Animal Science 1997, **64**: 1-15

1357-7298/97/60230001$20·00

Insulin-like growth factors and their binding proteins in domestic animals

K. L. Hossner[1], R. H. McCusker[2] and M. V. Dodson[3]

[1]*Department of Animal Sciences, Colorado State University, Fort Collins, CO 80523, USA*
[2]*Department of Animal Sciences, University of Illinois, Urbana, IL 61801-4737, USA*
[3]*Department of Animal Sciences, Washington State University, Pullman, WA 99164, USA*

Abstract

Insulin-like growth factors (IGFs) and their binding proteins play an essential rôle in regulating animal growth and metabolism. The initial portion of the current review focuses on the physiological effects of the IGFs and delineates their rôle as regulators of animal growth and metabolism. The rôle of IGFs as mediators of growth hormone effects, as insulin-like metabolic regulators and as foetal growth regulators is discussed. The remainder of the review is devoted to the IGF binding proteins, their modulation of IGF action and their rôle in foetal and postnatal regulation of growth.

Keywords: *binding proteins, insulin-like growth factor.*

Introduction

Historical background

Production of agricultural animals has long focused on enhancing productive efficiency. Increasing rate of gain and reducing food per kg of gain of farm animals has been accomplished by genetic selection, nutrient optimization and by using exogenous hormones. Early attempts at modifying animal production used inhibitors (Beeson *et al.*, 1947) or enhancers (Beeson *et al.*, 1949) of thyroid function, steroid hormones (Hancock *et al.*, 1991), and beta-adrenergic agents (beta agonists; Anderson *et al.*, 1991). Steroid implants and beta-agonists as well as antibiotics (Armstrong, 1986) are still important adjuncts for enhancing livestock production. Until the early 1980s, metabolic modifiers were limited to relatively small molecules which could be extracted from biological sources or chemically synthesized. Complex macromolecules such as protein or polypeptide hormones were not available. The advent of new techniques of inserting a single gene encoding for a specific protein or polypeptide into bacterial expression vectors has provided an effective method to produce large quantities of these complex molecules. Biologically important proteins such as growth hormone (GH) and the insulin-like growth factors (IGF) have been commercially produced using recombinant DNA methods. The availability of these and other proteins has provided research scientists and clinicians with new tools which may be used to alleviate disease or enhance animal productivity.

The current review focuses on the effects of the IGF system in regulating animal growth and metabolism. After a relatively cursory coverage of the biochemistry of IGFs and their receptors, the physiological effects of the IGFs *in vivo* are examined in the context of IGFs as mediators of GH action and as local tissue and endocrine growth factors. The second half of the review is devoted to the burgeoning field of IGF binding proteins, their chemistry and their effects on enhancing or inhibiting the actions of IGFs.

The IGF system

Biochemistry of IGF-1 and IGF-2

There are two forms of the IGFs, designated IGF-1 and IGF-2. Both are single chain polypeptides, similar in structure to proinsulin, with an intact c-peptide and three intramolecular disulphide bonds. Human IGF-1 consists of 70 amino acids and has a molecular weight of 7646 Da (Rinderknect and Humbel, 1978). IGF-1 has a basic isoelectric point (8·5) and lacks the amino acids histidine and tryptophan (Klapper *et al.*, 1983). The amino acid sequences of the IGFs are highly conserved in mammals: bovine and porcine IGF-1 are identical to human IGF-1 (Honegger and Humbel, 1986), while ovine IGF-1 differs only by a substitution of alanine

for proline at residue 66 (Francis *et al.*, 1989a). Human IGF-2 has an acidic isoelectric point (<6·5) and consists of 67 amino acids, with a molecular weight of 7470 Da (Humbel, 1990). Porcine IGF-2 differs from human IGF-2 by only one amino acid, while bovine and ovine IGF-2 differ from the human molecule by three and four amino acids, respectively (Francis *et al.*, 1989b). Avian IGF-1 differs from the human version by eight amino acid substitutions (Ballard *et al.*, 1990) while chicken IGF-2 differs by 12 amino acids (Upton *et al.*, 1995).

IGF receptors

IGF-1 and IGF-2 have distinct plasma membrane glycoprotein receptors (De Meyts *et al.*, 1994). The native type 1 IGF receptor is very similar to the insulin receptor and has a molecular weight of ~450 kDa and is composed of two 130 to 140 kDa alpha subunits and two 90 to 98 kDa beta subunits, linked by disulphide bonds (LeRoith *et al.*, 1995). The alpha subunits contain the extra-cellular ligand binding site and the smaller beta subunits transverse the plasma membrane and contain the cytoplasmic tyrosine kinase activity. These subunits are covalently bound by disulphide bonds into a heterotetrameric structure. Upon ligand activation, the beta subunits of the type 1 receptor are autophosphorylated on tyrosine residues and also catalyse phosphorylation of other cytoplasmic tyrosine-containing protein substrates (Zick *et al.*, 1983). The IRS-1 protein is a major substrate for both the type 1 IGF receptor and the insulin receptor (Myers *et al.*, 1993). Phosphorylated IRS-1 may activate several other intracellular messengers, including phosphoinositol-3′-kinase and other SH-2 domain-containing proteins (reviewed in LeRoith *et al.*, 1995). The IGF-2 receptor is composed of a single glycosylated protein chain, ~250 kDa, which is identical to the mannose-6-phosphate receptor (Kiess *et al.*, 1994). No definitive second messenger for the IGF-2 receptor has been identified and IGF-2 is thought to act through the type 1 IGF receptor (Ballard *et al.*, 1988). Chickens are unique in that the mannose-6-phosphate receptor does not bind IGF-2 (Canfield and Kornfeld, 1989) and the actions of IGF-1 and IGF-2 in chickens are apparently mediated solely by the type 1 IGF and insulin receptors.

IGF as mediator of GH action

While it has long been known that IGF-1 synthesis and secretion is stimulated by GH (Daughaday *et al.*, 1976), this effect has only been confirmed recently in domestic animals. When bovine GH was administered to Dorset/Suffolk crossbred sheep by osmotic pump at 2 or 4 mg/day for 43 days a time- and dose-dependent increase in circulating IGF-1 was observed (McLaughlin *et al.*, 1991). A doubling of IGF-1 serum levels was observed in lambs injected daily with ovine or bovine growth hormone for 6 weeks (McLaughlin *et al.*, 1993) and twin lambs (22 kg) injected daily with bovine GH for 11 weeks demonstrated an immediate and progressive increase in circulating IGF-1 (Pell, 1989). Although IGF-1 levels were increased proportionately by about 0·6 after the 1st week of treatment, after 10 weeks of GH treatment, serum levels of IGF-1 had tripled. In growing heifers, bovine GH injections (50 μg/kg per day) progressively increased serum levels of IGF-1 over a 6-week period (Lemal *et al.*, 1989) and beef steers demonstrated a dose-dependent increase in IGF-1 concentration when given bovine GH by daily injection (Mosely *et al.*, 1992) or as a slow-release implant (Dalke *et al.*, 1992; Schwarz *et al.*, 1993). Similarly, in pigs, IGF-1 levels increase in response to porcine GH given by daily injection (Owens *et al.*, 1990; Azain *et al.*, 1992) or as a sustained release implant (Azain *et al.*, 1992; Klindt *et al.*, 1992). Thus, the IGF-1 induction by GH in farm animals is similar to that of laboratory animals and humans. Birds appear to be exceptions to the regulation of IGF-1 by GH, as the treatment of young broiler chickens with chicken GH has little or no effect on circulating IGF-1 (Cogburn *et al.*, 1989; Moellers and Cogburn, 1995). Studies with chronic elevated circulating levels of GH in which IGF-1 levels have been progressively increased over time, suggest that the synthesis and secretion of IGF-1 (and its binding proteins) are occurring at a rate which overwhelms clearance mechanisms and leads to a continuous accumulation of systemic IGF-1.

Recently, transgenic animals have been used to examine the GH : IGF axis. Mice which express ovine GH under the control of the zinc-responsive metallothionein promotor have elevated levels of serum GH, increased hepatic GH receptors and elevated serum IGF-1 concentrations (Orain *et al.*, 1991; Mathews *et al.*, 1988b). The elevated growth rates and body sizes of these mice (2x normal) were correlated with IGF-1 concentrations.

Endocrine effects of IGFs

When the IGFs were originally discovered, they were believed to play solely an endocrine rôle, mediating the effects of GH. This hypothesis has been modified to include not only endocrine effects of the IGFs, but also to acknowledge that IGFs have local effects which are independent of the circulatory system. Local, tissue-level actions of IGFs likely complement the systemic actions of IGFs but the problems associated with studying localized effects of IGFs in isolation, especially *in vivo*, make endocrine studies attractive. The validity of such studies is reinforced by a body of data which suggests that circulating IGFs regulate the growth and metabolism of animals.

The pulsatile nature of GH secretion has made it difficult to correlate GH levels to growth characteristics (Davis *et al.*, 1984). On the other hand, serum IGF-I levels are relatively constant and reflect the GH status of animals. While it is necessary to collect multiple blood samples over a 6 to 8 h time period accurately to assess GH levels, a single blood sample suffices to assess IGF-1 concentrations. Thus, while it is technically challenging to measure GH status and correlate GH to growth parameters, IGF-1 serum concentrations are related to a number of growth characteristics. For example, IGF-1 levels have been correlated to the body weight of dogs (Eigenmann *et al.*, 1988), and with weight and growth rate in pigs (Buonomo *et al.*, 1987), sheep (Roberts *et al.*, 1990) and cattle (Bishop *et al.*, 1989; Davis and Bishop, 1991).

IGFs are involved in animal growth and cell metabolism from early in embryonic development. Transcripts for IGF-1 and -2 and the type 1 IGF receptor have been detected in sheep embryos from the one-cell to the blastocyst stage (Watson *et al.*, 1994). Later in development, foetal mesenchymal tissues produce both IGF-1 and IGF-2. As IGF-2 mRNA is produced more abundantly in the rat foetus than IGF-1 (Rotwein *et al.*, 1987), IGF-2 is considered the more important foetal IGF. Similarly, foetal serum levels of IGF-2 are much greater than IGF-1 in pigs (Lee *et al.*, 1993a), cattle (Holland, 1991) and sheep (Owens *et al.*, 1994). This pattern is reversed in the adult when IGF-1 predominates in the circulation. Postnatal IGF-1 concentrations increase almost four-fold in pigs between days 2 and 40 (Scanes *et al.*, 1987), progressively increase until weaning in calves (Breier *et al.*, 1988) and increase two- to four-fold at 4 to 6 weeks of age in chickens and turkeys (McMurtry *et al.*, 1994).

The observations that circulating levels of IGF-1 correlated with body size and growth rate provided the rationale for its use as an easily accessible marker for livestock selection. Attempts at using IGF-1 as a selection marker in livestock date back 20 years (Lund-Larsen and Bakke, 1975). Preliminary studies in mice showed that IGF-1 levels had a heritability of 0·4 at 35 days of age (Blair *et al.*, 1987) and that selecting mice on the basis of IGF-1 levels increased 6-week weights proportionately by about 0·2 after seven generations (Blair *et al.*, 1988), but did not affect body composition (Siddiqui *et al.*, 1990). Angus cattle selected for high food efficiencies had slightly higher IGF-1 levels than less efficient animals (Bishop *et al.*, 1989). Analysis of IGF-1 levels in Targhee sheep selected for high 120-day weaning weights showed that IGF-1 levels were too variable (CV = 0·36 to 0·50) to be useful as a selection tool (Medrano and Bradford, 1991). This variability is greatly reduced in cattle which have been selected on the basis of serum IGF-1 levels (Davis *et al.*, 1995), although IGF-1 levels were shown to have a strong non-genetic component, with season, sex and age of calf affecting IGF-1. Male cattle (Davis *et al.*, 1995; Kerr *et al.*, 1991) and lambs (Roberts *et al.*, 1990; Medrano and Bradford, 1991) generally have higher levels of IGF-1 than females. In addition, there is a strong nutritional influence on IGF-1 levels in all animals and IGF-1 increases in response to elevated protein and energy intake (Ellenberger *et al.*, 1989; Bass *et al.*, 1991). Thus, while the circulating levels of IGF-1 are correlated to animal growth in a variety of species, the utility of this parameter for genetic selection and improvement of livestock is still under investigation.

Autocrine/paracrine effects of IGFs

Circulating IGFs are synthesized and secreted primarily by the liver. In addition, several foetal and adult tissues synthesize the IGFs (D'Ercole *et al.*, 1984; Murphy *et al.*, 1987). As outlined in Figure 1, these extrahepatic sources of IGFs are believed to act primarily at the site of their synthesis, on adjacent cells (paracrine action) or on the same cells that produce them (autocrine action). Fibroblasts have been shown to secrete IGF-1 and IGF-2 (Adams *et al.*, 1983) and to respond to exogenous IGFs (Moats-Staats *et al.*, 1993). Skeletal muscle expresses IGF-1 and skeletal muscle IGF-1 mRNA is increased in response to GH injections (Isgaard *et al.*, 1988 and 1989).

Injection of small doses of GH directly into the tibial growth plate of hypophysectomized rats stimulated

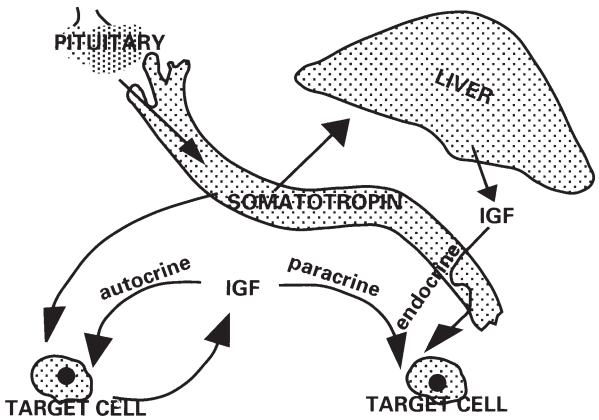

Figure 1 Endocrine, paracrine and autocrine activities of IGF-1, as regulated by growth hormone (from McGuire *et al.*, 1992, with permission). As locally produced factors like proteases, binding proteins and growth factors also regulate IGF-1 release from cells, a systemic and local mode of IGF-1 control has been proposed.

longitudinal bone growth of the treated leg (Isaksson *et al.*, 1982; Russell and Spencer, 1985). Later studies showed that IGF-1 or GH infusions into the rat hindlimb arterial supply also stimulated bone elongation, but the local effects of GH were abolished by the co-infusion of antiserum to IGF-1 (Schlechter *et al.*, 1986). Although circulating IGF-1 levels are usually well correlated to growth status, this is not always true. In rats treated with doses of GH which did not alter circulating serum IGF-1 concentrations, hepatic and renal IGF-1 levels were doubled and trebled, respectively, while body weights and tibial epiphyseal plate widths increased over control animals (Orlowski and Chernausek, 1988). Transgenic mice have been used to show that local concentrations of IGF-1 may be important in regulating muscle growth. When IGF-1 gene expression is limited to skeletal muscle by linking it to an actin promoter, there is a significant induction of muscle hypertrophy without an increase in body weight or serum IGF-1 concentrations (Coleman *et al.*, 1995). The above studies provide strong evidence that locally produced IGF plays an important rôle in the regulation of animal growth.

Effects of IGFs on animal growth and metabolism

Effects of IGF-1 on growth have been demonstrated in hypophysectomized rats, in which IGF-1 stimulates growth in a dose-dependent manner (Schoenle *et al.*, 1982; Guler *et al.*, 1988). More recently, mice transgenic for the IGF-1 gene have been developed. These animals, which over express IGF-1 in most tissues, have circulating IGF-1 which is 1·5 × normal (Mathews *et al.*, 1988a). These mice also demonstrated organomegaly, and growth of spleen, pancreas, kidney and brain were 1·4- to 2·0-fold higher than non-transgenic litter mates. In IGF-1 transgenic mice crossed with GH-deficient dwarf mice, body weight and linear growth was identical to that of normal litter mates (Behringer *et al.*, 1990), suggesting that IGF-1 can mediate all of GH's effects on somatic growth. A significant difference between GH and IGF-1 transgenic mice was that IGF-1 selectively increased brain size, while GH induced growth of the liver (D'Ercole, 1993). In castrated male sheep chronically treated with IGF-1 (150 μg/kg every 8 h for 8 weeks), there were no significant effects on body or carcass weights, long bone growth or wool growth. Although there was an increase in blood glucose and bone density, there were no effects on the fat in the carcass or hind leg (Cottam *et al.*, 1992). Thus, in well fed, pituitary-intact sheep, doubling the circulating levels of IGF-1 did not enhance growth performance.

Continuous osmotic pump infusion of IGF-1 into growing normal or dwarf chickens for 4 weeks had no effect on longitudinal growth but decreased body fat (Tixier-Boichard *et al.*, 1992). Passive immunization of chickens against IGF-1 did not affect growth, carcass composition or energy efficiency, while immunization against both IGF-1 and IGF-2 decreased body weight and abdominal fat (Spencer *et al.*, 1995). Thus, the IGFs may be more involved with fat metabolism in chickens than with regulation of growth. The latter study suggests an important rôle for IGF-2 in adipose tissue in chickens.

IGF-2 is thought to play a growth-promoting rôle primarily in foetal development, when circulating levels and tissue expression are elevated. This rôle has been directly confirmed in mice in which the IGF-2 gene was selectively disrupted (so-called 'knockout mice'). These mice were apparently normal and fertile, but were proportionately about 0·40 smaller than unaffected litter mates throughout postnatal growth (up to 30 days). This negative effect on growth was evident in 16-day mouse embryos (DeChiara *et al.*, 1990). Recent studies in which normal female rats were continuously infused with IGF-1 or IGF-2 for 14 days showed that both growth factors increased body-weight gain, food efficiency and nitrogen retention, but IGF-1 was two- to three-fold more potent than IGF-2 (Conlon *et al.*, 1995). Mice transgenic for IGF-2 did not exhibit increased body weights or lengths and had organ weights identical to age-matched controls (van Buul-Offers *et al.*, 1995). Only the thymus of transgenic IGF-2 mice was increased in weight. These studies demonstrate that IGF-1 and IGF-2 have direct effects on whole body growth and the similar actions, but lower potency, of IGF-2 suggests that it is acting in a manner similar to IGF-1, probably via the type 1 IGF receptor. A fundamental rôle in foetal growth has been demonstrated for IGF-2, as mice deficient in IGF-2 are proportionately stunted beginning prior to day 16 of embryonic development.

IGFs share structural and biological properties with insulin. Thus, at pharmacological concentrations, insulin interacts with the IGF-1 receptor and has growth-promoting properties in addition to its effects on glucose, lipid and protein metabolism. Likewise, elevated concentrations of IGF-1 can mimic the metabolic effects of insulin by acting through the insulin receptor. Guler *et al.* (1987) showed that intravenous injection of IGF-1 induced rapid hypoglycemia in humans. This effect of IGF-1 has led to its use to control blood glucose in diabetes (Zenobi *et al.*, 1993). In addition, IGF-1 has distinct effects on protein metabolism, and has been shown to stimulate protein synthesis and inhibit protein breakdown in skeletal muscle *in vitro* (Roeder *et al.*, 1988) and *in vivo* (Douglas *et al.*, 1991). This protein-sparing effect of IGF-1 may provide a useful clinical

adjunct for catabolic states such as infection, reduced food intake or premature birth. Indeed, the IGFs may prove to be most useful as clinical adjuncts for specific metabolic disorders rather than as growth promotants.

Insulin-like growth factor binding proteins

Insulin-like growth factors do not act alone in their modulation of growth and metabolism, but are part of a complex system which modulates and mediates the action of these growth factors. As described above, both IGFs interact with two IGF receptors (type 1 and type 2) as well as the insulin receptor. In addition, the activity of the IGFs is regulated not only by GH and other endocrine modulators which enhance or suppress local and systemic IGF levels, but also by the presence of IGF-specific binding proteins (IGFBP). These IGFBPs sequester circulating IGFs, prolong their half-life in the circulation and make the analysis of IGF levels difficult. It has long been evident that the IGF bound to IGFBPs is less active than 'free' (non-bound) IGF. More recently, several research groups have shown that IGFBPs also enhance the activity of the IGFs (see below). The following portion of this review focuses on the mechanisms by which IGFBPs modulate IGF activity and emphasizes that the IGFBPs are essential in regulating the actions of the IGFs.

Characteristics of the IGF binding proteins

Six IGFBPs (IGFBP-1 to 6) have been cloned and characterized in humans and rodents (Ballard *et al.*, 1989). It is presumed that a similar array of IGFBP exists in domestic animals, although they are not yet well characterized. In most species, total IGF levels in serum are greater than 500 µg/l throughout postnatal development. Since this is far above the range necessary to stimulate cell growth, IGF activity is controlled by pathways independent of changes in circulating IGF levels. Indeed, IGF action is largely influenced by the presence of IGFBPs. This is evident when considering that unbound (´free´) IGF in serum and most body fluids is difficult to measure (Frystyk *et al.*, 1994) and may be present only in low quantities (<0·4% of total).

The IGFBPs are similar in overall structure, consisting of 200 to 300 amino acids (see Table 1). The amino acid sequences of both the N and C-terminal thirds of the binding proteins are highly conserved between the IGFBPs and across species (Shimasaki and Ling, 1991). The major variation in amino acid number and composition occurs in the middle third of the proteins, suggesting that specific amino acids necessary for IGF-binding (Huhtala *et al.*, 1986; Brinkman *et al.*, 1991) are probably localized to the conserved end regions. Structurally, all but IGFBP-6 have at least 18 cysteines (conserved in number and spacing) which may be involved in intramolecular disulphide bond formation and ligand binding.

All six of the IGFBPs are found in human or rat serum (Shimasaki *et al.*, 1991; Baxter and Saunders, 1992; Baxter, 1994) forming binary complexes with the IGFs of 30 to 50 kDa. In contrast, IGFBP-3 either binds IGF to form a binary complex or combines with a larger protein, ALS (acid-labile subunit) and IGF to form a trinary complex of 150 kDa (Barreca *et al.*, 1995; Lee and Rechler, 1995). The half-life of ´free´ IGFs in serum is approximately 20 min, while the binary and trinary complexes have half-lives of approximately 2 and 6 to 12 h, respectively (Davis *et al.*, 1989; Hodgkinson *et al.*, 1989; Walton *et al.*, 1989; Francis *et al.*, 1990).

IGFBPs as modulators of IGF activity

The IGFBPs have multiple modes of action (endocrine, paracrine, autocrine) and two distinct mechanisms of action (stimulatory or inhibitory). The IGFBPs are expressed in almost all tissues with

Table 1 *Physical and biological properties of IGFBPs*†

	Size (kDa)	Cysteines (No.)	RGD	Heparin binding site	Glycosylation	Phosphorylation	Ligand preference	Levels (µg/l) in serum	
								Foetal	Adult
IGFBP-1	25·3	18	+	+	–	+	I II	25	12
IGFBP-2	31·4	18	+	+	–	?	I<II	640	180
IGFBP-3	28·7	18	–	+	+	+	I II	1000	3200
IGFBP-4	26·0	20	–	–	+	?	I II	?	450
IGFBP-5	28·6	18	–	+	+	?	I<II	?	?
IGFBP-6	22·8	16	–	+	+	?	I<<II	?	220

† Information is for human proteins; ? unknown; + present; – not present (Information in this table was drawn from Bang *et al.*, 1994; Baxter, 1994; Bach and Rechler, 1995; Honda *et al.*, 1996).

the exception of IGFBP-5 whose expression is very low or absent in the liver (Shimasaki *et al.*, 1991). Soluble IGFBPs largely attenuate IGF activity and those which are cell-associated may either attenuate or enhance IGF activity. An understanding of how these processes are controlled and manifested is essential in determining actions of the IGFs as anabolic agents.

Circulating IGFBPs have two overall functions in the intact animal. First, the IGF-IGFBP complexes establish and maintain a large circulating pool of IGF. The majority of systemic IGFs are bound to the high molecular weight IGFBP-3 trinary complex. This complex does not cross the capillary endothelium and provides a long-lived, stable reservoir of circulating IGFs. In addition to providing a relatively stable circulating form of IGF, the binary IGF-IGFBP complexes likely cross the capillary endothelium and transport bound IGF to target tissues (Bar *et al.*, 1990; Boes *et al.*, 1992).

Inhibitory activity. Soluble IGFBPs bind the IGFs with high affinity, preventing IGF association with cell surface receptors and inhibiting IGF activity in a dose-dependent manner (Ritvos *et al.*, 1988; Gopinath *et al.*, 1989; Lee *et al.*, 1993b) and blunting the metabolic and mitogenic effects of the IGFs (Zapf, 1995). A large molar excess of any soluble IGFBP will eliminate essentially all of the effects of the IGFs. Inhibition of IGF activity by sequestration by IGFBP was originally thought to be the major rôle of circulating IGFBPs. Such activity was thought to prevent rapid changes in non-bound IGF from causing metabolic effects such as hypoglycemia, which may occur in response to acute pharmacological levels of IGF-1 (Guler *et al.*, 1987). However, rapid fluctuations in serum IGF have not been reported and changes in IGF concentrations are quite slow (Thissen *et al.*, 1994). Thus, under most conditions, flux of IGF through the circulating pool is constant, in equilibrium, and equal to the rate of IGF secretion. Total circulating IGF pool size and flux through the circulating pool only changes during adaptation to stress (Figure 2).

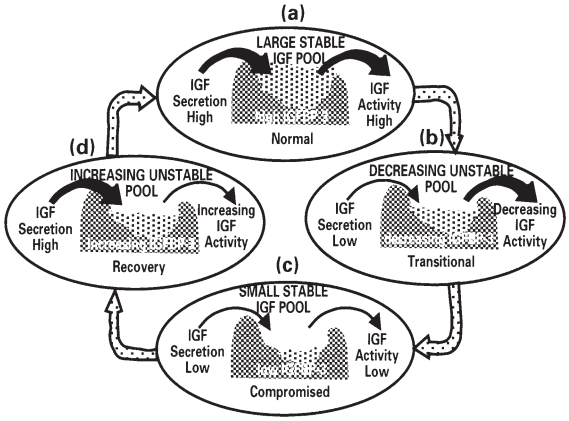

Figure 2 Endocrine activity of the IGFs is a function of secretory rate and pool stability. The endocrine activity of the IGFs is generally considered a function of the rate of secretion, transit time and rate of clearance into tissues. In unstressed conditions, when the IGFBPs (especially IGFBP-3) maintain a large stable pool of circulating IGFs, clearance (tissue availability) is equal to rate of secretion, both being high (a). After adaptation to physiological stress, IGF secretion is generally low but still equal to clearance rate (c). During adaptation to (b) or recovery from (d) stress, pool size is changing and rate of clearance and secretion differ. Only during these transient conditions do the IGFBPs temporarily affect IGF activity by controlling transit into tissues via changes in pool size.

Many cells secrete at least one form of IGFBP. It is assumed from studies *in vitro* that IGFBPs released from cells inhibit IGF activity by preventing receptor binding. This is supported by reports of IGF-1 analogues, with reduced IGFBP binding activity, having greater anabolic activities than IGF-1 (Cascieri and Bayne, 1994), although such studies are relatively short-lived. However, the inhibitory effect of IGFBPs *in vivo* can occur only until a steady pool size is established, a phenomenon which has not been mimicked *in vitro*. Thus, there is no known mechanism by which the IGFBPs can depress IGF activity over long periods of time *in vivo* by preventing type 1 IGF receptor binding. Indeed, transgenic mice which overexpress IGFBP-1 exhibit normal growth patterns (Dai *et al.*, 1994).

Stimulatory activity. IGFBP-1, 2, 3 and 5 enhance the mitogenic activity of the IGFs *in vitro* (Bourner *et al.*, 1992; Coleman and Etherton, 1994; Zapf, 1995). In contrast, IGFBP-4 exhibits only inhibitory effects, indicating that the forms and relative proportions of IGFBPs present in extracellular fluids may determine the net effect of the IGFs. IGFBP inhibitory activity is related to short-term reduced type 1 IGF receptor binding, whereas stimulating activity may be related to IGF - IGFBP complex association with cell surfaces. IGFBP-1 and IGFBP-2 enhanced the mitogenic activity of IGF-1 in porcine smooth muscle cells (Camacho-Hubner *et al.*, 1991; Bourner *et al.*, 1992), in which treatment with IGFBP-1 and IGF-1 together increased ^{3}H-thymidine incorporation four-fold above IGF-1 alone, whereas IGFBP-2 with IGF-1 was twice as potent as IGF-1 alone. The potentiating activity was related to the ability of the IGFBPs to adhere to cell surfaces and bind ^{125}I-IGF-1 (McCusker *et al.*, 1991a; Bourner *et al.*, 1992). IGFBP-1 adheres to cell surfaces better than does IGFBP-2 and is more effective in potentiating IGF effects (Busby *et al.*, 1988). The stimulation of skeletal muscle myoblast

proliferation by IGFBP-1 was proportionately enhanced by 0·35 in the absence of added IGF-1 (McCusker *et al.*, 1989a), suggesting that IGFBPs may potentiate the mitogenic activity of endogenously secreted IGFs. The ability of IGFBP-5 to potentiate osteoblast mitogenesis (Andress and Birnbaum, 1992; Andress *et al.*, 1993) and the correlation between IGFBP-5 synthesis and myoblast proliferation (McCusker and Clemmons, 1988), suggests that the IGFBPs have potential to increase muscle and bone growth.

Thus, the effects of the IGFBPs depend not only upon the concentration and relative proportions of IGFBPs in extracellular fluids, but on the relative distribution of IGFBPs between extracellular fluids and cell surfaces. Distinct yet undefined mechanisms permit expression of the proper form of IGFBP under the proper physiological condition to either depress or enhance IGF-mediated growth.

Mechanisms of IGFBP action

Recent information indicates that there are several mechanisms by which the IGFBPs associate with cells and either enhance type 1 IGF receptor-mediated signalling or cause a type 1 IGF receptor-independent signalling event. IGFBP-1 and IGFBP-2 have the amino acid sequence arginine-glycine-asparagine (RGD) near their carboxy termini (Shimasaki and Ling, 1991). This sequence is involved in ligand recognition by integrin receptors and is involved in IGFBP-1 binding to cultured cells (Jones *et al.*, 1993c). Cells transfected with IGFBP-1 migrate more rapidly (Jones and Clemmons, 1995), an effect mediated by the $\alpha5\beta1$ integrin receptor (Jones *et al.*, 1993a; Irving and Lala, 1995). The rôle of the integrin receptor in potentiating IGF-stimulated mitosis and the requirement of IGF-1 for IGFBP-1 stimulation of mitosis is undefined.

Although IGFBP-3 and IGFBP-5 are also found in association with cultured cells, they do not contain the RGD sequence. IGFBP-5 associates with the extracellular matrix (ECM) of cells (Jones *et al.*, 1993b) and IGFBP-3 can associate with cells via interactions with cell membrane glycosaminoglycans (Martin and Baxter, 1993; Hodgkinson *et al.*, 1994). Cell-associated IGFBP-3 (McCusker *et al.*, 1990; Conover, 1992) and IGFBP-5 have a markedly reduced affinity for the IGFs. An IGFBP-3 fragment with low ligand binding affinity potentiated IGF-1 activity, whereas intact high affinity IGFBP-3 depressed IGF-1 activity (Schmid *et al.*, 1991). Similarly, IGFBP-5 associated with collagen or ECM had a lower affinity for IGF-1 than soluble IGFBP-5, and potentiated IGF's mitogenic activity (Jones *et al.*, 1993a). Finally, carboxy-truncated IGFBP-5 with decreased ligand affinity also potentiated IGF activity (Andress *et al.*, 1993). This affinity-shift phenomenon permits two potentiating events to occur. First, by binding to cell membranes or the pericellular ECM, the high affinity IGF-IGFBP complex is delivered to the target cell. Secondly, the reduced binding affinity allows IGF to dissociate and increase type 1 IGF receptor activation. This phenomenon does not appear to involve any direct interaction between IGFBPs and the type 1 IGF receptor and is distinct from integrin-mediated signalling.

Regulation of IGFBP activity by phosphorylation or glycosylation

Recently it has been demonstrated that post-translational modifications of IGFBPs may affect biological activity. Naturally occurring IGFBP-1 was found to be phosphorylated on serine residues (Frost and Tseng, 1991; Jones *et al.*, 1991 and 1993a). The affinity for IGF-1 of phosphorylated IGFBP-1 is four- to six-fold higher than that of non-phosphorylated IGFBP-1 (Jones *et al.*, 1991; Koistinen *et al.*, 1993). IGF-1 induced wound healing was enhanced by the lower affinity non-phosphorylated recombinant IGFBP-1, but not by the high affinity phosphorylated IGFBP-1 (Jyung *et al.*, 1994). However, a mixture of high affinity phosphorylated IGFBP-1 isoforms potentiated the mitogenic activity of IGF-1 (Koistinen *et al.*, 1993). However, phosphorylated IGFBP-1 isoforms may potentiate IGF activity in cells which can remove phosphate groups, although this has not been studied. As IGFBP-1 is believed not to be dephosphorylated by cells which secrete it (Frost and Tseng, 1991; Jones *et al.*, 1991), these cells would not secrete IGFBP-1 to enhance their own growth, but would secrete IGFBP-1 to affect other tissues, acting primarily as a systemic factor. IGFBP-3 is also phosphorylated (Hoeck and Mukku, 1994) although a rôle in controlling its activity has not been established. Point-mutations of the two major phosphorylated serines did not affect IGFBP-3 affinity for IGF-1. To date, phosphorylated forms of IGFBP-2, 4, 5, and 6 have not been reported. Binding proteins 3 to 6 are glycosylated (Shimasaki and Ling, 1991), but this does not appear to affect ligand binding or activity *in vitro* (Conover, 1991).

Proteolysis and the IGF-IGFBP system

As discussed above, one of the original functions proposed for the IGFBPs was to prevent IGF degradation. Ironically, it appears that the IGFs may instead control IGFBP degradation. IGFBP-5 levels in conditioned medium of several cell types is increased by the addition of the IGFs. This effect is independent of the type 1 IGF receptor (Clemmons *et al.*, 1990; Camacho-Hubner *et al.*, 1992) and was shown to be due to IGF protection of IGFBP-5 from

degradation (Kanzaki *et al.*, 1994). Soluble IGFBP-5 appears to be rapidly degraded in the absence of IGFs, whereas IGFBP-5 within the ECM remains intact (Jones *et al.*, 1993b). Hence, newly secreted IGFs may bind ECM-associated intact IGFBP-5 but unsaturated intact IGFBP-5 is not present in extracellular fluids to inhibit IGF activity. This localization of IGF to the ECM-associated IGFBP-5 may have an autocrine potentiating effect. In contrast to the effect on IGFBP-5, IGF binding enhances the proteolysis of IGFBP-4 *in vitro* (Kanzaki *et al.*, 1994; Conover *et al.*, 1995a; Irwin *et al.*, 1995). Growth of cells which secrete an IGFBP-4 protease is not inhibited by IGFBP-4 (Conover *et al.*, 1995a). This may provide a mechanism to target IGF activity, as cells with the protease could release IGF for an anabolic effect.

IGFBP-3 proteolysis has been extensively studied. Partially degraded, 'nicked', IGFBP-3 is present in serum and has a reduced affinity for IGF. IGFBP-3 is degraded by several cell types *in vitro* (Fowlkes *et al.*, 1994; Conover *et al.*, 1995b; Tonner *et al.*, 1995) and an IGFBP-3 specific protease is found in serum (Guidice, 1995). Nicked IGFBP-3 can form the 150 KDa complex with ALS in serum (Lee and Rechler, 1995) and is thus still involved in IGF transport. Cells or tissues with the ability to nick IGFBP-3 may release some of the bound IGFs as a means to control IGF activity. This may be especially true during gestation or trauma when IGFBP-3 protease activity is elevated in serum (Baxter, 1994). A similar proteolysis of IGFBP-2 in serum occurs during food deprivation (McCusker *et al.*, 1991b).

Foetal v. postnatal IGFBPs

Human foetal serum levels of IGFBP-1 and -2 are at least two-fold higher than adult values (Table 1). Conversely, IGFBP-3 concentration is about 30% of adult levels. Thus, in the foetus, there is an abundance of low molecular weight IGFBPs which are developmentally regulated. Similarly, levels of foetal IGFBP-2 are high and increase throughout foetal development in sheep (Gallaher *et al.*, 1995) and pigs (McCusker *et al.*, 1989b and 1991b). It should be noted that IGFBP-2 preferentially binds IGF-2 (Baxter, 1994), which is thought to be an important regulator of foetal growth. IGFBP-1 levels rise until mid-gestation human foetuses (Lee *et al.*, 1993b) and throughout gestation in pigs (McCusker *et al.*, 1991b). Postnatally, serum levels of IGFBP-3 increase and levels of IGFBP-1 and -2 decline (Owens, 1991). This coincides with the increased sensitivity to GH which stimulates IGFBP-3 production and insulin, which inhibits IGFBP-1 (Lee *et al.*, 1993b). In the adult, IGFBP-3 is the predominant IGF carrier, binding proportionately more than 0·8 of systemic IGF.

Phosphorylation of IGFBPs during foetal development may also alter the actions of IGFs. In non-pregnant human adults, most IGFBP-1 is highly phosphorylated (Frost *et al.*, 1994; Westwood *et al.*, 1994). During pregnancy, IGFBP-1 is primarily in the non-phosphorylated form in serum, decidua and amniotic fluid (Koistinen *et al.*, 1993), while foetal serum contains a mix of non-phosphorylated and partially phosphorylated IGFBP-1 (Jones *et al.*, 1991). Thus, as discussed above, different forms of IGFBP-1 exist in different physiological states and may be important in regulating IGF activity. The predominance of the low affinity non-phosphorylated IGFBP-1 during early pregnancy and foetal growth may enhance the actions of IGF during embryonic and foetal development.

IGFBPs and compromised growth

The long-term regulation of IGFBP levels in serum after birth can be summarized by two statements. When growth is compromised one of two (or both) events occurs: (1) IGFBP-1/IGFBP-2 levels increase and/or IGFBP-3 levels decrease; (2) ALS levels decrease and IGFBP-3 forms binary complexes with the IGFs. Simply, the IGFBP serum profile returns to that found in the foetus. This is true for minor stresses such as changes in dietary protein levels and major physiological stresses such as pregnancy, starvation/fasting, infection, diabetes, hypercorticosteroidism, hypothyroidism and hyperthyroidism (McCusker and Clemmons, 1992; Lee *et al.*, 1993a). There are marked differences in the degree of change among these conditions and these statements are a simplification of a complex regulatory process. However they do indicate a general theme on how the body responds to a variety of conditions which depress growth.

These changes lead to IGFBP proportions which may accelerate IGF uptake by vital organs such as the heart (Bar *et al.*, 1990). This may shift the anabolic effects of the IGFs away from non-vital tissues, such as skeletal muscle. The stress-related changes in IGFBP profile decrease the circulating half-life of the IGFs and thus release circulating IGFs into target tissues (Figure 2). This short-term response temporarily helps to maintain the anabolic state. This response is adequate only until the IGF reserve is depleted, and then IGF activity is determined by the (attenuated) rate of IGF secretion (Figure 2). Therefore, growth is maintained at a low level due to the depressed rate of IGF secretion until the stress is removed.

Several aspects of IGF and IGFBP physiology discussed here warrant considerable interest, especially relative to domestic animal production. The most interesting aspect is the potential to target

and control IGF activity (Figure 3). Although only limited information is available, targeting may be performed by multiple means: using IGFBPs that transport IGFs to specific tissues, using IGFBPs that transport IGFs to specific cells within a tissue, regulating IGF activity via IGFBP degradation by specific cell types, regulation of IGFBP activity by post-translational modifications, and targeting activity via specific IGFBP cell surface receptors. For example, selection of animals with increased ability for muscle IGFBP-4 degradation or IGFBP-1 dephosphorylation may lead to increased growth performance or greater muscle mass relative to total body weight. These and many other possibilities are yet to be explored.

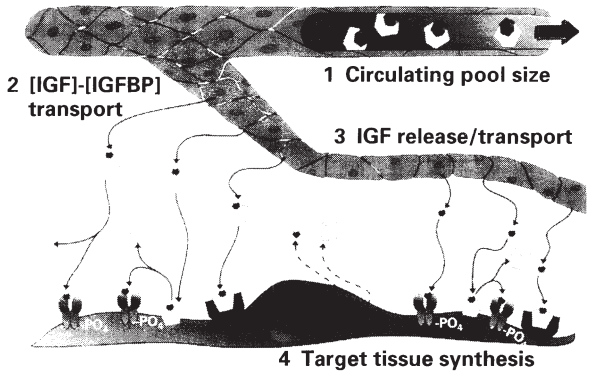

Figure 3 Sites for possible intervention in the IGF/IGFBP pathway for the augmentation of animal production. (1) The majority of IGFs in the circulation are part of an [IGF]-[IGFBP-3]-[ALS] trinary complex. Systemic intervention as a method of growth enhancement and/or metabolic regulation is currently being exploited in both animal and human trials. (2) IGFs may leave the circulation as binary [IGF]-[IGFBP] complexes making it possible to target IGF activity via tissue-specific uptake. Although a strong candidate for partitioning of tissue growth, exploitation of this pathway awaits further characterization of the system. Once outside the circulation, the binary complex may dissociate or bind intact to cell glycosyaminoglycans or ECM. Dissociation results in 'free' IGF for type 1 receptor activation whereas [IGF]-[IGFBP] complexes form a reservoir of IGFs in the pericellular environment. Enhanced IGFBP de-phosphorylation, proteolysis, de-glycosylation or cell-association increase the dissociation of the [IGF]-[IGFBP] complex, increasing 'free' IGF. Control of these processes is currently being investigated as a means to enhance IGF activity. Regulation of possible IGFBP receptor signalling may provide another means of growth regulation. (3) IGFs may leave the circulation 'free' of the IGFPBs. The 'free' IGFs could directly activate the type 1 IGF receptor or bind to IGFBPs at the cell surface forming an IGF reservoir. Controlling the amount of 'free' IGF in the circulation to alter IGF activity has yet to be explored. 4) as an alternative to step 1, the IGFs and IGFBPs are secreted by 'target' or vicinal cells resulting in autocrine/paracrine effects. Targeted over-expression of IGF/IGFBP activity as a means of growth regulation is as yet unexplored in agriculture.

Farm animal production and the IGF system

The purpose of this review was to examine the IGF/IGFBP system and its potential relative to production and domestic animals. The literature suggests that serum levels of both IGFs and IGFBPs change markedly with differing growth rate, age and state of nutrition. There is a large expanding list of references showing a positive correlation between IGF (especially IGF-1) levels and performance and a small but building literature on the relationship between IGFBP expression and growth. The possible efficacy of IGF administration as a systemic growth promotant is exciting although prematurely justified. Further work is needed using domestic animals as models for such studies. This group of growth factors may prove to be useful therapeutic agents during times of animal stress such as disease. In addition, there are still many aspects of the system that are not well understood and may be exploited relative to animal agriculture. Particularly promising is the possibility of agents (or IGF itself) which would enhance local IGF activity for the regulation of specific tissue metabolism, e.g. enhanced muscle growth. There are several means yet unexploited to target IGF activity which warrant future research (Figure 3), in particular the regulation of IGFBP activity by post-translational modifications. After years of study we still know very little about this system. This probably relates to the complexity of the system and the still unknown interactions between all of the components.

Acknowledgements

The authors are indebted to Vicky Malmkar and Pat Beebe for their excellent skills in typing numerous revisions of this manuscript. RHM and MVD are participants in the USDA Regional Research Project NC-131, 'Molecular mechanisms regulating skeletal muscle growth and differentiation', supported in part by Colorado State ARC project no. 629, Washington State ARC projects 0769 and 0913 and Illinois ARC project no. 0310. Correspondence, including reprint requests, should be directed to: Dr Kim L. Hossner, Department of Animal Sciences, Colorado State University, Fort Collins, CO 80523, USA.

References

Adams, S. O., Nissley, S. P., Handwerger, S. and Rechler, M. M. 1983. Developmental patterns of insulin-like growth factor-I and -II synthesis and regulation in rat fibroblasts. *Nature* **309:** 150-153.

Anderson, D. B., Veenhuison, E. L., Jones, E. J., Schroeder, A. L. and Hancock, D. L. 1991. The use of phenethanolamines to reduce fat and increase carcass

leanness in meat animals. In *Fat and cholesterol reduced foods: technologies and strategies* (ed. C. Huberstroh and C. E. Morris), pp. 43-73. *Advances in applied biotechnology series, vol. 12.* Portfolio, The Woodlands, TX.

Andress, D. L. and Birnbaum, R. S. 1992. Human osteoblast-derived insulin-like growth factor (IGF) binding protein-5 stimulates osteoblast mitogenesis and potentiates IGF action. *Journal of Biological Chemistry* **267:** 22467-22472.

Andress, D. L., Loop, S. M., Zapf, J. and Kiefer, M. C. 1993. Carboxy-truncated insulin-like growth factor binding protein-5 stimulates mitogenesis in osteoblast-like cells. *Biochemical and Biophysical Research Communications* **195:** 25-30.

Armstrong, D. G. 1986. Gut-active growth promoters. In *Control and manipulation of animal growth* (ed. P. J. Buttery, D. B. Lindsay and N. B. Haynes), pp. 21-37. Butterworths, London.

Azain, M. J., Bullock, K. D., Kasser, T. R. and Veenhuizen, J. J. 1992. Relationship of mode of porcine somatotropin administration and dietary fat to the growth performance and carcass characteristics of finishing pigs. *Journal of Animal Science* **70:** 3086-3095.

Bach, L. A. and Rechler, M. M. 1995. Insulin-like growth factor binding proteins. *Diabetes Reviews* **3:** 38-60.

Ballard, F. J., Baxter, R., Binoux, M., Clemmons, D., Drop, S., Hall, K., Hintz, R., Rechler, M., Rutanen, E. and Schwander, J. 1989. On the nomenclature of the IGF binding proteins. *Acta Endocrinologica* **121:** 751-752.

Ballard, F. J., Johnson, R. J., Owens, P. C., Francis, G. L., Upton, F. M., McMurtry, J. P. and Wallace, J. C. 1990. Chicken insulin-like growth factor-I: amino acid sequence, radioimmunoassay, and plasma levels between strains and during growth. *General and Comparative Endocrinology* **79:** 459-468.

Ballard, F. J., Ross, M., Upton, F. M. and Francis, G. L. 1988. Specific binding of insulin-like growth factors I and II to the type I and type II receptors respectively. *Biochemistry Journal* **249:** 721-726.

Bang, P., Westgren, M., Schwander, J., Blum, W. F., Rosenfeld, R. G. and Stangenberg, M. 1994. Ontogeny of insulin-like growth factor-binding protein-1, -2, and -3: quantitative measurements by radioimmunoassay in human fetal serum. *Pediatric Research* **36:** 528-536.

Bar, R. S., Boes, M., Dake, B. L., Sandra, A., Bayne, M. L., Cascieri, M. A. and Booth, B. A. 1990. Tissue localization of perfused endothelial cell IGF binding protein is markedly altered by association with IGF-I. *Endocrinology* **127:** 3243-3245.

Barreca, A., Ponzani, P., Arvigo, M., Giordano, G. and Minuto, F. 1995. Effect of the acid-labile subunit on the binding of insulin-like growth factor (IGF)-binding protein-3 to [I-125]IGF-I. *Journal of Clinical Endocrinology and Metabolism* **80:** 1318-1324.

Bass, J. J., Oldham, J. M., Hodgkinson, S. C., Fowke, P. J., Sauerwein, H., Molan, P., Breier, B. H. and Gluckman, P. D. 1991. Influence of nutrition and bovine growth hormone (GH) on hepatic GH binding, insulin-like growth factor-I and growth of lambs. *Journal of Endocrinology* **128:** 181-186.

Baxter, R. C. 1994. Insulin-like growth factor binding proteins in the human circulation: a review. *Hormone Research* **42:** 140-144.

Baxter, R. C. and Saunders, H. 1992. Radioimmunoassay of insulin-like growth factor-binding protein-6 in human serum and other body fluids. *Journal of Endocrinology* **134:** 133-139.

Beeson, W. M., Andrews, F. N. and Brown, P. T. 1947. The effect of thiouracil on the growth and fattening of yearling steers. *Journal of Animal Science* **6:** 16-23.

Beeson, W. M., Andrews, F. N., Perry, T. W. and Witz, H. L. Jr. 1949. The effect of thyroprotein and thiouracil on the growth and fattening of swine. *Journal of Animal Science* **8:** 508-517.

Behringer, R. R., Lewin, T. M., Quaife, C. J., Palmiter, R. D., Brinster, R. L. and D'Ercole, A. J. 1990. Expression of insulin-like growth factor I stimulates normal growth in growth hormone-deficient transgenic mice. *Endocrinology* **127:** 1033-1040.

Bishop, M. D., Simmen, R. C. M., Simmen, F. A. and Davis, M. E. 1989. The relationship of insulin-like growth factor-I with post-weaning performance in Angus beef cattle. *Journal of Animal Science* **67:** 2872-2880.

Blair, H. T., McCutcheon, S. N., Mackenzie, D. D. S., Gluckman, P. D. and Ormsby, J. E. 1987. Variation in plasma concentration of insulin-like growth factor-1 and its covariation with liveweight in mice. *Australian Journal of Biological Science* **40:** 287-293.

Blair, H. T., McCutcheon, S. N., Mackenzie, D. D. S., Ormsby, J. E., Siddiqui, R. A., Breier, B. H. and Gluckman, P. D. 1988. Genetic selection for insulin-like growth factor-1 in growing mice is associated with altered growth. *Endocrinology* **123:** 1690-1692.

Boes, M., Booth, B. A., Sandra, A., Dake, B. L., Bergold, A. and Bar, R. S. 1992. Insulin-like growth factor binding protein (IGFBP)4 accounts for the connective tissue distribution of endothelial cell IGFBPs perfused through the isolated heart. *Endocrinology* **131:** 327-330.

Bourner, M. J., Busby, W. H., Siegel, N. R., Krivi, G. G., McCusker, R. H. and Clemmons, D. R. 1992. Cloning and sequence determination of bovine insulin-like growth factor binding protein-2 (IGFBP-2): comparison of its structural and functional properties with IGFBP-1. *Journal of Cellular Biochemistry* **48:** 215-226.

Breier, B. H., Gluckman, P. D. and Bass, J. J. 1988. Plasma concentrations of insulin-like growth factor-I and insulin in the infant calf: ontogeny and influence of altered nutrition. *Journal of Endocrinology* **119:** 43-50.

Brinkman, A., Kortleve, D. J., Schuller, A. G. P., Zwarthoff, E. C. and Drop, S. L. S. 1991. Site-directed mutagenesis of the N-terminal region of IGF binding protein-1; analysis of IGF binding capability. *FEBS Letters* **291:** 264-268.

Buonomo, F. C., Lauterio, T. J., Baile, C. A. and Campion, D. R. 1987. Determination of insulin-like growth factor 1 (IGF1) and IGF binding protein levels in swine. *Domestic Animal Endocrinology* **4:** 23-31.

Busby, W. H., Klapper, D. G. and Clemmons, D. R. 1988. Purification of a 31000 dalton insulin like growth factor binding protein from human amniotic fluid. *Journal of Biological Chemistry* **263:** 14203-14210.

Buul-Offers, S. C. van, Haan, K. de, Reijnen-Gresnigt, M. G., Meinsma, D., Jansen, M., Oei, S. L., Bonte, E. J.,

Sussenbach, J. S. and Van den Brande, J. L. 1995. Overexpression of human insulin-like growth factor-II in transgenic mice causes increased growth of the thymus. *Journal of Endocrinology* **144:** 491-502.

Camacho-Hubner, C., Busby, W. H., McCusker, R. H., Wright, G. W. and Clemmons, D. R. 1992. Identification of the forms of insulin-like growth factor binding proteins produced by human fibroblasts and the mechanisms that regulate their secretion. *Journal of Biological Chemistry* **267:** 11949-11956.

Camacho-Hubner, C., McCusker, R. H. and Clemmons, D. R. 1991. Secretion and biological actions of insulin-like growth factor binding proteins in two human tumor-derived cell lines *in vitro*. *Journal of Cell Physiology* **148:** 281-289.

Canfield, W. M. and Kornfeld, S. 1989. The chicken liver cation independent mannose-6-phosphate receptor lacks the high affinity binding site for IGF-II. *Journal of Biological Chemistry* **264:** 7100-7103.

Cascieri, M. A. and Bayne, M. L. 1994. Analysis of the interaction of insulin-like growth factor I (IGF-I) analogs with the IGF-1 receptor and IGF-binding proteins. *Hormone Research* **41:** 80-86.

Clemmons, D. R., Cascieri, M. A., Camacho-Hubner, C., McCusker, R. H. and Bayne, M. L. 1990. Discrete alterations of the IGF-I molecule that alter its affinity for IGF binding proteins result in changes in bioactivity. *Journal of Biological Chemistry* **265:** 12210-12216.

Cogburn, L. A., Liou, S. S., Rand, A. L. and McMurtry, J. P. 1989. Growth, metabolic and endocrine responses of broiler cockerels given a daily subcutaneous injection of natural or biosynthetic chicken growth hormone. *Journal of Nutrition* **119:** 1213-1222.

Coleman, M. E., DeMayo, F., Yin, K. C., Lee, H. M., Geske, R., Montgomery, C. and Schwartz, R. J. 1995. Myogenic vector expression of insulin-like growth factor I stimulates muscle cell differentiation and myofiber hypertrophy in transgenic mice. *Journal of Biological Chemistry* **270:** 12109-12116.

Coleman, M. E. and Etherton, T. D. 1994. Porcine insulin-like growth factor (IGF)-binding protein-3 elicits bi-phasic effects on IGF-I stimulated DNA synthesis in neonatal porcine skin fibroblasts. *Domestic Animal Endocrinology* **11:** 299-305.

Conlon, M. A., Francis, G. L., Tomas, F. M., Wallace, J. C., Howarth, G. S. and Ballard, F. J. 1995. Continuous 14 day infusion of IGF-II increases the growth of normal female rats, but exhibits a lower potency than IGF-I. *Journal of Endocrinology* **144:** 91-98.

Conover, C. A. 1991. Glycosylation of insulin-like growth factor binding protein-3 (IGFBP-3) is not required for potentiation of IGF-I action: evidence for processing of cell-bound IGFBP-3. *Endocrinology* **129:** 3259-3268.

Conover, C. A. 1992. Potentiation of insulin-like growth factor (IGF) action by IGF-binding protein-3 — studies of underlying mechanism. *Endocrinology* **130:** 3191-3199.

Conover, C. A., Durham, S. K., Zapf, J., Masiarz, F. R. and Kiefer, M. C. 1995a. Cleavage analysis of insulin-like growth factor (IGF)-dependent IGF-binding protein-4 proteolysis and expression of protease-resistant IGF-binding protein-4 mutants. *Journal of Biological Chemistry* **270:** 4395-4400.

Conover, C. A., Perry, J. E. and Tindall, D. J. 1995b. Endogenous cathepsin D-mediated hydrolysis of insulin-like growth factor-binding proteins in cultured human prostatic carcinoma cells. *Journal of Clinical Endocrinology and Metabolism* **80:** 987-993.

Cottam, Y. H., Blair, H. T., Gallaher, B. W., Purchas, R. W., Breier, B. H., McCutcheon, S. N. and Gluckman, P. D. 1992. Body growth, carcass composition, and endocrine changes in lambs chronically treated with recombinantly derived insulin-like growth factor-I. *Endocrinology* **130:** 2924-2930.

Dai, Z. H., Xing, Y. Z., Boney, C. M., Clemmons, D. R. and D'Ercole, A. J. 1994. Human insulin-like growth factor-binding protein-1 (hIGFBP-1) in transgenic mice: characterization and insights into the regulation of IGFBP-1 expression. *Endocrinology* **135:** 1316-1327.

Dalke, B. S., Roeder, R. A., Kasser, T. R., Veenhuizen, J. J., Hunt, C. W., Hinman, D. D. and Schelling, G. T. 1992. Dose-response effects of recombinant bovine somatotropin implants on feedlot performance in steers. *Journal of Animal Science* **70:** 2130-2137.

Daughaday, W. H., Phillips, L. S. and Mueller, M. C. 1976. The effects of insulin and growth hormone on the release of somatomedin by the isolated rat liver. *Endocrinology* **98:** 1214-1219.

Davis, M. E. and Bishop, M. D. 1991. Preliminary results on between and within twin set variation in insulin-like growth factor I (IGF-1) and some relationships with performance traits in identical twin heifers. *Livestock Production Science* **27:** 255-262.

Davis, M. E., Bishop, M. D., Park, N. H. and Simmen, R. C. M. 1995. Divergent selection for blood serum insulin-like growth factor I concentration in beef cattle. 1. Nongenetic effects. *Journal of Animal Science* **73:** 1927-1932.

Davis, S. L., Hossner, K. L. and Ohlson, D. L. 1984. Endocrine regulation of growth in ruminants. In *Manipulation of growth in farm animals* (ed. J. F. Roche and D. O'Callaghan), pp. 151-183. Martinus Nijhoff, Boston.

Davis, S. R., Hodgkinson, S. C., Moore, L. G. and Gluckman, P. D. 1989. Improved estimates of clearance of ^{131}I-labelled insulin-like growth factor-I carrier protein complexes from blood plasma of sheep. *Journal of Endocrinology* **123:** 469-475.

DeChiara, T. M., Efstratiadis, A. and Robertson, E. J. 1990. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* **345:** 78-80.

De Meyts, R., Wallach, B., Christoffersen, C., Urso, B., Gronskov, K., Latus, L.-J., Yakushiji, F., Ilondo, M. and Shymko, R. 1994. The insulin-like growth factor-I receptor. *Hormone Research* **42:** 152-169.

D'Ercole, A. J. 1993. Expression of insulin-like growth factor-I in transgenic mice. *Annals of the New York Academy of Science* **692:** 149-160.

D'Ercole, A. J., Stiles, A. D. and Underwood, L. E. 1984. Tissue concentrations of somatomedin-C: further evidence for multiple sites of synthesis and paracrine or autocrine

mechanisms of actions. *Proceedings of the National Academy of Sciences of the USA* **81:** 935-939.

Douglas, R. G., Gluckman, P. D., Ball, K., Breier, B. H. and Shaw, J. H. F. 1991. The effects of insulin-like growth factor (IGF)I, IGF-II, and insulin on glucose and protein metabolism in fasted lambs. *Journal of Clinical Investigation* **88:** 614-622.

Eigenmann, J. E., Amador, A. and Patterson, D. F. 1988. Insulin-like growth factor I levels in proportionate dogs, chondrodystrophic dogs and in giant dogs. *Acta Endocrinologica* **118:** 105-108.

Ellenberger, M. A., Johnson, D. E., Carstens, G. E., Hossner, K. L., Holland, M. D., Nett, T. M. and Nockels, C. F. 1989. Endocrine and metabolic changes during altered tissue accretion rates in beef cattle. *Journal of Animal Science* **66:** 1446-1454.

Fowlkes, J. L., Enghild, J. J., Suzuki, K. and Nagase, H. 1994. Matrix metalloproteinases degrade insulin-like growth factor-binding protein-3 in dermal fibroblast cultures. *Journal of Biological Chemistry* **269:** 25742-25746.

Francis, G. L., McMurtry, J. P., Johnson, R. J. and Ballard, F. J. 1990. Plasma clearance of chicken and human insulin-like growth factor-I and their association with circulating binding proteins in chickens. *Journal of Endocrinology* **124:** 361-370.

Francis, G. L., McNeil, K. A., Wallace, J. C., Ballard, F. J. and Owens, P. C. 1989a. Sheep insulin-like growth factors I and II: sequences, activities and assays. *Endocrinology* **124:** 1173-1183.

Francis, G. L., Owens, P. C., McNeil, K. A., Wallace, J. C. and Ballard, F. J. 1989b. Purification, amino acid sequences and assay cross-reactivities of porcine insulin-like growth factor -I and -II. *Journal of Endocrinology* **122:** 681-687.

Frost, R. A., Bereket, A., Wilson, T. A., Wojnar, M. M., Lang, C. H. and Gelato, M. C. 1994. Phosphorylation of insulin-like growth factor binding protein-1 in patients with insulin-dependent diabetes mellitus and severe trauma. *Journal of Clinical Endocrinology and Metabolism* **78:** 1533-1535.

Frost, R. A. and Tseng, L. 1991. Insulin-like growth factor-binding protein-1 is phosphorylated by cultured human endometrial stromal cells and multiple protein kinases *in vitro*. *Journal of Biological Chemistry* **266:** 18082-18088.

Frystyk, J., Skjaerbaek, C., Dinesen, B. and Ørskov, H. 1994. Free insulin-like growth factors (IGF-I and IGF-II) in human serum. *FEBS Letters* **348:** 185-191.

Gallaher, B. W., Breier, B. H., Blum, W. F., McCutcheon, S. N. and Gluckman, P. D. 1995. A homologous radioimmunoassay for ovine insulin-like growth factor-binding protein-2: ontogenesis and the response to growth hormone, placental lactogen and insulin-like growth factor-I treatment in sheep. *Journal of Endocrinology* **144:** 75-82.

Gopinath, R., Walton, P. E. and Etherton, T. D. 1989. An acid stable insulin-like growth factor (IGF)-binding protein from pig serum inhibits binding of IGF-I and IGF-II to vascular endothelial cells. *Journal of Endocrinology* **120:** 231-236.

Guidice, L. C. 1995. IGF binding protein-3 protease regulation: how sweet it is. *Journal of Clinical Endocrinology and Metabolism* **80:** 2279-2281.

Guler, H.-P., Zapf, J. and Froesch, E. R. 1987. Short-term metabolic effects of recombinant human insulin-like growth factor I in healthy adults. *New England Journal of Medicine* **317:** 137-140.

Guler, H.-P., Zapf, J., Scheiwiller, E. and Froesch, E. R. 1988. Recombinant human insulin-like growth factor I stimulates growth and has distinct effects on organ size in hypophysectomized rats. *Proceedings of the National Academy of Sciences of the USA* **85:** 4889-4893.

Hancock, D. L., Wagner, J. F. and Anderson, D. B. 1991. Effects of estrogens and androgens in animal growth. *Advances in Meat Science* **7:** 255.

Hodgkinson, S. C., Davis, S. R., Moore, L. G., Henderson, H. V. and Gluckman, P. D. 1989. Metabolic clearance of insulin-like growth factor-II in sheep. *Journal of Endocrinology* **123:** 461-468.

Hodgkinson, S. C., Napier, J. R., Spencer, G. S. G. and Bass, J. J. 1994. Glycosaminoglycan binding characteristics of the insulin-like growth factor-binding proteins. *Journal of Molecular Endocrinology* **113:** 105-112.

Hoeck, W. G. and Mukku, V. R. 1994. Identification of the major sites of phosphorylation in IGF binding protein-3. *Journal of Cellular Biochemistry* **56:** 262-273.

Holland, M. D. 1991. Bovine fetal growth and development: whole-body, compositional and endocrine aspects. *Ph.D. dissertation, Colorado State University.*

Honda, Y., Landale, E. C., Strong, D. D., Baylink, D. J. and Mohan, S. 1996. Recombinant synthesis of insulin-like growth factor-binding protein-4 (IGFBP-4): development, validation and application of a radioimmunoassay for IGFBP-4 in human serum and other biological fluids. *Journal of Clinical Endocrinology and Metabolism* **81:** 1389-1396.

Honegger, A. and Humbel, R. E. 1986. Insulin-like growth factors I and II in fetal and adult bovine serum. *Journal of Biological Chemistry* **261:** 569-575.

Huhtala, M. L., Koistinen, R., Palomaki, P., Partanen, P., Bohn, H. and Seppala, M. 1986. Biologically active domain in somatomedin binding protein. *Biochemical and Biophysical Research Communications* **141:** 263-270.

Humbel, R. E. 1990. Insulin-like growth factors I and II. *European Journal of Biochemistry* **190:** 445-462.

Irving, J. A. and Lala, P. K. 1995. Functional role of cell surface integrins on human trophoblast cell migration: regulation by TGF-beta, IGF-II, and IGFBP-1. *Experimental Cell Research* **217:** 419-427.

Irwin, J. C., Dsupin, B. A. and Giudice, L. C. 1995. Regulation of insulin-like growth factor-binding protein-4 in human endometrial stromal cell cultures: evidence for ligand-induced proteolysis. *Journal of Clinical Endocrinology and Metabolism* **80:** 619-626.

Isaksson, O. G. P., Jansson, J.-O. and Gause, I. A. M. 1982. Growth hormone stimulates longitudinal bone growth directly. *Science* **216:** 1237-1239.

Isgaard, J., Carlsson, L., Isaksson, O. G. P. and Jansson, J.-O. 1988. Pulsatile intravenous growth hormone (GH) infusion to hypophysectomized rats increases insulin-like growth factor I messenger ribonucleic acid in skeletal tissues more effectively than continuous GH infusion. *Endocrinology* **123:** 2605-2610.

Isgaard, J., Nilsson, A., Vikman, K. and Isaksson, O. G. P. 1989. Growth hormone regulates the level of insulin-like growth factor-I and mRNA in rat skeletal muscle. *Journal of Endocrinology* **120:** 107-112.

Jones, J. I., Busby, W. H., Wright, G., Smith, C. E., Kimack, N. M. and Clemmons, D. R. 1993a. Identification of the sites of phosphorylation in insulin-like growth factor binding protein-1 — regulation of its affinity by phosphorylation of serine-101. *Journal of Biological Chemistry* **268:** 1125-1131.

Jones, J. I. and Clemmons, D. R. 1995. Insulin-like growth factors and their binding proteins: biological actions. *Endocrine Review* **16:** 3-34.

Jones, J. I., D'Ercole, A. J., Camacho-Hubner, C. and Clemmons, D. R. 1991. Phosphorylation of insulin-like growth factor (IGF)-binding protein-1 in cell culture and *in vivo*: effects on affinity for IGF-I. *Proceedings of the National Academy of Sciences of the USA* **88:** 7481-7485.

Jones, J. I., Gockerman, A., Busby, W. H., Camacho-Hubner, C. and Clemmons, D. R. 1993b. Extracellular matrix contains insulin-like growth factor binding protein-5-potentiation of the effects of IGF-I. *Journal of Cell Biology* **121:** 679-687.

Jones, J. I., Gockerman, A., Busby, W. H., Wright, G. and Clemmons, D. R. 1993c. Insulin-like growth factor binding protein-1 stimulates cell migration and binds to the alpha 5 beta 1 integrin by means of its arg-gly-asp sequence. *Proceedings of the National Academy of Sciences of the USA* **90:** 10553-10557.

Jyung, R. W., Mustoe, T. A., Busby, W. H. and Clemmons, D. R. 1994. Increased wound-breaking strength induced by insulin-like growth factor I in combination with insulin-like growth factor binding protein-1. *Surgery* **115:** 233-239.

Kanzaki, S., Hilliker, S., Baylink, D. J. and Mohan, S. 1994. Evidence that human bone cells in culture produce insulin-like growth factor-binding protein-4 and -5 proteases. *Endocrinology* **134:** 383-392.

Kerr, D. E., Laarveld, B., Fehr, M. E. and Manns, J. G. 1991. Profiles of serum IGF-I concentrations in calves from birth to eighteen months of age and in cows throughout the lactation cycle. *Canadian Journal of Animal Science* **71:** 695-705.

Kiess, W., Yang, Y., Kessler, U. and Hoeflich, A. 1994. Insulin-like growth factor II (IGF-II) and the IGF-II/mannose-6-phosphate receptor: the myth continues. *Hormone Research* **41:** (suppl. 2) 66-73.

Klapper, D. G., Svoboda, M. E. and Van Wyk, J. J. 1983. Sequence analysis of somatomedin-c: confirmation of identity with insulin-like growth factor I. *Endocrinology* **112:** 2215-2217.

Klindt, J., Buonomo, F. C. and Yen, J. T. 1992. Administration of porcine somatotropin by sustained-release implant: growth and endocrine responses in genetically lean and obese barrows and gilts. *Journal of Animal Science* **70:** 3721-3733.

Koistinen, R., Angervo, M., Leinonen, P., Hakala, T. and Seppala, M. 1993. Phosphorylation of insulin-like growth factor-binding protein-1 increases in human amniotic fluid and decidua from early to late pregnancy. *Clinica Chimica Acta* **215:** 189-199.

Lee, C. Y., Chung, C. S. and Simmen, F. A. 1993a. Ontogeny of the porcine insulin-like growth factor system. *Molecular and Cellular Endocrinology* **93:** 71-80.

Lee, C. Y. and Rechler, M. M. 1995. A major portion of the 150-kilodalton insulin-like growth factor-binding protein (IGFBP) complex in adult rat serum contains unoccupied, proteolytically nicked IGFBP-3 that binds IGF-II preferentially. *Endocrinology* **136:** 668-678.

Lee, P. D. K., Conover, C. A. and Powell, D. R. 1993b. Regulation and function of insulin-like growth factor-binding protein-1. *Proceedings of the Society for Experimental Biology and Medicine* **204:** 4-29.

Lemal, D., Renaville, R., Claes, V., Ruelle, L., Fabry, J., Burny, A., Underwood, L. and Ketelslegers, J.-M. 1989. Effect of pituitary somatotropin injections on plasma insulin-like growth factor I and somatotropin levels in growing heifers. *Journal of Animal Science* **67:** 2715-2723.

LeRoith, D., Werner, H., Beitner-Johnson, D. and Roberts, C. T. Jr. 1995. Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocrine Reviews* **16:** 143-163.

Lund-Larsen, T. R. and Bakke, H. 1975. Growth hormone and somatomedin activities in lines of pigs selected for rate of gain and thickness of backfat. *Acta Agriculturae Scandinavica* **25:** 231-234.

McCusker, R. H., Busby, W. H., Camacho-Hubner, C. and Clemmons, D. R. 1989a. Secretion of insulin-like growth factor binding proteins (IGF-BPs) by muscle cells *in vitro*. In *Insulin-like growth factor binding proteins* (ed. S. L. S. Drop and R. L. Hintz), pp. 257-265. *Excerpta Medica international congress series 881.* Elsevier Science Publishers B. V., Amsterdam.

McCusker, R. H., Busby, W. H., Dehoff, M. H., Camacho-Hubner, C. and Clemmons, D. R. 1991a. Insulin-like growth factor (IGF) binding to cell monolayers is directly modulated by the addition of IGF-binding proteins. *Endocrinology* **129:** 939-949.

McCusker, R. H., Camacho-Hubner, C., Bayne, M. L., Cascieri, M. A. and Clemmons, D. R. 1990. Insulin-like growth factor (IGF) binding to human fibroblast and glioblastoma cells: the modulating effect of cell released IGF binding proteins (IGFBPs). *Journal of Cell Physiology* **144:** 244-253.

McCusker, R. H., Campion, D. R., Jones, W. K. and Clemmons, D. R. 1989b. The insulin-like growth factor-binding proteins of porcine serum: endocrine and nutritional regulation. *Endocrinology* **125:** 501-509.

McCusker, R. H. and Clemmons, D. R. 1988. Insulin-like growth factor binding proteins (IGFBPs) secretion by muscle cells: effect of cellular differentiation and proliferation. *Journal of Cell Physiology* **137:** 505-512.

McCusker, R. H. and Clemmons, D. R. 1992. The insulin-like growth factor binding proteins: structure and biological functions. In *The insulin-like growth factors: structure and biological functions* (ed. P. N. Schofield), pp. 110-150. Oxford University Press, Oxford.

McCusker, R. H., Cohick, W. S., Busby, W. H. and Clemmons, D. R. 1991b. Evaluation of the developmental and nutritional changes in porcine insulin-like growth factor-binding protein-1 and -2 serum levels by immunoassay. *Endocrinology* **129:** 2631-2638.

McGuire, M. A., Vicini, L. J., Bauman, D. E. and Veenhuizen, J. J. 1992. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. *Journal of Animal Science* **70:** 2901-2910.

McLaughlin, C. L., Hedrick, H. B.,Veenhuizen, J. J., Finn, R. F., Hintz, R. L., Hartnell, G. F., Kasser, T. R. and Baile, C. A. 1993. Comparison of performance, clinical chemistry, and carcass characteristics of finishing lambs treated with recombinant ovine or bovine somatotropins. *Journal of Animal Science* **71:** 1453-1463.

McLaughlin, C. L., Rogan, G. J., Buonomo, F. C., Cole, W. J., Hartnell, G. F., Hudson, S., Kasser, T. R., Miller, M. A. and Baile, C. A. 1991. Finishing lamb performance responses to bovine and porcine somatotropins administered by Alzet pumps. *Journal of Animal Science* **69:** 4039-4048.

McMurtry, J. P., Francis, G. L., Upton, F. Z., Rosselot, G. and Brocht, D. M. 1994. Developmental changes in chicken and turkey insulin-like growth factor-I (IGF-I) studied with homologous radioimmunoassay for chicken IGF-I. *Journal of Endocrinology* **142:** 225-234.

Martin, J. L. and Baxter, R. C. 1993. Release of fibroblast IGFBPs by TGF-beta and IGF-I occurs through distinct mechanisms. *Growth Regulation* **3:** 62-65.

Mathews, L. S., Hammer, R. E., Behringer, R. R., D'Ercole, A. J., Bell, G. I., Brinster, R. L. and Palmiter, R. D. 1988a. Growth enhancement of transgenic mice expressing human insulin-like growth factor I. *Endocrinology* **123:** 2827-2833.

Mathews, L. S., Hammer, R. E., Brinster, R. L. and Palmiter, R. D. 1988b. Expression of insulin-like growth factor I in transgenic mice with elevated levels of growth hormone is correlated with growth. *Endocrinology* **123:** 433-437.

Medrano, J. F. and Bradford, G. E. 1991. Growth performance and plasma insulin-like growth factor I concentrations in sheep selected for high weaning weight. *Journal of Animal Science* **69:** 1912-1918.

Moats-Staats, B. M., Retsch-Bogart, G. Z., Price, W. A., Jarvis, H. W., D'Ercole, A. J. and Stiles, A. D. 1993. Insulin-like growth factor-I (IGF-I) antisense oligodeoxynucleotide mediated inhibition of DNA synthesis by WI-38 cells: evidence for autocrine actions of IGF-I. *Molecular Endocrinology* **7:** 171-180.

Moellers, R. F. and Cogburn, L. A. 1995. Chronic intravenous infusion of chicken growth hormone increases body fat content of young broiler chickens. *Comparative Biochemistry and Physiology, A Comparative Physiology* **110A:** 47-56.

Mosely, W. M., Paulissen, J. B., Goodwin, M. C., Alaniz, G. R. and Claflin, W. H. 1992. Recombinant bovine somatotropin improves growth performance in finishing beef steers. *Journal of Animal Science* **70:** 412-425.

Murphy, L. J., Bell, G. I. and Friesen, H. G. 1987. Tissue distribution of insulin-like growth factor-I and II messenger ribonucleic acid in the adult rat. *Endocrinology* **120:** 1279-1282.

Myers, M. G. Jr., Sun, X. J., Cheatham, B., Jachna, B. R., Glasheen, E. M., Backer, J. M. and White, M. F. 1993. IRS-1 is a common element in insulin and insulin-like growth factor-I signaling to the phosphatidylinositol 3'-kinase. *Endocrinology* **132:** 1421-1430.

Orain, J. M., Snibson, K., Stevenson, J. L., Brandon, M. R. and Herrington, A. C. 1991. Elevation of growth hormone (GH) and prolactin receptors in transgenic mice expressing ovine GH. *Endocrinology* **128:** 1238-1246.

Orlowski, C. C. and Chernausek, S. D. 1988. Discordance of serum and tissue somatomedin levels in growth hormone-stimulated growth in the rat. *Endocrinology* **122:** 44-49.

Owens, J. A. 1991. Endocrine and substrate control of fetal growth: placental and maternal influences and insulin-like growth factors. *Reproduction, Fertility and Development* **3:** 501-517.

Owens, J. A., Kind, K. L., Carbone, F., Robinson, J. S. and Owens, P. C. 1994. Circulating insulin-like growth factors-I and -II and substrates in fetal sheep following restriction of placental growth. *Journal of Endocrinology* **140:** 5-13.

Owens, P. C., Johnson, R. J., Campbell, R. G. and Ballard, F. J. 1990. Growth hormone increases insulin-like growth factor-I (IGF-I) and decreases IGF-II in plasma of growing pigs. *Journal of Endocrinology* **124:** 269-275.

Pell, J. M. 1989. Growth promoting properties of recombinant growth hormone. In *Biotechnology in growth regulation* (ed. R. B. Heap, C. G. Prosser and G. E. Lamming), pp. 85-96. Butterworths, London.

Rinderknect, E. and Humbel, R. 1978. The amino acid sequence of insulin-like growth factor I and its structural homology with proinsulin. *Journal of Biological Chemistry* **235:** 2769-2776.

Ritvos, O., Ranta, T., Jalkanen, J., Suikkari, A. M., Voutilainen, R., Bohn, H. and Rutanen, E. M. 1988. Insulin-like growth factor (IGF) binding protein from human decidua inhibits the binding and biological action of IGF-I in cultured choriocarcinoma cells. *Endocrinology* **122:** 2150-2157.

Roberts, C. A., McCutcheon, S. N., Blair, H. T., Gluckman, P. D. and Breier, B. H. 1990. Developmental patterns of plasma insulin-like growth factor-1 concentrations in sheep. *Domestic Animal Endocrinology* **7:** 457-464.

Roeder, R. A., Hossner, K. L., Sasser, R. G. and Gunn, J. M. 1988. Regulation of protein turnover by recombinant human insulin-like growth factor-I in L6 myotube cultures. *Hormone and Metabolic Research* **20:** 698-700.

Rotwein, P., Pollock, K. M., Watson, M. and Milbrandt, J. D. 1987. Insulin-like growth factor gene expression during rat embryonic development. *Endocrinology* **121:** 2141-2144.

Russell, S. and Spencer, M. 1985. Local injections of human or rat growth hormone or of purified human somatomedin C stimulate unilateral tibial epiphyseal growth in hypophysectomized rats. *Endocrinology* **116:** 2563-2567.

Scanes, C. G., Lazarus, D., Bowen, S., Buonomo, F. C. and Gilbreath, R. L. 1987. Postnatal changes in circulating concentrations of growth hormone, somatomedin C and thyroid hormones in pigs. *Domestic Animal Endocrinology* **4:** 253-257.

Schlechter, N. L., Russell, S. M., Spencer, E. M. and Nicoll, C. S. 1986. Evidence suggesting that the direct growth-promoting effect of growth hormone on cartilage *in vivo* is mediated by local production of somatomedin. *Proceedings of the National Academy of Sciences of the USA* **83:** 7932-7934.

Schmid, C., Rutishauser, J., Schlapfer, I., Froesch, E. R. and Zapf, J. 1991. Intact but not truncated insulin-like growth factor binding protein-3 (IGFBP-3) blocks IGF-I-stimulation of osteoblasts: control of IGF signalling to bone cells by IGFBP-3-specific proteolysis? *Biochemical and Biophysical Research Communications* **179:** 579-585.

Schoenle, E., Zapf, J., Humbel, R. E. and Froesch, E. R. 1982. Insulin-like growth factor I stimulates growth in hypophysectomized rats. *Nature* **296:** 252-253.

Schwarz, F. J., Schams, D., Röpke, R., Kirchgessner, M., Kögel, J. and Matzke, P. 1993. Effects of somatotropin treatment on growth performance, carcass traits, and the endocrine system in finishing beef heifers. *Journal of Animal Science* **71:** 2721-2731.

Shimasaki, S. and Ling, N. 1991. Identification and molecular characterization of insulin-like growth factor binding proteins (IGFBP-1,-2,-3,-4,-5 and -6). *Progress in Growth Factor Research* **3:** 243-266.

Shimasaki, S., Shimonaka, M., Zhang, H.-P. and Ling, N. 1991. Identification of five different insulin-like growth factor binding proteins (IGFBPs) from adult rat serum and molecular cloning of a novel IGFBP-5 in rat and human. *Journal of Biological Chemistry* **266:** 10646-10653.

Siddiqui, R. A., Blair, H. T., McCutcheon, S. N., Mackenzie, D. D. S., Gluckman, P. D. and Breier, B. H. 1990. Developmental patterns of plasma insulin-like growth factor-I (IGF-I) and body growth in mice from lines divergently selected on the basis of plasma IGF-I. *Journal of Endocrinology* **124:** 151-158.

Spencer, G. S., Decuypere, E., Buyse, J., Hodgkinson, S. C., Bass, J. J. and Zeman, M. 1995. Passive immunization of insulin-like growth factor (IGF)-1 and of IGF-1 and IGF-2 in chickens. *Comparative Biochemistry and Physiology, C Pharmacology, Toxicology, Endocrinology* **110C:** 29-33.

Thissen, J. P., Ketelslegers, J. M. and Underwood, L. E. 1994. Nutritional regulation of the insulin-like growth factors. *Endocrine Review* **15:** 80-101.

Tixier-Boichard, M., Huybrechts, L. M., Decuypere, E., Kuhn, E. R., Monvoisin, J. L., Coquerelle, G., Charrier, J. and Simon, J. 1992. Effects of insulin-like growth factor-I (IGF-I) infusion and dietary tri-iodothyronine (T3) supplementation on growth, body composition and plasma hormone levels in sex-linked dwarf mutant and normal chickens. *Journal of Endocrinology* **133:** 101-110.

Tonner, E., Beattie, J. and Flint, D. J. 1995. Production of insulin-like growth factor binding protein (IGFBP), IGFBP-3 protease, and expression of IGF-I receptors by cells of the sheep immune system. *European Journal of Endocrinology* **132:** 118-122.

Upton, Z., Francis, G. L., Kita, K., Wallace, J. C. and Ballard, F. J. 1995. Production and characterization of recombinant chicken insulin-like growth factor-II from *Escherichia coli*. *Journal of Molecular Endocrinology* **14:** 79-90.

Walton, P. E., Gopinath, R., Burleigh, B. D. and Etherton, T. D. 1989. Administration of recombinant human insulin-like growth factor I to pigs: determination of circulating half-lives and chromatographic profiles. *Hormone Research* **31:** 138-142.

Watson, A. J., Watson, P. H., Arcellana-Panlilio, M., Warnes, D., Walker, S. K., Schultz, G. A., Armstrong, D. T. and Seamark, R. F. 1994. A growth factor phenotype map for ovine preimplantation development. *Biology of Reproduction* **50:** 725-733.

Westwood, M., Gibson, J. M., Davies, A. J., Young, R. J. and White, A. 1994. The phosphorylation pattern of insulin-like growth factor-binding protein-1 in normal plasma is different from that in amniotic fluid and changes during pregnancy. *Journal of Clinical Endocrinology and Metabolism* **79:** 1735-1741.

Zapf, J. 1995. Physiological role of the insulin-like growth factor binding proteins. *European Journal of Endocrinology* **132:** 645-654.

Zenobi, P. D., Holzmann, P., Glatz, Y., Riesen, W. F. and Froesch, E. R. 1993. Improvement of lipid profile in type-2 (non-insulin-dependent) diabetes-mellitus by insulin-like growth factor-I. *Diabetologia* **36:** 465-469.

Zick, Y., Sasaki, N., Rees-Jones, R. W., Grunberger, G., Nissley, S. P. and Rechler, M. M. 1983. Insulin-like growth factor-I (IGF-I) stimulates tyrosine kinase activity in purified receptors from a rat liver cell line. *Biochemical and Biophysical Research Communications* **119:** 6-13.