THE NUTRITIONAL REGULATION OF GROWTH HORMONE ACTION

J. M. PELL

Department of Endocrinology & Animal Physiology, AFRC Institute of Grassland and Environmental Research, Hurley, Berks SL6 5LR

AND P. C. BATES

Nutrition Research Unit, London School of Hygiene & Tropical Medicine, 4 St Pancras Way, London NW1 2PE

CONTENTS

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INTRODUCTION

Growth hormone (GH) has widespread and varied actions in vivo and in vitro, influencing skeletal and muscle growth, adipose tissue accumulation and mammary function via direct and indirect effects on protein, lipid and carbohydrate metabolism. The net results of this, in terms of benefits to agriculture and medicine, have now largely been characterized thanks to the availability of recombinantly-derived material. However, while exogenous GH treatment can yield useful information on GH action it is not really relevant in the context of the present review and will therefore only be quoted when important for understanding the physiological relationship between nutrition and GH action. Rather, we will concentrate on the mechanisms which mediate GH action, discussing nutritional regulation where this has been investigated. First, the known events leading to the mediation of GH action will be outlined, along with a summary of the subsequent physiological 'end-products'; the more detailed mechanisms of GH action can then be considered in context.

SITES OF CONTROL FOR GH ACTION

Fig. 1. shows a schematic summary of the events known to date which are involved in GH action. The rate of release from the pituitary into blood is largely controlled by GH-releasing hormone (promotes release) and somatostatin (inhibits release) and a complex series of positive and negative feedback systems; this expanding field has been reviewed by Buchanan & Ross (1990). The appearance of GH into the circulation is not constant but pulsatile, and peak frequency and amplitude and trough concentrations are therefore important, although mean concentration can often be related to physiological state. Possible modulators of the activity of circulating GH are the GH-binding proteins, whose role is not clear at present.

GH promotes a physiological response by direct interaction with its receptors, indirect stimulation of insulin-like growth factor-1 (IGF-1) production, or modulation of the concentration or activity of other hormones such as insulin. The regulation of receptor populations for GH, in terms of tissue distribution, density and affinity is therefore critical. Post-receptor events for GH are largely unexplored at present.

The control of IGF-1 activity is possibly more complex than that of GH because it can be synthesized in most tissues in the body, and particularly in the liver. This raises the possibility that blood IGF-1 either represents an endocrine 'message' from the liver to peripheral tissues, or is merely the 'waste' IGF-1 which has been released after local activity at its site of production. To complicate matters further, several circulating IGF-1-binding proteins (BP) exist, and these certainly regulate tissue availability. The distribution and nature of IGF-1 receptors might provide some insight into this endocrine *v.* autocrine/paracrine controversy.

Last, there can be no doubt that GH and IGF-1 action must be co-ordinated and orchestrated with the activity of other hormones necessary for such complex processes as muscle and bone growth, adipose tissue accumulation and lactation.

PHYSIOLOGICAL EFFECTS OF GH: NUTRITIONAL MODULATION

THE ROLE OF GH IN VIVO

GH is a single polypeptide chain of approximately 190 amino acids containing two intra-chain disulphide bridges. It consists mainly of four anti-parallel α-helices which are

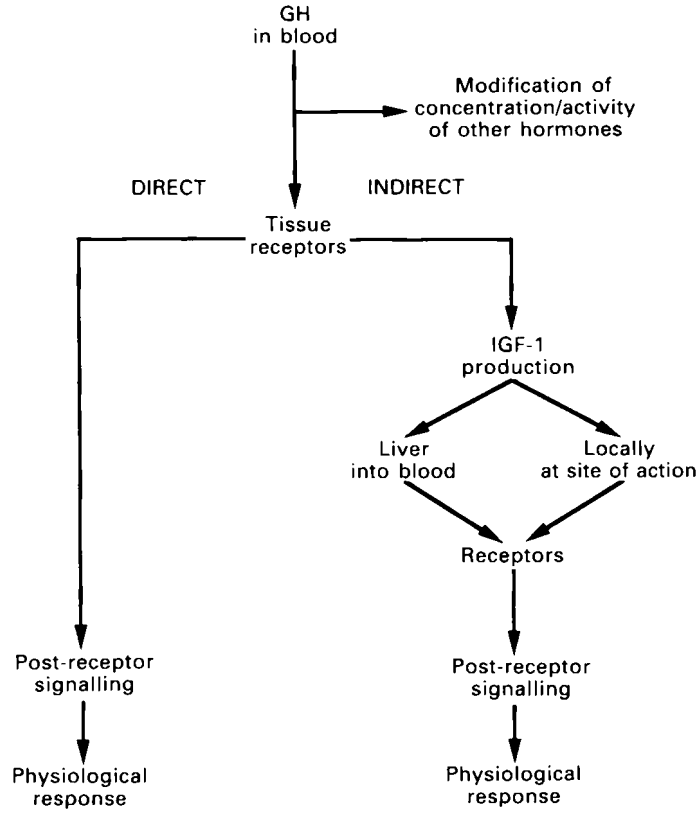

Fig. 1. Schematic representation of potential control sites for growth hormone (GH) action. IGF-1, insulin-like growth factor-1.

arranged in a left-twisted, tightly packed helical bundle (Abdel-Meguid *et al.* 1987). The amino acid sequences of GH isolated from different species exhibit a varying degree of homology, their relationship to a hypothetical common 'placental ancestor' being determined by their individual rates of evolutionary divergence. For example, the rate of evolution of primate GH is far greater than that for non-primate GH and this is reflected in their actions; primate GH is active in most non-primates but not vice versa (Wallis, 1989).

The range of biological processes which GH can influence is impressive, varying from cellular events such as mitosis and differentiation to net protein anabolism, net fat catabolism, insulin resistance and mineral metabolism (summarized by Etherton, 1989). Of necessity, many of these actions have been determined in vitro, which may not be representative of the events which occur in vivo. Broadly, the effects of GH in normal animals can be summarized into two categories: short- and long-term. In man and in ruminants, the short-term function of GH is basically one of protein sparing. For example, during fasting for as little as 24 h mean GH concentrations will increase and body protein is spared at the expense of fat degradation which is used to meet the energy deficit.

The long-term role of GH is to channel nutrients towards some 'target' process (Bauman *et al.* 1982); the nature of this effect depends on the developmental stage of the animal. It has long been established that GH is essential for the normal growth of young animals and the primary role of GH prepubertally is to promote skeletal and lean tissue growth and

differentiation. Later on, growth is clearly no longer a physiological priority for an animal, and GH is more concerned with the regulation of body composition. In ruminant animals, GH is also essential for mammary development and lactation; in primates, GH can influence lactational performance but it is the structurally related hormone, prolactin, which is most important (Cowie *et al.* 1980).

GROWTH AND BODY COMPOSITION

Laboratory animal models

The early work on the growth-promoting properties of crude extracts of exogenous GH in rats have been difficult to repeat and this is well illustrated by the relative paucity of recent information on the anabolic actions of GH in normal rats. Increases in live-weight gain have only been reported following administration of pharmacological doses of GH (e.g. Groesbeck *et al.* 1987). It is not surprising, therefore, that the preferred animal model for the investigation of GH action is one in which circulating GH concentrations are low or absent.

The most obvious GH-deficient animal is the hypophysectomized rat. Since this involves removal of the pituitary gland, the complex series of hormones normally produced by the pituitary are absent and serum insulin concentrations are also decreased (Brown *et al.* 1981) limiting their usefulness for growth studies. An alternative model is the dwarf mouse; these have an autosomal recessive mutation, resulting in a defective pituitary gland which secretes very low amounts of GH, prolactin, thyroid stimulating hormone (TSH) and ACTH. Homozygotes only grow to 25–35% of the weight of their heterozygote littermates and correspond to congenital GH deficiency in man (Brook *et al.* 1986) and will respond to exogenous GH in a sensitive, dose-dependent manner (Wallis & Dew, 1973). Concentrations of other hormones are apparently normal (Van Buul-Offers, 1983), implying that dwarf mice are a more physiological model for the study of GH action than are hypophysectomized rats. Exogenous GH not only induces increases in skeletal growth and live-weight gain but also increases lean tissue mass while decreasing fat accumulation (Holder *et al.* 1988). A more specific model for GH deficiency may be found in the homozygous little mouse, which cannot synthesize or secrete GH (Cheng *et al.* 1983). One major problem with dwarf and little mice is their small size, which can make some physiological studies, for example involving frequent blood sampling, impossible. In this regard, Charlton *et al.* (1988) have described a new strain of dwarf rats which specifically have low, but not negligible, serum GH concentrations.

Pigs

The most comprehensive work to date on responses in vivo to GH in normal intact animals has been performed on pigs. The response of pigs to increasing doses of porcine (p) GH is illustrated in Fig. 2 (Boyd & Bauman, 1989). Protein accretion increased by at least 50% to a maximum plateau whereas lipid accretion decreased dramatically in a dose-dependent manner. Changes in rates of ash accumulation were modest but increased in a curvilinear manner. Daily gain was increased at the lower doses of pGH but began to decline at the higher doses. Voluntary feed intake was depressed in GH-treated swine so that efficiency, in terms of feed/gain, was improved. It is interesting that the dose–response relationships of protein and fat accretion were different, implying either that they are controlled by separate mechanisms or that nutrient intake becomes limiting for protein deposition at the highest doses of pGH. In fact, it has recently been confirmed that the decline in daily gain at the highest doses of pGH was due to a deficiency in amino acid

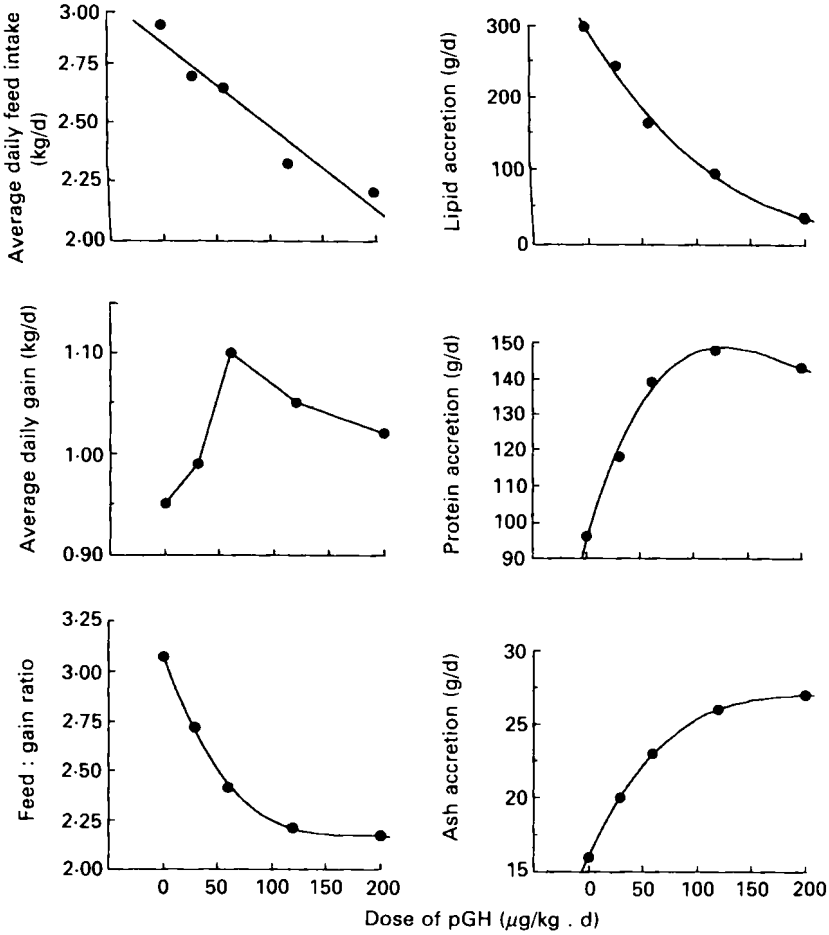

Fig. 2. Feed intake, average daily gain, composition of gain and feed:gain ratio in pigs treated with various doses of porcine growth hormone (pGH) (from Boyd & Bauman, 1989).

intake which occurred as a result of the decreased feed consumption, thus limiting protein accretion (R. D. Boyd, personal communication).

Responsiveness to pGH is dependent on several factors. The fractional changes in lipid and protein accretion are greater in older, and therefore heavier, pigs than in young pigs (Etherton *et al.* 1987*b*; Campbell *et al.* 1989). This is probably because older pigs are laying down more fat and there is a greater opportunity for GH to perturb metabolism towards lean tissue growth. Androgen status will influence responsiveness to pGH. In untreated pigs, boars exhibit the greatest rate of lean tissue deposition, followed by barrows and then gilts; an inverse relationship exists for fat accumulation and feed intake. These differences are largely abolished by pGH treatment (Campbell *et al.* 1989). The effects of reduced energy intake on GH action have been investigated by Campbell *et al.* (1988). In control pigs, greater energy intake increased linear growth rate and fat deposition and did not change feed/gain; GH induced increased live-weight gain, protein deposition and decreased feed/gain with concomitant decreases in lipid accumulation at all levels of feed intake. The findings imply that the effects of pGH were independent and additive to those of changing energy intake.

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Table 1. *Effect of growth hormone (GH) on empty-body-weight (kg) and carcass components (kg) in lambs offered a restricted (30 g/kg live weight) or* ad lib. *(approximately 50 g/kg live weight) concentrate diet*

	Restricted				*ad lib.*			
	C	GH	SED	Statistical significance of difference: *P*	C	GH	SED	Statistical significance of difference: *P*
Empty-body-wt	23·3	25·3	1·3	> 0·1	39·4	42·4	1·57	> 0·1
Carcass: Wt	12·46	13·48	0·68	> 0·1	21·55	22·55	0·78	> 0·1
Water	7·28	8·06	0·41	0·066	11·28	12·52	0·44	0·009
Ash	0·57	0·61	0·04	> 0·1	0·92	0·97	0·07	> 0·1
Fat	2·64	2·55	0·21	> 0·1	6·35	5·56	0·38	0·05
Protein	2·00	2·28	0·11	0·018	3·23	3·68	0·12	0·001

C, control; SED, standard error of difference.

Ruminant animals

Generally the effects of exogenous GH on growth and body composition in sheep and cattle have been more varied than those found for pigs, even though they have been qualitatively similar. This variability may be related to nutrient supply. Zainur *et al.* (1989*a*, *b*) reported dose responses to GH in lambs fed on low- and high-quality diets respectively. On the high-quality diet, GH treatment decreased body fat and increased protein accretion whereas only protein accretion was stimulated in the lambs with the poor-quality diet. Pell *et al.* (1989) examined the interaction between dietary protein and energy and responsiveness to GH in lambs (9 weeks old) which were offered diets containing either 120, 160 or 200 g crude protein/kg, either *ad lib.* or at 30 g/kg live weight. Half the lambs from each dietary group were administered GH (0·1 mg/kg per d) for 10 weeks. Changing dietary protein content had little effect on empty-body-weight or carcass composition and the findings are therefore summarized in terms of energy intake and GH status in Table 1. For the restricted lambs, carcass protein content increased by 14%, accompanied by a tendency for water content to increase; fat content was unchanged. However, carcass water and protein increased significantly and carcass fat decreased significantly in *ad lib.*-fed lambs. Since *ad lib.*-fed lambs had almost 2·5 times more carcass fat than restricted lambs this supports the suggestion that exogenous GH can exert greater effects on carcass composition when it has the opportunity to prevent lipid accumulation, i.e. in well-fed old or high-body-weight animals. These findings, confirming those of Campbell *et al.* (1988), also indicate an interaction between energy intake and GH-induced changes in carcass composition.

Man

The earliest studies investigating the effects of GH in man were attempts to correct hypopituitary dwarfism (Raben, 1958) and many investigations have illustrated the increase in height velocity which can occur when GH-deficient children are treated with exogenous human GH (e.g. Albertsson-Wikland *et al.* 1988). Responsiveness depends on dose rate and frequency, the increase in height velocity being maximal when administered as frequent injections (Smith *et al.* 1988) mimicking the pulsatile GH secretion found in normal children. Treated GH-deficient children tend to increase their energy intake by

approximately 20%, although there is great variation between individuals (Kaplowitz & Jennings, 1987); this is not surprising since at least a doubling in height velocity can occur. Body composition may also change, lean body mass increasing by almost 7% and body fat decreasing by approximately 5·5% over 1 year of treatment (Linarelli, 1989).

Even though one group have reported that the level of dietary energy does not influence the actions of GH (Manson *et al.* 1988), Snyder *et al.* (1989) have found that dietary fat or carbohydrate content can indeed determine responsiveness to GH during energy restriction; nitrogen excretion was less on a high-carbohydrate diet than on a high-lipid diet, and GH reduced N excretion on the high-carbohydrate diet but not the high-lipid diet. Absolute fasting in humans or in rats causes GH resistance and therefore a minimum dietary carbohydrate level must be necessary for GH action.

LACTATION

In ruminant animals, GH is essential for the induction and maintenance of lactation. It is possible, even, that GH status can influence mammary growth in prepubertal ruminants. The poor milk yields often obtained from dairy animals which have been fed at a high plane of nutrition during development may be related to an inhibition of mammary parenchymal growth (Little & Kay, 1979). Johnsson & Hart (1985) investigated this further in prepubertal lambs and found that a high plane of nutrition decreased the rate of allometric growth of mammary parenchyma; this was related to lower plasma GH concentrations in animals on a higher plane of nutrition (Johnsson *et al.* 1985). Furthermore, when lambs fed on a high plane of nutrition were treated with exogenous GH the inhibition of parenchymal growth was, in part, reversed (Johnsson *et al.* 1986). Sandles *et al.* (1987) confirmed these findings for prepubertal heifers; they also observed an increased udder volume at calving in GH-treated heifers, but this was not related to any subsequent increase in milk yield.

The galactopoietic action of exogenous GH in adult ruminants has been reviewed recently (e.g. Peel & Bauman, 1987). The first comprehensive study on the effects of GH on lactation was published by Bauman *et al.* (1985). Milk yield increased in a dose-dependent manner by between 23 and 41% in GH-treated cows. Although yield increased during the first week of treatment, voluntary food intake increased only gradually which meant that the cows were in negative energy balance until week 10 of treatment. Over all, gross efficiency as milk output per unit net energy intake was increased by an average of 15%. Later studies have confirmed these findings (e.g. Soderholm *et al.* 1988; Bauman *et al.* 1989) for dairy cows and also for lactating ewes (Sandles *et al.* 1988).

The relationship between feed intake, the yield and composition of milk output, body weight and body composition is complex. When the GH-induced increase in milk yield causes negative energy balance milk fat content increases, probably due to a net breakdown of body lipid reserves and an increase in plasma non-esterified fatty acid (NEFA) concentrations; if the cows remain in positive energy balance then milk fat content is unchanged (Peel & Bauman, 1987). Milk protein content can increase in GH-treated cows; this coincides with the increase in voluntary feed intake and may therefore be related to protein supply (Phipps *et al.* 1990). However, DeBoer & Kennelly (1989*a*) could find no further stimulation of milk output in GH-treated cows fed on a diet containing 160 g crude protein (N × 6·25)/kg, as opposed to 110 g crude protein/kg. It is possible that energy and protein intake must increase for milk protein output to be stimulated. Certainly, milk protein content decreases when lactating cows are in negative N balance (Tyrrell *et al.* 1982).

The effects of GH on mammary metabolism are thought to be indirect. No receptors for GH have been demonstrated in mammary tissue (Keys & Djiane, 1988) and direct infusions

of GH into the mammary arterial supply do not stimulate increases in yield until peripheral blood GH concentrations are elevated (McDowell *et al.* 1987*b*). It has been suggested that the GH-induced increase in milk yield is a passive response due to increased nutrient supply to the mammary gland; McDowell *et al.* (1987*a*) have demonstrated that nutrient availability is indeed greater. However, increasing nutrient supply via post-ruminal nutrient supplementation does not change milk yield.

METABOLIC RESPONSES

Since GH co-ordinates homeorhetic responses, partitioning nutrients towards specific processes, continued exposure to increased GH concentrations (whether of endogenous or exogenous origin) would be expected to modulate the balance of metabolites and other hormones. GH does not appear to influence the efficiency of nutrient absorption as digesta flow through the gastrointestinal tract (Pell *et al.* 1990) and apparent digestibility of dry matter is unchanged in both meat- (Eisemann *et al.* 1986*b*, 1989; Pell *et al.* 1990) and milk- (Sechen *et al.* 1989) producing animals, thus the actions of GH are essentially post-absorptive. Exogenous GH generally increases blood flow to the mammary gland and to muscle, thus increasing nutrient supply to body tissues (McDowell *et al.* 1988; Elcock & Pell, 1989; Fullerton *et al.* 1989).

GH can modulate N metabolism by altering N excretion or its distribution in body tissues. In growing animals, GH increases whole-body protein accretion largely by decreasing urinary N excretion (Eisemann *et al.* 1986*b*, 1989; Pell *et al.* 1990), although of course changes in feed intake may also occur. This adaptation in N metabolism is illustrated by the decrease in blood urea concentration which typically occurs in GH-treated animals and presumably reflects a reduction in urea synthesis, which has been confirmed in GH-treated hypopituitary children (Dahms *et al.* 1989). Dietary energy intake can influence urea metabolism in response to GH; McShane *et al.* (1989) fed beef heifers high- and low-energy diets and, even though those on the high-energy diet had increased urea concentrations compared with those on the low-energy diet, GH treatment still reduced the urea concentrations further.

Nutrition is also a major determinant in the response of NEFA metabolism to GH. In well-fed growing cattle, pigs and sheep, NEFA concentrations are unchanged over a range of GH dose rates (Peters, 1986; Etherton *et al.* 1987*b*; Pell *et al.* 1990) implying that net fat mobilization does not occur in these animals, assuming that rates of utilization are unchanged. If dietary intake is decreased, NEFA concentrations tend to increase (McShane *et al.* 1989) in response to exogenous GH, and Eisemann *et al.* (1986*a*) reported increased NEFA concentrations and turnover in GH-treated steers fed only slightly above maintenance requirements.

Modification of glucose metabolism is complex; GH may directly stimulate gluconeogenesis and can indirectly influence glucose utilization by changing insulin sensitivity or possibly by direct action. In GH-treated well-fed animals, signifiant increases in plasma concentrations of both insulin and glucose have been observed (Johnsson *et al.* 1987; Pell *et al.* 1990), although some studies have only reported increased insulin concentrations (Etherton *et al.* 1987*b*). McShane *et al.* (1989) obtained increased glucose concentrations in GH-treated beef heifers, provided that they were fed on a high-energy diet.

Simultaneous increases in plasma glucose and insulin concentrations seem paradoxical; however, GH has an insulin-antagonistic action, being able to stimulate glucose synthesis, while at the same time directing its disposal. Glucose production from isolated hepatocytes was increased by acute incubation with GH, using tissue from hypophysectomized rats (Blake & Clarke, 1989) or preparations from lambs treated with GH in vivo (Elcock & Pell,

1989). Glucagon-stimulated glucose production was greater in GH-treated cows than control cows (DeBoer & Kennelly, 1989*b*). Gopinath & Etherton (1989) have observed that the decrease in plasma glucose concentrations which occurs during an insulin tolerance test is suppressed and both glucose turnover and synthesis rate are increased in GH-treated pigs. McDowell *et al.* (1987*a*) have reported a decrease in glucose uptake by the hind limb of lactating cows treated with GH, in spite of increased blood flow. A role for GH in glucose counter-regulation has been suggested for man (DeFeo *et al.* 1989). These studies all confirm a role for GH in the regulation of glucose synthesis as well as its disposal, such that glucose is conserved.

MUSCLE AND CONNECTIVE TISSUE

Muscle mass may increase by one or a combination of mechanisms. Each multinucleated muscle fibre may simply increase in size or satellite cells may proliferate and daughter cells can then become incorporated into existing fibres. Goldspink & Goldberg (1975) reported that GH stimulated incorporation of [^{3}H]thymidine into DNA in skeletal muscle from hypophysectomized rats, indicating cellular proliferation. Increased fibre diameter and cellular proliferation have also been reported for adult plateaued rats induced to grow by relatively large doses of GH (Ullman & Oldfors, 1989). Ayling *et al.* (1989) found that hypophysectomy reduced the number of type 1 fibres and that this could be reversed by GH treatment. However, GH treatment of normal animals does not influence fibre-type proportions, although energy intake may independently influence fibre size and relative proportions (Soloman *et al.* 1988).

In hypophysectomized animals, muscle protein synthetic rate is decreased and this may be partly restored by GH administration in vivo (Flaim *et al.* 1978). Reeds *et al.* (1971) incubated muscles with GH and insulin in vitro and found that even though both could stimulate synthetic rate, only insulin increased amino acid uptake; they proposed that insulin might be necessary for the optimization of GH action. Exogenous GH also increases protein synthetic rate in hypopituitary Snell dwarf mice (Bates & Holder, 1988). In normal GH-treated farm animals, muscle protein turnover is increased but with the increase in synthesis rate exceeding that for degradation (Pell & Bates, 1987; Eisemann *et al.* 1989). Schwartz (1982) demonstrated a role for GH in the maintenance of synthesis in normal rat muscle by inducing a fall in synthetic rate with injections of anti-rat GH antiserum.

Several conflicting reports exist concerning the mechanism of GH-induced increases in protein synthesis. In some cases a general increase in the protein synthetic capacity has been found, indicated by a significant increase in total RNA concentrations (Pell & Bates, 1987; Bates & Holder, 1988). However, Kostyo & Rillema (1971) and Flaim *et al.* (1978) proposed that the primary effect of GH was to increase the efficiency of protein synthesis by increasing the amount of protein synthesized per unit RNA. It is likely that such differences may be due to dose and method of administration of GH and to the physiological state of the animal before treatment.

Since one of the primary functions of GH, at least in young animals, is to promote protein growth, it might be expected that GH would also induce increases in the tensile strength of connective tissue. Hydroxyproline excretion, indicative of collagen turnover, is increased in growth-retarded children treated with growth hormone and the increase is correlated with the overall growth rate (Wit & Van den Brande, 1984). GH induced an increase in collagen content and the mechanical strength in skin of GH-treated rats, and may also have increased the number of cross-links between collagen fibres or the diameter of the fibres (Jorgensen *et al.* 1989*b*). Increased collagen synthesis rate, though not content, was found in red-type, but not white-type, muscle of GH-treated lambs (Pell & Bates, 1987)

whereas increased synthetic rate and content were found in the skin of these lambs (Pell *et al.* 1987). Clearly GH has an important function in maintaining connective tissue mass.

ADIPOSE TISSUE

A major component of GH action is the inhibition of nutrient storage as lipid reserves in adipose tissue and, if necessary, net mobilization of these reserves. Thus, GH ensures nutrient availability for muscle and bone growth or for lactation. GH action may be direct, since GH receptors are present on adipocytes (Herington, 1981), but IGF-1 receptors have not been detected (Massague & Czech, 1982; DiGirolamo *et al.* 1986), other than in one recent study (Kern *et al.* 1989), making IGF-1 action equivocal. The actions of GH on adipocyte function have recently been reviewed in some detail (Vernon & Flint, 1989).

Animals treated with GH in vivo often exhibit a decreased rate of lipogenesis in adipose tissue samples incubated in vitro immediately following slaughter (Pell, 1989; Sinnett-Smith & Woolliams, 1989) and rates of lipogenesis were decreased when hypophysectomized rats were treated with GH (Goodman, 1963). However, this inhibition is not always observed (Peters, 1986; Walsh *et al.* 1990) for reasons which are not readily apparent. These findings demonstrate the complexity which may exist in adaptation to GH.

Antilipogenic actions of GH can be demonstrated in vitro but are dependent on the length of exposure to GH and are probably mediated via a change in insulin sensitivity rather than by GH itself. Thus, although long-term (24–72 h) incubation with GH induces an inhibition of insulin-stimulated lipid synthesis in cultured adipocytes, short-term (2–4 h) exposure to GH did not change, or even increased, insulin-stimulated lipid accumulation (Etherton *et al.* 1987*a*; Schwartz & Carter-Su, 1988). These actions of GH require differentiated cells and are correlated with glucose uptake (Schwartz & Carter-Su, 1988) and are also thought to be mediated by a post-receptor effect as GH does not influence insulin binding (Foster *et al.* 1988*a*). It is difficult to identify a physiological role for the short-term action of GH on lipogenesis but its long-term actions can be readily associated with its role in nutrient partitioning. The GH-induced insulin resistance ensures that nutrients are not channelled into adipose tissue but rather towards a 'target' process such as tissue growth or lactation. For example, adipose tissue fatty acid synthesis decreases in ruminants during lactation and GH:insulin ratios increase, implying that GH may have a role in mediating this adaptation (Vernon & Finlay, 1988).

Changes in rates of lipolysis in vivo in GH-treated animals have been implied by increased plasma concentrations of NEFA and glycerol (Goodman & Schwartz, 1974; Vernon, 1980). However, this response does depend on the nutritional status of the animal. If energy intake is sufficient, then NEFA concentrations are not changed by GH treatment (Peters, 1986; Pell *et al.* 1990), but when intake is restricted or animals are in negative energy balance, NEFA concentrations are elevated (Eisemann *et al.* 1986*a*; Peters, 1986). In contrast to these indirect findings obtained in vivo, incubation of adipose tissue from GH-treated animals in vitro has generally failed to demonstrate increased rates of lipolysis (Eisemann *et al.* 1986*a*; Peters, 1986; Walton *et al.* 1986; Sinnett-Smith & Woolliams, 1989; Walsh *et al.* 1990). It is possible that any lipolytic actions of GH are not direct but mediated via adipose tissue sensitivity to catecholamines. Increased responsiveness to a noradrenaline challenge has been observed in GH-treated animals, in terms of elevated NEFA concentrations (Peters, 1986; Vernon *et al.* 1987; Wray-Cahen *et al.* 1987).

It is clear from the foregoing discussion that the actions of GH on adipose tissue are not unequivocally established. Undoubtedly, GH stimulates net fat breakdown but the extent to which this is due to direct action, or to modulation of the activity of other hormones, is not yet clear. In addition, GH may inhibit lipogenesis or stimulate lipolysis, depending

on length of exposure to GH and nutritional status. In well-fed animals, when rates of lipogenesis will be high, synthesis may be the primary site of action, whereas GH action is likely to be targeted on enhancing the elevated rates of lipolysis which occur in animals in negative energy balance.

BONE AND CARTILAGE

It was initially thought that GH had no direct action on bone, based on observations that explanted cartilage fragments responded little to addition of GH to the incubation medium (Salmon & Daughaday, 1957; Isaksson *et al.* 1987) and it was postulated that some other 'factor' mediated GH action; this factor is, of course, now known to be IGF-1. However, direct actions of GH on bone and cartilage have been demonstrated recently. Local injection of GH into the proximal tibial epiphyseal plate of one leg of hypophysectomized rats induced increased bone growth in the injected side (Isaksson *et al.* 1982; Russell & Spencer, 1985). Local administration of GH, via a catheter implanted in the bony epiphysis, also induced dose-dependent bone growth (Isgaard *et al.* 1986) and arterial infusion of GH into one hind limb of hypophysectomized rats stimulated local growth (Schlechter *et al.* 1986).

Longitudinal bone growth is achieved via the production of new cells in the epiphyseal growth plate, followed by their differentiation, proliferation and maturation. The presence of both GH and IGF-1 in vitro potentiates the formation of colonies of epiphyseal chondrocytes (Lindahl *et al.* 1986) and bovine adult articular chondrocytes (Smith *et al.* 1989). GH stimulates the formation of large-size colonies and IGF-1 small-size colonies (Lindahl *et al.* 1987*a*). In addition, GH only stimulates the formation of colonies isolated from the proximal part of the growth plate, but IGF-1 is effective on cells from both the proximal and intermediate zones (Lindahl *et al.* 1987*c*). These observations indicate that GH may interact with only a limited number of cells having a high proliferative potential in the growth plate. If hypophysectomized rats are pretreated with GH in vivo, then colony formation in response to IGF-1 in vitro is enhanced; pretreatment with IGF-1 does not induce this sensitivity (Lindahl *et al.* 1987*b*). It has, therefore, been proposed that GH treatment of some cell types can render them sensitive to the mitogenic actions of IGF-1.

Clearly systemic GH has a fundamental role in the control of bone growth. Most research to date has concentrated on control systems in vitro, and little information is therefore available on the modification of GH action by nutritional status; we have no knowledge on 'priorities' of GH action on bone and connective tissue *v.* muscle anabolism in different conditions.

DIRECT ACTIONS OF GH AND THEIR REGULATION

CIRCULATING CONCENTRATIONS OF GH

GH turnover is approximately 21 min in man (Jorgensen *et al.* 1989*a*) and GH status may therefore change rapidly. Even though little is known about GH degradation, the pulsatile nature of GH synthesis has been studied extensively (for review, see Buchanan & Ross, 1990). In male rats, serum GH concentrations exhibit a predictable pattern with a peak every 3–4 h (Tannenbaum & Martin, 1976). In man and sheep, GH release is episodic but is intermittent and unpredictable (Davis *et al.* 1977; Ho *et al.* 1988). The episodic nature of GH production is important for its action. Clark *et al.* (1985) observed that GH action, in terms of weight gain and tail length growth, was greater when GH was administered in a pulsatile manner than as a continuous infusion into hypophysectomized rats. Not all

aspects of GH action are dependent on pulsatility; Maiter *et al.* (1988) observed that plasma IGF-1 concentrations are significantly greater when a given dose of GH is administered in a pulsatile manner *v.* a continuous infusion, whereas GH-induced increases in hepatic receptor binding of GH only responded to the continuous infusion. Similarly, there is some evidence for tissue specificity since mRNA concentrations for IGF-1 are greater in skeletal muscle and rib growth plate when stimulated by pulsatile rather than continuous GH, whereas no such additional increase occurred in the liver (Isgaard *et al.* 1988*a*).

Even though blood GH concentrations are difficult to quantify because of the pulsatility, several attempts have been made to correlate them with growth rates. Verde & Trenkle, (1987) reported that GH concentrations were increased in steers with high rather than low growth potentials. Arbona *et al.* (1988) could only detect increased baseline, rather than peak, GH concentrations in pigs which had been selected for high as opposed to low growth rates. In beef steers which had been fed on diets containing a range of crude protein concentrations so that final carcass composition was varied, GH concentrations were negatively correlated with carcass fat content (Anderson *et al.* 1988). Albertsson-Wikland & Rosberg (1988) have reported that height of prepubertal children was highly correlated with blood GH concentrations, measured as area above baseline levels. Therefore, circulating GH concentrations may be indicative of growth potential.

Blood GH concentrations are very sensitive to nutritional status. In rats, GH concentrations generally decrease during undernutrition (unlike other species) which makes them a poor model for GH action during fasting. Fasting induces an increase in average GH concentrations in man (Ho *et al.* 1988) and in sheep (Bassett, 1974). Concentrations decline on refeeding; this was mimicked by Tindal *et al.* (1985) by distension of the cranial sac of the rumen in goats using a water-filled balloon, implying that physical consequences of feed intake are important. Trenkle (1989) was unable to repeat this, although response of GH concentrations to a GH-releasing factor challenge was inhibited by rumen distension. GH concentrations are modified by the dietary composition as well as total intake. When cattle are fed on diets containing high energy from grain, serum GH concentrations are low. However, if the energy is supplied in association with fibre supplementation, the depression in GH concentrations is overcome (Houseknecht *et al.* 1988).

GH-BINDING PROTEINS

Proteins in human serum which could specifically bind human GH have been identified by two independent research groups (Ymer & Herington, 1985; Baumann *et al.* 1986). This implies that the physiology of circulating GH is more complex than was previously thought; however, only limited information has been accumulated to date on their role. To complicate matters further, Barnard & Waters (1988) suggested that GH-BP and membrane and cytosolic GH receptors were antigenically identical, leading to the possibility that GH-BP were a cleaved form of GH receptors. This was later confirmed (Leung *et al.* 1987; Spencer *et al.* 1988) and it is now thought that the BP forms the extracellular domain of the GH receptor, or could be produced by altered processing of a precursor of receptor mRNA (Leung *et al.* 1987).

BP decreases the interaction of [^{125}I]GH with tissue receptors (Herington *et al.* 1986) and could therefore have a role in modulating the delivery of GH to tissues. The metabolic clearance rate of GH is slower when it is associated with BP (Baumann *et al.* 1987). Also the distribution volume of GH is equivalent to that for the extracellular volume when in the free form, and to the vascular volume when complexed (Baumann *et al.* 1987); thus, GH-BP protect serum GH. In fact, two GH-BP may exist (Baumann, 1989) with high and

low affinities for GH. The high-affinity protein is far more abundant and most studies have related to this protein. Up to 50% of 22000 mol. wt hGH and 30% of the 20000 mol.wt hGH is bound at basal concentrations, and most is associated with the high-affinity BP (Baumann *et al.* 1988). At GH concentrations of above about 20 ng/ml, serum BP become saturated and the proportion of free GH therefore increases. This may be relevant for GH action because free GH concentrations will fluctuate more dramatically than will total GH. GH complexed to the high-affinity BP is fully immunoreactive with polyclonal antiserum against human GH but the lower-affinity BP does have reduced immunoreactivity (Baumann, 1989).

To date, no major nutritional studies of GH-BP have been published. In man, GH-BP activity increases with age, being very low in premature infants and neonates (Silbergeld *et al.* 1989), and appears to correlate with the ontogeny of GH receptors (Daughaday *et al.* 1987). Silbergeld *et al.* (1989) reported a significant relationship between GH-BP activity and height and weight standard deviation scores for children before puberty, suggesting a role in modulating the growth-promoting action of GH. It is known that Laron dwarfs have an impaired responsiveness to GH, having normal serum GH levels but low IGF-1 concentrations, and are thought to lack functional GH receptors; they also have little or no GH-BP activity (Baumann *et al.* 1987; Daughaday & Trivedi, 1987) which is consistent with their known structural origin. Clearly many questions remain concerning the physiological function of GH-BP in relation to GH action.

GH RECEPTORS

GH receptors are distributed widely, being found in many tissues. When radiolabelled GH is injected in vivo, the liver has the highest concentration of binding after 2 h but, when tissue mass is taken into account, skeletal muscle is quantitatively the major site of GH binding (Wallis, 1980). Two major classes of cellular binding site have been identified for GH: somatogenic and lactogenic, which are associated with growth and lactation respectively. However, there is now considerable evidence to suggest that 'somatogenic' receptors exhibit structural heterogeneity, so that this classification is an oversimplification. Thomas *et al.* (1987) and Barnard *et al.* (1985), using monoclonal antibodies against hGH and the rabbit liver GH receptor respectively, showed that different populations of receptors may occur. The recent purification, cloning and expression of GH receptors from rabbit and human liver (Leung *et al.* 1987) have demonstrated a very high degree of homology between species, 84%, which is greater than that for rabbit and human GH, implying that receptor structure has been remarkably conserved. The receptor has distinct extracellular, transmembrane and intracellular components and represents a new class of transmembrane receptors with an amino acid sequence dissimilar to that of any other known proteins except for the prolactin receptor which may function in a similar manner (Waters *et al.* 1989). It is not yet clear whether one gene is responsible for the GH receptor subtypes or not, or whether GH receptors in other tissues are unique.

GH may have a role in regulating its own receptor number which decreases in hypophysectomized animals and can be induced by chronic GH treatment in rats and sheep (Posner *et al.* 1980) and in pigs (Chung & Etherton, 1986). Gluckman & Breier (1989) have investigated oGH binding in the liver of bGH-treated lambs and identified two receptor types: high affinity and low affinity. The high-affinity site is thought to be important for GH action and it is the capacity of this site which increases in GH-treated lambs. In an attempt to identify the effects of a rapid pulse of GH, which occurs in vivo, Maiter *et al.* (1988) injected a single dose of GH into rats and reported a down-regulation, consistent with ligand-induced internalization and degradation of the receptor. Bick *et al.* (1989*a*) have

demonstrated a cycling in binding activity which is related to GH pulsatility; spontaneous surges of GH secretion were followed by an immediate decline in the number of free binding sites which were restored 2 h later. The opposite effects of chronic and acute GH administration on receptor number are difficult to reconcile unless a new steady-state of free receptors can be established after exposure to GH. Therefore, rate of receptor turnover may be an important modulator of GH action.

Hepatic GH receptor capacity is regulated by nutritional status, being decreased with fasting in rats (Baxter *et al.* 1981; Maes *et al.* 1983) and restored on refeeding. The fall in receptor capacity is correlated with serum IGF-1 concentrations (Maes *et al.* 1983). The nutritional regulation of hepatic GH receptors has been examined in steers which were maintained on high or low planes of nutrition (Breier *et al.* 1988*c*). On the low plane, presence of the high-affinity GH receptor could not be demonstrated, whereas it was apparent in the well-fed animals; weight gain was proportional to the capacity of the high-affinity binding site. When steers were injected with a single dose of bGH, on high or low planes of nutrition, only those on the high plane responded with an increase in circulating IGF-1 concentrations (Breier *et al.* 1988*b*), confirming the functional importance of the high-affinity site. As this high-affinity site is almost completely saturated at normal serum GH levels, it has been proposed that receptor number may be crucial in the regulation of GH action (Gluckman & Breier, 1989). Hepatic receptor capacity is decreased in protein–energy malnutrition and treatment of protein-restricted rats with GH-induced hepatic GH binding but did not restore serum IGF-1 concentrations, suggesting a post-receptor defect (Thissen *et al.* 1990). It is possible that insulin has a role in the regulation of the GH receptor; the loss of somatogenic sites which occurs during fasting can be reversed by insulin (Baxter *et al.* 1980) but this role for insulin has not been examined rigorously.

GH RECEPTOR PROCESSING AND CELL SIGNALLING

Few studies have been performed on the fate of GH after receptor binding, and the role of nutrition on receptor processing has not been investigated; current knowledge has been reviewed by Roupas & Herington (1989). GH receptor turnover appears to depend on occupancy, its half-life being decreased when GH is bound. The exact mechanism of internalization is unknown but it is assumed that this is via cell surface aggregation into coated pits which then internalize, as has been shown for other peptide hormones. Once internalized, most GH–receptor complex remains bound and passes through two endocytic compartments before incorporation into lysosomes and degradation (Husman *et al.* 1988). Bick *et al.* (1989*b*) have demonstrated that binding of radiolabelled GH to Golgi membranes is correlated with GH pulsatility. A small proportion of the receptor-bound GH is recycled to the cell membrane, GH being released from the cell.

As yet, no unequivocal second messenger has been identified for GH, although several have been suggested. Using 3T3-F422A cells, which undergo GH-stimulated differentiation from fibroblasts to adipocyts, Foster *et al.* (1988*b*) demonstrated that the binding of GH to its receptor stimulates phosphorylation of a tyrosine residue. No evidence was found, though, for a conventional tyrosine kinase (protein-tyrosine kinase; *EC* 2.7.1.112) receptor in the amino acid sequence of the hepatic GH receptor (Leung *et al.* 1987) but there is some evidence that GH may activate phospholipase C (*EC* 3.1.4.3). In Ob1771 cells, GH-stimulated diacylglycerol formation was consistent with activation of phospholipase C, but no effect on inositol phosphate production was detected. In addition, regulation of IGF-1 gene expression appeared to be unrelated to this mechanism (Doglio *et al.* 1989). Rogers & Hammerman (1989) suggested that GH activates protein kinase C (*EC* 2.7.1.37), using canine renal tubule membranes, but also detected production of

diacylglycerol and inositol triphosphate. Clearly, a unified mechanism for GH cell signalling is not yet apparent. Even though these investigations have used tissue from different sources it seems unlikely that a wide range of signal transduction mechanism would exist.

INDIRECT ACTIONS OF GH: ROLE OF IGF-1

CO-ORDINATION OF GH AND IGF-1 ACTION

It was proposed (Daughaday *et al.* 1972) that the anabolic actions of GH were mediated via circulating IGF-1 produced in the liver in response to GH and transported in blood to its site of action as an endocrine hormone. This indirect action of GH has been challenged: Green *et al.* (1985) proposed that both GH and IGF-1 action were required at target tissues for complex GH-dependent processes such as growth. Since then, much evidence has accumulated to confirm this, most of which has been focused on the control of bone growth (see p. 173). According to Isaksson *et al.* (1987) GH can directly stimulate differentiation of prechondrocytes or young differentiating cells. During this process, cells become responsive to IGF-1 and, at the same time, the gene encoding IGF-1 is expressed. The newly-synthesized IGF-1 is then released from the cells and stimulates them, or adjacent cells, to proliferate, acting in an autocrine or paracrine manner. Thus, GH has stimulated differentiation and IGF-1 production which has then induced clonal expansion. Evidence for direct action of GH has been reported by Nilsson *et al.* (1989) who demonstrated the presence of GH receptors in chondrocytes. GH can also stimulate mRNA for IGF-1 in bone growth plate (Isgaard *et al.* 1988*b*), skeletal muscle (Turner *et al.* 1988) and cultured hepatocytes (Norstedt & Möller, 1987). Co-operation between GH and IGF-1 has been proposed for anabolic actions in many cell types, for example, in cultured human fibroblasts (Cook *et al.* 1988), although direct actions of GH on cultured growth plate and circulating chondrocytes have now been challenged by Trippel *et al.* (1989) who claim that IGF-1 itself stimulated differentiation. This is supported by Allen & Boxhorn (1989) and Tollefsen *et al.* (1989) for differentiating cultured muscle cells.

The importance of locally produced IGF-1 has been emphasized by several studies. Skottner *et al.* (1987) were able to demonstrate acute insulin-like actions of exogenous IGF-1 on serum glucose concentrations in hypophysectomized rats, but were able to demonstrate only small increases in bone growth. They concluded that locally produced, rather than systemic, IGF-1 must be important. IGF-1 mRNA is present in many tissues, although it is 50-fold more abundant in the liver than elsewhere (Murphy *et al.* 1987). Orlowski & Chernausek (1988) reported that serum IGF-1 concentrations changed little in GH-stimulated growth in hypophysectomized rats, whereas tissue IGF-1 concentrations increased two- to three-fold.

Since locally produced IGF-1 appears to be important, the relevance of circulating IGF-1 must be considered. It is possible that this merely represents 'waste' IGF-1 which has been produced by various tissues; this would still be compatible with the correlations of growth rate and serum IGF-1 concentrations which are often observed. However, the liver does synthesize and release large amounts of IGF-1 into the circulation and it is difficult to imagine that this has no physiological significance. It is likely that both systemic and locally produced IGF-1 have a role in mediating GH action.

PHYSIOLOGICAL EFFECTS OF IGF-1

IGF-1 is structurally related to insulin and both are derived from a common evolutionary precursor. However, IGF-1 has retained the C-peptide region, found in proinsulin, and also

has an elongated A chain. IGF-1 exist in several forms in vivo—either free or bound to various specific proteins (see p. 180). Initial studies investigating IGF-1 action involved bolus intravenous injections which induced rapid hypoglycaemia in rats and man (Zapf *et al.* 1986; Guler *et al.* 1987). These insulin-like responses were probably mediated via the insulin receptor and probably represent an unlikely physiological role, rarely occurring in vivo.

As discussed earlier, IGF-1 functions independently and in conjunction with GH to stimulate tissue growth and differentiation. Serum IGF-1 concentrations have been correlated with size in different breeds of poodles (Eigenmann *et al.* 1984), pygmies *v.* normal man (Merimee *et al.* 1981) and in mice selected and bred for high and low serum IGF-1 concentrations for seven generations (Blair *et al.* 1988). In all these studies serum GH concentrations were normal, emphasizing the importance of IGF-1 in the control of growth. In further support of this, Mathews *et al.* (1988*b*) have reported that elevated mRNA levels for IGF-1, and serum IGF-1 concentrations, precede the increase in growth rate which occurs in transgenic mice expressing the GH gene.

The growth-promoting action of IGF-1 has been investigated in hypopituitary animal models. Schoenle *et al.* (1982) reported that purified IGF-1 increased body-weight gain, bone length growth and [^{3}H]thymidine uptake into the costal cartilage of hypophysectomized rats. IGF-1 also stimulated weight gain and bone growth in Snell dwarf mice (Van Buul-Offers *et al.* 1986) and bone growth but with no change in muscle mass in hypophysectomized rats (Guler *et al.* 1988). Considerable increases in kidney and spleen, and a decrease in epididymal fat pad weights were also obtained in these rats. In contrast, Mathews *et al.* (1988*a*) obtained an increased weight gain, due to organomegaly and increased muscle mass, but could not demonstrate significant increases in bone growth in transgenic mice expressing the IGF-1 gene. At present, these differences in responsiveness to IGF-1 cannot be explained, although it should be noted that IGF-1-BP distribution is probably different in hypopituitary *v.* transgenic mice.

On a quantitative basis, all studies comparing the growth-promoting properties of GH and IGF-1 have found that GH is more potent. This could be due to a requirement for the presence of GH for optimal IGF-1 action. Guler *et al.* (1988) investigated the dependence of IGF-1 and GH for growth promotion by treating hypophysectomized rats for a prolonged period of time; they reasoned that if IGF-1 action required co-operation with GH, this would become apparent as a diminished response to IGF-1 with time. Linear growth rates, as body-weight gain, were obtained over an 18 d period, similar to those for GH-treated rats. Skottner *et al.* (1987) investigated whether priming of hypophysectomized rats with GH would enhance responsiveness to IGF-1; bone growth was not significantly increased by the addition of IGF-1 to an infusate of GH, even though IGF-1 alone could stimulate bone growth. They suggest that GH and IGF-1 act independently in some tissues. The interaction of GH and IGF-1 has been studied further in Snell dwarf mice (Pell & Bates, 1989) which were treated with IGF-1 alone or in combination with very low or moderate doses of bGH. Both GH and IGF-1 increased whole-body-weight gain and in skeletal muscle both stimulated protein synthesis rates; these effects were additive. In liver, only GH increased rates of protein synthesis, IGF-1 having, if anything, a negative effect (Fig. 3). These findings indicate that, even though GH may act via IGF-1 in muscle, it certainly acts directly and by a different mechanism in the liver. This focuses attention on the receptor populations for GH and IGF-1 in different tissues.

All findings discussed so far have been concerned with the anabolic actions of IGF-1 in IGF-deficient models. Conflicting results have been obtained in intact animals. Hizuka *et al.* (1986) reported a stimulation of weight gain and bone growth in intact rats treated with IGF-1 for 7 d, although the growth stimulation was modest (approximately 10%).

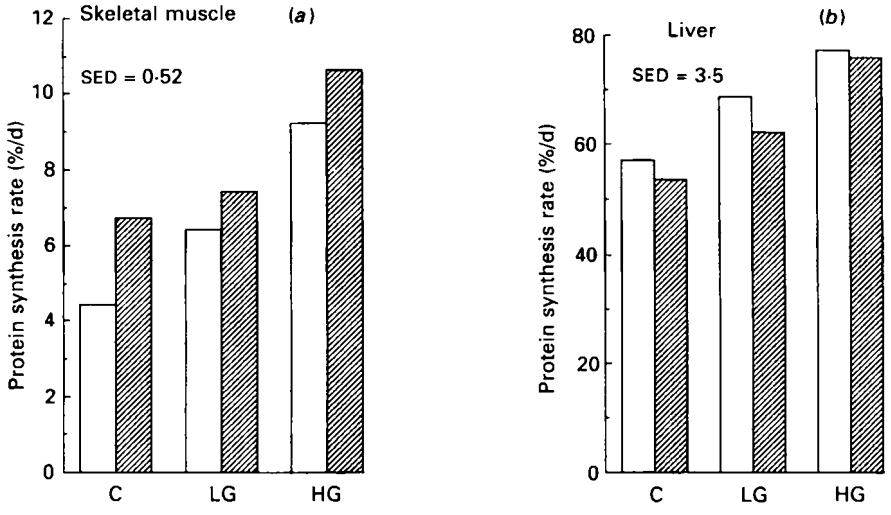

Fig. 3. Rates of protein synthesis in (*a*) skeletal muscle (gastrocnemius plus plantaris) and (*b*) liver from dwarf mice treated with saline (9 g sodium chloride/l) (□) or insulin-like growth factor-1 (IGF-1; 20 μg/d) (▨) in conjunction with varying doses of bovine growth hormone (bGH) (C, saline only; LG, 1·5 μg/d; HG, 20 μg/d). Both GH and IGF-1 stimulated muscle protein synthesis ($P < 0·001$) but only GH increased hepatic protein synthesis ($P < 0·001$). SED, standard error of difference.

Zapf *et al.* (1989*a*) treated mini-poodles with IGF-1 for 130 d; no convincing evidence for growth stimulation was obtained. However, GH responses to GRF were decreased in IGF-1 treated dogs and IGF-1 might therefore exert a considerable negative feedback on endogenous hormone secretion, confounding its anabolic effects.

The role of IGF-1 in mammary development and lactation, and its function in mediating the galactopoietic actions of GH, are equivocal. IGF-1 is present in higher concentrations in mammary secretions prepartum, and decline during the first 5 weeks after birth in cows (Collier *et al.* 1989). Specific receptors for IGF-1 have been found in bovine (Collier *et al.* 1989) and ovine (Winder & Forsyth, 1987) mammary tissue and IGF-1 stimulates mammary epithelial cell proliferation at physiological concentrations (Winder *et al.* 1989). In untreated cows, IGF-1 is present in mammary tissue and is associated with stromal elements; concentrations increase in GH-treated goats (Prosser *et al.* 1989) and cows, where it is then also distributed in epithelial cells (Glimm *et al.* 1988). The origin of this IGF-1 is not yet clear; certainly some is derived from blood but local secretion is also possible, although it is unlikely that this is stimulated directly by GH. There are two reports on the effects of IGF-1 administration on milk yield. Prosser *et al.* (1988) infused IGF-1 directly into the mammary gland of the goat via close-arterial infusion and reported modest increases in milk yield. However, Davis *et al.* (1989) could not demonstrate a galactopoietic action of IGF-1 whether given as a daily subcutaneous injection or via local infusion. Thus the importance of IGF-1 in mediating GH action on the mammary gland is uncertain. Most indirect evidence to date would imply that it is more important for mammary growth than for secretion.

Diabetic rats exhibit decreased serum concentrations of GH and poor rates of growth, possibly related to the resultant decrease in circulating IGF-1 concentrations. Scheiwiller *et al.* (1986) treated diabetic rats with GH or IGF-1. GH was ineffective in restoring either growth or IGF-1 concentrations whereas IGF-1 increased growth without alleviating the other symptoms of diabetes, such as hyperglycaemia. They suggested a new model for the control of growth in which IGF-1 was the most important stimulator of cellular growth,

and GH and insulin merely modulated IGF-1 synthesis. This theory has been disputed by Carlsson *et al.* (1989) who suggest that diabetes results in intracellular starvation leading to a loss of pulsatile GH secretion. Treatment with insulin or IGF-1 restores cellular nutrient uptake which leads to the restoration of GH secretion. In either case, interpretation of findings from diabetic rats must be considered with caution since GH concentrations increase during diabetes in other species, such as man (Hayford *et al.* 1980).

IGF-BINDING PROTEINS

The IGF are unusual because, to date, they are the only growth factors which are complexed to BP in blood and extracellular fluids. In blood, virtually no IGF-1 exists in the free form. Current knowledge on the structure of, and interrelationships between, the various BPs has been reviewed by several authors recently (Baxter, 1988; Ooi & Herington, 1988; Baxter & Martin, 1989; Clemmons, 1989; Povoa *et al.* 1989). The most abundant BP in serum has a molecular weight of about 150000, binds about 80% of serum IGF-1 and is fully saturated in normal conditions (Povoa *et al.* 1989). It is synthesized as two subunits (acid-labile and acid-stable) and, like IGF-1, is predominantly of hepatic origin. The acid-stable subunit has a molecular weight of about 50000, is responsible for IGF-1 binding and is known as IGFBP-3. The two subunits only aggregate in serum in the presence of IGF-1 (Baxter, 1988). Most of the remaining IGF-1 in blood is bound to a smaller protein, IGFBP-1, which has a molecular weight ranging from 25000–30000; it is abundant in human amniotic fluid (Povoa *et al.* 1989) and is synthesized by the liver and many other cell types such as fibroblasts or vascular endothelial cells.

There are several theories regarding the functions of the binding proteins. IGF-1 is synthesized by many tissues of the body but is released immediately; therefore, the pool of protein-bound serum IGF-1 could act as a storage depot, since the BP certainly prolong the half-life of IGF-1 (Zapf *et al.* 1986; Hodgkinson *et al.* 1987). Blum *et al.* (1989) have shown that IGF-1 which had been precomplexed to IGFBP-3 was a better mitogen than was free IGF-1 for fibroblasts. They suggested that the BP was acting as a reservoir, continuously releasing small amounts of IGF-1 and maintaining receptor occupancy.

The insulin-like actions of IGF-1 might be prevented by BP since, even though IGF-1 has a lower potency than insulin, its circulating pool is so much greater that it could have 50-fold more insulin-like activity than insulin itself (Baxter, 1988). Walton & Etherton (1989) also demonstrated that IGFBP-3 can inhibit the insulin-like actions of IGF-1 in porcine adipose tissue. Partly purified preparations of BP have also been reported to inhibit IGF-1 activity itself (Drop *et al.* 1979; Ooi & Herington, 1986). This inhibition is supported by the relative potencies of IGF-1 and a naturally occurring truncated form of IGF-1, des-(1-3)-IGF-1 which lacks the N-terminal tripeptide and has much lower affinity for the BP. This is more active than IGF-1 itself in several conditions in vitro (Cascieri *et al.* 1988; Carlsson-Skwirut *et al.* 1989; Ross *et al.* 1989) and it was concluded that BP limit availability of serum IGF-1 for receptor binding. The physiological relevance of the truncated form of IGF-1 is not clear at present but it appears to be a way of expressing potent IGF-1 activity without BP interference. Mohan *et al.* (1989) have also suggested an inhibitory role for a novel 25000 mol.wt BP, isolated from human bone cells, which prevented bone cell proliferation and presents the possibility of tissue specific regulation of IGF-1 activity.

Alternatively, it has been reported that some BP may specifically enhance IGF-1 action. Elgin *et al.* (1987) purified IGFBP-1 from amniotic fluid; when complexed with IGF-1, it potentiated the replication of several cell types compared with IGF-1 alone. IGFBP-1 has an Arg-Gly-Asp sequence near the carboxy terminus which is important for cell attachment of matrix proteins (Brewer *et al.* 1988). This BP could therefore have a role in the

association of IGF-1 with the cell surface; the potentiating effects observed by Elgin *et al.* (1987) are not apparent if the BP does not associate with the cell surface or matrix (Busby *et al.* 1988*a*). Therefore, IGFBP-1 may be associated with paracrine functions of IGF-1, transporting it from one cell type to another in a tissue. Evidence for this has originated from the cellular localization of IGF-1 and IGF-1 mRNA (Clemmons, 1989). It is also possible that IGFBP-1 and the 150000 mol.wt BP may co-operate to transport IGF-1 from blood to target cells, since IGFBP-1 is present in extracellular fluid in higher concentrations whereas IGFBP-3 is the predominant BP in blood.

Both IGFBP-1 and IGFBP-3 are thought to be under hormonal control. IGFBP-3 is, like IGF-1, largely GH-dependent (Scott *et al.* 1985; Walton & Etherton, 1989). IGFBP-1 concentrations increase during GH deficiency, possibly to compensate for the lack of IGFBP-3, and decrease with GH treatment (Hintz *et al.* 1981). Recently Zapf *et al.* (1989*b*) have suggested that IGF-1 itself can induce the production of IGFBP-3 but Walton & Etherton (1989) could not demonstrate this in pigs. IGFBP-1 exhibits a marked diurnal rhythm, having elevated concentrations at night and low concentrations in the day (Baxter & Cowell, 1987; Busby *et al.* 1988*b*; Holly *et al.* 1988). This diurnal variation is due to nutritional status, IGFBP-1 concentrations increasing during fasting and declining on refeeding. Insulin may be responsible for the decrease in BP concentration (Busby *et al.* 1988*b*; Holly *et al.* 1988; Suikkari *et al.* 1989) since the response is observed when euglycaemic insulinaemia is induced. However, Cotterill *et al.* (1987) have suggested that blood glucose, and not insulin, is the primary inhibitor. IGFBP-1 could moderate IGF-1 activity to supplement insulin activity, the decrease in BP concentrations after a meal allowing IGF-1 to exert its insulin-like effects.

IGF RECEPTORS

The amino acid sequences for receptors for IGF-1 and insulin exhibit many similarities, as do the sequences for insulin and IGF-1 themselves. However, each receptor stimulates different processes when bound to ligand; insulin receptors modulate metabolic processes such as glucose uptake and oxidation whereas IGF-1 receptors stimulate cellular proliferation and differentiation. IGF-1 will, in fact, bind to three receptor types: the insulin receptor and the type 1 and type 2 growth factor receptors (Rosenfeld, 1989). The type 1 growth factor receptor binds IGF-1 more readily than does the type 2 receptor, the latter being the predominant IGF-2 binding site.

Few investigations have been carried out on the control of type 1 receptor function in relation to physiological or nutritional state. It is interesting that IGF-1 receptors are not present in adult rat or human hepatocytes but are induced during the liver regeneration which occurs following subtotal hepatectomy (Caro *et al.* 1988). This may explain why liver protein synthesis is unresponsive to exogenous IGF-1 (Pell & Bates, 1989). The competence of the IGF-1 receptors was investigated in insulin-resistant patients: muscle receptor binding activity was similar to that of normal subjects, even though capacity of the insulin receptor was decreased (Livingston *et al.* 1988) and the type 1 receptor does not therefore appear to be involved in non-insulin-dependent (type 2) diabetes. Following IGF-1 binding, type 1 receptors are down-regulated and internalized. The endocytosed hormone–receptor complex is degraded, at least in part, in lysosomes (Furlanetto, 1988). Therefore, the type 1 receptor behaves in a similar manner to insulin and other polypeptide hormone receptors. Binding of ligand to the type 1 receptor also induces phosphorylation of tyrosine, and activation of receptor kinases (Czech, 1989) although the role of this phosphorylation and further cell signalling in the biological activity of IGF-1 is not fully understood at present.

NUTRITION AND IGF-1

The importance of IGF-1 in different nutritional states has been investigated mainly with respect to its serum concentrations. Shapiro & Pimstone (1977) first reported that GH could not stimulate growth in malnourished rats. This apparent GH resistance has been investigated using GH challenges. The increase in serum IGF-1 concentrations which occurs when well-nourished animals are injected with GH was abolished or attenuated in man (Merimee *et al.* 1982), heifers (Elasasser *et al.* 1989) and cattle (Breier *et al.* 1988*b*). Many studies have shown that serum IGF-1 concentrations decrease in fasted animals, for example, Maes *et al.* (1983). This is due, at least in part, to a decrease in hepatic concentration of mRNA for IGF-1 which is restored on refeeding (Emler & Schalch, 1987). IGF-1 concentrations are also diminished in protein–energy malnutrition and correlate with the degree of weight deficiency (Soliman *et al.* 1986). The decrease in circulating IGF-1 is thought to be due partly to a loss of hepatic GH receptors in fasted rats but not in rats fed on a low-protein diet, implying that a post-receptor defect may be important (Thissen *et al.* 1990). Since insulin concentrations also decrease in malnutrition, Maiter *et al.* (1989) have investigated the role of insulin in protein–energy malnutrition, using insulin-maintained diabetic rats on high- and low-protein diets. In protein-restricted diabetic rats which had been treated with insulin, circulating IGF-1 concentrations and weight gain were still suppressed. The authors concluded that the effects of protein restriction were independent of those for insulin.

Blood IGF-1 concentrations are very responsive, not only to the level of nutrition but also to the composition of the diet. Prewitt *et al.* (1982) fed rats on diets containing three levels of protein and at three energy levels: serum IGF-1 concentrations were correlated with protein and energy content, protein intake appearing to be more important. This has been confirmed in cattle (Elsasser *et al.* 1989) and in sheep (Pell *et al.* 1989) although, of course, the range of protein and energy intakes must influence the apparent responsiveness of IGF-1 concentration. Yahya *et al.* (1987) have reported the influence of dietary protein and energy on plasma levels of IGF-1, insulin and T3 in rats fed on either graded protein-deficient diets, restricted-energy but isonitrogenous diets and in rats treated with corticosterone. Plasma levels of IGF-1 were shown to be under the separate influence of dietary protein and energy intakes, plasma insulin and corticosterone concentrations. In calves, weaning onto a primarily concentrate diet induces a decrease in IGF-1 concentration which may be mediated by the decrease which also occurs in insulin concentration (Breier *et al.* 1988*a*). It is possible that these decreases in hormonal concentration are due to a degree of undernutrition which can occur on weaning when the animals must change to a ruminant digestion.

In order to further define the role of IGF-1 in undernutrition, O'Sullivan *et al.* (1989) and Jacob *et al.* (1989) have administered IGF-1 to fasting mice and rats respectively. In the former study, IGF-1 inhibited weight loss after starvation had continued for more than 28 h, indicating that IGF-1 can be anabolic in severe catabolic states. In agreement with this sparing role for IGF-1, Jacob *et al.* (1989) reported a decreased leucine turnover in IGF-1-treated fasted rats and concluded that IGF-1 had suppressed whole-body protein degradation. Based on this information, it is possible to formulate a function for blood IGF-1 during undernutrition. It is important that available nutrients are only utilized for essential processes during dietary restriction. Since IGF-1 is primarily a growth-promoting anabolic hormone, it is logical that it should be depressed in these conditions. This will allow the catabolism of non-essential tissues, such as muscle and adipose tissue, for the preservation of vital functions. In support of this, IGF-1 concentrations increase during overfeeding when muscle and adipose tissue have accumulated (Forbes *et al.* 1989).

The relationships between circulating and tissue IGF-1 concentrations and rates of proteoglycan synthesis in muscle and bone have been investigated by Yahya *et al.* (1990). Plasma IGF-1 concentrations decreased considerably in rats fed on low-protein diets and this was poorly related to mean tissue (skeletal muscle and bone) concentrations, implying that the endocrine role for IGF-1 in undernutrition has been overemphasized. However, mean tissue IGF-1 concentrations were correlated with proteoglycan synthesis in skeletal muscle but not in bone. The relative insensitivity of bone IGF-1 concentrations to nutritional status was also found for low-energy diets and with corticosterone treatment (Yahya *et al.* 1989). This raises the possibility that either IGF-1 is not important in the regulation of bone growth in conditions of poor nutrition (IGF-1 resistance) or that some local modulator of IGF-1 action exists, such as the inhibitory BP, for example, described by Mohan *et al.* (1989). It cannot be ruled out, though, that changes in IGF-1 concentrations in the tibial epiphysis may be confined to very discrete areas and could not therefore be detected using mean tissue concentrations.

Many investigations have reported that IGF-1 and insulin concentrations are well correlated in both well-fed, undernourished and GH-treated animals (e.g. Pell *et al.* 1990; Yahya *et al.* 1987). It is likely that these two essentially anabolic hormones cooperate together to co-ordinate metabolism and growth in different nutritional states. It has also been suggested that such hormonal communication may be necessary to achieve an anabolic response to GH (Pell *et al.* 1990) and this concept of an 'anabolic drive' has been discussed by Millward (1990); on the basis of findings in rats it is suggested that the role of IGF-1 and GH in growth regulation may be to mediate the homeorhetic regulation of growth in response to a sustained nutrient input. The major direct role of insulin in this scheme is suggested as mediating homeostatic regulation by controlling the reversible deposition of protein within the myofibre, since insulin does not play a direct role in the regulation of either protein or proteoglycan synthesis in bone (Bates *et al.* 1987). Whether insulin is involved in regulating the levels of the inhibitory IGF-1-BP in bone described by Mohan *et al.* (1989), as has been suggested to occur for the IGFBP-1 (see p. 180), has yet to be determined.

FUTURE PERSPECTIVES

Even though research on GH and IGF-1 has expanded rapidly over the past few years, this has been biased towards whole-body and organ responses; knowledge at the cellular level is limited, particularly in terms of nutritional modulation. Altered nutritional status does not merely change nutrient availability but, of course, induces complex adaptations in the hormonal and metabolic balance of an animal. It is only when each of these has been investigated in terms of fundamental processes such as receptor population and cell signal transduction that an integrated assessment of GH action can be made in relation to nutritional control.

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