Proceedings of the Nutrition Society (1997), **56**, 607–619

Molecular mechanisms involved in the nutritional and hormonal regulation of growth in pigs

BY JOHN M. BRAMELD

Department of Applied Biochemistry and Food Science, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD

Nutritional and hormonal factors are major determinants of animal growth and, postnatally at least, pituitary-derived growth hormone (GH), via its stimulatory effects on insulin-like growth factor-I (IGF-I) production, is one of the major regulators of growth. The production of GH by the pituitary is under the local control of growth-hormone-releasing factor (GRF) and growth-hormone-release-inhibiting factor (somatostatin). However, diet also has a major effect on circulating GH concentrations, such that nutritional restriction leading to growth arrest is associated with a decrease in plasma IGF-I concentration, but an increase in plasma GH concentration in most species, including the pig (Buonomo & Baile, 1991), the rat being the exception.

The molecular mechanisms for this growth arrest, in the presence of high concentrations of this positive regulator of growth (GH), are proposed to be via dietary effects on the components of the GH–IGF axis, and in particular IGF-I, and form the subject for the present review.

COMPONENTS OF THE GROWTH HORMONE–INSULIN-LIKE GROWTH FACTOR AXIS

Growth hormone receptor

This resistance to GH action in situations of poor nutrition suggests nutritionally-sensitive control at the level of tissue responsiveness. The first step in the action of GH is in binding to the GH receptor (GHR), and ligand-binding studies have identified two classes of receptor with low and high binding affinities (Breier & Sauerwein, 1995). The low-affinity receptor has been proposed to be due to GH binding to only one GHR molecule at low receptor concentrations, whereas the high-affinity receptor has been proposed to be due to GH binding to two distinct GHR molecules at high receptor concentrations (see Breier & Sauerwein, 1995). The actual activation of the receptor and subsequent signal transduction requires the binding to two GHR molecules, resulting in receptor dimerization (for review, see Kelly *et al.* 1994), and this binding then promotes association of an intracellular tyrosine kinase (JAK2), and results in phosphorylation of both the GHR and JAK2 molecules. The subsequent steps in the second messenger pathway are as yet unclear. It would seem likely, therefore, that the first site for nutritional control may be in controlling the expression and/or production or second messenger signalling pathway of the GHR.

The GHR gene has been cloned in many species including pig (Cioffi *et al.* 1990), with the gene found to encode for a protein of about 638 amino acids with a relative molecular mass of about 70 000, and, due to sequence similarities, is a member of the cytokine–haematopoietic receptor superfamily (Kelly *et al.* 1991). The GHR molecule contains both intra- and extracellular domains, relating to GH binding (extracellular) and JAK2 association (intracellular). A plasma GH-binding protein (GHBP) has recently been identified in most species, including pig (Davis *et al.* 1992), and has been shown to have an identical amino acid sequence to that of the extracellular domain of the GHR. In rats and

mice, the mechanism for production of this GHBP appears to be via an alternate transcript, as there are two RNA transcripts for GHR found in rat liver (Baumbach *et al.* 1989). However, in pigs and other species there is only one RNA transcript for GHR in liver (Louveau & Etherton, 1992), and it has been shown in cultured cells that the extracellular domain of the GHR can be released by proteolytic cleavage of the membrane-bound receptor (Trivedi & Daughaday, 1988). Thus, there are two possible mechanisms for nutritional–hormonal effects on growth, first via the GHR, which will affect GH action, and second via the GHBP, which may alter the half-life of GH, or affect its availability for binding to the GHR.

Insulin-like growth factors

The IGF (IGF-I and -II) are two polypeptide growth factors which are structurally related to proinsulin. They are made up of seventy (IGF-I) and sixty-seven (IGF-II) amino acids, with a molecular mass of 7·65 and 7·47 kDa respectively, and share about 60 % homology (for review, see Sussenbach *et al.* 1992). They have been shown to stimulate cell proliferation and differentiation, and to inhibit cell death (for review, see Jones & Clemmons, 1995). The IGF have also been shown to stimulate protein synthesis, amino

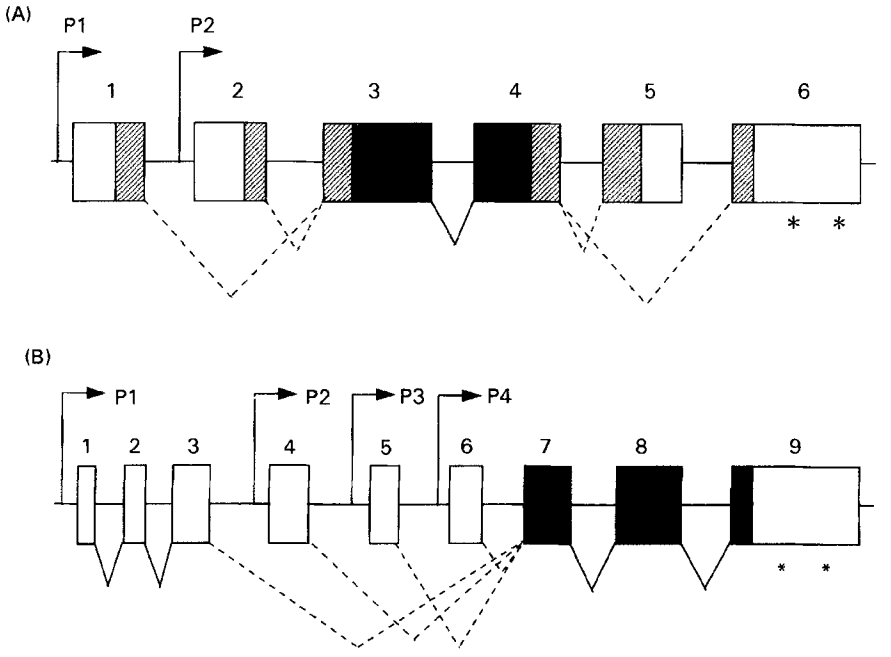

Fig. 1. Structures of the mammalian (A) insulin-like growth factor (IGF)-I and (B) IGF-II genes (■, ▨, □) showing the exons (1–6 for IGF-I and 1–9 for IGF-II); (—, – – –), the introns (not to scale). (■), Coding sequence for the mature peptide; (▨), coding sequence for the unprocessed peptide (prohormone); (□), untranslated sequence. (→; P1–P2 (IGF-I), P1–P4 (IGF-II)), Alternative promoter initiation sites. *, Alternative polyadenylation sites. Primary mRNA molecules are derived from the splice permutations between alternatively-used exons (– – –), and from the invariant splices (—) between exons 3 and 4 for IGF-I, and between exons 1, 2 and 3, and 7, 8 and 9 for IGF-II. (Adapted from Gilmour, 1994 and Sussenbach *et al.* 1992.)

acid and glucose uptake, and to inhibit protein degradation in skeletal muscle cells in culture (Harper *et al.* 1987; Roe *et al.* 1989, 1995).

IGF-I has been shown to mediate many of the growth-promoting effects of GH, and is produced postnatally, mainly by the liver, under GH control and secreted into the circulation. However, it is now well established that most tissues are capable of producing IGF-I, which may then act in an autocrine or paracrine manner to promote tissue growth. Very few fetal tissues express IGF-I, presumably due to the very low levels of GHR expression, and, therefore, IGF-I is considered to be mainly a postnatal growth factor. The gene for IGF-I has been cloned in many species including pig (Weller *et al.* 1993), and has been shown to span nearly 90 kb DNA, and to consist of six exons (Fig. 1(A)). The first two exons (1 and 2) represent alternative pro-peptide domains and 5′-untranslated regions, which have associated transcriptional promoters, and result in two classes of transcript (the exon 1 promoter gives rise to class 1 transcripts). Exons 3 and 4 encode for the mature IGF-I polypeptide, and are present, therefore, in all transcripts, whereas exons 5 and 6 give rise to alternative E-domains and 3′-untranslated regions, likely to be involved in the processing and progress through the cell (see Sussenbach *et al.* 1992; Gilmour, 1994).

IGF-II is produced mainly in the fetus by a variety of tissues, and this high level of expression in the fetus has led to the suggestion that it is a fetal growth factor. The gene for IGF-II has been cloned in few species, with the only large farm animal apparently being the sheep (Li *et al.* 1993). The gene contains nine exons (Fig. 1(B)), but this time the first six exons represent alternative 5′-untranslated regions and have four associated promoters. The first promoter (exons 1–3) is only active in the postnatal liver, whereas the other three promoters (exons 4, 5 and 6) are only active in the fetus. Exons 7, 8 and 9 encode for the IGF-II precursor, and exon 9 also gives rise to two alternative 3′-untranslated regions (see Sussenbach *et al.* 1992).

Insulin-like growth factor-binding proteins

The IGF, like GH, circulate in the blood and extracellular fluid bound to specific binding proteins, and, to date, there are known to be six distinct IGFBP (IGFBP-1–IGFBP-6). Five of these have been described in pigs (McCusker *et al.* 1985; Coleman *et al.* 1991). They differ in the factors which regulate them, and also in their relative affinities for the two IGF, with IGFBP-1, -3 and -4 having similar affinities for IGF-I and IGF-II, but IGFBP-2, -5 and -6 having a higher affinity for IGF-II. The predominant IGFBP in blood is IGFBP-3, which is associated with both the IGF and an acid-labile subunit (ALS) to form a 150 kDa complex; other IGFBP found in pig blood include IGFBP-1, -2 and -4 (Coleman *et al.* 1991; Dauncey *et al.* 1993). Unlike many hormones, the IGF are not stored intracellularly, but are in fact synthesized and then released from the cell. It has been suggested, therefore, that the large 150 kDa complex of IGF, ALS and IGFBP-3 may be a mechanism for storing the IGF and then transporting it to the site of action. The actual effects of the IGFBP on IGF activity remains unclear, with various IGFBP found to inhibit, enhance or have no effect on IGF activity in cultured cells, depending on conditions and relative times of addition of the IGFBP and IGF (for review, see Zapf, 1995). The genes for all six IGFBP have been cloned, and the gene structures are at varying stages of understanding, with sequences available to make molecular probes for virtually all IGFBP (for reviews, see Rechler & Brown 1992; Jones & Clemmons, 1995). However, no work has been published on the IGFBP genes in pigs.

Insulin-like growth factor receptors

The actions of the IGF are mediated via two independent receptors (for review, see Jones & Clemmons, 1995). The type-1 IGF-receptor (IGF-1R) is structurally similar to the insulin receptor and indeed binds insulin with a low affinity. The comparative binding of the three ligands to IGF-1R is IGF-I > IGF-II ≫ insulin and, therefore, supraphysiological concentrations of insulin are able to exert IGF actions via the IGF-1R. The second receptor, the IGF-2R, has been shown to be the cation-independent mannose-6-phosphate receptor, and binds IGF-II with a greater affinity than IGF-I, and does not bind insulin at all. The elucidation of the second messenger pathways linking ligand binding to the various intracellular actions is still in its infancy. However, the IGF-1R is known to involve a tyrosine kinase activity and receptor phosphorylation, whereas the IGF-2R is reported to couple to a GTP-binding protein. There is a lack of published work on the regulation of IGF-receptor levels (protein or mRNA) in the pig, despite their obvious importance in the control of growth and metabolism. The studies carried out to date on the IGF-1R are in rats (Dardevet *et al.* 1991) or sheep (Glassford *et al.* 1995; Oldham *et al.* 1996), and all seem to indicate the major regulator of IGF-1R to be IGF-I itself. Any condition that results in decreased IGF-I concentrations (e.g. fasting, feed restriction or diabetes) results in increased IGF-1R expression (for review, see Werner *et al.* 1995).

WHOLE-ANIMAL PIG STUDIES

A number of groups have studied the effects of dietary manipulation and/or GH administration on pig growth, but few have tried to identify the mechanisms involved. The present review will look at the effects of diet and/or GH on the various components of the GH–IGF system, at both the gene and protein levels, and in particular at IGF-I, which is thought to be the main growth factor responsible for the changes in growth rate.

Effects of manipulating energy availability

Recent studies have demonstrated effects on growth and expression of GHR and IGF-I genes with increasing energy availability via intake and environmental temperature in young growing pigs (Dauncey *et al.* 1994; Weller *et al.* 1994). GHR expression in the liver was found to increase with food intake, whereas expression in *longissimus dorsi* (LD) muscle decreased with increased food intake and temperature (Dauncey *et al.* 1994). Strong positive correlations were observed between liver GHR mRNA and both plasma IGF-I concentration and growth rate, whereas a negative correlation was noted between LD muscle GHR mRNA and growth rate. Thus, the effects of intake and temperature on growth rate appeared to be mediated via control of liver GHR expression. However, it should be noted that plasma triiodothyronine (T_3) concentrations were affected by these manipulations, and that liver GHR mRNA and plasma T_3 concentrations were also positively correlated. Thus, whether the dietary effects observed are direct nutrient effects on gene expression, or indirect via T_3 remains to be answered.

A similar study was also carried out to study the effects of energy availability on IGF-I expression (Weller *et al.* 1994). The results were similar to the effects on GHR expression, in that expression in the liver increased with both intake and temperature, whereas expression in LD muscle decreased with increased intake and temperature. It was also found that major levels of class 2 transcripts (exon 2-containing transcripts) of IGF-I were only present in the liver, a result also found in other whole-animal pig studies looking at

differences with age (Brameld *et al.* 1995*a*) and breed of pig (Brameld *et al.* 1996*a*). Increased growth rates were accompanied by an increase in the proportion of class 2 IGF-I mRNA in the liver, suggesting a role for class 2 IGF-I in the endocrine growth response. It had previously been demonstrated that these dietary and temperature manipulations had no marked effects on circulating GH concentrations (Dauncey & Buttle, 1990), but that there were significant effects on plasma concentrations of IGF-I (Dauncey *et al.* 1990), IGFBP-3 and IGFBP-2 (Dauncey *et al.* 1993), with both IGF-I and IGFBP-3 increasing with intake and temperature, whereas IGFBP-2 tended to decrease.

These results could all be explained by the hypothesis that dietary energy increases liver GHR expression, thereby increasing the ability of the liver to respond to the circulating GH. However, this hypothesis cannot be tested employing *in vivo* studies and, therefore, requires further study using *in vitro* cell culture methods.

Effects of exogenous growth hormone

A number of studies have been carried out to investigate the effects of GH administration on various components of the GH–IGF axis in pigs. Chronic administration of GH to pigs has been shown to increase GH binding to liver membranes (Chung & Etherton, 1986; Ambler *et al.* 1992), but not to adipose tissue membranes (Sorensen *et al.* 1992), and to increase GHBP levels in pig serum, with the two being positively correlated (Ambler *et al.* 1992), suggesting that serum GHBP levels may reflect hepatic GHR numbers. We (Brameld *et al.* 1996*b*) have subsequently demonstrated GH-dependent increases in expression of both intra- and extracellular domains of the GHR in liver and skeletal muscle (both LD and *semitendinosus*), but not in adipose tissue (subcutaneous, omental or perirenal).

Exogenous GH has been shown to significantly increase plasma IGF-I concentrations in well-fed animals (Evock *et al.* 1990; Owens *et al.* 1990), and to either decrease (Owens *et al.* 1990) or have no effect on plasma IGF-II (Evock *et al.* 1990). Administration of GH to pregnant sows has also been shown to result in increased plasma IGF-I concentrations in their neonatal offspring (20 kg), but this effect is lost by the time the offspring reach market weight (Kelley *et al.* 1995). At the gene level, administration of GH has been shown to result in dose-dependent increases in IGF-I expression in both liver and subcutaneous adipose tissue, but not in LD muscle (Coleman *et al.* 1994). Studies at Nottingham (Brameld *et al.* 1996*b*) have similarly demonstrated GH-dependent increases in class 1 transcripts of IGF-I in liver, all three adipose tissue depots, and also in *semitendinosus* muscle, but not in LD muscle. Class 2 transcripts of IGF-I were only found in the livers of GH-treated pigs. Time-dependent increases in IGF-I mRNA have been shown as a result of a single GH injection in liver and subcutaneous and perirenal adipose tissues, with a slight increase also being seen in *vastus lateralis* muscle, but no effect in LD or *semitendinosus* muscles (Ramsay *et al.* 1995). Thus, different muscles appear to respond differently. GH has also been shown to decrease IGF-II expression in subcutaneous adipose tissue, and this effect was lessened by feeding an extra 100 g fat/kg diet (Wolverton *et al.* 1992). The addition of dietary fat was shown to have no effect on the GH-stimulation of IGF-I expression in the same tissue.

As well as increasing circulating IGF-I concentrations, GH administration has been shown to increase IGFBP-3 and to decrease IGFBP-2 concentrations in serum (Coleman & Etherton, 1991). The GH effects on IGFBP-2, however, appear to be dependent on dietary protein levels (Guan *et al.* 1997; see p. 612), with IGFBP-1 and -4 also being increased by GH (Guan *et al.* 1997).

Thus, GH affects many of the components of the GH–IGF axis, not just IGF-I expression and production.

Effects of manipulating dietary protein intake and growth hormone status

Studies of the interaction between dietary protein intake and exogenous GH administration on plasma IGF-I concentrations in pigs have shown contrasting results, with increased protein shown to increase the response of plasma IGF-I to GH (Campbell *et al.* 1990; Caperna *et al.* 1990), or to have no effect on the GH response (Seve *et al.* 1993; Brameld *et al.* 1996*b*). However, the differences in dietary protein content in these studies were not restrictive (ranging from 83 to 270 g/kg diet), unlike many of the studies carried out in rats, where crude protein (N × 6·25) levels as low as 55 g/kg have been used (Dardevet *et al.* 1991). The lack of effect of increasing dietary protein on GH-dependent increases in plasma IGF-I concentration was accompanied by a lack of effect on IGF-I expression (both class 1 and 2 transcripts) in pig liver (Grant *et al.* 1991; Brameld *et al.* 1996*b*). Increasing dietary protein intake (from 99 to 194 g/kg, however, was found to significantly increase class 1 transcripts of IGF-I in all three adipose tissue depots, and also to a smaller degree in *semitendinosus* muscle (Brameld *et al.* 1996*b*), but not in LD muscle (Grant *et al.* 1991; Brameld *et al.* 1996*b*). A significant positive interaction between dietary protein content and GH administration was observed on IGF-I expression in the adipose tissue depots (Brameld *et al.* 1996*b*).

Similar interactions were also observed on expression of GHR in liver, skeletal muscle and adipose tissue (Brameld *et al.* 1996*b*). However, the direction of the interaction was tissue specific, resulting in the highest level of GHR mRNA being in the high-protein–GH-treated group for liver, but in the low-protein–GH-treated group for muscle (LD and *semitendinosus*) and adipose tissue (subcutaneous, omental and perirenal).

Recent work at Nottingham has looked at the effects of dietary protein content and GH administration on the circulating IGFBP (Guan *et al.* 1997). IGFBP-3 and -1 were both increased at high dietary protein intake, but the dietary protein intake did not interact with the stimulatory effects of exogenous GH. The effect of exogenous GH on IGFBP-2 appears to be inhibited by increasing dietary protein intake, such that GH increased IGFBP-2 levels in the pigs fed on the lower-protein diets, but decreased IGFBP-2 levels in the pigs fed on the highest-protein diet (194 g/kg). However, these effects were not statistically significant. Coleman & Etherton (1991) reported that GH administration decreased plasma IGFBP-2 levels, but they do not state what diet the pigs were fed. It might be expected that a high-nutrient (both energy and protein) diet would be given in order to meet the extra demands for growth.

Thus, dietary protein seems to affect both GHR and IGF-I expression, often in tissue-specific ways, as well as altering the circulating IGFBP levels, but further study is required using *in vitro* cell culture methods to identify whether these are direct nutrient effects on gene expression or indirect via manipulation of hormonal status.

HEPATOCYTE CELL CULTURE STUDIES

It can be seen that the whole-animal studies demonstrate effects of both dietary energy and protein on various components of the GH–IGF system in pigs, with some effects being seen at the mRNA level. However, the true mechanism for these nutrient and hormone effects cannot be determined *in vivo*, since the dietary manipulations also affect various circulating hormone levels, including GH, insulin, thyroid hormones and glucocorticoids. Thus, it is

impossible to identify whether there are direct nutrient–gene interactions, or whether the observed effects are indirect via these circulating hormones.

The liver is the major site of IGF-I expression and production and, more specifically, the parenchymal cells (hepatocytes) are the main cellular site for expression of both GHR and IGF-I (van Neste *et al.* 1988; Uchijima *et al.* 1995). The liver is also a sensor of nutritional and metabolic status and, therefore, a primary site for nutrient–gene interactions. For these reasons we have established a primary pig hepatocyte culture system (Brameld *et al.* 1995*b*), in order to investigate the direct effects of various hormones and nutrients on the expression of GHR and IGF-I genes. For these studies it was essential to use a serum-free culture medium, and to identify which factors were necessary for maintenance of cell viability, in order to reduce the components of the basal medium to a minimum. Very little work in this area has been carried out using pig hepatocytes, with the majority of investigators using rat or chicken hepatocytes. It will be necessary, therefore, to widen the scope for the present discussion to include those species.

Hormonal effects on hepatocyte expression and production

We have demonstrated stimulatory effects of dexamethasone (Dex; a synthetic glucocorticoid) and both thyroid hormones (T_3 and thyroxine (T_4)) on GHR expression, and also of Dex on IGF-I expression by pig hepatocytes, with T_4 appearing to decrease IGF-I expression (Brameld *et al.* 1995*b*). When each of these hormones was added in combination with GH, there was significant enhancement of the increases in class 1 transcripts obtained with GH alone, and the appearance of class 2 transcripts of IGF-I, presumably due to an increased number of GHR. Similar increases in class 1 transcripts, and the appearance of class 2 transcripts, were observed in the livers of GH-treated pigs (see p. 611). Studies in rat hepatocytes have produced similar effects of Dex on GH binding (Niimi *et al.* 1991), but conflicting effects of Dex on IGF-I, with Dex shown to either increase IGF-I expression and production (Phillips *et al.* 1991; Arany *et al.* 1993), or to have no effect on IGF-I expression and production (Miura *et al.* 1992; Uchijima *et al.* 1995). However, T_3 alone has been shown to have no effect on IGF-I expression or secretion (Uchijima *et al.* 1995), a similar result to that observed in the pig hepatocytes.

Studies using rat (Boni-Schnetzler *et al.* 1991; Phillips *et al.* 1991; Denver & Nicoll, 1994) or chicken hepatocytes (Houston & O'Neill, 1991) have demonstrated stimulatory effects of insulin on IGF-I expression (Boni-Schnetzler *et al.* 1991; Phillips *et al.* 1991) or production (Houston & O'Neill, 1991; Denver & Nicoll, 1994), with the effects of insulin and GH being additive when in combination (Boni-Schnetzler *et al.* 1991; Houston & O'Neill, 1991). However, the pig hepatocytes died during prolonged culture without insulin, therefore insulin was always present in the cultures, and indeed appears to be the only essential hormone. The effect of insulin on pig hepatocyte IGF-I and/or GHR expression is, therefore, still unclear, especially as insulin has been shown to be essential for maintenance of protein synthesis and to increase both total and poly(A)$^+$ RNA levels in rat hepatocytes (Hsu *et al.* 1992).

Glucagon has been shown to either inhibit IGF-I production, including GH-stimulated production and/or expression (Arany *et al.* 1993; Denver & Nicoll, 1994), or to stimulate IGF-I expression and production, with an additive effect when in combination with GH (Kachra *et al.* 1991).

Last, epidermal growth factor (EGF) has been shown to increase IGF-I production in a dose-dependent manner (Barreca *et al.* 1992), which may be important in the process of liver regeneration after extensive organ mass loss.

Hepatocytes have been shown to secrete mainly IGFBP-1 and -4, but not IGFBP-3, which is only expressed in the non-parenchymal cells (Uchijima *et al.* 1995), with the various hormones described previously controlling their secretion into the culture medium. Culture medium concentrations of IGFBP-1 have been shown to be increased by Dex (Miura *et al.* 1992; Arany *et al.* 1993; Uchijima *et al.* 1995), glucagon (Kachra *et al.* 1991; Denver & Nicoll, 1994; Uchijima *et al.* 1995) and EGF (Barreca *et al.* 1992), and to be decreased by insulin (Miura *et al.* 1992; Arany *et al.* 1993; Denver & Nicoll, 1994; Uchijima *et al.* 1995), with T_3 shown to have no effect (Uchijima *et al.* 1995) and GH to either have no effect (Uchijima *et al.* 1995), or to decrease secretion (Kachra *et al.* 1991; Miura *et al.* 1992; Thissen *et al.* 1994). IGFBP-4 secretion was found to be increased by Dex (Arany *et al.* 1993), T_3 (Uchijima *et al.* 1995), insulin (Uchijima *et al.* 1995) and glucagon (Kachra *et al.* 1991; Uchijima *et al.* 1995), and to be decreased by GH (Kachra *et al.* 1991).

Effects of glucose on hepatocyte expression and production

The major energy source in non-ruminants is glucose, and glucose has been shown to inhibit the gradual decline in GH binding to rat hepatocytes seen when they are cultured in serum-free conditions (Niimi *et al.* 1991). A similar effect was observed in pig hepatocytes (Brameld *et al.* 1995*b*), with high glucose levels reducing the decline in GHR expression with time in culture. Recent work has involved investigating the effects of glucose on GHR expression by pig hepatocytes, and we have demonstrated dose-dependent increases in GHR expression with increased glucose concentration both in the presence and absence of T_3 and Dex (Brameld, unpublished results), but only following an initial overnight pre-incubation without glucose, in order to deplete the hepatocytes of their stored glucose. A lack of glucose was found to be dominant over the added hormones, in that the stimulatory effect of T_3 and Dex was only seen in the presence of high concentrations of glucose, or in cells that had not been depleted of glucose. Whether glucose has any effect on IGF-I expression by the pig hepatocytes remains to be seen, but increased glucose has been found either to have no effect (Arany *et al.* 1993), or to increase (Luo & MacRae, 1990) IGF-I production by rat hepatocytes.

The only published study on the effects of glucose on IGFBP expression and/or secretion by rat hepatocytes (Arany *et al.* 1993) shows a decrease in IGFBP-1 mRNA with increased glucose, but no effect on secretion. There was, however, an interaction between glucose and insulin on IGFBP-1 secretion, such that as the glucose concentration increased so insulin became less effective in reducing IGFBP-1 secretion. Thus, at lower (2·7 and 6·5 mM) glucose concentrations, insulin reduced IGFBP-1 levels in a dose-dependent manner; but, at higher (11·1 mM) glucose concentrations, insulin had no effect on IGFBP-1 levels.

Direct stimulatory effects of glucose, or one of its metabolites, on hepatocyte gene expression have previously been described for a number of genes, including fatty acid synthase (*EC* 2.3.1.85; FAS; Prip-Buus *et al.* 1995), liver pyruvate kinase (*EC* 2.7.1.40; LPK; Kang *et al.* 1996), S_{14} (Jacoby *et al.* 1989), and glucose transporter type 2 (GLUT 2; Rencurel *et al.* 1996), with glucose also being shown to directly stimulate expression of insulin mRNA by β-cells in the pancreas (for review, see Docherty & Clark, 1994). Many of these genes also seem to be dependent on the presence of insulin (FAS, Prip-Buus *et al.* 1995; LPK, Kang *et al.* 1996), and to some extent T_3 and Dex (Prip-Buus *et al.* 1995), all of which have some effect on pig GHR expression (see p. 613). DNA-binding studies and transfection of suspected gene promoter constructs have revealed glucose response elements (GlRE; for review, see Vaulont & Kahn, 1994), which have a consensus

sequence of CACGGG or CACGTG. Whether the pig GHR gene has a similar GlRE in one of its promoters remains to be seen, since the actual number and sequence(s) of GHR promoters in any species remains to be established.

Effects of amino acids on hepatocyte expression and production

No studies of the effects of altering culture medium amino acid concentrations on pig hepatocyte expression or production of GH–IGF components have been reported to date. However, effects of altering the concentrations of total amino acids in rat hepatocyte culture medium have been studied. Conflicting results have been published on the effects of increasing amino acids on the expression and production of IGF-I and IGFBP-1. Two studies have shown increases in IGF-I mRNA (Phillips *et al.* 1991; Thissen *et al.* 1994) and decreases in IGFBP-1 mRNA and production with increased amino acids (Thissen *et al.* 1994), but another study showed the opposite, with decreases in IGF-I secretion and increases in IGFBP-1 mRNA and production with increased amino acids (Arany *et al.* 1993). The most recent study (Thissen *et al.* 1994) also demonstrated interactions between the amino acid concentrations and GH on both IGF-I and IGFBP-1 expression, such that the stimulatory effects of GH on IGF-I expression were enhanced in the presence of the highest amino acid concentrations, whereas the inhibitory effects of GH on IGFBP-1 expression were diminished in the presence of the highest amino acid concentrations. Studies carried out using rat hepatoma cells (a liver-derived cell line) have demonstrated increases in IGFBP-1 mRNA following removal of a single essential amino acid (phenylalanine, methionine, leucine, or tryptophan) from the serum-free culture medium for 24 h (Straus *et al.* 1993).

The current understanding of amino acid-dependent control of gene expression in mammalian cells is very limited, although the evidence suggests that deprivation of one or a number of amino acids leads to an increase in the expression and production of a putative regulatory protein, which in turn alters target gene expression (for review, see Kilberg *et al.* 1994). However, the identification of this (or these) regulatory proteins is at a very early stage.

CONCLUSIONS

The molecular mechanisms by which nutrients and hormones interact to control expression of the genes for the various components of the porcine GH–IGF system and, therefore, growth are likely to be very complex. Both the energy component and the protein (amino acid) component of the diet appear to directly control gene expression, in combination with various endocrine hormones also regulated by the diet. No single component of the GH–IGF system is likely to be dominant over the rest and, therefore, it is the overall effect on IGF bioactivity that is important. The energy component (glucose) would appear to be the major regulator of GHR expression, with various hormones also interacting. The protein component (amino acids) has been postulated to directly affect IGF-I expression, but this would appear to be dependent on the GH status of the animal. However, GH is the only factor that has been shown to directly increase the rate of transcription of IGF-I. The functions of the IGFBP are still not clear, but they are also affected, be that directly or indirectly, by diet. The primary regulator of the IGF-1R appears to be IGF-I itself; however, further study of nutrient effects is obviously necessary. Thus, the mechanisms relating to nutrient control of gene expression

are still unclear, but there is increasing evidence for direct interactions of nutrients in the control of expression of various growth regulatory genes.

The author is grateful to Professor P. J. Buttery and Dr J. M. Dawson for their helpful comments, and the BBSRC for their financial support.

REFERENCES

Ambler, G. R., Breier, B. H., Surus, A., Blair, H. T., McCutcheon, S. N., Silbergeld, A. & Gluckman, P. D. (1992). The interrelationship between and the regulation of hepatic growth hormone receptors and circulating GH binding protein in the pig. *Acta Endocrinologica* **126**, 155–161.

Arany, E., Strain, A. J., Hube, M. J., Phillips, I. D. & Hill, D. J. (1993). Interactive effects of nutrients and hormones on the expression of insulin-like growth factor binding protein-1 (IGFBP-1) mRNA and peptide, and IGF-I release from isolated adult rat hepatocytes. *Journal of Cellular Physiology* **155**, 426–435.

Barreca, A., Voci, A., Minuto, F., de Marchis, M., Cecchelli, E., Fugassa, E., Giordano, G. & Gallo, G. (1992). Effect of epidermal growth factor on insulin-like growth factor-I (IGF-I) and IGF-binding protein synthesis by adult rat hepatocytes. *Molecular and Cellular Endocrinology* **84**, 119–126.

Baumbach, W. R., Horne, D. L. & Logan, J. S. (1989). The growth hormone-binding protein in the rat serum is an alternatively spliced form of the rat growth hormone receptor. *Genes & Development* **3**, 1199–1205.

Boni-Schnetzler, M., Schmid, C., Meier, P. J. & Froesch, E. R. (1991). Insulin regulates insulin-like growth factor I mRNA in rat hepatocytes. *American Journal of Physiology* **260**, E846–E851.

Brameld, J. M., Atkinson, J. L., Budd, T. J., Saunders, J. C., Pell, J. M., Salter, A. M., Gilmour, R. S. & Buttery, P. J. (1996*a*). Expression of insulin-like growth factor-I (IGF-I) and growth hormone receptor (GHR) mRNA in liver, skeletal muscle and adipose tissue of different breeds of pig. *Animal Science* **62**, 555–559.

Brameld, J. M., Atkinson, J. L., Saunders, J. C., Pell, J. M., Buttery, P. J. & Gilmour, R. S. (1996*b*). Effects of growth hormone administration and dietary protein intake on insulin-like growth factor-I (IGF-I) and growth hormone receptor (GHR) mRNA expression in porcine liver, skeletal muscle and adipose tissue. *Journal of Animal Science* **74**, 1832–1841.

Brameld, J. M., Weller, P. A., Pell, J. M., Buttery, P. J. & Gilmour, R. S. (1995*a*). Ontogenic study of insulin-like growth factor-I and growth hormone receptor mRNA expression in porcine liver and skeletal muscle. *Animal Science* **61**, 333–339.

Brameld, J. M., Weller, P. A., Saunders, J. C., Buttery, P. J. & Gilmour, R. S. (1995*b*). Hormonal control of insulin-like growth factor-I (IGF-I) and growth hormone receptor (GHR) mRNA expression by porcine hepatocytes in culture. *Journal of Endocrinology* **146**, 239–245.

Breier, B. H. & Sauerwein, H. (1995). Regulation of growth in ruminants by the somatotropic axis. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. Proceedings of the Eighth International Symposium on Ruminant Physiology*, pp. 451–474 [W. V. Engelhardt, S. Leonhard-Marek, G. Breves and D. Giesecke, editors]. Stuttgart, Germany: Ferdinand Enke Verlag.

Buonomo, F. C. & Baile, C. A. (1991). Influence of nutritional deprivation on insulin-like growth factor I, somatotropin, and metabolic hormones in swine. *Journal of Animal Science* **69**, 755–760.

Campbell, R. G., Johnson, R. J., King, R. H., Taverner, M. R. & Meisinger, D. J. (1990). Interaction of dietary protein content and exogenous porcine growth hormone administration on protein and lipid accretion rates in growing pigs. *Journal of Animal Science* **68**, 3217–3225.

Caperna, T. J., Steele, N. C., Komarek, D. R., McMurtry, J. P., Rosebrough, R. W., Solomon, M. B. & Mitchell, A. D. (1990). Influence of dietary protein and recombinant porcine somatotropin administration in young pigs: Growth, body composition and hormone status. *Journal of Animal Science* **68**, 4243–4252.

Chung, C. S. & Etherton, T. D. (1986). Characterization of porcine growth hormone (pGH) binding to porcine liver microsomes: Chronic administration of pGH induces pGH binding. *Endocrinology* **119**, 780–786.

Cioffi, J. A., Wang, X. & Kopchick, J. J. (1990). Porcine growth hormone receptor cDNA sequence. *Nucleic Acid Research* **18**, 6451.

Coleman, M. E. & Etherton, T. D. (1991). Effects of exogenous growth hormone on serum insulin-like growth factor-binding proteins in growing pigs. *Journal of Endocrinology* **128**, 175–180.

Coleman, M. E., Pan, Y.-C. E. & Etherton, T. D. (1991). Identification and NH_2-terminal amino acid sequence of three insulin-like growth factor-binding proteins in porcine serum. *Biochemical and Biophysical Research Communications* **181**, 1131–1136.

Coleman, M. E., Russell, L. & Etherton, T. E. (1994). Porcine somatotropin (pST) increases IGF-I mRNA abundance in liver and subcutaneous adipose tissue but not in skeletal muscle of growing pigs. *Journal of Animal Science* **72**, 918–924.

Dardevet, D., Manin, M., Balage, M., Sornet, C. & Grizard, J. (1991). Influence of low- and high-protein diets on insulin and insulin-like growth factor-1 binding to skeletal muscle and liver in the growing rat. *British Journal of Nutrition* **65**, 47–60.

Dauncey, M. J., Burton, K. A., White, P., Harrison, A. P., Gilmour, R. S., Duchamp, C. & Cattaneo, D. (1994). Nutritional regulation of growth hormone receptor gene expression. *FASEB Journal* **8**, 81–88.

Dauncey, M. J. & Buttle, H. L. (1990). Differences in growth hormone and prolactin secretion associated with environmental temperature and energy intake. *Hormone and Metabolic Research* **22**, 524–527.

Dauncey, M. J., Rudd, B. T., White, D. A. & Shakespear, R. A. (1993). Regulation of insulin-like growth factor binding proteins in young growing animals by alteration of energy status. *Growth Regulation* **3**, 198–207.

Dauncey, M. J., Shakespear, R. A., Rudd, B. T. & Ingram, D. L. (1990). Variations in somatomedin-C/insulin-like growth factor-I associated with environmental temperature and nutrition. *Hormone and Metabolic Research* **22**, 261–264.

Davis, S. L., Graf, M., Morrisson, C. A., Hall, T. R. & Swift, P. J. (1992). Identification and partial purification of serum growth hormone binding protein in domestic animal species. *Journal of Animal Science* **70**, 773–780.

Denver, R. J. & Nicoll, C. S. (1994). Pancreatic hormones differentially regulate insulin-like growth factor (IGF)-I and IGF-binding protein production by primary rat hepatocytes. *Journal of Endocrinology* **142**, 299–310.

Dougherty, K. & Clark, A. R. (1994). Nutrient regulation of insulin gene expression. *FASEB Journal* **8**, 20–27.

Evock, C. M., Walton, P. E. & Etherton, T. D. (1990). Effect of GH status on IGF-I and IGF-II concentrations and serum IGF binding profiles in pigs. *Journal of Animal Science* **68**, 1953–1964.

Gilmour, R. S. (1994). The implications of insulin-like growth factor mRNA heterogeneity. *Journal of Endocrinology* **140**, 1–3.

Glassford, J., Flick-Smith, H. & Pell, J. M. (1995). Regulation of insulin-like growth factor type-1 receptor (IGF1R) and insulin receptor (IR) gene expression by nutritional status. *Journal of Endocrinology* **147**, Suppl., O40.

Grant, A. L., Helferich, W. G., Kramer, S. A., Merkel, R. A. & Bergen, W. G. (1991). Administration of growth hormone to pigs alters the relative amount of insulin-like growth factor-1 mRNA in liver and skeletal muscle. *Journal of Endocrinology* **130**, 331–338.

Guan, X. F., Brameld, J. M. & Buttery, P. J. (1997). Effects of dietary protein levels and porcine growth hormone administration on plasma concentrations of insulin-like growth factor binding proteins in growing pigs. *Proceedings of the Nutrition Society* **56**, 168A.

Harper, J. M. M., Soar, J. B. & Buttery, P. J. (1987). Changes in protein metabolism of ovine primary muscle cultures on treatment with growth hormone, insulin, insulin-like growth factor-I or epidermal growth factor. *Journal of Endocrinology* **112**, 87–96.

Houston, B. & O'Neill, I. E. (1991). Insulin and growth hormone act synergistically to stimulate insulin-like growth factor-I production by cultured chicken hepatocytes. *Journal of Endocrinology* **128**, 389–393.

Hsu, C.-J., Kimball, S. R., Antonetti, D. A. & Jefferson, L. S. (1992). Effects of insulin on total RNA, poly(A)$^+$ RNA, and mRNA in primary cultures of rat hepatocytes. *American Journal of Physiology* **263**, E1106–E1112.

Jacoby, D. B., Zilz, N. D. & Towle, H. C. (1989). Sequences within the 5′-flanking region of the S_{14} gene confer responsiveness to glucose in primary hepatocytes. *Journal of Biological Chemistry* **264**, 17623–17626.

Jones, J. I. & Clemmons, D. R. (1995). Insulin-like growth factors and their binding proteins: Biological actions. *Endocrine Reviews* **16**, 3–34.

Kachra, Z., Barash, I., Yannopoulos, C., Khan, M. N., Guyda, H. J. & Posner, B. I. (1991). The differential regulation by glucagon and growth hormone of insulin-like growth factor (IGF)-I and IGF binding proteins in cultured rat hepatocytes. *Endocrinology* **128**, 1723–1730.

Kang, R., Yamada, K., Tanaka, T., Lu, T. & Noguchi, T. (1996). Relationship between concentrations of glycolytic intermediates and expression of the L-type pyruvate kinase gene in cultured hepatocytes. *Journal of Biochemistry* **119**, 162–166.

Kelley, R. L., Jungst, S. B., Spencer, T. E., Owsley, W. F., Rahe, C. H. & Mulvaney, D. R. (1995). Maternal treatment with somatotropin alters embryonic development and early postnatal growth of pigs. *Domestic Animal Endocrinology* **12**, 83–94.

Kelly, P. A., Djiane, J., Postel-Vinay, M. C. & Edery, M. (1991). The prolactin/growth hormone receptor family. *Endocrinology Reviews* **12**, 235–251.

Kelly, P. A., Goujon, L., Dinerstein, H., Esposito, N., Edery, M., Finidori, J. & Posten-Vinay, M. C. (1994). The GH receptor and signal transduction. *Hormone Research* **42**, 133–139.

Kilberg, M. S., Hutson, R. G. & Laine, R. O. (1994). Amino acid-regulated gene expression in eukaryotic cells. *FASEB Journal* **8**, 13–19.

Li, J., Saunders, J. C., Gilmour, R. S., Silver, M. & Fowden, A. L. (1993). Insulin-like growth factor-II messenger ribonucleic acid expression in fetal tissues of the sheep during late gestation: Effects of cortisol. *Endocrinology* **132**, 2083–2089.

Louveau, I. & Etherton, T. D. (1992). Partial purification of somatotropin receptors from pig liver: they arise from a single somatotropin receptor messenger RNA transcript. *Journal of Animal Science* **70**, 3421–3428.

Luo, Q. J. & MacRae, J. C. (1990). Production of insulin-like growth factor-1 by rat hepatocytes is sensitive to nutrients but not growth hormone. *Biochemical Society Transactions* **18**, 1197–1198.

McCusker, R. H., Campion, D. R., Jones, W. K. & Clemmons, D. R. (1985). The insulin-like growth factor-binding proteins of porcine serum: endocrine and nutritional regulation. *Endocrinology* **125**, 501–509.

Miura, Y., Higashi, Y., Kato, H., Takahashi, S.-I. & Noguchi, T. (1992). Effects of dexamethasone on the production of insulin-like growth factor-I and insulin-like growth factor binding proteins in primary cultures of rat hepatocytes. *Bioscience Biotechnology and Biochemistry* **56**, 1396–1400.

Niimi, S., Hayakawa, T., Tanaka, A. & Ichihara, A. (1991). Glucose regulation of growth hormone receptors in primary cultured rat hepatocytes. *Endocrinology* **129**, 2734–2739.

Oldham, J. M., Martyn, J. A. K., Kirk, S. P., Napier, J. R. & Bass, J. J. (1996). Regulation of type 1 insulin-like growth factor (IGF) receptors and IGF-I mRNA by age and nutrition in ovine skeletal muscles. *Journal of Endocrinology* **148**, 337–346.

Owens, P. C., Johnson, R. J., Campbell, R. G. & 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.

Phillips, L. S., Goldstein, S. & Pao, C.-I. (1991). Nutrition and somatomedin XXVI. Molecular regulation of IGF-I by insulin in cultured rat hepatocytes. *Diabetes* **40**, 1525–1530.

Prip-Buus, C., Perdereau, D., Foufelle, F., Maury, J., Ferre, P. & Girard, J. (1995). Induction of fatty-acid-synthase gene expression by glucose in primary culture of rat hepatocytes: Dependency upon glucokinase activity. *European Journal of Biochemistry* **230**, 309–315.

Ramsay, T. G., Chung, I. B., Czerwinski, S. M., McMurtry, J. P., Rosebrough, R. W. & Steele, N. C. (1995). Tissue IGF-I protein and mRNA responses to a single injection of somatotropin. *American Journal of Physiology* **269**, E627–E635.

Rechler, M. M. & Brown, A. L. (1992). Insulin-like growth factor binding proteins: Gene structure and expression. *Growth Regulation* **2**, 55–68.

Rencurel, F., Waeber, G., Antoine, B., Rocchiccioli, F., Maulard, P., Girard, J. & Leturque, A. (1996). Requirement of glucose metabolism for regulation of glucose transporter type 2 (GLUT2) gene expression in liver. *Biochemical Journal* **314**, 903–909.

Roe, J. A., Haji Baba, A. S., Harper, J. M. M. & Buttery, P. J. (1995). Effects of growth factors and gut regulatory peptides on nutrient uptake in ovine muscle cell cultures. *Comparative Biochemistry and Physiology* **110**A, 107–114.

Roe, J. A., Harper, J. M. M. & Buttery, P. J. (1989). Protein metabolism in ovine primary muscle cultures derived from satellite cells—effects of selected peptide hormones and growth factors. *Journal of Endocrinology* **122**, 565–571.

Seve, B., Ballevre, O., Ganier, P., Noblet, J., Prugnaud, J. & Obled, C. (1993). Recombinant porcine somatotropin and dietary protein enhance protein synthesis in growing pigs. *Journal of Nutrition* **123**, 529–540.

Sorensen, M. T., Chaudhuri, S., Louveau, I., Coleman, M. E. & Etherton, T. D. (1992). Growth hormone binding proteins in pig adipose tissue: number, size and effects of pGH treatment on pGH and bGH binding. *Domestic Animal Endocrinology* **9**, 13–24.

Straus, D. S., Burke, E. J. & Marten, N. W. (1993). Induction of insulin-like growth factor binding protein-1 gene expression in liver of protein-restricted rats and in rat hepatoma cells limited for a single amino acid. *Endocrinology* **132**, 1090–1100.

Sussenbach, J. S., Steenbergh, P. H. & Holthuizen, P. (1992). Structure and expression of the human insulin-like growth factor genes. *Growth Regulation* **2**, 1–9.

Thissen, J.-P., Pucilowska, J. B. & Underwood, L. E. (1994). Differential regulation of insulin-like growth factor-I (IGF-I) and IGF binding protein-1 messenger ribonucleic acids by amino acid availability and growth hormone in rat hepatocyte primary culture. *Endocrinology* **134**, 1570–1576.

Trivedi, B. & Daughaday, W. H. (1988). Release of growth hormone binding protein from IM-9 lymphocytes by endopeptidase digestion is dependent on sulfydryl group inactivation. *Endocrinology* **123**, 2201–2206.

Uchijima, Y., Takenaka, A., Takahashi, S.-I. & Noguchi, T. (1995). Production of insulin-like growth factors and their binding proteins in primary cultures of rat liver parenchymal and nonparenchymal cells. *Bioscience Biotechnology and Biochemistry* **58**, 1503–1515.

van Neste, L., Husman, B., Moller, C., Andersson, G. & Norstedt, G. (1988). Cellular distribution of somatogenic receptors and insulin-like growth factor-I mRNA in the rat liver. *Journal of Endocrinology* **119**, 69–74.

Vaulont, S. & Kahn, A. (1994). Transcriptional control of metabolic regulation genes by carbohydrates. *FASEB Journal* **8**, 28–35.

Weller, P. A., Dauncey, M. J., Bates, P. C., Brameld, J. M., Buttery, P. J. & Gilmour, R. S. (1994). Regulation of porcine insulin-like growth factor-I and growth hormone receptor mRNA expression by energy status. *American Journal of Physiology* **266**, E776–E785.

Weller, P. A., Dickson, M. C., Huskisson, N. S., Dauncey, M. J., Buttery, P. J. & Gilmour, R. S. (1993). The porcine insulin-like growth factor-I gene: characterization and expression of alternate transcription sites. *Journal of Molecular Endocrinology* **11**, 201–211.

Werner, H., Hernandez-Sanchez, C., Karnieli, E. & LeRoith, D. (1995). The regulation of IGF-I receptor gene expression. *International Journal of Biochemistry and Cell Biology* **27**, 987–994.
Wolverton, C. K., Azain, M. J., Duffy, J. Y., White, M. E. & Ramsay, T. G. (1992). Influence of somatotropin on lipid metabolism and IGF gene expression in porcine adipose tissue. *American Journal of Physiology* **263**, E637–E645.
Zapf, J. (1995). Physiological role of the insulin-like growth factor binding proteins. *European Journal of Endocrinology* **132**, 645–654.