Bovine Growth Hormone and Milk Fat Synthesis: from the Body to the Molecule[1]
– Review –

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ABSTRACT : Injection of bovine growth hormone (bGH) to lactating dairy cows increases milk yield and yields of milk components including fat. It is generally believed that most of the anabolic effects derived from bGH in animal tissues are primarily mediated by IGF-1. IGF-1 is a strong anabolic peptide in the plasma of animals and exerts mitogenic and metabolic effects on target cells. Contrary to most protein hormones, the majority of IGF-1 in circulation is bound to the binding proteins (IGFBPs) which are known to be responsible for modifying the biological actions of IGF-1, thus making determinations of IGF-1 actions more difficult. On the other hand, fat is a major milk component and the greatest energy source in milk. Currently, the fat content of milk is one of the major criteria used in determining milk prices. It has been known that flavor and texture of dairy products are mainly affected by milk fat and its composition. Acetyl-CoA carboxylase (ACC) is the rate limiting enzyme which catalyzes the conversion of acetyl-CoA to malonyl-CoA for fatty acid synthesis in lipogenic tissues of animals including bovine lactating mammary glands. In addition to the short-term hormonal regulation of ACC by changes in the catalytic efficiency per enzyme molecule brought about by phosphorylation and dephosphorylation of the enzyme, the long-term hormonal regulation of ACC by changes in the number of enzyme molecules plays an essential role in control of ACC and lipogenesis. Insulin, at supraphysiological concentrations, binds to IGF-1 receptors, thereby mimicking the biological effects of IGF-1. The receptors for insulin and IGF-1 share structural and functional homology. Furthermore, epidermal growth factor increased ACC activity in rat hepatocytes and adipocytes. Therefore, it can be assumed that IGF-1 mediating bGH action may increase milk fat production by stimulation ACC with phosphorylation (short term) and/or increasing amounts of the enzyme proteins (long term). Consequently, the main purpose of this paper is to give the readers not only the galactopoietic effects of bGH, but also the insight of bGH action with regard to stimulating milk fat synthesis from the whole body to the molecular levels.

(**Key Words**: Bovine Growth Hormone, Insulin-Like Growth Factor-1, Milk Fat Synthesis, Acetyl-CoA Carboxylase)

INTRODUCTION

It has been well documented that bGH stimulates milk production via the action of IGF-1, somatomedin (Bauman and McCucheon, 1986; Johnson and Hart, 1986; Peel and Bauman, 1987; Bauman, 1992; Forsyth, 1996). This classical somatomedin hypothesis of bGH is that bGH triggers the liver to secrete IGF-1, which then transported though blood to act on specific target tissues including mammary glands. We do also believe that bGH performs the dual actions, with which it acts on some tissues to exert catabolic functions, such as stimulating lipolysis in adipose tissues by the direct manner, whereas acts on other tissues to give rise to anabolic functions, such as increasing milk components in mammary glands by the indirect way, using the bGH mediator, IGF-1.

Even though this dogmatic hypothesis has been tremendously studied and modified, it has not been fully elucidated mainly because the actions of IGF-1 are complex, and the proper cell culture system is not established. Two phenomena in the IGF-1 properties may explain the complexity of IGF-1 actions. One is that IGF-1 is performed by endocrine, paracrine and/or autocrine actions, by which the IGF-1 level in serum does not indicate the exact concentration of acting IGF-1 on the

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Received April 27, 1997; Accepted July 25, 1997

target tissues. The other is that specific IGF-binding proteins (IGFBPs) exist in serum that modulate IGF-1 actions and its clearance from serum, which makes the researchers to interpret IGF-1 data very difficult. Regardless of the improper elucidation of the mode of bGH action, recombinantly derived bGH (rbGH) has been commercialized throughout the world except the European countries.

Even then, unfortunately, no one have explored the entire possible mechanism of bGH action from the whole body to the cellular and/or molecular levels. Therefore, with the coverage of the galactopoietic effects of bGH at the whole body level, we disscussed the possible mode of bGH action via IGF-1 by examining the IGF-1 effects specifically on milk fat synthesis at the body and tissue level as well as acetyl-CoA carboxylase (ACC) at the molecular level.

BOVINE GROWTH HORMONE (bGH) AND LACTATION IN DAIRY CATTLE

Biochemical nature

Growth hormone (GH) is produced by somatotropes, a subclass of pituitary acidophilic cells in the anterior pituitary glands of animals, and released in a pulsatile manner (Ross and Buchanan, 1990). GH secretion is primarily controlled by a dynamic equilibrium between the inhibitory and stimulatory hypothalamic peptides, somatostatin and GH-releasing factor. Secretion of these hypothalamic peptides and controlled by neuropeptides and neurotransmitters which are, in turn, influenced by GH feedback, age, sex, hormones and nutritional status (Buonomo and Baile, 1990).

bGH has been isolated from the pituitary gland and its amino acid sequences were elucidated as two different amino acid sequences containing either 190 or 191 amino acids (Wallis, 1975; Kostyo and Reagan, 1976). It was also observed that bGH has an allelic variation at position 126, which leads to a 2:1 ratio of leucine to valine (Wood et al., 1989). Therefore, four different bGH are produced by the pituitary gland. bGH contains two intra-chain disulfide bridges and consists mainly of four anti-parallel α-helices which are arranged in a left-twisted, tightly packed helical bundle (Abdel-Meguid et al., 1987).

In terms of physiological roles of GH, it has been known that GH exerts, either directly or indirectly, anabolic responses such as bone growth, cell division and protein synthesis, increases the oxidation of lipid, and inhibits the transport of glucose into body tissues (Pell and Bates, 1990). A primary physiological role of GH action is assumed to be the preservation of body protein, particularly during periods of energy deficit, by reducing proteolysis and stimulating the incorporation of amino acids into muscle while diverting glucose and fatty acids away from tissue deposition, thus making them available as energy sources (Pell and Bates, 1990).

Effects on lactation

Asimov and Krouze (1937) reported for the first time that injections of crude extracts of bovine anterior pituitary glands enhanced milk production in lactating cows. Young (1947) showed that the galactopoietic response was due to bGH in the pituitary extracts. Later, Cowie et al. (1964) demonstrated that bGH was the major galactopoietic hormone was essential to the maintenance of normal lactation in ruminants. In contrast, prolactin plays the role of major galactopoietic hormone in the rat (Sinha et al., 1974).

Among the early studies, Brumby and Hancock (1955) conducted the most comprehensive evaluation of the galactopoietic effect of GH in lactating cows. Daily injections of 50 mg bGH for 12 weeks beginning in the second month of lactation increased milk production by 72% while milk production increased 44% when treatments were initiated the ninth month of lactation. The results of this study were verified when Machlin (1973) administered 90 mg of highly purified bGH to lactating cows three times a week. After an initial dramatic increase in milk production the lactational profiles declined in parallel with those of control cows. Treatment resulted in a sustained increase of 25-30% in milk production over a 10 week period.

Further progress in bGH research was limited until the early 1980s mainly because of shortages and impurities of pituitary gland extracts from slaughtered animals (Johnsson and Hart, 1986). However, recombinant DNA technology finally made large amounts of pure bGH availabel to the researchers, resulting in stimulating research into re-examinations of the galactopoietic role of bGH and of biological actions of the hormone in the lactating cows (Peel and Bauman, 1987). Taking advantage of the availability of this product, Bauman et al. (1982) conducted the first study with dariy cows using rbGH. They showed that rbGH was as effective as pituitary-derived bGH in enhancing milk production. Since then, a tremendous amount of the research with rbGH (more than 1,000 bGH studies using over 20,000 dairy cows) has been conducted (Bauman, 1992). Milk production has been increased up to 40% with daily injections of rbGH (Peel and Bauman, 1987). Most experiments have utilized rbGH doses between 10 to 50 mg/d with maximum milk response achieved at a rbGH

daily injection of approximately 30-40 mg/d (Johnsson and Hart, 1986; Peel and Bauman, 1987).

Effects on milk fat production

Milk fat is mainly composed of triglycerides which make up 97 to 98% of bovine milk fat (Bauman and Davis, 1974). The fatty acids in milk triglycerides are derived from two sources, circulating lipid in blood and de novo synthesis in mammary secretory cells (Moore and Christie, 1981). It has been experimentally determined that the fatty acids of milk fat with carbon chains of C_4-C_{14} (short- and medium- chain fatty acids) and 50% of C_{16} arise from de novo synthesis within the mammary gland. In contrast, long-chain fatty acids such as $C_{18:1}$ are derived from circulating lipids and arise from either dietary sources or body fat reserves (Christie, 1981). Approximately 40% by weight or 60 molar % of milk fatty acids are synthesized in the mammary gland (Bauman and Davis, 1974). It has been well documented that administration of either pituitary-derived or recombinant-DNA-derived bGH enhance milk production and milk components, including milk fat in dairy cows (Johnsson and Hart, 1986; Peel and Bauman, 1987). Studies involving both short-(less than 30 d) and long-term (more than 30 d) treatments with bGH or rbGH increase milk fat yields by 10-40% (Peel and Bauman, 1987).

Peel et al. (1981) examined effects of highly-purified bGH on milk fat during early lactation in high-yielding dairy cows. bGH 944 mg/d) was injected to Holstein cows for 11 days beginning on day 74 of lactation. The cows treated with bGH increased milk fat production (22.7%). This increase significantly surpassed the increase in milk production (9.5%). The concentration of NEFA in plasma was remarkedly elevated as a result of bGH treatment when cows were in negative energy balance during the experiment period. A similar study conducted by the same group of researchers (Peel et al., 1983) in which bGH (44 mg/d) was administered to lactating Holstein cows in early (wk 12) and late lactation (wk 35) for 10 days. In early lactation. bGH caused an increase of milk fat yield (17%) which was proportional to the increase in milk yield (15%). However, in late lactation, the increase in milk fat yield (42%) by bGH treatment markedly exceeded the increase in milk yield (31%). During control periods, cows were in similar positive energy balance at each stage. However, the reduction in feed intake concurrent with bGH treatments in late lactation resulted in negative energy balances during treatment, whereas during early lactation cows remained in positive energy balance. bGH significantly increased NEFA concentration (68%) of plasma in late lactation, however, only a slight increase in NEFA concentration occurred in early lactation.

Based on the above early studies, researchers have discussed the hypothesis that the energy status of the cow at the time of treatment determines effects of bGH on milk fat. Therefore, bGH increases milk fat yield more than milk yield when cows are in negative energy balance, but proportionally enhances the production of milk and fat, when cows are in positive energy balance. This hypothesis has been further supported by subsequent studies in which bGH markedly enhanced milk fat yields exceeding increases in milk yields when cows were in negative energy balance (Bitman et al., 1984; Eppard et al., 1985; McCutcheon and Bauman, 1986; Lynch et al., 1992), while increased milk fat yields induced by bGH were similar to increase in milk yield when cows were in positive energy balance (Fronk et al., 1983; Bauman et al., 1985, Eppard et al., 1985; Lynch et al., 1992).

Examination of effects of bGH on the fatty acid composition of milk fat provides insight to the mechanism of bGH stimulation of milk fat synthesis. Bitman et al. (1984) reported that short-term administration (14d) of bGH (44 mg/d) to lactating Holstein cows in negative energy balance resulted in less short- and medium- chain fatty acids and more long chain fatty acids, mailnly $C_{18:1}$, in milk fat. The increase in milk fat yields from preformed fatty acids (long-chain fatty acids) with bGH treatment was about three times that of those synthesized de novo. These workers also suggested that the enhanced portion of $C_{18:1}$ in milk fat possibly were derived from increased mobilization of lipid reserves since diet composition did not change. Another study (Eppard et al., 1985) demonstrated that bGH shifted the fatty acid composition of milk fat to an increase in the percentage of $C_{18:1}$. When bGH was administered at 30.9 mg/d to lactating Holstein cows beginning in 15 wk postpartum and continuing for the duration of the lactation, a decrease in the percentages of short- and medium- chain fatty acids and increase in the percentages of long-chain fatty acids was observed (Baer et al., 1989). Differences in fatty acid compositions between bGH-treated and control cows were greatest during the first 8 wk of bGH administration. These difference became smaller as bGH treatment continued.

The comprehensive study was recently completed by Lynch et al. (1992) with nine Holstein cows administered biweekly with a prolonged-release formulation of rbGH commencing at 9 wk postpartum and continued for the full lactation. Administration of rbGH increased milk fat production by increasing output of both short- and

medium-chain fatty acids (de novo synthesis) and long-chain fatty acids (preformed fatty acids). Although average fatty acid composition of milk fat was not affected by rbGH treatment, during the first 13 wk of treatment the percentage of $C_{18:1}$ was higher in milk from rbGH-treated cows. The difference gradually declined as energy balance became less negative. Injection of bGH consistently decreased the percentage of C_{18} in the milk fat. They reported that a possible explanation for a differential effect of bGH on C_{18} versus $C_{18:1}$ is that rbGH treatment might enhance the activity of mammary stearyl-CoA desaturase.

The mode of bGH action underlying effects on milk fat production

At the onset of lactation the mammary gland has metabolic priority over other tissues through coordinated changes, resulting in increasing blood flow to the gland, decreasing the utilization of substrates by peripheral tissues and increasing the metabolic capacity of mammary tissues. This coordination of nutrient partitioning is accomplished by integrated changes in hormone secretion and receptor populations (Bauman and Currie, 1980; Dehoff et al., 1988). In the dairy cow, plasma bGH concentrations increase abruptly at parturition (Ingalls et al., 1793; Oda et al., 1989). It has been generally believed that GH acts as a homeorhetic controller that shifts partitioning of nutrients in support of milk synthesis. Therefore, bGH effects are primarily upon directing the availabilities of absorbed nutrients to the mammary gland. This involves coordinating the metabolism of various body organs and tissues (Johnsson and Hart, 1986; Peel and Bauman, 1987; Vernon, 1989).

One of the major effects of bGH on milk fat production seems to be due to change in capacity of adipose tissues. An increase in plasma concentrations of NEFA with bGH administration *in vivo* and an increase in NEFA release in short-term incubations of bovine adipose tissue *in vitro* (Kronfeld, 1965; Bines and Hart, 1982) support this suggestion. It seems that effects of bGH on adipose tissue are direct. Hart (1983) reported that goats treated with ovine GH dramatically reduced the degree to which hypoglycemia is induced by an insulin challenge. *In vitro* studies with bovine and ovine adipose tissue demonstrated that incubation for a few days with GH antagonized the responsiveness of adipose tissue to insulin (Vernon, 1982; Etherton et al., 1987; Marinchenko et al., 1992). When bGH was administered to lactating cows, the increase in NEFA following an epinephrine challenge is more than doubled relative to controls (McCutcheon and Bauman, 1986). Therefore, it was suggested that bGH treatment alters change the responsiveness of adipose tissue to hormonal signals, presumably by altering numbers and/or affinities of receptors or degree of enzyme activation (Peel and Bauman, 1987). This hypothesis is supported by the studies demonstrated that an increase in the activity of hormone sensitive lipase and decreases in lipoprotein lipase and acetyl-CoA carboxylase in rats administered GH (Bunyan and Greenbaum, 1965; Goodman, 1963; Toshio et al., 1981). Although it is known that bovine adipose thissue has bGH receptors (Collier et al., 1984), no clear cellular mechanisms have been elucidated regarding effects of bGH on bovine adipose tissues.

Rates of blood flow are one of the major factors affecting the intracellular availability of substrates (NEFA, acetate and 3-hydroxybutyrate) for milk fat synthesis in bovine mammary glands. Since rates of extraction of many milk precursors are high, there is close correlation between mammary blood flow per unit weight of tissue and milk production (Linzell, 1974). The increase in milk output during GH treatment has been shown to be associated with proportional increases in mammary blood flow in goats (Hart et al., 1980; Mepham et al., 1984) and cows (Davis et al., 1983; McDowell et al., 1984). McDowell et al. (1984) indicated that changes in blood flow contributed more to the increased uptake of glucose, acetate and 3-hydroxybutyrate than changes in plasma concentrations or extraction percentages across the gland. Overall, increases in cardiac output and peripheral blood flow appear to be general actions of GH in both lactating and non-lactating ruminants (Johnsson and Hart, 1986). Cellular mechanisms responsible for these effects of bGH are not clear. It has been assumed that bGH, acting indirectly via IGF-1, increases the metabolic activity of mammary glands resulting in increased O_2 requirements and CO_2 production, resulting in increasing rates of blood flow to the mammary gland (Bauman and McCutcheon, 1986).

The early hypothesis that the enhanced rate of milk production during bGH injection may be only a passive response to the availabilities of additional nutrients to the mammary gland as a result of GH effects on other tissues was tested by the study of Peel et al. (1982) which demonstrated that milk component yields including fat were not increased, when lactating cows were supplied with additional nutrients postruminally. This study was confirmed by Lough et al. (1984) who reported that there was no increase in milk synthesis when additional nutrients were supplied in combination with bGH as compared to bGH alone. Thus, nutrient availability, expressed in terms of arterial concentrations, may not be

rate-limiting to increases in milk component yields, including fat, during GH administration. In addition, Knight and Peaker (1984) suggested that the decrease in milk yield during the course of a normal lactation is assumed to be due to a decline in the number and/or activity of secretory cells in the mammary gland which occurs in spite of relative improvements in nutrient balance in later lactation. Therefore, one may suggest that bGH may enhance the metabolic capacity of mammary glands to increase milk production due to an increase in the number or activity of epithelial cells of bovine mammary glands.

The mode of action of bGH upon the mammary gland has not been elucidate. Although bGH receptor mRNA has been detected in bovine mammary epithelial cells (Glim et al., 1990), bGH receptors have not been detected on the membranes of epithelial cells in bovine mammary glands (Akers, 1985; Kazmer et al., 1986). In addition, infusion of bGH directly into the mammary artery did not increase milk production in sheep (McDowell and Hart, 1984). Therefore, it appears that bGH does not directly acts on mammary glands.

Many of the anabolic effects derived from bGH have been suggested to be mediated by IGF-1 (Pell and Bates, 1990). bGH administration to lactating dairy cows increased the plasma concentrations of IGF-1 (Davis et al., 1984; Peel et al., 1985; Glimm et al., 1988). The presence of high affinity receptors for IGF-1 in bovine mammary glands has been demonstrated (Campbell and Baumrucker, 1986; Dehoff et al., 1988; Glimm et al., 1988). Further, lactation is associated with increases in the concentrations of IGF-1 receptors in bovine mammary glands (Dehoff et al., 1988). Thus, it is highly possible that bGH acts on the mammary gland indirectly via its positive effects upon IGF-1 synthesis and secretion by liver.

IGF-1 AS A MEDIATOR OF GH

IGF-1

It has been well knowned that IGF-1 has both metabolic and mitogenic activities in many cells and tissues (Van Wyk, 1984; Lowe, 1991; Cohick and Clemmons, 1993). IGF-1 contains a number of insulin-like properties, primarily a strong hypoglycemic effect, thus has been named as "insulin-like growth factor" (Ross and Buchanan, 1990). Like insulin, IGF-1 is composed of B and A domains containing 70 amino acids with molecular weights of about 7,500 Da and shows high structural homology to insulin (49%) (Rinderknecht and Humbel, 1978). The amino acid sequences of the mature circulating form of IGF-1 is more than 92% conserved among mammalian species (Foyt and Roberts, 1991). The amino acid sequence of IGF-1 purified from bovine serum is identical to that purified from human serum (Honegger and Humbel, 1986).

While the majority of circulating IGF-1 is of hepatic origin (Froesch et al., 1985), many other tissues contain significant concentrations of IGF-1, as a results of local production (D'Ercole et al., 1984). Thus, synthesis of IGF-1 at a lot of locations suggests that is may function in an autocrine/paracrine fashion in addition to having the classical endocrine mode of action. In the bovine, IGF-1 has been also detected in colostrum and milk (Malven et al., 1987; Francis et al., 1988), follicular fluid (Spicer et al., 1988), and mammary lymph (Lacasses et al., 1991).

Relationship between bGH and IGF-1

The observation that cartilage from hypophysectomized rats was unresponsive to GH added *in vitro,* but incorporated sulphate in response to normal rat serum and serum from GH-treated hypopysectomized rats (Salmon and Daughaday, 1957) led to the original IGF-1 hypothesis. This hypothesis suggested that GH from the pituitary exerts its effects by stimulating IGF-1 release from the liver which then mediates anabolic actions in target tissues.

However, two lines of evidences argued against the original hypothesis. One of these arose from studies demonstrating that many tissues other than liver produce significant levels of IGF-1 (D'Ercole, 1984). This observation has been confirmed by demonstrations of the presence of IGF-1 mRNA in many extrahepatic tissues (Murphy et al., 1987; Han et al., 1988). The other evidence is that GH has direct anabolic actions on some tissues mediated via GH receptors instead of via IGF-1. For example, local administration of GH into the proximal tibial epiphyseal plates of hypophysectomized rats stimulates longitudinal bone grwoth on the injected side (Russell et al., 1985). Local administration of GH through cannulae implanted into the bony epiphysis produces a consistent, dose-dependent response suggesting that GH effectively interacts with its target cells by this route of administration (Isgaard et al., 1986). In addition, GH stimulates differentiation of pre-chondrocytes in the epiphyseal growth plate which then undergo chonal expansion in response to locally expressed IGF-1, suggesting that GH and IGF-1 are both required for bone growth (Isaksson et al., 1987. Further, IGF-1 mRNA levels has been shown to be regulated by GH in many extrahepatic tissues (Holly and Wass, 1989).

These observations have led to modification of the original IGF-1 hypothesis which difined IGF-1 as the

endocrine mediator of GH action. However, significant amounts of IGF-1 (more than 50% of total body content) are present in the circulation and originated from hepatic production of IGF-1 in rats (Schwander et al., 1983). This together with the observation that IGF-1 receptors are clearly absent from adult liver (Caro et al., 1988) supports the original hypothesis that hepatic IGF-1 is not acting locally within the liver but enters the circulation to perform some endocrine function. The recent observation of Hodgkinson et al. (1991) that n-methionyl IGF-1 administered to lactating sheep was unequally distributed to target tissues suggests that blood borne IGF-1 may conduct specific endocrine functions in selected tissues. In this study, it was also demonstrated that 85% of IGF-1 in mammary epithelial cells is derived from circulating IGF-1. Therefore, the endocrine function of IGF-1 might be prominent in some tissue and, especially, in mammary glands.

Ronge et al. (1988) reported that in dairy cows, IGF-1 concentrations in circulation were low at peak lactation when GH concentrations were concomitantly enhanced. They also demonstrated a negative relationship between blood IGF-1 concentrations and milk yield over the entire duration of lactation. In contrast, it has been well documented that administration of bGH significantly increases the circulating concentrations of IGF-1 in dairy cattle (Davis et al., 1987; Cohick et al., 1989; Prosser et al., 1989, Cisse et al., 1991). Furthermore, when the distribution pattern of immunoreactive IGF-1 was determined in both normal and bGH-treated lactating bovine mammary tissues, a prominent elevation in immunoreactive IGF-1 were observed in the cytoplasm of mammary epithelial cells of tissue from bGH-treated cows (Glimm et al., 1988).

Biological actions on mammary glands

The mechanisms associated with bGH actions in enhancing milk production are not entirely elucidated. It is obvious from the discussion presented in the previous sections that bGH effects may be mediated, at least in part, by IGF-1. Thus, IGF-1 may act directly on the mammary gland to increase synthesis of milk components. The number and metabolic activities of secretory cells of the mammary glands are the major factors determining the ability of a cow to produce milk (Johnsson and Hart, 1986). However, currently little is known concerning the mitogenic and metabolic effects of IGF-1 on bovine mammary secretory cells.

In vitro studies have shown that IGF-1 stimulated [^{3}H]-thymidine incorporation into DNA of mammary gland explants from virgin heifers (Shamay et al., 1988) and lactating cows (Baumrucker and Stemberger, 1989). In these studies, it was not clear whether or not increased DNA synthesis induced by IGF-1 resulted in increased secretory cell numbers or larger cells since the mitogenic effect of IGF-1 was evident in all of the heterogeneous cell types in the mammary gland explants. McGrath et al. (1991) demonstrated that IGF-1 enhances cell proliferation in cultures of mammary gland epithelial cells isolated from non-lactating heifers was demonstrated. In addition, IGF-1 stimulates cell proliferation in a human breast cancer cell line (Myal et al., 1984) and the COMMA-D mouse mammary cell line (Riss and Sirbasku, 1987). It is well documented that IGF-1 is capable of exerting mitogenic effects in a variety of cell types of various species (Lowe, 1991). Therefore, the above findings suggest, but do not prove, that IGF-1 acts as a mediator of bGH by increasing secretory cell numbers rather by increasing metabolic capacities of mammary secretory cells.

In contrast, a number of data suggest that IGF-1 exerts metabolic effects on other bovine cell types. When bovine adrenal fasculata cells are treated with IGF-1, corticosterone biosynthesis is increased in a dose-dependent manner. This augmentation of steroidogenesis is apparently due to increases in the activities of several enzymes in the pathway of steroid biosynthesis (Penhoat et al., 1988). Dopamine β-hydroxylase activity in bovine adrenal chromaffin cells is enhanced by IGF-1 regardless of the mitogenic activity of IGF-1 (Wilson, 1991). Moreover, IGF-1 treatment of bovine granulosa cells stimulates the release of oxytocin and increases oxytocin mRNA levels (Holtorf et al., 1989). In bovine pulmonary artery smooth muscle cells, IGF-1 increases the synthesis of an mRNA encoding tropoelastin (Badesch et al., 1989). It has been also well documented that IGF-1 exerts metabolic effects associated with the stimulation of protein and mRNA synthesis in various cell types of many other species (Lowe, 1991).

Some *in vivo* studies support the possible metabolic effects of IGF-1 on the mammary gland. In a study with goats (Fleet et al., 1988), IGF-1 was infused via the public artery directly supplying either the left or right mammary gland. Infusion of IGF-1 for 6 h caused a 30% increase in the rate of milk secretion in the infused gland compared with a 15% increase in the non-infused gland. Increase in milk secretion on the infused side increased within 4 h of infusion. Further, data on effects of IGF-1 upon metabolic activity per secretory cell are, essentially, limited to the data of Middleton et al. (1988; largely unpublished) which indicated, not always statistically significant, increases in RNA, enzyme and metabolic

activities per unit DNA and/or per total mammary glands from bGH as compared to control cows. If IGF-1 causes increases in milk synthesis by direct actions on the mammary gland, one can assume that IGF-1 increases metabolic activities of mammary secretory cells, resulting from enhancing the activities of rate-limiting enzymes in the pathways of milk component synthesis. Available data support this view but are very limited as noted above.

IGFBPs: Modulators of IGF-1 action

Unlike most polypeptide hormones, the majority of IGF-I in circulation is bound to high affinity, soluble binding proteins (Baxter and Martin, 1988). IGFBPs function in various ways to modify the biological actions of IGF-1, thus making determinations of IGF-1 actions more complicated (Clemmons, 1991; Cohick and Clemmons, 1993; Jones and Clemmons, 1995). IGFBPs have been detected in the plasma as well as the other biological fluids of a variety mammals (Clemmons, 1991) including cows (Ronge and Blum, 1989). Furthermore, it has been demonstrated that media from bovine tissues, cells, and cell lines in culture also contain IGFBPs (Conover, 1990; Campbell et al., 1991; Cohick and Clemmons, 1991; McGrath et al., 1991).

Six forms of IGFBPs in rats and humans, designated as IGFBP-1 through-6, have been cloned and their complete primary structures has been determined (Cohick and Clemmons, 1993). In bovine, IGFBP-2 (Bourner et al., 1992) and -3 (Spratt et al., 1991) have been cloned and sequenced. Although six different IGFBPs have been identified, five forms are detected using ligand blotting methods in sera of several species (Rechler and Nissley, 1990). Bovine serum contains the five forms of IGFBPs which are present in other species (Cohick et al., 1992). Two forms with molecular weights of 43 Kd and 39 Kd have been identified as IGFBP-3, representing the acid-stable subunit of a high molecular weight complex (150 Kd) which binds most of the circulating IGF-1 (Baxter and Martin, 1989). These two forms appear to be different glycosylation variants of the same gene products (Wood et al., 1988). The 34 Kd band present in human and bovine serum corresponds to IGFBP-2 (Upton et al., 1990). A 29 Kd band in human and rat sera has been identified as IGFBP-1 (Hardouin et al., 1989; Murphy et al., 1991) and the 24 Kd band present in sera of most species has been identified as IGFBP-4 (Shimonaka et al., 1989; Shimasaki et al., 1990).

The functions of IGFBPs have not been completely elucidated. It has been proposed that functions of IGFBPs are: 1) to regulate IGF-1 transport in blood; 2) to regulate the amount and rate of IGF-1 transport from the vascular compartment; 3) to direct the transport of IGF-1 to specific tissues and cell types; 4) to control the binding of IGF-1 to its receptors; 5) to regulate the half-life of IGF-1; and, 6) to regulate IGF-1 actions on target cells by inhibiting or potentiating IGF-1 mediated cell growth responses and metabolic actions (Clemmons, 1991; Jones and Clemmons, 1995).

It appears that IGFBPs have a crucial role in the regulation of the bioavailabilty of IGF-1. The 150 Kd of ternary complex (IGF-1 · IGFBP-3 · acid-labile unit), which is not able to cross the capillary barrier, protects IGF-1 from enzymatic degradation and rapid clearance from the circulation thereby prolonging the serum half-life of IGF-1 in the circulation (Hodgkinson et al., 1987; Binoux et al., 1991). It has been also reported that IGFBPs may modulate IGF-1 actions at the cellular level. Addition of purified IGFBP-1, -2, -3, or -4 to various cells in culture in culture inhibits binding of IGF-1 to cell surfaces and thus decreases IGF-1 bioactivity by sequestering IGF-1 and preventing receptor interaction (Cohick and Clemmons, 1993). The biological effects of purified bovine IGFBP-3 on IGF-1 action in bovine fibroblasts have been investigated. Co-incubation of bovine fibroblasts with IGFBP-3 inhibits IGF-1 actions on aminoisobutyric acid uptake and thymidine incorporation, while preincubation with IGFBP-3 is potentiating (Conover et al., 1990).

Administration of bGH to cows elevated IGFBP-3 concentrations in sera (Vicini et al., 1991; Cohick et al., 1992). This effect is likely to be mediated by IGF-1 because direct infusion of IGF-1 into hypophysectomized rats increases IGFBP-3 (Zapf et al., 1989) and IGFBP-3 levels are increased due to overexpression of an IGF-1 transgene in either normal of GH-deficient transgenic mice (Camacho-Hubner et al., 1991). In contrast to the increase observed in IGFBP-3, circulating IGFBP-2 concentrations in cows declined in response to bGH administration (Vicini et al., 1991; Cohick et al., 1992). This suppressive effect may be due to a direct action of bGH since plasma IGFBP-2 levels are elevated approximately threefold in hypophysectomized rats (Orlowski et al., 1990) and infusion of IGF-1 into two human subjects did not raise the levels of IGFBP-2 when these subjects were treated with GH (Zapf et al., 1990). Therefore, one can assume that the reduction of IGFBP-2 and increase in IGFBP-3 and the acid-labile subunit might cause a decrease in the rate of IGF-1 transport from circulation. Indeed, the decrease in IGFBP-2 concentrations in cow plasma observed during bGH treatment was paralleled by a decrease in IGFBP-2 concentrations in afferent mammary lymph, whereas

IGFBP-3 concentrations did not change (Cohick et al., 1992).

Like the production of IGF-1, liver is not the only source of IGFBPs. IGFBPs are secreted by extrahepatic tissues and several types of cultured cells (Clemmons, 1991). Bovine mammary explants from pregnant non-lactating and lactating non-pregnant cows were found to synthesize and secrete IGFBPs including four forms ranging in molecular weight from 29 Kd to 46 Kd (Campbell et al., 1991). Similar forms of IGFBPs were detected in media from bovine mammary epithelial cells grown *in vitro* on collagen matrices (McGrath et al., 1991).

Syntheses of IGFBPs by various cell types in culture are known to be regulated by hormonal factors including IGF-1 and insulin (Cohick and Clemmons, 1993). Bovine mammary epithelial cells cultured in the presence of IGF-1 produced 20-25 ng/ml IGFBP-2 while control cultures secreted approximately 1.0 ng/ml, whereas insulin did not increase IGFBP-2 production (McGrath et al., 1991). On the other hand, Conover (1990) reported that both IGF-1 and insulin increased the production of IGFBP-1, -2, and -4 in a dose-dependent manner and induced IGFBP-3 when bovine fibroblasts were cultured in serum-free media. Moreover, IGFBP-2 synthesis by a bovine kidney epithelial cell line was enhanced by insulin and IGF-1 (Cohick and Clemmons, 1991). These findings are consistent with observations in human fibroblasts (Hill et al., 1989) and rat muscle cells (McCusker and Clemmons, 1988), in which insulin and IGF-1 enhanced IGFBP production. However, one can not rule out the opposite effects of insulin and IGF-1 on the IGFBP production because of the observation (Clemmons, 1991) that depending on cell type and IGFBP species, IGF-1 and insulin can either enhance or inhibit IGFBP syntheses.

Signal transduction

From the discussion in the previous sections, it appears that the potential involvement of IGF-1 in mediating the galactopoietic effects of bGH may be to increase the metabolic activities of existing secretory cells in the mammary gland, thereby enhancing rates of synthesis of milk components. Metabolic effects of IGF-1 are the result of a chain of events which are initiated by the binding of IGF-1 ligand to the extracellular portion of its receptor. Conformational changes in the receptor structure caused by the ligand binding activate the cytoplasmic tyrosine kinase-containing domain of the molecule, resulting in transduction of the IGF-1 signal through a mode of action which remains obscure (Yarden and Ullrich, 1988).

The receptors for IGF-1 and insulin have similar morphology, consisting of two a subunits and two b subunits that are connected by disulfide bonds to form the functional β-α-α-β heterotetrameric receptor complex (Rechler and Nissley, 1985). The similarity of the receptors becomes most remarkable in the tyrosine kinase domain which is the functional part of the receptor (Czech, 1989). Further, both receptors bind IGF-1 and insulin, although they bind their specific ligands with 100- to 1000- fold higher affinities than the cross-reacting ligand (Moxham et al., 1989). These similarities and the similar metabolic effects of IGF-1 and insulin made researchers to postulate that the two receptors mediate similar biological responses (Czech, 1989; Werner et al., 1991).

Since both receptors contain intrinsic tyrosine kinase activity, it seems likely that the signalling cascade from these receptors is initiated by tyrosine phosphorylation of substrate proteins and that sequential phosphorylation events may lead to the biological responses of IGF-1 and insulin (Werner, 1991). Indeed, IGF-1 and insulin stimulate phosphorylation of tyrosine residues in a 175 Kd protein (named as insulin receptor substrate-1) in a rat thyroid cell line, possibly by the receptors (Condorelli et al., 1989). A recent study demonstrated that the tyrosine-phosphorylated 175 Kd protein (receptor substrate-1) binds tightly to phosphatidylinositol 3-kinase, thus activating this enzyme (Giorgetti et al., 1993).

Short-term metabolic effects of insulin result from regulation of key enzymes, at least in part, due to enzyme phosphorylation (Brownsey and Denton, 1987). For example, it is generally believed that insulin increases the activity of acetyl-CoA carboxylase (ACC) by phosphorylating serine/threonine residues of the enzyme at a site distinct from the cAMP-dependent protein kinase phosphorylations site (Brownsey and Denton, 1987). Later, Kim (1989) suggested that activation of different serine/threonine kinases by distinct hormones (insulin and IGF-1 vs glucagon and epinephrine) is responsible for phosphorylating separate serine/threonine residues on ACC. This explains the paradox that both activation and inhibition of ACC can occur by phosphorylation of serine/threonine residues.

No information is available about ACC phosphorylation by IGF-1. However, it was reported that epidermal growth factor activates ACC activity by phosphorylating the enzyme on the same phosphorylation sites as does insulin in rat adipocytes (Haystead and Hardie, 1986a). Taken together, it is reasonable to postulate that IGF-1 may be involved in short-term regulation of ACC, the limiting enzyme in the synthetic pathway of milk fatty

acids (see below) by phosphorylating the enzymes as described for insulin.

In terms of long-term metabolic effects, insulin and growth factors increase phosphorylations of serine residues on ribosomal protein S6 by a phosphorylated and activated ribosomal protein S6 kinase (Smith et al., 1979). This may increase rates of translation since phosphorylation prevents dissociation of 40S ribosomal subunits from polysomes (Thomas et al., 1980; Duncan and McConkey, 1982). Ribosomal protein S6 is a component of the eukaryotic 40S ribosomal subunit and likely to be involved in initiation of translation (Terao and Ogata, 1979).

Isozaki and Kohn (1987) reported that when FRTL-5 rat thyroid cells were treated with IGF-1, c-myc and c-fos known as transcriptional factors were induced without increasing cell numbers. During preadipocyte differentiation, IGF-1 also increases a c-fos protien which in turn induces expression of specific genes related to lipid synthesis including glycerol phosphate dehydrogenase (Distel et al., 1987). Meanwhile, Oemar et al. (1991) observed that stimulation of mesangial cells with IGF-1 results in the tyrosyl phosphorylation of a transcriptional factor c-jun and other nuclear proteins. It has been well documented that transcription factor functions are directly regulated by phosphorylation (Hunter and Karin, 1992).

Therefore, it is possible that in the long-term effects IGF-1 are mediated via activation of transcriptional factors by tyrosine or serine/threonine phosphorylations. These then stimulate transcription of genes for the lactogenic enzymes, such as acetyl-CoA carboxylase, in the mammary gland.

ACETYL-CoA CARBOXYLASE (ACC)

Biochemical nature

Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, which is the first step in fatty acid synthesis. It is generally accepted that ACC is the rate-limiting enzyme in fatty acid synthesis (Volpe and Vagelos, 1976; Wakil et al., 1983; Numa and Tanabe, 1984; Kim et al., 1989). The observations that tissue concentrations of malonyl-CoA vary in parallel with rates of fatty acid synthesis and that ACC activity changes in response to the lipogenic needs of the cell reflect the rate-limiting nature of ACC (Kim et al., 1989).

In terms of cow mammary fatty acid synthesis, two original studies demonstrated the regulatory role of ACC. Mellenberger et al. (1973) reported a correlation of 0.97 between ACC activity and lipogenic capacity in tissue biopsies obtained at four time points from 30 days prepartum to 40 days postpartum in cows. Activities of other bovine mammary enzymes including fatty acid synthetase did not correlate (Baldwin, 1966; Mellenberger et al., 1973).

In general, ACC in homogenized tissue samples is found in the cytoplasm following subcellular fractionation (Volpe and Vagelos, 1976). However, in subcellular fraction studies of rabbit mammary tissue, it was demonstrated that ACC was present in both the cytosol and microsomal fraction (Easter and Dils, 1968). Thus, *in vivo*, ACC may be loosely associated with the microsomal particles. Aslo, it was found that rat liver contains substantial quantities of a relatively inactive but immunologically precipitable form of ACC bound to the mitochondrial outer membrane (Allred and Roman-Lopez, 1988). Roman-Lopez et al. (1989) suggested that the concentration of the active form of the cytosolic ACC increased at the expense of mitochondrial forms when fasted rats were refed.

In ACC preparations from various animal tissues, analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has shown that a molecular weight of ACC ranges from 220 Kd to 260 Kd (Numa and Tanabe, 1984). Some of this variability may be explained by the use of different molecular weight standards; by the enzyme from the different animals having slightly different molecular weights; or by proteolytic cleavage artifacts (Brownsey and Denton, 1987). The complete amino acid sequence of ACC from rat mammary glands was deduced by cloning and sequence analysis of DNA complementary to its mRNA (Lopez-Casillas et al., 1988). The complete coding sequence containing 7035 bases encodes a polypeptide chain of 2345 amino acids having a molecular weight of about 265 Kd. Recently, a unique biotin-containing cytosolic protein of a molecular weight 280 Kd that is distinct from the 265 Kd form has been identified in rat mammary glands and is considered an isozymic form of ACC (Bianchi et al., 1990).

There is general agreement that ACC exists in at least two major forms which are an active polymer and an inactive protomer (Numa and Tanabe, 1984; Kim et al., 1989). The inactive protomer (dimer form) has a molecular weight of 500 Kd containing two identical subunits (Beaty and Lane, 1983). Each subunit contains one biotin and active sites for the enzyme action. The polymerized active form represents a large filamentous polymer with a molecular weight of up to 10,000 Kd which corresponds to the aggregation of up to 20 inactive dimers to give filaments of up to 0.5 mm in length

(Brownsey and Denton, 1987). Dissociation to the protomeric form is favored by low protein concentration, NaCl, pH greater than 7.5, palmityl-CoA, and malonyl-CoA, whereas the equilibrium toward the aggregated polymeric form is favored by citrate, acetyl-CoA, high protein concentration, and pH 6.5-7.0 (Lane et al., 1974).

Enzyme preparations are routinely preincubated with 10-20 mM citrate at temperatures between 25℃ and 30℃ for about 30 min to establish full activation of ACC since no activity of the enzyme is detected without citrate (Lane et al., 1974). Citrate is one of the notable metabolites which mediate the polymerization of ACC. Thus, it has been postulated that citrate functions as a positive activator by inducing polymerization of the enzyme (Kim et al., 1989). This hypothesis has been questioned by the observations that the citrate concentration in the cytosol is an order of magnitude lower than that required for ACC activation *in vitro* and that there is no apparent correlation between lipogenesis and citrate concentration (Kim et al., 1989).

Reaction mechanisms

In the reactions of ACC, biotin bound to the protein becomes carboxylated in a reaction that requires ATP and bicarbonate. In the second step, the carboxyl group of carboxybiotin is trans ferred to an acceptor, usually acetyl-CoA forming malonyl-CoA (Knowles, 1989). These reactions can be summarized:

Biotin-Enz. + ATP + HCO3 → COO--Biotin-Enzyme + ADP + Pi ..... (1)

COO-Biotin-Enz. + Acetyl-CoA → Malonyl-CoA + Biotin-Enzyme ..... (2)

Overall: Acetyl-CoA + ATP + HCO3 → Malonyl-CoA + ADP + Pi ..... (3)

The reaction mechanism of ACC has been relatively well elucidated in Escherichia Coil. ACC in E. Coli is readily separated into three active subunits: a biotin-carboxyl carrier protein (44 Kd), a biotin carboxylase (98 Kd), and a carboxyltransferase (130 Kd) (Volpe and Vagelos, 1976). The first reaction (1) involves the carboxylation of biotin. The biotin prosthetic group is attached by linkage of its valeric acid side chain to a lysine residue of a biotin-carboxyl carrier protein and plays an essential role in the carboxylation reaction (Alberts and Vagelos, 1972). Carboxylation of the biotinyl moiety of the biotin-carboxyl carrier protein by bicarbonate is catalyzed by the ATP-dependent biotin carboxylase. It has been proven that the carboxylated site of biotin is the l'-nitrogen of the ureido ring (Volpe and Vagelos, 1976). The most favored possible mechanism of ATP-dependent biotin carboxylase involves the activation of bicarbonate by ATP to produce carboxyphosphate, then this reactive carboxyphosphate carboxylates the l'-nitrogen of biotin (Knowles, 1989).

In the second reaction (2), carboxyltransferase catalyzes the transfer of the activated carboxyl group from carboxybiotin to acetyl-CoA to form malonyl-CoA (Alberts and Vagelos, 1972). Prior to the transfer of the active carboxyl group to acetyl-CoA, the carboxybiotin must be shifted to the active site of carboxyltransferase (Numa and Tanabe, 1984). The swinging arm theory accounting for this movement is that the length, rotation and flexibility of the link between biotin and its carrier protein allow the activated carboxyl group to move from one active site to another (Numa and Tanabe, 1984).

In contrast, the eukaryotic ACC is composed of one kind of polypeptide which carries out the functions of the biotin-carboxyl carrier protein, biotin carboxylase, and carboxyltransferase as well as the regulatory function, thus exhibiting a single, integrated multifunctional polypeptide (Wakil et al., 1983). Recently, the cDNA coding for ACC in the rat mammary gland was cloned and sequenced (Lopez-Casillas et al., 1988). On the basis of this sequence identity and availabilities of cDNAs for the other biotin-containing enzymes, the functional domains of ACC have been tentatively identified. The functional domains of the ATP and HCO3-interaction site, and acetyl-CoA and biotin binding sites have been proposed in the deduced amino acid sequence of rat mammary ACC (Lopez-casillas, 1988). Two proline residues at positions away from the biotin- binding site could serve as hinges for bending the protein so that the ATP and HCO3-interaction site comes close to the biotin site, thus possibly allowing a biotin-containing arm to flip-flop between the carboxyl donor and acceptor sites (Kim et al., 1989).

HORMONAL REGULATION OF ACC

Short-term

Short-term hormonal regulation of ACC is achieved by modulating enzyme activity covalently resulting from phosphorylation and dephosphorylation of the enzyme by kinases and phosphatases, respectively (Kim, 1983; Brownsey and Denton, 1987). These effects can be observed within a few minutes of exposure of cells to hormones and thus are very unlikely to involve changes in amount of enzyme (Kim, 1983; Brownsey and Denton, 1987). Incubation of rat adipocytes with ^{32}P phosphate results in the incorporation of ^{32}P into ACC (Brownsey et

al., 1977). Carefully prepared ACC from both rat liver and mammary glands contains multiple phosphates which are as many as 8 moles of phosphate per mole of ACC (Witters and Vogt, 1981; Thampy and Wakil, 1985). These observations indicate that ACC in animal tissues is intrinsically phosphorylated.

In rat mammary glands, inhibition of fatty acid synthesis by cyclic AMP (cAMP)-elevating hormones such as glucagon and epinephrine has not been demonstrated (Plucinski and Baldwin, 1982, Clegg et al., 1986). The mammary gland is refractory to the usual events initiated by an increase in intracellular cAMP (Clegg et al., 1987) and glucagon receptors are absent from rat mammary epithelial cells (Robson et al., 1984). In contrast, it is well established that glucagon induced increases in cAMP inhibits fatty acid synthesis in rat hepatocytes (Witters et al., 1979; Holland et al., 1984). In adipocytes, epinephrine (Brownsey et al., 1979; Zammit and Corstorphine, 1982) and glucagon (Zammit and Corstorphine, 1982) have similar effects.

Lee and Kim (1979) preincubated rat adipocytes with ^{32}P phosphates for 3 hr and then examined the effects of epinephrine on both phosphorylation and ACC activity after 30 minute more incubation During the 30 minute period no further incorporation of ^{32}P into ACC of the control tissue observed. In contrast, in the presence of epinephrine, the incorporations of ^{32}P into ACC almost doubled within 30 minutes while the enzyme was inactivated by 61%. Witters et al. (1979) also provided evidence of a relationship between phosphorylation and inactivation of rat hepatocyte ACC following glucagon treatment. Subsequently, similar observations that glucagon and epinephrine stimulate phosphorylation of ACC and decrease enzyme activity have been documented in rat hepatocytes by glucagon (Holland et al., 1984; Mabrouk et al., 1990) and epinephrine (Mabrouk et al., 1990), and in adipocytes by epinephrine (Witters et al., 1983; Haystead et al., 1990) and glucagon (Holland et al., 1985).

Rat liver ACC which has been phosphorylated and inactivated with a protein kinase can be dephosphorylated and reactivated by incubating with a protein phosphatase (Carlson and Kim, 1973). This finding was supported by the subsequent obserbvations that incubating purified ACCs from lactating rat and rabbit mammary glands with protein phosphatases resulted in dephosphorylation and activation of ACC (Hardie and Cohen, 1979; Hardie and Guy, 1980).

The physiological significance of the relationship of phosphorylation status and ACC activity has been investigated. Citrate is a required activator of animal ACC with half maximal activation observed at concentrations of 2-10 mM (Kim, 1979). This citrate concentration is significantly higher than intracellular concentration of 0.15-0.45 mM and thus may not be an appropriate indicator of the rate of fatty acid synthesis (Kim, 1983). Holland et al. (1984) demonstrated that glucagon causes a significant decrease in fatty acid synthesis by phosphorylation of ACC in rat hepatocytes without a concomitant decrease in the cellular content of citrate. Some studies demonstrated that the dephosphorylated ACC requires less citrate for maximum activation than the phosphorylated forms (Carlson and Kim, 1974; Hardie and Guy, 1980). In detail, phosphorylated ACC has a Km for citrate of 2.4 mM, whereas the Km of the active dephosphorylated form is 0.2 mM (Calrson and Kim, 1974).

Further, the phosphorylated forms of ACC are more susceptible to inactivation and inhibition by various negative effectors such as palmityl-CoA, avidin and ATP than the dephosphorylated forms (Carlson and Kim, 1974; Brownsey et al., 1979). Taken together, these observations suggest that covalent phosphorylation of ACC is prerequisite for the functions of the allosteric mechanisms in the presence of metabolites and support the hypothesis that changes in the phosphorylation status of ACC can influence activity at physiological concentrations of citrate (Kim et al., 1989).

In addition, it has been observed that phosphorylation and inactivation of ACC are accompanied by the conversion of active polymers into inactive protomers (Lent et al., 1978; Shiao et al., 1981), whereas dephosphorylation of ACC by a phosphatase results in polymerization of ACC (Thampy and Wakil, 1988). Mabrouk et al. (1990) showed that there are more inactive protomer forms of ACC in the livers of rats injected glucagon or epinephrine and that these protomers have a higher phosphate content than protomers isolated from the livers of control animals.

The signal transduction system responsible for phosphorylation of ACC by cAMP-elevating hormones has been intensively investigated. Acetylj-CoA carboxylases purified from rat mammary glands (Hadie and Cohen, 1978; Hadie and Guy, 1980; Munday and Hardie, 1984) and rat livers (Tipper and Witters, 1982; Witters et al., 1983) are phosphorylated and inactivated by cAMP-dependent protein kinases. These observations are consistent with those found after exposure of intact adipocytes and hepatocytes to epinephrine and glucagon as described previously. Mapping of tryptic or chymotryptic ^{32}P-peptides isolated by high performance liquid chromatography (HPLC) revealed that the major ^{32}P-

peptide phosphorylated by epinephrine in rat adipocytes (Brownesey and Hardie, 1980; Witters et al., 1983; Holland et al., 1985) co-migrated with the major ^{32}P-peptide phosphorylated by cAMP-dependent protein kinase. These findings suggest that cAMP-elevating hormones inactivate ACC via activation of the cAMP-dependent protein kinase.

Three cAMP-independent ACC kinases which completely lack sensitivity to protein inhibitors of cAMP-dependent protein kinases were isolated from lactating mammary glands (Munday and Hardie, 1984). Among these kinases, ACC kinase 3 is known to cause inactivation of ACC (Munday et al., 1988b). This enzyme is stimulated by phosphorylation and AMP (Carling et al., 1989) and is now termed AMP-activated protein kinase (Hardie et al., 1989). Recent studies (Munday et al., 1988a; Davis et al., 1990) demonstrated that cAMP-dependent protein kinase which phosphorylates at ser-77 and ser-1200 caused modest inactivation while AMP-activated protein kinase which phosphorylates at ser-79 and ser-1200 causes a much more dramatic inactivation in ACC purified from lactating rat mammary glands. Phosphorylation of ser-77 and ser-79 was responsible for the observed inhibition of the activity (Munday et al., 1988a; Davis et al., 1990). Further, when ACC was isolated from rat hepatocytes and adipocytes which had been incubated with glucagon and epinephrine and ^{32}P-peptide containing ser-77 and ser-79 was sequenced, Ser-79 was phosphorylated but ser-77 was not (Sim and Hardie, 1988; Haystead et al., 1990). This lack of phosphorylation of ser-77 demonstrated that although cAMP-dependent protein kinase phosphorylates ACC in cell-free extracts, this phosphorylation does not occur in intact cells (Haystead et al., 1990). This observation strongly suggests that ACC may not be a physiological substrate for cAMP-dependent protein kinase, and that cAMP-elevating hormones may cause phosphorylation and inactivation of ACC through AMP-activated protein kinase, which is, in turn, activated by a 'kinase kinase' system (Haystead et al., 1990).

On the other hand, it is well established that insulin stimulates fatty acid synthesis by increasing ACC activity within a few minutes in rat adipocytes (Brownsey and Denton, 1982; Zammit and Corstorphine, 1982), hepatocytes (Witters et al., 1979; Buechler et al., 1984), and lactating mammary acini (Williamson et al., 1983). In ruminants, studies of insulin responses of the lipogenic tissues have been greatly hampered by the generally low and variable response of the tissue to insulin during short-term (up to 3 hr) incubations (Vernon and Sasaki, 1989). However, Yang and Baldwin (1973) demonstrated that insulin caused a doubling of rates of fatty acid synthesis in bovine adipocytes during the short-term incubations. Although lactating bovine mammary glands appear to be refractory to insulin effects on fatty acid synthesis (Laarveld et al., 1985), insulin is able to bind to bovine mammary glands due to the presence of insulin receptors (Oscar et al., 1986).

Although insulin and cAMP-elevating hormones have contrasting effects on catalytic activity of ACC and fatty acid synthesis, insulin activation of ACC is also associated with an increase in the overall extent of phosphorylation of the enzyme in rat hepatocytes (Witters, 1981; Holland and Hardie, 1985) and adipocytes (Brownsey et al., 1977; Brownsey and Denton, 1982; Haystead and Hardie, 1986; Haystead and Hardie, 1988).

However, two-dimensional analyses of tryptic peptides derived from ^{32}P-labelled ACC isolated from rat adipocytes by immunoprecipitation demonstrated that the principle sites exhibiting increased phosphorylation in the adipocytes exposed to insulin was quite distinct from those in cells exposed to epinephrine (Brownsey and Denton, 1982). The major effect of insulin was to cause a marked increase in the phosphorylation of a peptide (I-peptide), whereas epinephrine resulted in increased phosphorylation of a separate group of two to three peptides with similar mobilities (Brownsey and Denton, 1982). Similar results were obtained by Witters et al. (1983) and Holland and Hardie (1985) using rat hepatocytes and adipocytes, respectively. Further, the polymerized form of ACC, which is acctive in the absence of citrate, was found to exhibit increased phosphorylation within the 'I'-peptide in rat adipocytes (Borthwick et al., 1987). Taken together, these studies demonstrated that exposure of rat adipocytes to insulin and epinephrine led essentially to phosphorylation of different sites on ACC and suggesting that these different patterns of phosphorylation by different protein kinases might be associated with opposite changes in enzyme activity.

It has been in dispute whether the increased phosphorylation by insulin causes activation of the enzyme since the effect of insulin on the enzyme activity does not persist during enzyme purification, although the phosphorylation state is preserved (Brownsey and Denton, 1982; Haystead and Hardie, 1986b; Witters et al., 1988). This contrasts with the inactivating effects of cAMP-elevating hormones, which are quite stable to purification (Holland et al., 1984 and 1985). Further, it was reported that insulin activation of ACC apparently involves the phosphorylation of low-molecular-weight effector and not ACC itself in rat adipocytes (Haystead and Hardie, 1986b).

Recently, Mabrouk et al. (1990) reported that ACC from livers of untreated rats was relatively low in activity and high in phosphate content. Whereas the enzyme from livers from rats that received insulin has higher activity and lower phosphate content. Thus, this study suggests that insulin causes activation of the hepatic ACC by dephosphorylation of ACC.

Borthwick et al., (1990) first showed that parallel increases in phosphorylation and activity of ACC occur in rat adipocytes exposed to insulin. In this study, the co-purification of insulin-sensitive ACC kinase with the low-molecular-weight component allowed demonstration of parallel increase in phosphorylation and activity of ACC under conditions where phosphorylation of the ACC subunit appears to be occurring primarily in serine residues of the 'I'-peptide. Therefore, it was suggested that the insulin-sensitive ACC kinase apparently requires the presence of low-molecular-weight component which might have been removed during conventional purification techniques (Borthwick et al., 1990).

It has also been reported that epidermal growth factor stimulates both fatty acid synthesis and phosphorylation of ACC in rat hepatocytes (Holland and Hardie, 1985) and adipocytes (Haystead and Hardie, 1986a) within a few minutes. Further, Holland and Hardie (1985) demonstrated that epidermal growth factor and insulin showed the same phosphorylation patterns in ACC when tryptic digests were analyzed by reverse-phase HPLC. These observations imply that other growth factors including IGF-1 may stimulate fatty acid synthesis by phosphorylation and activation of ACC in major lipogenic tissues including lactating mammary glands. This seems reasonable since the plasma-membrane receptor for insulin is known to share many similarities with receptors for growth factors, including amino acid sequence homologies and the cytoplasmic regions of both receptors contain a tyrosine kinase which is activated by binding of insulin or growth factors (Ullrich et al., 1985).

Long-term

In addition to the short-term hormonal regulation of ACC by changes in the catalytic efficiency per enzyme molecule brought by phosphorylation/dephosphorylation, the long-term hormonal regulation of ACC by changes in the number of enzyme molecules plays an essential role in the control of ACC (Numa and Tanabe, 1984). It has been well documented that ACC activity of a variety of animal tissues undergoes long-term regulatory changes in accord with the rate of fatty acid synthesis under a variety of nutritional, hormonal, genetic and development conditions (Numa and Yamashita, 1974; Volpe and Vagelos, 1976; Numa and Tanabe, 1984). The changes in ACC activity that occur over a several-hour to several-day period of time have been shown to result from a change in the amount of enzyme protein (Lakshmanan et al., 1975; Kitajima, 1975). Thus, long-term hormonal regulation of ACC involves changes in the amount of ACC through modification of rates of synthesis and/or degradation of the enzyme protein (Porter and Swenson, 1983). In contrast to the abundance information on the short-term hormonal regulation of ACC, our knowledge of its long-term hormonal regulation is limited.

In ruminants, the mammary gland and adipose tissue appears refractory to short-term effects of insulin on fatty acid synthesis (Vernon and Sasaki, 1989). However, Baldwin et al. (1972) reported that chronic insulin administration to lactating cow significantly increased fatty acid synthesis in mammary glands. When incubations of bovine adipocytes are continued for 24 hr or longer, insulin causes a highly reproducible increases in fatty acid synthesis (Vernon et al., 1985; Etherton and Evock, 1986) and ACC activity (Vernon and Flint, 1989). Jindal and Pandey (1988) demonstrated that insulin, in combination with prolactin and cortisol, increases ACC activity due to the increased synthesis of the enzyme in explants of goat mammary tissue incubated for 36 hr in the media containing insulin. Recent studies indicate that prolactin also increases rate of fatty acid synthesis in mammary explants from pregnant sheep, concomitant with the increases in activity and amount of ACC when the explants are cultured for 48 hr (Barber et al., 1990 and 1991). In addition, GH exerts its effects on lipogenesis in adipose tissue from lactating sheep by reducing ACC activity, together with a decrease in the amount of enzyme (Vernon et al., 1988).

Unlike ruminants, nonruminants, especially rodents, have been extensively utilized to elucidate the long-term hormonal regulation of ACC. A role for insulin in the long term regulation of ACC is suggested by the observation of markedly decreased hepatic ACC activity in alloxan-induced diabetic rats (Nakanishi and Numa, 1970). In this study, the rate the enzyme synthesis was measured by injecting animals with a dose of [^{3}H] leucine and determining isotope incorporation into enzyme which was precipitated using a specific antibody. Rates of degradation were measured by following loss of isotope from prelablled enzyme. This study demonstrated that the decrease in enzyme content in diabetic rats was due solely to a corresponding change in the rate of synthesis of the enzyme. Recovery of ACC activities and amounts in liver or adipose tissue occurred with insulin replacement therapy (Volpe and Vagelos, 1974; Pape et

al., 1988; Katsurada et al., 1990).

It was demonstrated that glucagon prevents the marked increase in hepatic ACC activity that results when fasted rats and refed a fat-free diet (Volpe and Marasa, 1975). Rats made thyrotoxic by 7-9 days of injection of triiodothyronine (T3) exhibit approximately twofold-higher ACC activities in liver and adipose tissue (Diamant et al., 1972). T3-mediated stimulation (24 to 72 h following T3 treatment) of hepatic ACC in rats was inhibited by cycloheximide and actinomycin D (Das, 1980). In addition, hypophysectomized rats showed a marked reduction in hepatic ACC activity (Volpe and Marasa, 1975).

In an attempt to gain further insight into the molecular mechanism underlying the regulation of synthesis of ACC, Nakanishi et al. (1976) estimated the relative content of polysomes involved in the synthesis of ACC in the livers of rats subjected to alloxan-diabetes with or without insulin treatment by determining the binding of 125I-labelled antibody to isolated liver polysomes. This study demonstrated that the rates of synthesis of ACC and the amount of 125I-anti ACC bound to to liver polysomes were significantly higher in diabetic rats treated with insulin than in untreated diabetic rats, suggesting that increased synthesis of ACC by insulin during a long-term period could be due to changes in the amount of mRNA coding for the enzyme.

The complex physiological interactions imposed on cells *in vivo* make it difficult to asses the direct role of hormones in the long-term regulation of ACC, thus cell culture provides a useful system for studying the regulation of ACC under defined conditions (Dakshinamuri and Chauhan, 1988). Using rat hepatocyte culture, Kaze and Ick (1981) demonstrated that ACC activity was enhanced approximately two fold between 24 hr and 48 hr in culture in the presence of insulin. The increase in ACC activity by insulin was blocked by either cordycepin or cycloheximide, suggesting that the activity enhancements might be due to the increased enzyme synthesis. A similar observation was reported by Spence and Pitot (1982). A subsequent study demonstrated that insulin-dependent enhancement of ACC activity in long-term (48 hr) primary culture of rat hepatocytes were due to an increased amount of enzyme (Giffhorn and Kaze, 1984). In this study, incorporation of [^{32}S] methionine and immunoprecipitation of the enzyme revealed that the increase in ACC content was due to an increased rate of enzyme synthesis rather than decreased rate of enzyme degradation. Further, this insulin-dependent induction of ACC was prevented by addition of a-aminitin, implying that insulin may regulate ACC activity at the transcriptional level.

Fisher and Goodridge (1978) showed that insulin causes a twofold increase in the relative synthesis of ACC and addition of glucagon plus insulin to the cell culture media abolished the insulin-mediated increase in relative synthesis of ACC. Shafrir and Bierman (1981) demonstrated that human skin fibroblasts grown in lipid-poor medium showed a twofold increase in ACC activity upon addition of insulin, and that this increase was abolished in the presence of cycloheximide. Dexamethasone had a permissive effect on insulin induction of ACC in primary cultures of rat hepatocytes (Salati and Clark, 1986). On the other hand, addition of glucagon to Hela cell culture medium caused a 50% decrease in ACC activity accompanied by a corresponding decrease in the relative synthesis of ACC as measured using immunological techniques (Bhullar and Dakash-inamuri, 1985).

Collectively, the studies discussed indicate that regulation of the amount of ACC during certain hormonal conditions can be accounted for regulation of the rate of the enzyme synthesis. However, the relationship between changes in RNA metabolism and control of enzyme synthesis was not demonstrated in these studies. Recently, the cloning of cDNAs for ACCs allowed researchers to investigate ACC gene activation by hormones through measurement of the abundance of ACC mRNA.

Paper et al. (1988) reported that the amount of ACC mRNA decreased markedly in epididymal fat tissue of diabetic rats as compared to nondiabetic rats, however the mRNA level returned to that of the nondiabetic rats 6 hr after insulin injections. This study also showed that the mRNA of ACC corresponded to changes in the activity and amount of the enzyme. In livers from diabetic rats the transcription rate, mRNA concentration and enzyme induction of ACC markedly decreased as compared to the normal level but, with insulin injection, were increased to the normal level (Katsurada et al., 1990). In this study, the insulin-dependent increase in transcription rate and mRNA concentration of ACC were abolished by cycloheximide treatment, suggesting that de novo synthesis of protein is responsible for the mRNA induction of the ACC gene since relative concentration of B-actin mRNA was not significantly altered by insulin or cycloheximide treatment. This result is in line with the observations that insulin and growth factors, including IGF-1, are able to induce transcriptional factors in some cell line cultures (Stumpo and Blackshear, 1986; Isosaki and Kohn, 1987).

Recently, rat hepatocytes in primary culture were utilized for further studies on the mechanism regulating

the gene expression of ACC by insulin (Fukuda et al., 1992). When the effects of insulin on the mRNA level of ACC were examined in rat hepatocytes during the process of induction, the addition of insulin to culture media markedly enhanced the concentration of ACC mRNA in 16 hr (Fukuda et al., 1992). This study also demonstrated that induction of ACC mRNA was the most sensitive to insulin among the lipogenic enzymes. Further, Foufelle et al. (1992) reported that insulin stimulates ACC gene expression, resulting in enhanced concentrations of ACC mRNA in cultured rat adipocytes.

CONCLUSION

After reviewing the previous studies, We may conclude that bGH stimulates milk production in diverse ways not by disturbing the homeostasis. In terms of the stimulatory effects of IGF-1 mediating the bGH action on milk fat synthesis, we postulated that for the short term IGF-1 may increase ACC activity by phosphorylating the enzyme, and for the long term it may increase ACC activity by enhancing the enzyme amount resulting from activation of transcriptional factors by tyrosine or serine/threonine phosphorylation followed by stimulating transcription of ACC genes, in bovine mammary secretory cells. We hope that this scenario must be elucidated in the very near future. In order to do that, we must put our energy primarily to overcome the current dilemma, such as, the proper establishment of primary culture system of bovine mammary secretory cells, more detail information about the importance of paracrine and/or autocrine functions of IGF-1 as well as IGFBPs, and implications of other signalling molecules/effectors (hormones, growth factors) in the actions of bGH and IGF-1.

REFERENCES

Abdel-Mequid, S. S., H. S. Shieh, W. W. Smith, H. E. Dayringer, B. N. Violand and L. A. Bentle. 1987. Three dimensional structure of a genetically engineered variant of porcine growth hormone. Proc. Natl. Acad. Sci. U. S. A. 84:6434.

Akers, R. M. 1985. Lactogenic hormones: binding sites, mammary grwoth, secretory cell differentiation, and milk biosynthesis in ruminants. J. Dairy Sci. 68:501.

Alberts, A. W. and P. R. Vagelos. 1972. Acyl carrier protein: VIII. Studies of acyl carrier protein and enzyme A in Escherichia coli pantothenate or a-alanine auxotrophs. J. Biol. Chem. 241:5201.

Allred, J. B. and C. R. Roman-Lopez. 1988. Enzymatically inactive forms of acetyl-CoA carboxylase in rat liver mitochondria. Biochem. J. 251:881.

Asimov, G. T. and N. K. Krouze. 1937. The lactogenic preparations from the anterior pituitary and the increase of milk yield in cows. J. Dairy Sci. 20:289.

Badesch, D. B., P. D. Lee, W. C. Parks, and K. R. Stenmark. 1989. Insulin-like growth factor I stimulates elastin synthesis by bovine pulmonary arterial smooth muscle cells. Biochem. Biophys. Res. Commun. 160:382.

Baer, R. J., K. M. Tieszen, D. J. Schingoethe, D. P. Casper, W. A. Eisenbeisz, R. D. Shaver and R. M. Cleale. 1989. Composition and flavor milk produced by cows injected with recombinant; bovine somatotropin. J. Dairy Sci. 71:1424.

Baldwin, R. L. 1966. Enzymatic activities in mammary glands of several species. J. Dairy Sci. 49:1533.

Baldwin, R. L., E. Osborne, J. Reichl, N. E. Smith and Y. T. Yang. 1972. Effect of chronic insulin administration and a high concentrate diet on cow mammary glucose oxidation and lipogenesis. Fed. Proc. 31:675(Abstr. 2594).

Barber, M. C., E. Finley, A. T. Vallanse, and R. G. Vernon. 1990. The effect of prolactin on acetyl-CoA carboxylase activity on mammary explants from pregnant sheep. Biochem. Soci. Transactions. 18:1259.

Barber, M. C., E. Finley and R. G. Vernon. 1991. Mechanisms whereby prolactin modulates lipogenesis in sheep mammary gland. Horm. Metab. Res. 23:143.

Bauman, D. E., P. J. Eppard, M. J. DeGeeter and G. M. Lanza. 1985. Responses of high-producing dairy cows to long-term treatment with pituitary somatotropin and recombinant somatotropin. J. Dairy Sci. 68:1352.

Bauman, D. E. and S. N. McCutcheon. 1986. The effects of growth hormone and prolactin on metabolism. In Control of Digestion and Metabolism in Ruminants. pp. 436-455. L. P. Milligan, W. L. Grovum, and A. Dobson, ed. Prentice-Hall, Englewood, Cliffs. NJ.

Bauman, D. E., M. J. DeGeeter, C. J. Peel, G. M. Lanza, R. C. Gorewit and R. W. Hammond. 1982. Effect of recombinantly derived bovine growth hormone (bGH) on lactational performance of high yielding dairy cows. J. Dairy Sci. 65(Suppl. 1):121(Abstr.)

Bauman, D. E. 1992. Bovine somatotropin: Review of an emerging animal technology. J. Dairy Sci. 75:3432.

Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanism involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514.

Bauman, D. E. and C. L. Davis. 1974. Biosynthesis of milk fat. In Lactation. Vol. 2 pp. 31-69. B. L. Larson and V. R. Smith, ed. Academic Press, New york, NY.

Baumrucker, C. R. and B. H. Stemberger. 1989. Insulin and insulin-like growth factor-I stimulate DNA synthesis in bovine mammary tissue *in vitro*. J. Anim. Sci. 67:3503.

Baxter, R. C. and Martin, J. L. 1989. Binding proteins for the insulin-like growth factors: structure, regulation and function. Prog. Growth Factor Res. 1:49.

Beaty, N. B. and M. D. Lane. 1983. Kinetics of activation of acetyl-CoA carboxylase from rat liver. J. Biol. Chem. 263:6447.

Bhullar, R. P. and K. Dakshinanmurti. 1985. Regulation of acetyl-CoA carboxylase by glucagon in Hela cells. Biosci. Rep. 5:491.

Bianchi, A., J. L. Evans, A. J. Iverson, A. C. Nordlund, J. D.

Watts and L. A. Witters. 1990. Identification of an isozymic form of acetyl-CoA carboxylase. J. Biol. Chem. 265:1502.

Bines, J. A. and I. C. Hart. 1982. Metabolic limits to milk production, especially roles of growth hormone and insulin. J. Dairy Sci. 65:1375.

Binoux, M., M. Roghani, P. Hossenlopp, S. Hardouin and M. Gourmelen. 1991. Molecular forms of human IGF-1 binding proteins: physiologices implications. Acta. Enocrinolgica. 124(suppl. 2):41.

Bitman, J., D. L. Wood, H. F. Tyrrell, D. E. Bauman, C. J. Peel, A. C. G. Brown, and P. J. Reynolds. 1984. Blood and milk lipid responses induced by growth hormone administration in lactating cows. J. Dairy Sci. 67:2873.

Borthwick, A. C., N. J. Edgell and R. M. Denton. 1990. Protein-serine kinase from rat epididymal adipose tissue which phosphorylates and activates acetyl-CoA carboxylase. Biochem. J. 270:795.

Borthwick, A. C., N. J. Edgell and R. M. Denton. 1987. Use of rapid gel-permeation chromatography to explore the inter-relationships between polymerization phosphorylation and activity of acetyl-CoA carboxylase (Effects of insulin and phosphorylation by cyclic AMP-dependent protein kinase) Biochem. J. 241:773.

Bouner, M. J., W. H. Busby, N. R. Siegel, G. G. Krivi, R. H. McCusker and D. R. Clemmons. 1992. Cloning and sequence determination of bovine insulin-like growth factor binding protein-2 (IGFBP-2): Comparison of its structural and functinal properties with IGFBP-1. J. Cell Biochem. 48:215.

Brownsey, R. W., W. A. Hugher and R. M. Denton. 1979. Adrenaline and the regulation of acetyl-CoA carboxylase in rat epididymal adipose tissue. Biochem. J. 184:23.

Brownsey, R. W. and D. G. Hardie. 1980. Regulation of acetyl-CoA carboxylase: Identity of sites phosphorylated in intact cells treated with adrenaline and *in vitro* by cyclic AMP-Dependent protein kinase. FEBS Lett. 120:67.

Brownsey, R. W., W. A. Hughes and R. M. Denton. 1977. Demonstration of the phosphorylation of Acetyl-Coenzyme A carboxylase within intact rat epididymal fat cells. Biochem. J. 168:441.

Brownsey, R. W. and R. M. Denton. 1982. Evidence that insulin activates fat cell acetyl-CoA carboxylase by increased phosphorylation at a specific site. Biochem. J. 202:77.

Brownsey, R. W. and R. M. Denton. 1987. Acetyl-CoA carboxylase. In The enzymes Vol. XVIII. pp. 123-143. Academic Press.

Brumby, P. J. and J. Hancock. 1955. The galactopoietic role of growth hormone in dairy cattle. N. Z. J. Sci. Technol. 36A:417.

Buechler, K. F., A. C. Beynen and M. T. H. Geelen. 1984. Studies on the assay, activity and sedimentation behavior of acetyl-CoA carboxylase from isolated hepatocytes incubated with insulin or glucagon. Biochem. J. 221:869.

Bunyan, P. J. and A. L. Greenhaum. 1965. The effects of treatment of rats with pituitary GH on the activity of some enzymes involved in fatty acid degradation and synthesis. Biochem. J. 96:432.

Buonomo, F. C. and C. A. Baile. 1990. The neurophysiological regulation of growth hormone secretion. Domestic Anim. Endocrinol. 7:435.

Camacho-Hubner, C., D. R. Clemmons and A. J. D'Ercole. 1991. Regulation of insulin-like growth factor binding proteins in transgenic mice with altered expression of growth hormone and IGF-I. Endocrinol. 129:1201.

Campbell, P. G. and C. R. Baumrucker. 1986. Characterization of insulin-like growth factor-I receptors in the bovine mammary gland. J. Dairy Sci. 69(Suppl. 1):163(Abstr.).

Campbell, P. G., T. C. Skaar, J. R. Vega and C. R. Baumrucker. 1991. Secretion of insulin-like gorwth factor-I (IGF-I) and IGF-binding proteins from bovine mammary tissue *in vitro*. J. Endocrinol. 128:219.

Carling, D. P. R. Clarke, V. A. Zammit and D. G. Hardie. 1989. Purification and characterization of the AMP-activated protein kinase. Eur. J. Biochem. 186:129.

Carlson, C. A. and K. H. Kim. 1974. Differential effects of metabolites on the active and inactive forms of hepatic acetyl CoA carboxylase. Arch. Biochem. Biophys. 164:490.

Carlson, C. A. and K. H. Kim. 1973. Regulation of hepatic acetyl-CoA carboxylase by phosphorylation and dephosphorylation. J. Biol. Chem. 248:378.

Caro, J. F., J. Poulos, O. Hoop, W. F. Portes and E. G. Flickinger, M. K. Sinha. 1988. Insulin-like growth factor-I binding on hepatocytes from human liver, human hepatoma and normal regenerating and fetal rat liver. J. Clin. Invest. 81:976.

Christie, W. W. 1981. The effects of diet and other factors on the lipid composition of ruminant tissues and milk. In Lipid Metabolism in Ruminant Animals. pp. 193. W. W. Christie, ed. Pergamon Press Ltd., Elmsford, NY.

Cisse, M., Y. Chilliard, V. Coxam, M. J. Davicco and B. Remond. 1991. Slow release somatotropin in dairy heifers and cows fed two levels of energy concentrate. 2. plasma hormones and metabolites. J. Dairy Sci. 74:1382.

Clegg, R. A., I. Mullany, N. A. Robson and V. A. Zammit. 1986. Modulation of intracellular cyclic AMP content and rate od lipogenesis in mammary acini *in vitro*. Biochem. J. 240:13.

Clegg, R. A., D. W. West, R. E. D. Aitchson. 1987. Protein phosphorylation in rat mammary acini and in cytosol preparations *in vitro*. Biochem. J. 241:447.

Clemmons, D. R. 1991. Insulin-like growth factor binding proteins. In Insilin-like Growth Factors Molecular and Cellular Aspects. pp. 49-85. D. Leroith, ed. Boca Rator, CRC Press.

Cohick, W. S. and D. R. Clemmons. 1993. The insulin-like growth factors. Annu. Rev. Phsiol. 55:131.

Cohick, W. S., K. Plaut, S. J. Sechen and D. E. Bauman. 1989. Temporal pattern of insulin-like growth factor-I response to exogenous bovine somatotropin in lactating cows. Domest. Anim. Endocrinol. 6:263.

Cohick, W. S. and D. R. Clemmons. 1991. Regulation of insulin-like growth factor binding protein synthesis and secretion in a bovine epithelial cell line. Endocrinol. 129:1347.

Cohick, W. S., M. A. McGurie, D. R. Clemmons and D. E. Bauman. 1992. Regulation of insulin-like growth factor binding proteins in serum and lymph of lactating cows by somatotropin. Endocrinol. 130:1514.

Collier, R. J., J. P. McNamara, C. R. Wallac and M. H. Dehoff. 1984. A review of endocrine regulation of metabolism during lactation. J. Anim. Sci. 59:498.

Condorelli, G., P. Formisano, G. Villone, R. J. Smith and F.

Beguinot. 1989. Insulin and insulin-like growth factor I (IGF-I) stimulate phosphorylation of a Mr 175,000 cytoskeleton-associated protein in intact FRTL5 cells. J. Biol. Chem. 264:12633.

Conover, C. A., M. Ronk, F. Lombana and D. R. Powell. 1990. Structural and biological characterization of bovine insulin-like growth factor binding protein-3. Endocrinol. 127:2795.

Conover, C. A. 1990. Regulation of insulin-like growth factor (IGF)-binding protein synthesis by insulin and IGF-1 in cultured bovine fibroblasts. Endocrinol. 126:3139.

Gowie, A. T., G. S. Knaggs and J. S. Tindal. 1964. Complete restoration of lactation in the goat after hypophysectomy. J. Endocrinol. 28:267.

Czech, M. 1989. Signal transmission by the insulin-like growth factors. Cell. 59:235.

D'Ercole, A. J., A. D. Stiles and L. E. Underwood. 1984. Tissue Concentrations of Somatomedin-C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms. Proc. Natl. Acad. Sci. U. S. A. 81:1984.

Dakshinamurti, K. and J. Chauhan. 1988. Regulation of biotin enzymes. Ann. Rev. Nutr. 8:211.

Das, D. K. 1980. Regulation of hepatic fatty acid-synthesizing enzyme of diabetic animals by thyroid hormone. Archiv. Biochem. Biophs. 203:25.

Davis, S. R., R. J. Collier, J. P. McNamara and H. H. Head. 1983. Effect of growth hormone and thyroxine of dairy cows on milk production, cardiac output and mammary blood flow. Proc. Endocr. Soc. Aust. 26(Suppl. 2):31 (Abstr.).

Davis, S. R., P. D. Gluckman, I. C. Hart and H. V. Henderson. 1987. Effects of injecting growth hormone or thyroxine on milk production and blood plasma concentrations of insulin-like growth factors I and II in diary cows. J. Endocrinol. 114:17.

Davis, S. P., A. T. R. Sim and D. G. Hardie. 1990. Location and function of three sites phosphorylated on rat acetyl-CoA carboxylase by the AMP-activated protein kinase. Eur. J. Biochem. 187:183.

Davis, S. R., P. D. Gluckman and I. C. Hart. 1984. Effect of growth hormone and thyroxine treatment of lactating cows on milk production and plasma concentrations of IGF-I and IGF-II. Proc. Endocrinol. Soc. Asut. 27(Suppl. 1):16(Abstr.).

Dehoff, M. H., R. G. Elgin, R. J. Collier and D. R. Clemmons. 1988. Both type I and II insulin-like growth factor receptor binding increase during lactogenesis in bovine mammary tissue. Endocrinol. 122:2412.

Diamant, S., E. Gorin and E. Shafrir. 1972. Enzyme activities related to fatty-acid synthesis in liver and adipose tissue of rats treated with triiodothyronine. Eur. J. Biochem. 26:553.

Distel, R. J., H. S. Ro, B. S. Rosen, D. L. Groves, B. M. Spiegelman. 1987. Nucleoprotein complexes that regulate gene expression in adipocyte differentiation: direct participation of c-fos. Cell. 49:835.

Duncan, R. and E. H. McConkey. 1982. Rapid alterations ininitiation rate and recruitment of inactive RNA are temporally correlated with S6 phosphorylation. Eur. J. Biochem. 123:539.

Easter, D. J. and R. Dils. 1968. Fatty acid biosynthesis. IV. Properties of acetyl-CoA carboxylase in lactating rabbit mammary gland. Biochem. Biophys. Acta. 152:653.

Eppard, P. J., D. E. Bauman, J. Bitman, D. Wood, R. M. Akers and W. A. House. 1985. Effect of dose of bovine growth hormone on milk composition: -lactalbumin, fatty acids and mineral elements. J. Dairy Sci. 68:3047.

Etherton, T. D. and C. M. Evock. 1986. Stimulation of lipogenesis in bovine adipose tissue by insulin-like growth factor. J. Anim. Sci. 62:357.

Etherton, T. D., C. M. Evock and R. S. Kensinger. 1987. Native and recombinant bovine growth hormone antagonize insulin action in culture bovine adipose tissue. Endocrinol. 121:699.

Fisher, P. W. E. and A. G. Goodridge. 1978. Coordinate regulation of acetyl Coenzyme a carboxylase and fatty acid synthetase in liver cells of the developing chick *in vivo* and in culture. Arch. Biochem. Biophys. 190:332.

Fleet, I. R., C. G. Prosser, A. N. Corps, E. R. Froesch and R. B. Heap. 1988. Increase in milk secretion and mammary blood flow by an intra-arterial infusion of insulin-like growth factor I into the mammary gland of the goat. J. Endocrinol. 117(Suppl. 1):248.

Forsyth, I. A. 1996. The insulin-like growth factor and epidermal growth factor families in mammary cell growth in ruminant: Action and Interaction with hormones. J. Dairy Sci. 79:1085.

Foufelle, F. B. Gouhot, J. P. Pe'gorier, O. Perdereau, J. Girard and P. Ferre'. 1992. Glucose stimulation of lipogenic enzyme gene expression in cultured white adipose tissue. J. Biol. Chem. 267:20543.

Foyt, H. L. and C. T. Roberts, Jr. 1991. The IGF-I gene: structure, expression, and gene products. In Insulin-like Growth Factors: Molecular and Cellular Aspects. pp. 1-12. D. LeRoith, ed. Boca Raton, CRC Press.

Francis, G. L., F. M. Upton, F. J. Ballard, K. A. McNeil and J. C. Wallace. 1988. Insulin-like growth factors 1 and 2 in bovine colostrum. Sequences and biological activities compared with those of a potent truncated form. Biochem. J. 251:95.

Froesch, E. R., C. Schmid, J. Schwander and J. Zapf. 1985. Actions of insulin-like growth factors. Anu. Rev. Physiol. 47:443.

Fronk, T. J., C. J. Peel, D. E. Bauman and R. C. Gorewit. 1983. Composition of different pattems of exogenous growth hormone administration on milk production in Holstein cows. J. Anim. Sci. 57:699.

Fukuda, H., A. Katsurada and N. Iritani. 1992. Nutritional and hormonal regulation of mRNA levels of lipogenic enzymes in primary cultrue of rat hepatocytes. J. Biochem. 111:25.

Giffhon, S., N. R. Katz. 1984. Glucose-dependent induction of acetyl-CoA carboxylase in rat hepatocyte cultures. Biochem. J. 221:343.

Gioregetti, S., R. Ballotti, A. Kowalski-Chauvel, S. Tartare and E. V. Obberghen. 1993. The insulin and insulin-like growth factor-I receptor substrate IRS-I associates with and activates phosphatidylinositol 3-kinase *in vitro*. J. Biol. Chem. 268:7358.

Glimm, D. R., V. E. Baracos and J. J. Kennelly. 1988. Effect of bovine somatotropin on the distribution of immunoreactive insulin-like grwoth factor-1 in lactating bovine mammary tissue. J. Dairy Sci. 71:2923.

Glimm, D. R., V. E. Baracos and J. J. Kennelly. 1990. Molecular evidence for the presence of growth hormone

receptors in the bovine mammary gland. J. Endocrinol. 126:R5.

Goodman, H. M. 1963. Effects of chronic growth hormone treatment on lipogenesis by rat adipose tissue. Endocrinol. 72:95.

Han, V. K. M., P. K. Lund, D. C. Lee and A. J. D'Ercole. 1988. Expression of somatomedin/insulin-like growth factor messenger ribonucleic acids in the human fetus: identification, characterization and tissue distribution. J. Clinic. Endocrinol. 66:422.

Hardie, D. G., D. Carling and A. T. R. Sim. 1989. The AMP-activated protein kinase: a multisubstrate regulator of lipid metaboslism. Trends Biochem. Sci. 14:20.

Hardie, D. G. and P. Cohen. 1979. Dephosphorylation and activation of acetyl-CoA carboxylase from lactating rabbit mammary gland. FEBS. Lett. 103:33.

Hardie, D. G. and P. Cohen. 1978. The regulation of fatty acid biosynthesis. FEBS Lett. 91:1.

Hardie, D. G. and P. S. Guy. 1980. Reversible phosphorylation and inactivation of acetyl-CoA carboxylase from lactating rat mammary glands by cyclic AMP-Dependent protein kinase. Eur. J. Biochem. 110:167.

Hardouin, S., M. Gourmelen, P. Noguiez, D. Seurin, M. Roghani, Y. Le Bouc, G. Povoa, T. J. Merimee, P. Hossenlopp and M. Binoux. 1989. Molecular forms of serum insulin-like growth factor (IGF)-binding proteins in man: Relationship with growth hormone and IGFs and physiological significance. J. Clin. Endocrinol. Metab. 69:1291.

Hart, I. C., J. A. Bines and S. V. Morant. 1980. The secretion and metabolic clearance rates of growth hormone, insulin and prolactin in high- and low-yielding cattle at four stages of lactation. Lif. Sci. 27:1839.

Hart, I. C. 1983. Endocrine control of nutrient partition in lactating ruminants. Proc. Nutr. Sci. 42:181.

Haystead, T. A. and D. G. Hardie. 1986a. Both insulin and epidermalgrowth factor stimulate lipogenesis and acetyl-CoA carboxylase activity in isolated adipocytes. Biochem. J. 234:279.

Haystead, T. A. J. and D. G. Hardie. 1986b. Evidence that activation of acetyl-CoA carboxylase by insulin in adipocytes is mediated by a low-Mr effector and not by increased phosphorylation. Biochem. J. 240:99.

Haystead, T. A. and D. G. Hardie. 1988. Insulin and phorbol eater stimulate phosphorylation of acetyl-CoA carboxylase at similar sites in isolated adipocytes. Eur. J. Biochem. 175:339.

Haystead, T. A., F. Moore, P. Cohen and D. G. Hardie. 1990. Roles of the AMP-activated and cyclic-AMP-dependent protein kinases in the adrenaline-induced inactivation of acetyl-CoA carboxylase in rat adipocytes. Eur. J. Biochem. 187:199.

Hill, D. J., C. Comacho-Hubner, P. Rashid, A. J. Strain and D. R. Clemmons. 1989. Insulin-like growth factor binding protein secretion by human fibroblasts: dependence on cell density and IGF peptides. J. Endocrinol. 122:87.

Hodgkinson, S. C., G. S. G. Spencer, J. J. Bass, S. R. Davis, P. D. Gluckman. 1991. Distribution of circulating insulin-like growth factor-I (IGF-I) in tissues. Endocrinol. 129:2085.

Hodgkinson, S. C., S. R. Davis, B. D. Burleigh, H. V. Henderson and P. D. Gluckman. 1987. Metabolic clearance rate of insulin-like growth factor-I in fed and starved sheep. J. Endocrinol. 115:233.

Holland, R., L. A. Witters and D. G. Hardie. 1984. Glucagon inhibits fatty acid synthesis in isolated hepatocytes via phosphorylation of acetylj-CoA carboxylase by cyclic-AMP-dependent protein kinase. Eur. J. Biochem. 140:325.

Holland, R., D. G. Hardie, R. A. Clegg and V. A. Zammit. 1985. Evidence that glucagon-mediated inhibition of acetyl-CoA carboxylase in isolated adipocytes involves increased phosphorylation of the enzyme by cyclic AMP-dependent protein kinase. Biochem. J. 226:139.

Holland, R. and D. G. Hardie. 1985. Both insulin and epidermal growth factor stimulate fatty acid synthesis and increase phosphorylation of acetyl-CoA carboxylase and ATP-citrate lyase in isolated hepatocytes. FEBS Lett. 181:308.

Holly, J. M. P. and J. A. H. Wass. 1989. Insulin-like growth factors; autocrine, paracrine or endocrine? New perspectives of the somatomedin hypothesis in the light of recent developments. J. Endocrinol. 122:611.

Holtorf, A. P., K. Furuya, R. Ivell and C. A. McArdle. 1989. Oxytocin production and oxytocin messenger ribonucleic acid levels in bovine granulosa cells are regulated by insulin-like growth factor-I: dependence on the developmental status of the ovarian follicle. Endocrinol. 123:2612.

Honegger, A. and R. E. Humbel. 1986. Insulin-like growth factors I and II in fetal and adult bovine serum: purification, primary structures, and immunological cross-reactivities. J. Biol. Chem. 261:560.

Hunter, J. and M. Karin. 1992. The regulation of transcription by phosphorylation. Cell. 70:375.

Ingalls, W. G., E. M. Convey and H. D. Hafs. 1973. Bovine serum LH, GH and prolactin during late pregnancy, parturition and early lactation. Proc. Soc. Exp. Biol. Med. 143:161.

Isaksson, O. G. P., A. Lindahl, A. Nilsson and Isgaard. 1987. Mechanism of the stimulatory effect of growth hormone on longitudinal bone growth. Endocrine Rev. 8:426.

Isgaard, J., A. Nilson, A. Lindahl, J. D. Jansson and O. G. P. Isaksson. 1986. Effects of local administration of GH and IGF-I on longitudinal bone growth in rats. Am. J. Physiol E367.

Isozaki, O. and L. D. Kohn. 1987. Control of c-fos and c-myc proto-oncogene induction in rat thyroid cells in culture. Mol. Endocrino. 1:839.

Jindal, H. K. and R. S. Pandey. 1988. Studies on the regulation of enzymes related to fatty acid synthesis in goat mammary gland using explant culture system. Indian J. Biochem. Biophys. 25:428.

Johnsson, I. D. and I. C. Hart. 1986. Manipulation of milk yield with growth hormone. In Recent Advances in Animal Nutrition. pp. 105-123. W. Haresign and D. J. A. Cole, ed. Butterworths, London, England.

Jones, J. J. and D. R. Clemmons. 1995. Insulin-like growth factors and thier binding proteins: Biological action. Endo. Rev. 16:3.

Katsurada, A., N. Iritani, H. Fukuda, Y. Matsumura, N. Nishimoto, T. Noguchi and T. Tanaka. 1990. Effects of nutrients and hormones on transcriptional and post-

transcriptional regulation of acetyl-CoA carboxylase in rat liver. Eur. J. Bichem. 190:435.

Katz, N. R. and M. Ick. 1981. Induction of acetyl-CoA carboxylase in primary rat hepatocyte cultures by glucose and insulin. Biochem. Biophys. Res. Commun. 100:703.

Kazmer, G. W., M. A. Barnes, R. M. Akers and W. D. Whittier. 1986. Lactogenic hormone receptors in mammary membrane preparations from prepartum and 60 and 180 day post-partum Holstein cattle. J. Endocrinol. 109:175.

Kim, K. H. 1979. Control of acetyl-CoA carboxylase by covalent modification. Mol. Cell. Biochem. 28:27.

Kim, K. H. 1983. Regulation of acetyl-CoA carboxylase. Curr. Top. Cell. Regul. 22:143.

Kim, K. H., F. Lopez-casillas, D. H. Bai, X. Luo and M. E. Pape. 1989. Role of reversible phosphorylation of acetyl-CoA carboxylase in long-clain fatty acid synthesis. FASEB. J. 3:2250.

Kitajima, K., S. I. Tashiro and S. Numa. 1975. Acetyl-CoA carboxylase in cultured hepatocytes. Effect of exogenous fatty acid on the content, synthesis, and degradation of the enzyme. Eur. J. Biochem. 54:373.

Knight, C. H. and M. Peaker. 1984. Mammary development and regression during lactation in goats in relation to milk secretion. Quarterly J. Experi. Physiol. 69:331.

Knowles, J. R. 1989. The mechanism of biotin-dependent enzymes. Annu. Rev. Biochem. 58:195.

Kostyo, J. L. and R. C. Reagan. 1976. The biology of somatotropin. Parmacol. Theriogenol. 2:591.

Kronfeld, D. S. 1965. Growth hormone-induced ketosis in the cow. J. Dairy Sci. 48:342.

Laarveld, B., R. K. Chaplin and R. P. Brockman. 1985. Effects of insulin on the metabolism of acetate, hydroxybutyrate and triglycerides by the bovine mammary gland. Comp. Biochem. Physiol. 828:265.

Lacasse, P., Y. Couture, R. Gorewait, E. Block and D. Peticlerc. 1991. Changes in lymphatic IGF-I concentrations of dairy cows during the dry period and onset of lactation. J. Anim. Sci. 69(Sully. 1):449(Abstr.).

Lakshmanan, M. R., C. M. Nepokroeff, M. Kim and J. W. Porter. 1975. Adaptive synthesis of fatty acid synthetase and acetyl-CoA carboxylase by isolated rat liver cells. Archiv. Biochem. Biophys. 169:737.

Lane, M. D., J. Moss and S. E. Polakis. 1974. Acetyl-CoA carboxylase. Curr. Top. Cell. Regul. 8:139.

Lee, K. H. and K. H. Kim. 1979. Stimulation by epinephrine of *in vivo* phosphorylation and inactivation of acetyl coenzyme a carboxylase of rat epididymal adipose tissue. J. Biol. Chem. 254:1450.

Lent, B. A., K. H. Lee and K. H. Kim. 1978. Regulation of rat liver acetyl-CoA carboxylase. Stimulation of phosphorylationand subsequent inactivation of liver acetyl-CoA carboxylase by 3':5"-monophosphate and effect on the structure of the enzyme. J. Biol. Chem. 253:1897.

Linzell, J. L. 1974. p. 143. In Lactation. Vol. I. B. L. Larson and V. R. Smith, ed. Academic Press, New York, NY.

Lopez-Casellas, F., D. H. Bai, X. Luo and I. S. Kong. 1988. Structure of cooling sequence and primary amino acid sequence of acetyl-CoA carboxylase. Proc. Natl. Acad. Sci. USA. 85:5784.

Lough, D. S., L. D. Muller, R. S. Kensinger, T. F. Sweeney, L. G. Griel and T. D. Etherton. 1984. Effect of dietary fat and exogenous growth hormone on the performance of early lactation Holstein cows. J. Dairy Sci. 67(Suppl. 1):115 (Abstr.).

Lowe, W. L. Jr. 1991. Biological actions of the insulin-like growth factors. In Insulin-like Growth Factros: Molecular and Cellular Aspects. pp. 49-85. D. LeRoith, ed. Boca Rator, CRC Press.

Lynch, J. M., D. M. Barbano, D. E. Bauman, G. F. Hartnell and N. A. Nemeth. 1992. Effect of a prolonged-release formulation of N-methionyl bovine somatotropin (Sometribove) on milk fat. J. Dairy Sci. 75:1794.

Mabrouk, G. M., I. M. Helmy, K. G. Thamy and S. J. Wakil. 1990. Acute hormonal control of acetyl-CoA carboxylase. The role of insulin, glucagon, and epinephrine. J. Biol. Chem. 265:6330.

Machlin, L. J. 1973. Effect of growth hormone on milk production and feed utilization in dairy cows. J. Dairy Sci. 56:575.

Malven, P. V., H. H. Head, R. J. Collier and F. C. Buonomo. 1987. Periparturient changes in secretion and mammary uptake of insulin and in concentrations of insulin and insulin-like growth factors in milk of dairy cows. J. Dairy Sci. 70:2254.

Marinchenko, G. V., J. P. McNamara, B. Becker-Kjaleel and K. Parmley. 1992. Growth hormone alters metabolic effects and proteolysis of insulin in adipose tissue during lactation. Soc. Experi. Biol. Med. 200:57.

McCusker, R. H. and D. R. Clemmons. 1988. Insulin-like growth factor binding protein secretion by muscle cells: effects of cellular differentiation and proliferation. J. Cell physiol. 137:505.

McCutcheon, S. N. and D. E. Bauman. 1986. Effect of chronic growth hormone treatment on responses to epinephrine and thyrotropin releasing hormone in lactation cows. J. Dairy Sci. 69:44.

McDowell, G. H., D. Leenanuruksa, J. M. Gooden, M. Jois and E. F. Annison. 1984. Effects of growth hormone on muscle and mammary metabolism in cows. Proc. Nutr. Soci. Aust. 9:164(Abstr.).

McDowell, G. F. and I. C. Hart. 1984. Responses to infusion of growth hormone into the mammary arteries of lactating sheep. Can. J. Anim. Sci. 64(Suppl):306(Abstr.).

McGrath, M. F., R. J. Collier, D. R. Clemmons, W. H. Busby, C. A. Sweeny and G. G. Krivi. 1991. The direct *in vitro* effect of insulin-like growth factors (IGFs) on normal bovine mammary cell proliferation and production of IGF binding proteins. Endocrinol. 129:671.

Mellenberger, R. W., D. E. Bauman and D. R. Nelson. 1973. Metabolic adaptations during lactogenesis. Biochem. J. 136:741.

Mepham, T. B., S. E. Lawrence, A. R. Peters and I. C. Hart. 1984. Effects of exogenous growth hormone on mammary function in lactating goats. Horm. Metab. Res. 16:248.

Middleton, S. C., R. L. Baldwin and C. C. Calvert. 1988. Patterns of glucose metabolism in lactating cow mammary glands. J. Dairy Sci. 71(Suppl. 1):247.

Moore, J. H. and W. W. Christie. 1981. Lipid metabolism in the mammary gland of ruminant animals. In Lipid Metabolism in Ruminant Animals. pp. 227-276. W. W. Christie, ed.

Pergamon press Ltd., Elmsford, NY.

Moxham, C. P., V. Duronio and S. Jacobs. 1989. Insulin-like growth factor I receptor -subunit heterogeneity. J. Biol. Chem. 264:13238.

Munday, M. R. and D. G. Hardie. 1984. Isolation of three cyclic-AMP-independent acetyl-CoA carboxylase kinase from lactating rat mammary gland and characterization of their effects on enzyme activity. Eur. J. Biochem. 141:617.

Munday, M. R., D. G. Campbell, D. Carling and D. G. Hardie. 1988a. Identification by amino acid sequencing of three major regulatory phosphorylation sites on rat acetyl-CoA carboxylase. Eur. J. Biochem. 175:331.

Munday, M. R., D. Carling and D. G. Hardie. 1988b. Negative interactions between phosphorylation of acetyl-CoA carboxylase by the cyclic AMP-dependent and AMP-activated protein kinase. FEBS Lett. 235:144.

Murphy, L. J., C. Seneviratne, P. Moreira and R. E. Reid. 1991. Enhanced expression of insulin-like growth factor binding protein I in the fasted rat: the effects of insulin and growth hormone administration. Endocrinol. 128:689.

Murphy, L. J., G. I. Bell and H. G. Friesen. 1987. Tissue distribution of insulin-like growth factor I and II messenger ribonucleic acid in the adult rat. Endocrinol. 120:1279.

Myal, Y., R. P. C. Shiu, B. Bhaumick and M. Bala. 1984. Receptor binding and growth-promoting activity of insulin-like growth factor in human breast cancer cells (T-470) in culture. Cancer Res. 44:5486.

Nakanishi, S., T. Tanabe, S. Horikawa, and S. Numa. 1976. Dietary and hormonal regulation of the content of acetyl-coenzyme A carboxylase-synthesizing polysomes in rat liver. Proc. natl. Aced. Sci. U. S. A. 73:2304.

Nakanishi, S. and S. Numa. 1970. Purification of rat liver acetyl-CoA carboxylase and immunochemical studies on its synthesis and degradation. Eur. J. Biochem. 16:161.

Numa, S. and T. Tanabe. 1984. Acetyl-CoA carboxylase and its regulation. In Fatty acid metabolism and its regulation. pp. 1 -27. S. Numa, ed. Elsevier, New York, NY.

Numa, S. and S. Ymashita. 1974. Regulation of lipogenesis in animal tissues. Curr. Top. Regul. 8:197.

Oda, S., H. Satoh, T. Sugawara, N. Matsunaga, T. Kuhara, K. Katoh, Y. Shoji, A. Nihei, M. Ohta, and Y. Sasaki. 1989. Insulin-like growth factor-1, GH, insulin and glucagon concentrations in bovine colostrum and plasma of dairy cows and neonatal calves around parturition. Comp. Biochem. Physiol. 94A:805.

Oemar, B. S., N. M. Law and S. A. Rosenzweig. 1991. Insulin-like growth factor-I induces tyrosyl phosphorylation of nuclear proteins. J. Biol. Chem. 266:24241.

Orlowski, C. C., A. L. Brown, G. T. Ooi, Y. H. Yang, L. Y. Tseng and M. M. Rechler. 1990. Tissue, developmental, and metabolic regulation of messenger ribonucleic acid encoding a rat insulin-like growth factor binding protein. Endocrinol. 126:644.

Oscar, T. P., C. R. Baumrucker and T. D. Etherton. 1986. Insulin binding to bovine mammary membranes. Composition of microsomes versus smooth membranes. J. Anim. Sci. 62:179.

Pape, M. E., F. Lopez-Casillas, and K. H. Kim. 1988. Physiological regulation of acetyl-CoA carboxylase gene expression: Effects of diet, diabetes, and lactation on acetyl-CoA carboxylase mRNA. Archiv. Biochem. Biophys. 267:104.

Peel, C. J. and D. E. Bauman. 1987. Somatotropin and lactation. J. Dairy Sci. 70:474.

Peel, C. J., T. J. Fronk, D. E. Bauman and R. C. Gorewit. 1982. Lactational response to exogenous growth hormone and abomasal infusion of a glucose-sodium caseinate mixture in high-yielding dairy cows. J. Nutr. 112:1770.

Peel, C. J., J. J. Fronk, D. E. Bauman and R. C. Gorewit. 1983. Effect of exogenous growth hormone in early and late on lactational performance of dairy cows. J. Dairy Sci. 66:776.

Peel, C. J., L. D. Sandles, K. J. Quelch and A. C. Herington. 1985. the effects of long term administration of bovine growth hormone on the lactational performance of identical twin dairy cows. Anim. Prod. 41:135.

Peel, C. J., D. E. Bauman, R. C. Gorewit and C. J. Sniffen. 1981. Effect of exogenous growth hormone on lactational performance in high-yielding dairy cows. J. Nutr. 111:1662.

Pell, J. M. and P. C. Bates. 1990. The nutritional regulation of growth hormone action. Nut. Res. Rev. 3:163.

Penhoat, A., P. G. Chatelain, C. Jaillard and J. M. Saez. 1988. Characterization of insulin-like growth factor I and insulin receptors on cultured bovine adrenal fasciculata cells. Role of these peptides on adrenal cell function. Endocrinol. 122:2518.

Plucinski, T. M. and R. L. Baldwin. 1882. Effects of hormones on mammary adenosine 3', 5'-monophosphate levels and metabolism in normal and adrenalectomized lactating rats. Endocrinal. 111:2062.

Porter, J. W. and T. L. Swenson. 1983. Induction of fatty acid synthetase and acetyl-CoA carboxylase by isolated rat liver cells. 53:307.

Prosser, C. G., I. R. Fleet and A. N. Corps. 1989. Increased secretion of insulin-like growth factor I into milk of cows treated with recombinantly derived bovine growth hormone. J. Dairy Res. 56:17.

Rechler, M. M. and S. P. Nissley. 1985. the nature and regulation of the receptors for insulin-like growth factors. Annu. Rev. Physiol. 47:425.

Rechler, C. G. and S. P. Nissley. 1990. Insulin-like growth factors. In Hand book of Experimental Pharmacology. Vol. 95/I. pp. 263-367. M. B. Sporn and A. B. Roberts, ed. Spring-Verag, Berlin, FRG.

Rinderknecht, E. and R. E. Humbel. 1978. The amino acid sequence of human insulin-like growth I and structural homology with proinsulin. J. Biol. Chem. 253:2769.

Riss, T. L. and D. A. Sirbasku. 1987. Growth and continuous passage of comma-D mouse mammary epithe lial cells in hormonally defined serum-free medium. Cancer Res. 47:3776.

Robson, N. A., R. A. Clegg and V. A. Zammit. 1984. Regulation of peripheral lipogenesis by glucagon (Inability of the hormone to inhibit lipogeneses in rat mammary acid *in vitro* in the presence or absence of agents which after its effects on adipocytes). Biochemical. J. 217:743.

Roman-Lopez, C. R., B. J. Shriver, C. R. Jeseph and J. B. Allred. 1989. Mitochondrial acetyl-CoA carboxylase time course of mobilization in liver of refed rats. Biochem. J. 260:927.

Ronge, H. J., J. Blum, C. Clement, F. Jans, H. Leuenberger and

H. Binder. 1988. Somatomedin C in dairy cows related to energy and protein supply and to milk production. Anim. Prod. 47:165.

Ronge, H. and J. W. Blum. 1989. Insulin-like growth factor I binding proteins in dairy cows, calves and bulls. Acta Endocrinol. 121:153.

Ross, R. J. M. and C. R. Buchanan. 1990. Growth hormone secretion: its regulation and influence of nutritional factors. Nut. Res. Rev. 3:143.

Russell, S. M., E. M. Spencer. 1985. Local injections of human or rat growth hormone or of purified human somatomedin-C stimulate unilateral tibial epiphyseal growth in hypophysectomized rats. Endo crinol. 16:2563.

Salati, L. M. and S. D. Clarke. 1986. Fatty acid inhibition of hormonal induction of acetyl-Coenzyme A carboxylase in hepatocyte monolayer. Arch. Biochem. Biophys. 246:82.

Salmon, W. D., Jr. and W. H. Daughaday. 1957. A hormonally controlled serum factor which stimulates sulfate incorporation by carillage *in vitro*. J. lab. Clin. Med. 49:825.

Schwander, J. C., C. Hauri, J. Zapf and E. R. Froesch. 1983. Synthesis and secretion of insulin-like growth factor and its binding by the perfused rat liver: dependence on growth hormone status. Endocrinol. 113:297.

Shafrir, E. and E. L. Bierman. 1981. Acetyl-CoA carboxylase activity in cultured human fibroblasts. Induction by insulin in relation to cell growth and triacylglycerol metabolism. Biochem. biophys. Acta. 663:432.

Shamay, A., N. Cohen, M. Niwa and A. Gertler. 1988. Effect of insulin-like growth factor I on deoxyribonucleic acid synthesis and galactopoiesis in bovine undifferentiated and lactating mammary tissue *in vitro*. Endocrinol. 123:804.

Shiao, M. S., R. F. Drong and J. W. Porter. 1981. The purification and properties of protein kinase and the partial purification of a phosphoprotein phosphatase that inactivate and activate acetyl-CoA carboxylase. Biochem. Biophys. Res. Commun. 81:139.

Shimasaki, S., F. Uchlyama, S. Motoyuki and N. Ling. 1990. Molecular cloning of the complementary DNAs encoding novel insulin-like growth factor binding protein from rat and human. Mol. Endocrinol. 4:1451.

Shimonaka, M., R. Schroeder, S. Shimasaki, N. Ling. 1989. Identification of a novel binding protein for insulin-like growth factors in adult rat serum. Biochem. Biophys. Res. Commun. 165:189.

Sim, A. T. R. and D. G. Hardie. 1988. The low activity of acetyl-CoA carboxylase in basal and glucagon-stimulated hepatocytes is due to phosphorylation by the AMP-activated protein kinase and cyclic AMP-dependent protein kinase. FEBS Lett. 233:294.

Sinha, Y. N., F. W. Selby and W. P. Vanderlaan. 1974. Relationship of prolactin and growth hormone to mammary function during pregnancy and lactation in the C3H-ST mouse. J. Endocrinol. 61:219.

Smith, C. J., P. J. Weiksnora, J. R. Warmer, C. S. Rubin and O. M. Rosen. 1979. Insulin-Stimulated protein phosphorylation in 3T3-LI preadipocytes. Proc. Natl. Acad. Sci. U. S. A. 76:2725.

Spence, J. J. and H. C. Pitot. 1982. Induction of lipogenic enzymes in lipogeneses and carboHydrate metabolsim. Eur. J. Biochem. 128:15.

Spicer, L. J., S. E. Echternkamp, S. F. Canning and J. M. Hammond. 1988. Relationship between concentrations of immunoreactive insulin-like growth factor-I in follicular fluid and various biochemical markers of differentiation in bovine antral folicles. Biol. Reprod. 39:573.

Spratt, S. K., G. P. Tatsuno and A. Sommer. 1991. Cloning and characterization of bovine insulin-like growth factor binding protein-3 (IGFBP-3). Biochem. Biophys. Res. Commun. 177:1025.

Stumpo, D. J. and P. J. Blackshear. 1986. Insulin and growth factor effects on c-fos expression in normal and protein kinase C-deficient 3T3-L1 fibroblasts and adipocytes. Proc. Natl. Acad. Sci. U. S. A. 83:9453.

Terao, K. and K. Ogata. 1979. Proteins of small subunits of rat liver ribosomes that interact with poly(u). J. Biochem. 86:605.

Thampy, K. G. and S. J. Wakil. 1988. Regulation of Acetyl-Coenzyme A carboxylase. J. Biol. Chem. 263:6454.

Thampy, K. G. and S. J. Wakil. 1985. Activation of acetyl-CoA carboxylase purification and properties of a Mn-dependent phosphatase. J. Biol. Chem. 250:6318.

Thomas, G., M. Siegmann, A. M. Kubler, J. Gordon and L. Jimenez-de-Asua. 1980. Regulation of 40S ribosomal protein S6 phosphorylation in swiss mouse 3T3 cells. Cell. 19:1015.

Tipper, J. P. and L. A. Witters. 1982. *In Vitro* phosphorylation and inactivation of rat liver Acetyl-CoA carboxylase purified by affinity chromatography. Biochem. Biophys. Acta. 715:162.

Toshio, M., Y. Nobuhiro and F. Matsuzake 1981. The *in vitro* effect of growth hormone on adipose tissue lipoprotein lipase in rats. Lif. Sic. 28:199.

Ullrich, A., T. R. Bell, E. Y. Chen, R. Herrera, L. M. Petruzzelli, T. G. Dull, A. Gray, L. Coussens, Y. C. Liao, M. Tsubokawa, A. Mason, P. H. Seeburh, C. Grunfeld, O. M. Koson and J. Ramachandran. 1985. Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. Nature. 313:756.

Upton, F. Z., L. Szabo, J. C. Walker, F. J. Ballard. 1990. Characterization and cloning of insulin-like growth factor binding protein. Mol. Endocrinol. 5:77.

Van Wyk, J. J. 1984. The somatomedins: biological actions and physiologic control mechanisms. In Hormonal Proteins and Peptides. Vol. XII pp. 81-125. C. H. Lee, ed. Academic Press.

Vernon, R. G. and S. Sasaki. 1989. Control of responsiveness of tissue to hormones. In Physiological Aspects of Digestion and Metabolism in Ruminants. pp. 155-182. T. Tsuda, Y. Sasaki and R. Kawashima, ed. Academic Press.

Vernon, R. G., R. A. Clegg and D. J. Flint. 1985. Adaptation of adipose tissue metabolism and number of insulin receptors in pregnant sheep. Comp. Biochem. Physiol. 81B:909.

Vernon, R. G. 1989. Influence of somatotropin on metabolism. In Use of Somatotropin in live-stock Production. p. 31 K. Sejrsen, M. Vestergaard, and A. Neimann-Sorensesn, ed. Elsevier Appl. Sci. New York. NY.

Vernon, R. G., M. Barber, E. Finley and M. R. Grigor. 1988. Endocrine control of lipogenic enzyme activity in adipose tissue from lactating ewes. Proc. Nutr. Soc. 47:100A.

Vernon, R. G. and D. J. Flint. 1989. Role of growth hormone in

the regulation of adipocyte growth and function. In Biotechnology in Growth and Regulation. pp. 57-71. R. B. Heap, C. G. Prosser, and G. E. Lamming, ed. Butterworths, London, England.

Vernon, R. G. 1982. Effect of growth hormone on fatty acid synthesis in sheep adipose tissue. Int. J. Biochem. 14:255.

Vicini, J. L., F. C. Buonomo, J. J. Veenhuizein, M. A. Miller, D. R. Clemmons and R. J. Collier. 1991. Effect of nutritional balance and stage of lactation in dairy cows on insulin, insulin-like growth factors I and II and insulin-like growth factor binding protein 2 responses to somatotropin administration. J. Nutr. 121:1656.

Volpe, J. J. and J. C. Marasa. 1975. Hormonal regulation of fatty acid synthetase, Acetyl-CoA carboxylase and fatty acid synthesis in mammalian adipose tissue and liver. Biochem. Biophys. Acta. 380:454.

Volpe, J. J. and P. R. Vagelos. 1974. Regulation of mammalian fatty acid synthetase. The roles of carboxylate and insulin. Proc. Natl. Acad. Sci. U. S. A. 71:889.

Volpe, J. J. and P. R. Vagelos. 1976. Mechananisms and regulation of biosynthesis of saturated fatty acid. Physiol. Rev. 56:339.

Wakil, S. J., J. K. Stoops and V. C. Joshi. 1983. Fatty acid synthesis and its regulation. Ann. Rev. Biochem. 52:537.

Wallis, M. 1975. The molecular evolution of pituitary hormones. Bio. Rev. 50:35.

Werner, H., M. Woloschak, B. Stannard, Z. Shen-orr, C. T. Roberts and D. LeRoith. 1991. The insulin-like growth factor I receptor: Molecular biology, heterogeneity, and regulation. In Insulin-like Growth Factors: Molecular and Cellular Aspects. pp. 17-47. D. LekRoith, ed. Boca Ratar, CRC Press.

Williamson, D. H., M. R. Munday, R. G. Jones, A. F. C. Roberts and A. J. Ramsey. 1983. Short-term dietary regulation of lipogenesis in the lactating mammary glands of the rat. Adv. Enzyme Regul. 21:135.

Wilson, S. P. 1991. Insulin-like growth factor I enhances proenkephalin synthesis and dopamine b-hydroxylase activity in adrenal chromaffin cells. Lif. Sci. 49:269.

Witters, L. A., J. P. Tiper and G. W. Bacon. 1983. Stimulation of site-specific phosphorylation of acetyl Coenzyme A carboxylase by insulin and epinephrine. J. Biol. Chem. 258:5643.

Witters, L. A., E. M. Kowaloff and J. Avruch. 1979. Glucagon regulation of protein phosphorylation. J. Biol. Chem. 254:245.

Witters, L. A. and B. Vogt. 1981. A new method for isolation of rat liver acetyl-CoA carboxylase. J. Lipid Res. 22:364.

Witters, L. A. 1981. Insulin stimulates the phosphorylation of acetyl-CoA carboxylase. Biochem. Biophys. Res. Commun. 100:872.

Witters, L. A., T. D. Watts, D. L. Daniels and J. L. Evans. 1988. Insulin stimulates the dephosphorylation and activation of acetyl-CoA carboxylase. Proc. Natl. Acad. Sci. U. S. A. 85:5473.

Wood, W. I., G. Cachianes, W. J. Henzel, G. A. Winslow, S. A. Spencer, R. Hellmiss, J. Martin and R. C. Baxter. 1988. Cloning and expression of the growth hormone-dependent insulin-like frowth factor-bindng protein. Mol. Endocrinol. 2:1176.

Wood, D. C., W. J. Salsgiver, T. R. Kasser, G. W. Lange, E. Rowold, B. N. Violand, A. Johnson, R. M. Leimgruber, G. R. Parr, N. R. Siegel, N. M. Kimack, C. E. Smith, J. F. Zobel, S. M. Ganguli, J. R. Garbow, G. Bild, and G. G. Krivi. 1989. Purification and characterization of pituitary bovine somatotropin. J. Biol. Chem. 246:14741.

Yang, Y. T. and R. L. Baldwin. 1973. Preparation and metabolism of isolated cells from bovine adipose tissue. J. Dairy. Sci. 56:350.

Yarden, Y. and A. Ullrich. 1988. Growth factor receptor tyrosine kinases. Annu. Rev. Biochem. 57:443.

Young, F. G. 1947. Experimental stimulation (galactopoiesis) of lactation. Br. Med. Bull. 5:155.

Zammit, V. A. and C. G. Corstorphrine. 1982. Inhibition of acetyl-Ca-A carboxylase activity in isolated rat adipocytes incubated with glucagon. Biochem. J. 208:783.

Zapf, J., C. Hauri, M. Waldvogei. E. Futo, K. Hasler, K. Binz, H. P. Guler, C. Schmid and E. R. Froesch. 1989. Recombinant human insulin-like growth factor I induces its own specific carrier protein in hypophysectomized and diabetic rats. Proc. Natl. Acad. Sci. U. S. A. 86:3818.

Zapf, J., C. Schmid, H. P. Guler, M. Waldvogel, Haurich, E. Futo, P. Hossenlopp and M. Binoux. 1990. Regulation of binding proteins for insulin-like gorwth factor-2 in humans: increased expression of IGF binding proteins during treatment of healthy adults and in patients with extrapancreatic tumor. J. Clinic. Invest. 86:952.