose tissue from bST-treated and control animals. These results raised the possibility that the major mechanism by which growth hormone altered lipolysis might involve the antilipolytic system of adipocytes. Adenosine was a likely candidate because it is an autocrine/paracrine factor that exerts an acute antilipolytic effect via the G_i system. Indeed, chronic treatment with growth hormone decreases the antilipolytic effects of adenosine in adipose tissue (Lanna et al., 1995 and Houseknecht and Bauaman, 1997).

Effects on carbohydrate metabolism

Hepatic rates of gluconeogenesis are increased with growth hormone treatment of dairy cows as demonstrated by *in vivo* and *in vitro* studies (Knapp et al., 1992 and Pocius and Herbein, 1986). Mechanisms include a decreased ability of insulin to inhibit gluconeogenesis. Thus the reduction in hepatic response to insulin in bST-treated cows allows the liver to sustain an increased rate of gluconeogenesis that is critical to support the increase in the synthesis of milk components. When bST treatment is initiated, glucose turnover increases and glucose oxidation decreases (Bauman et al., 1988). In contrast, growth hormone treatment had no effect on liver glycogen concentration in lactating cattle in positive energy balance (Pocius and Herbein, 1986) although growth hormone treatment did induce a small decrease in cows in negative energy balance (Knapp et al., 1992). Liver glycogen reserves are to limit to sustain increased glucose output by the liver in lactating cows.

Effects on protein metabolism

The effects of growth hormone on growth and protein metabolism depend on an interaction between growth hormone and somatomedins, which are polypeptide growth factors (70 amino acids) secreted by the liver and other tissues in response to stimulation by growth hormone. Little is known about the effects of growth hormone on protein metabolism of domestic animals compared to lipid or carbohydrate metabolism. It is clear that growth hormone treatment increases muscle protein accretion in growing animals and milk protein synthesis in lactating cows. However, the precise mechanisms are not clear, and the extents to which the effects of growth hormone on protein metabolism are direct or mediated by insulin-like growth factor -1 (IGF-1) remain unclear.

Effects on mammary gland metabolism

Treatment with bST causes a dramatic increase in the uptake and utilization of nutrients for the synthesis of milk. However, it has proven to be difficult to document specific mechanisms. At the cellular level, the magnitude of the biochemical changes would likely be small, and mammary epithelial cells, which are actively secreting milk components, are difficult to maintain *in vitro* because of their high rates of metabolic activity (Etherton and Bauman, 1998). Nevertheless, the pattern of response to bST and the change in the shape of the lactation curve indicate that the bST effects involve both an increase in the rates of milk component synthesis per cell and improved maintenance of secretory cells.

Baldwin and Knapp (1993) demonstrated that bST-treated cows had increased protein synthetic capacity as indicated by an increase RNA per gland. Knight et al (1990) observed that the decline in mammary cell numbers that normally occurs during lactation was prevented in goat that received growth

hormone for 22 wk. Kleinberg et al. (1990) reported that rat growth hormone was more potent than human prolactin (hPRL) in stimulating mammary development in hypophysectomized castrated male rats supplemented with 17β-estradiol. In addition, local implantation of bovine or mouse growth hormone in the mammary gland stimulated end bud formation in female mice. The bST is also mammogenic in dairy cows. Sejrsen et al. (1986) found that systemic administration of bST to growing heifers increased the proliferation of mammary growing tissues.

The mechanism by which growth hormone affects mammary gland function is still uncertain but appears to be indirect, involving the IGF system. Several lines of evidence indicate that exogenous somatotropin does not act directly on the mammary gland (Peel and Bauman, 1987). Bovine research has been focused on the association between bST and IGF-1 primarily. Implicating IGF-1 in bovine galactopoiesis includes observations of chronically elevated IGF-1 concentrations in blood and lactating mammary tissue during periods of bST administration (Glimm et al., 1988 and Prosser et al., 1989). Administration of bST to lactating cows causes an increase in concentration of IGF-1 in blood (Davis et al., 1987). Sharma et al. (1994) found that bST increased serum growth hormone of late lactation cows by more than two folds and increased serum IGF-1 concentration two folds above those of late lactation controls. IGF_s have both acute metabolic and long-term growth promoting effects. IGF_s probably act as local tissue growth factors rather than as circulating hormone.

The concept of local regulation of blood flow is well accepted for other tissues, but has received only minimal investigation in the mammary gland. The IGF might also be locally produced factors that could regulate MBF; IGF-1 mRNA has been detected in mammary tissue of lactating cows (Glimm et al., 1992) and IGF-1 immunoreactivity has been detected by indirect immunofluorescence in blood vessels within lactating bovine mammary tissue (Glimm et al., 1988). Its origin appears to be in the systemic circulation but increased concentrations of radiolabelled IGF-1 in the local environment of individual mammary glands have

been shown to be reflected in an increased concentration in milk from that gland (Prosser et al., 1991a). In addition, increased concentrations of IGF-1 in milk appear to correlate with increased levels in mammary secretory tissue (Prosser et al., 1991b).

Most IGF-1 circulates bound to binding protein (BP; 95%), which exist in numerous molecular forms and are produced from a variety of tissues (McGuire et al., 1992 and Prosser et al., 1989). Two dominant forms of IGFBP exist in most species. One form is dependent on growth hormone for its secretion from the liver (Mr of 150000 when complexes with IGF-1) whereas the other form is growth hormone-independent (Mr of 50000 when complexes with IGF-1). The growth hormone-dependent BP at binds most liver-produced IGF-1 or near the time of secretion but the BP status of IGF-1 acting in an autocrine or paracrine fashion is not well defined and may be tissue-specific. Considering the complexity of normal regulation of mammary cell physiology, it is probable that the growth hormone does not act alone during galactopoiesis. Rather, growth hormone may also induce regulatory molecules, which direct and maintain lactation (Burton et al., 1994).