STUDIES ON THE REGULATION OF GROWTH HORMONE SECRETION IN MAN

by

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ABSTRACT

The availability of recombinant peptides, involved in linear growth, has invigorated the investigation of the neuroregulation of growth. The potentially unlimited quantities of GH available has led to an expansion in criteria for therapy to encompass children with growth failure from causes other than classical growth hormone deficiency, adults with growth hormone deficiency, and the elderly. Synthetic GHRH is a potent tool in the investigation of the growth axis and an alternative to GH therapy. Further understanding of the dynamics of growth should result from the recent availability of recombinant IGF-I for therapy of diseases associated with growth hormone resistance, and for the investigation of growth physiology. The studies described in this thesis take advantage of these advances to further understanding of the neuroregulation of GH secretion in man.

In a cohort of healthy male volunteers, a single subcutaneous injection of 40 μg/kg of IGF-I was not associated with side-effects, in particular hypoglycaemia was not a problem. Frequent venous sampling over 24 hours revealed a fall in plasma TSH, but secretion of the other pituitary hormones was unperturbed. IGF-I did not alter the pulsatile nature and quantity of GH secretion over 24 hours, but did result in potentiation of the GH response to GHRH.

The regulation of linear growth is dependent on many factors. An experimental paradigm of pharmacological tests and overnight sampling compared GH dynamics in groups of tall and short young men. Insulin-induced hypoglycaemia and GHRH were unable to differentiate between the two groups. Overnight profiles of spontaneous GH secretion suggested mean pulse amplitude to be greater in taller individuals although the difference did not reach significance.

Plasma growth hormone levels correlate with plasma oestrogen levels and are therefore greater in young women than men. In a group of healthy young men and women, no difference existed in either the GH responses to GHRH in a group of young men and women, or in the women at different stages of the menstrual cycle, indicative of a hypothalamic site of action for oestrogens.

Many characteristics of the adult GH deficiency syndrome are associated with "normal" aging. Spontaneous GH secretion decreases with age as did the response to GHRH in the subjects studied here. Modulation of cholinergic-regulated somatostatin secretion, with the anticholinesterase, pyridostigmine, augmented the GH response in all ages. However, the influence of pyridostigmine also diminished with age.

Elevated circulating glucocorticoids are associated with impaired GH secretion and growth failure in children. Pretreatment with a potent synthetic glucocorticoid, dexamethasone, virtually obliterated the GH response to GHRH. However, pyridostigmine significantly increased the GH response, indicative that increased somatostatin secretion is responsible for the acute glucocorticoid-induced diminished GH secretion.

GHRH therapy is of proven efficacy in the treatment of short stature and offers a theoretical advantage over GH of restoring pulsatile GH secretion. However, the short half-life of GHRH and the necessity of, at least, twice daily injection, limit its use. DC-21-346 was designed as a superpotent, degradation-resistant GHRH analogue, and in rats was fifty times as potent as conventional GHRH. Unfortunately, in normal volunteers DC-21-346 was only equipotent with conventional GHRH and therefore has no therapeutic future.

It is anticipated that these findings will be the foundation of further studies of the neuroregulation of GH secretion and ultimately improved management of disorders of the growth axis.

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

AIMS

The research presented in this thesis was designed to explore aspects of the neuroregulation of growth hormone secretion in man and the relationship between quantitative growth hormone secretion and adult height.

Insulin-like growth factor-I is a potent metabolic regulator and mediator of many functions ascribed to GH. Plasma IGF-I is principally hepatic in origin and predominantly regulated by GH. The availability of recombinant IGF-I offers new opportunities for therapy of disorders associated with GH resistance and investigation of IGF-I's physiological role. *In vivo* and *vitro* studies indicate IGF-I can autoregulate its plasma levels via feedback on GH secretion. A strategy of frequent sampling of the anterior pituitary hormones over 24 hours, and measurement of GH after GHRH stimulation, enabled the consequences of IGF-I for pituitary function to be delineated.

Normal linear growth is dependent on a complex genetic and environmental milieu, which can be disturbed by a multiplicity of factors such as psychological stress, incidental illness, malnutrition, and gonadal or thyroidal dysfunction. Growth hormone commands a pivotal role in the stimulation of linear growth, although its secretion and efficacy are sensitive to influence by many factors. The dynamics of GH secretion and its relationship to growth velocity have been extensively studied in children, predominantly those with abnormal growth. Investigation of growth in truly normal children is limited by ethical considerations and the cross-sectional nature of studies in normal children. By studying young adults and

defining the population by a solid endpoint, namely final height, it was possible to circumvent the problems inherent in investigating children. The experimental design combined measurement of spontaneous and pharmacologically-stimulated GH secretion in groups of tall and short normal individuals with the intention of defining the relationship of quantitative GH secretion to final height.

A positive correlation exists between plasma growth hormone and oestrogen levels and hence GH levels are higher in young women than men. However, controversy persists as to the influence of oestrogens and gender on the GH response to GHRH; it is variously reported that the response is either higher or lower or the same in women compared to men. Further topics of interest are the age-related decline which occurs in GH secretion and the inhibition by glucocorticoids of GH secretion. The nature and aetiology of the influence of gender, age and glucocorticoids on GH secretion were examined by an amalgamation of intravenous boluses of $GHRH_{(1-29)}NH_2$, in combination with modulation of the cholinergic nervous system using the anti-cholinesterase pyridostigmine.

GHRH treatment is a proven alternative to GH therapy for growth failure, with the theoretical attraction of producing a more physiological pattern of plasma GH levels than recombinant GH. Currently, the necessity of at least twice daily injection limits the use of GHRH therapy. DC-21-346 was synthesised as a superpotent, degradation-resistant analogue of $GHRH_{(1-29)}NH_2$. It is fifty times as potent at stimulating GH secretion from rat somatotrophs as conventional GHRH. The initial human studies of this peptide are included here.

CLAIM TO ORIGINALITY

All studies described in this thesis were conceived and supervised by the author. In particular, all protocols and submissions to the Ethical Committee were designed by the author, who also typed the thesis and created the artwork. All clinical trials were personally supervised. The data were collected and processed by the author who performed the statistical analysis, except in the study of IGF-I on anterior pituitary function.

The author has experience in performing the GH assay. Research nurses assisted in the collection of blood at St. Bartholomew's Hospital, and endocrine research fellows in Sardinia and Genoa.

The studies reported here are original for the following reasons. This is the first study of the effect in man, of IGF-I, by the therapeutic route, on anterior pituitary function. The study found TSH secretion to be inhibited and the GH response to GHRH to be potentiated by IGF-I. The investigation of a relationship between adult height and quantitative GH secretion is novel. The thesis addresses aspects not previously studied of the influence of age, gender and glucocorticoids on GH secretion. This thesis includes the first use in man of the new GHRH analogue DC-21-346.

ETHICAL CONSIDERATIONS

A full protocol for all studies was approved by the Ethical Committee of St Bartholomew's Hospital prior to the start of any study. The Ethical Committee is composed of 25 members and includes representatives from the University, medical staff, nursing staff, community health council, legal profession and the lay public. The committee takes a special interest in informed consent, and also takes advice from a Professor of Toxicology when studies concern the use of unlicensed drugs.

Volunteers gave fully informed written consent. This was witnessed by an adult not directly involved in the research.

Volunteers were paid an honorarium for participating in the studies which was between £25 and £200 per study.

ABBREVIATIONS

ACTH, adrenocorticotrophin

AUC, area under the curve

CV, coefficient of variation

FSH, follicle stimulating hormone

GH, growth hormone

GHBP, growth hormone-binding protein

GHD, growth hormone deficiency

GHRH, growth hormone-releasing hormone

IC-GH, integrated concentration of growth hormone

IGF-I, insulin-like growth factor-one

IGF-II, insulin-like growth factor-two

IRMA, immunoradiometric assay

ITT, insulin tolerance test

IV, intravenous

LH, luteinising hormone

NR, normal range

RIA, radioimmunoassay

SC, subcutaneous

SDS, standard deviation score

SEM, standard error of mean

TSH, thyrotrophin

ACKNOWLEDGEMENTS

I am grateful to the Joint Research Board of St. Bartholomew's Hospital for enabling me to return to St Bartholomew's to undertake this work. These studies would not have been possible without the generous provision of GHRH by Serono, and of GHRH and IGF-I by Pharmacia. Professor David Coy provided the GHRH analogue, DC-21-346, and collaborated in the design of the clinical study.

I am indebted to Professors Delitala (Sardinia) and Giusti (Genoa) for their encouragement and for making the resources of their Departments available to me, as well as generous hospitality and continuing friendship. I was ably assisted in sampling by Mario Palermo and Paolo Tomasi. The patronage of the British Council and Wellcome Foundation supported my trips to Italy and is gladly acknowledged.

The department of Clinical Endocrinology at St. Bartholomew's is blessed by superb support from the laboratories of the departments of Chemical Endocrinology and Reproductive Physiology and of the North East Thames Radioimmunoassay unit (NETRIA). I was fortunate to obtain excellent tutoring and guidance while performing assays, and in the handling of over 11,000 samples which were generated by the research undertaken for this project. All of the staff were welcoming and cooperative but special thanks are due to Doctors Sami Medbak (Chemical Endocrinology), Les Perry (Reproductive Endocrinology) and Ray Edwards (NETRIA).

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The successful outcome to these studies is in large measure due to the meticulous assistance of the research nurses in the department of Clinical Endocrinology. Without the willing support and good-natured encouragement offered by our nurses, it would not have been possible to carry out 24-hour sampling profiles. The Department is an inspiring and stimulating environment in which to practise endocrinology. I am grateful to my many colleges for their time, genuine interest and constructive comments, in particular to Jeremy Kirk and Ashley Grossman.

Most of all, I am indebted to Professor G. M. Besser for the opportunity to pursue my research in his Department, and to benefit from his personal guidance and constant encouragement. His prodigious knowledge of endocrinology which has been freely placed at my disposal, together with his boundless energy and enthusiasm for the subject, have been of incalculable value to my own work in this field.

I must also thank my long-suffering wife and children who have so often accepted my recluse existence with cheerful forbearance at times when families prefer to be together; and of course Frances has never wanted for ideas to fill each moment of the day.

INTRODUCTION

The discovery of growth hormone-releasing hormone (GHRH) in 1982 provided a powerful tool for investigating the regulation of growth hormone (GH) secretion and the treatment of short stature. In the intervening decade, synthetic GHRH has been used successfully to treat both children with GH- and non-GH deficient short stature (Thorner *et al.* 1988; Brain *et al.* 1990; Duck *et al.* 1992; Ross *et al.* 1987c; Kirk *et al.* 1994). The studies described in this thesis have used synthetic GHRH to investigate the relationship between quantitative GH secretion and final height, and aspects of the regulation of GH secretion.

GROWTH HORMONE-RELEASING HORMONE

Pituitary comes from the Latin "pituita" meaning mucus. For centuries it was thought that the pituitary was responsible for the regulation of the secretion of phlegm. Our modern understanding of pituitary function started with the speculation, more than three hundred years ago, by Richard Lower (1631-1691) that substances passed from the brain to the pituitary and were then distilled into the blood. In 1886, the French neurologist Pierre Marie (1853-1940), in his original description of acromegaly, noted the enlargement of the pituitary. However, Marie failed to appreciate the significance of his observation, believing it to be a secondary phenomenon paralleling the enlargement of other organs. Early this century, the observations and experiments of Harvey Cushing, a Baltimore and Boston neurosurgeon (1869-1939), laid the foundations of modern clinical neuroendocrinology.

He postulated the causative link between pituitary tumours and acromegaly, and in the syndrome that bears his name. He performed hypophysectomies on dogs and recorded the consequences, thereby establishing the true function of the pituitary. He coined the words hypo- and hyper-pituitarism to describe the pathological consequences of disturbed pituitary function. (Cushing, 1910). A decade later, proof that the anterior pituitary contained a growth promoting substance came from the observation that the intraperitoneal administration of crushed bovine pituitary tissue into rats stimulated somatic growth (Evans & Long, 1921).

In 1930, a Hungarian anatomist, Popa, identified the vascular nature of the connection between the hypothalamus and pituitary. He mistakenly believed the flow was from the pituitary to the hypothalamus. This misconception was corrected by Wislocki and King (1936). In 1947, Green and Harris proffered the first evidence supporting and extending Lower's theory of pituitary function and regulation. They and others suggested that the central nervous system controlled pituitary activity via a neuro-humoral relay (Scharrer & Scharrer, 1954). Reichlin (1961) showed a reduction in GH content of the pituitary, and also growth, in rats with lesions of the ventral hypothalamus. This demonstrated the importance of the hypothalamus in regulating GH secretion. Deuben and Meites in 1964 established that rat hypothalamic extracts could stimulate GH release from rat pituitary *in vitro*. Despite the evidence for the existence of a hypothalamic factor that acted on the pituitary to stimulate GH secretion, it proved the most elusive of the hypothalamic factors to identify. Between 1969 and 1981, thyrotrophin-releasing hormone, gonadotrophin-

releasing hormone, somatostatin (vide infra) and corticotrophin-releasing hormone were isolated from ovine and porcine hypothalami (Rezek & Novin, 1977; Schally *et al.* 1971; Boler *et al.* 1969; Burgus *et al.* 1970; Vale *et al.* 1981; Seeburg & Adelman, 1984). In addition, dopamine was identified as the tonic inhibitor of prolactin secretion (MacLeod & Lehmeyer, 1974; MacLeod *et al.* 1970). The characterisation of GHRH in 1982 was exceptional for its extraction from human tissue taken from only two patients with acromegaly due to the ectopic production of GHRH from neuroendocrine tumours of the pancreas. This contrasts with the 50 tons of fresh frozen ovine tissue processed between 1964 and 1973 in the hunt for the hypothalamic secretagogues.

As early as 1960 an association between acromegaly and a bronchial carcinoid tumour had been noted and it was subsequently reported that, in similar patients, the acromegaly could be cured by removal of the carcinoid tumour (Southern, 1960; Sonksen *et al.* 1976). In 1979 two groups demonstrated GH-releasing activity in the tumour extracts of patients with carcinoid tumours and acromegaly (Shalet *et al.* 1979; Saeed uz Zafar *et al.* 1979). Thorner in 1982 reported an acromegalic patient in whom somatotroph hyperplasia was found at transsphenoidal hypophysectomy. Subsequent investigation revealed a pancreatic tumour, the removal of which cured the acromegaly. Portions of the tumour were provided to both the groups of Vale and Guillemin. Guillemin also obtained pancreatic neuroendocrine tumour of the carcinoid type from another patient (Sassolas *et al.* 1983). Within one year both groups had extracted and sequenced GHRH and reports from four of these studies appeared in November 1982 (Guillemin *et al.* 1982; Esch *et al.* 1982; Spiess

et al. 1982; Rivier *et al.* 1982).

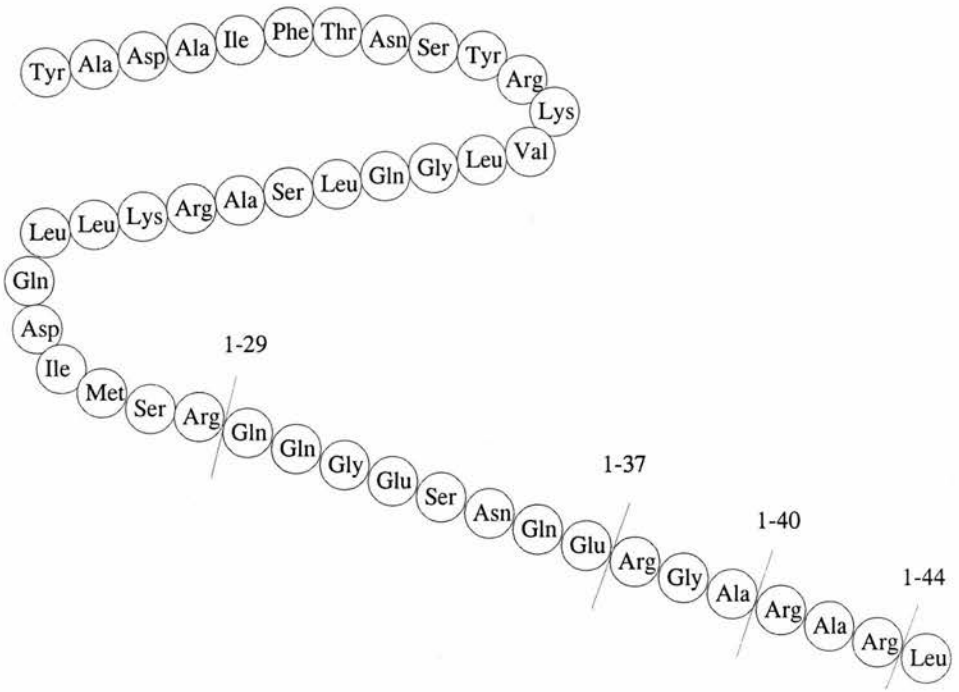

Figure 1

The structure of GHRH(1-29)NH_2 and its C-terminal shortened analogues

Three forms of GHRH were identified from the two tumours: GHRH(1-40)OH, GHRH(1-44)NH_2 and GHRH(1-37)NH_2 (Figure 1). Both 40 and 44 residue peptides identical to that from the pancreatic tumours were subsequently extracted from human hypothalami (Lin *et al.* 1984). Rat GHRH consists of 43 amino acids and differs from the human by 15 residues (Speiss *et al.* 1983). GHRH immunostaining has been demonstrated in the arcuate nucleus of the hypothalamus with fibres projecting to the median eminence and ending in contact with the portal vessels (Bloch *et al.* 1983). The human GHRH gene has been mapped to chromosome 20 (Mayo *et al.* 1985). It has been demonstrated, *in vitro*, that GHRH

stimulates transcription of the GH gene as well as GH secretion (Barinaga *et al.* 1985). There is evidence from *in vitro* studies in rats of at least two functional compartments for storage of GH in the somatotroph: a readily releasable pool, and a pool that responds to more prolonged stimulation (Stachura & Tyler, 1987). GHRH stimulates release of GH from the readily releasable pituitary pool of GH (Chao *et al.* 1988). The biological activity of GHRH appears to reside in the first 29 amino acids, as analogues of this length with deletions from the C-terminal end of the intact hormone retain their full GH-releasing activity (Lance *et al.* 1984), but the N-terminal residues need to be preserved (Ling *et al.* 1984). At doses of 1-3 μg/kg, all three analogues when given intravenously, selectively stimulated GH secretion in normal males (Gelato *et al.* 1983; Rosenthal *et al.* 1983; Grossman *et al.* 1983), eliciting an identical pattern of GH secretion (Grossman *et al.* 1984b; Losa *et al.* 1984). Dose-response studies established 1 μg/kg intravenously to be a supra-maximal dose (Grossman *et al.* 1983). The only side-effect of intravenous GHRH is facial flushing, which typically occurs within one minute and resolves in five.

Further understanding of the action of GHRH should follow the recent cloning and sequencing of its receptor. The GHRH receptor is homologous to other peptide receptors, notably secretin, vasoactive intestinal peptide and ACTH. In common with these receptors, it is a cell surface G protein-coupled receptor with seven trans-membrane domains (Mayo, 1992). The G-protein complex activates adenylate cyclase and results in both increased gene transcription and hormone release. GH release is cyclic AMP and calcium-dependent while GH transcription is cAMP-dependent but calcium independent. Constitutively active

mutations of the Gα subunit have been found in 40% of GH secreting pituitary tumours (Spada *et al.* 1993).

SOMATOSTATIN

The discovery of somatostatin was a by-product of the search for a growth hormone-releasing factor. While looking for a growth hormone-releasing factor in rat hypothalami, a substance that inhibited GH release was unexpectedly detected (Krulich *et al.* 1968). At about the same time, Hellman and Lernmark described a factor that inhibited pancreatic insulin secretion (Hellman & Lernmark, 1969a; Hellman & Lernmark, 1969b). Brazeau et al. (1973) extracted and sequenced somatostatin from ovine hypothalamic extracts and established it to be a cyclical tetradecapeptide (Figure 2). The tetradecapeptide is one of a family of somatostatin-related peptides and is synthesised as part of a larger precursor, preprosomatostatin, which is approximately eight times the size. In addition to the tetradecapeptide, post-transcriptional modification of preprosomatostatin results in several variants that include an amino-terminal extended 28 residue variant which is fully biologically active and prosomatostatin (Takahara *et al.* 1975). The physiological role of the different post-transcriptional products is uncertain, but evidence exists for differences between tissues in post-transcriptional modification.

Somatostatin-14 is synthesised in many tissues including the brain, hypothalamus and pancreatic islet cells, and is highly conserved between species. Evidence exists for

somatostatin as a neurotransmitter. Somatostatin positive neurons are to be found in the cerebral cortex, hippocampus, thalamus, caudate, brain stem nuclei and spinal cord (Robbins, 1988). Much remains to be understood of the multiple functions of somatostatin within the central nervous system. Evidence from rats suggests a role in thermoregulation and, within the dorsal hypothalamic area, to inhibit adrenomedullary adrenaline secretion (Brown, 1983; Brown *et al.* 1981). Decreased cerebrospinal fluid somatostatin levels have been noted in numerous diseases of the cerebral cortex, including Alzheimer's disease, multiple sclerosis and Huntington's chorea, although the physiological significance of these observations is uncertain (Robbins, 1988). Centrally administered somatostatin has analgesic properties and other opiate-like effects (Rezek *et al.* 1978).

The somatostatin neurones involved in the regulation of GH secretion are located in the anterior periventricular nucleus of the hypothalamus. Nerve fibres originating in these neurons project to the median eminence from where somatostatin is released into the hypothalamo-pituitary portal circulation.

The evidence for a physiological role of somatostatin in the control of GH secretion comes from many sources. In humans, exogenous somatostatin blocks the GH response to exercise, arginine, hypoglycaemia and GHRH (Hall *et al.* 1973; Mortimer *et al.* 1973; Wehrenberg *et al.* 1982b; Davies *et al.* 1985). The administration of anti-somatostatin antiserum increases trough levels of GH and reverses the growth hormone-inhibitory effect of stress and starvation in rats (Arimura *et al.* 1976; Terry & Martin, 1981; Wehrenberg *et*

al. 1982a). The interaction of GHRH and somatostatin in the regulation of GH secretion is discussed below.

The gastrointestinal system is rich in somatostatin-secreting cells and the peptide acts as both a neurotransmitter and hormone. The endocrine cells of the fundus and antrum of the stomach are the main source of circulating somatostatin. Somatostatin is also to be found in the neural structures in the deep submucosa, muscularis and muscularis mucosa. Intravenous somatostatin or the long-acting analogue octreotide inhibit secretion of many gastrointestinal peptides, namely, insulin, glucagon, vasoactive intestinal peptide, gastrin, secretin, pancreatic polypeptide, cholecystokinin (CCK), secretin, GIP and motilin. Exogenous somatostatin has inhibitory actions apparently unrelated to peptide secretion on gastric emptying, pancreatic exocrine function, gallbladder contraction and intestinal motility (Johansson *et al.* 1978; Boden *et al.* 1975). The physiological function of somatostatin in these sites is unclear; the relative contribution of endocrine, paracrine or neural mechanisms remains to be clarified. The presence of somatostatin in the D cells of the islets of Langerhans in such close proximity to the glucagon and insulin-containing α and β cells is intriguing, and it seems probable that somatostatin has a paracrine action inhibiting glucagon and insulin release (Unger & Orci, 1977). The diverse biological functions of the somatostatin peptides are mediated by a family of somatostatin receptors. Five subtypes have been cloned and characterised. As with the GHRH receptor, each has a seven transmembrane hydrophobic region coupled to G-proteins. The amino acid homology varies between 42 and 60% and they differ in their ability to couple G-proteins

and inactivate adenyl cyclase activity. Differences in affinity exist between receptors for various somatostatin analogues. The four amino acids in positions 7-10 at the β-turn are most important for receptor binding (Veber *et al.* 1978; Veber *et al.* 1981). Octreotide binds with greatest affinity to the type 2 and 5 receptors (Raynor *et al.* 1993b; Raynor *et al.* 1993a). The details of the relationship between the families of somatostatin peptides and receptors remain to be clarified.

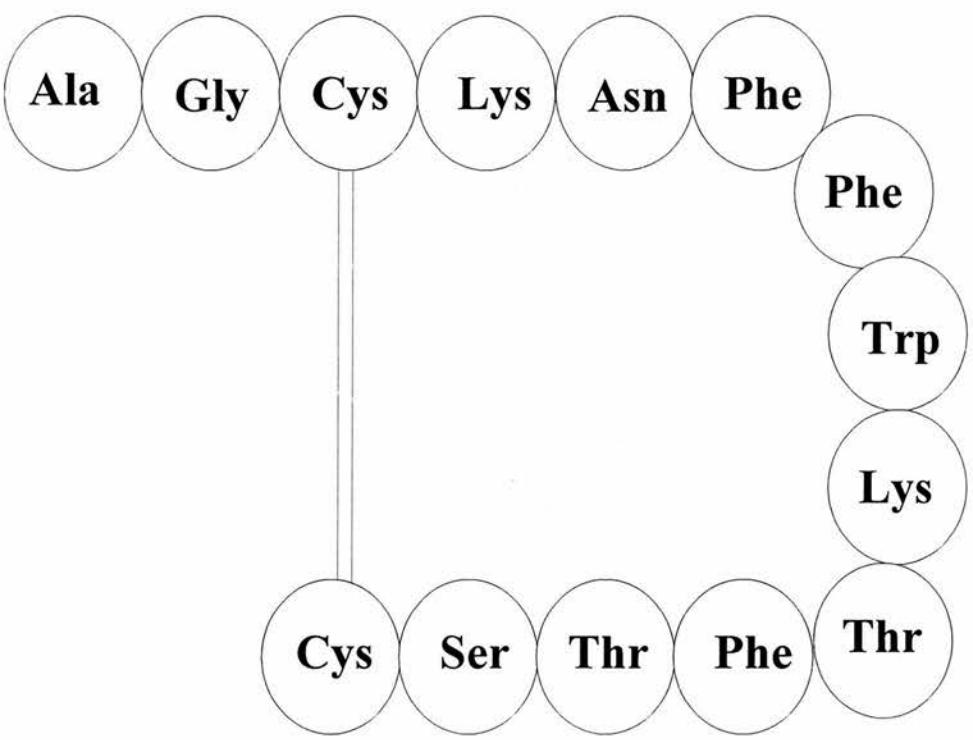

Figure 2

The amino acid sequence and structure of somatostatin-14

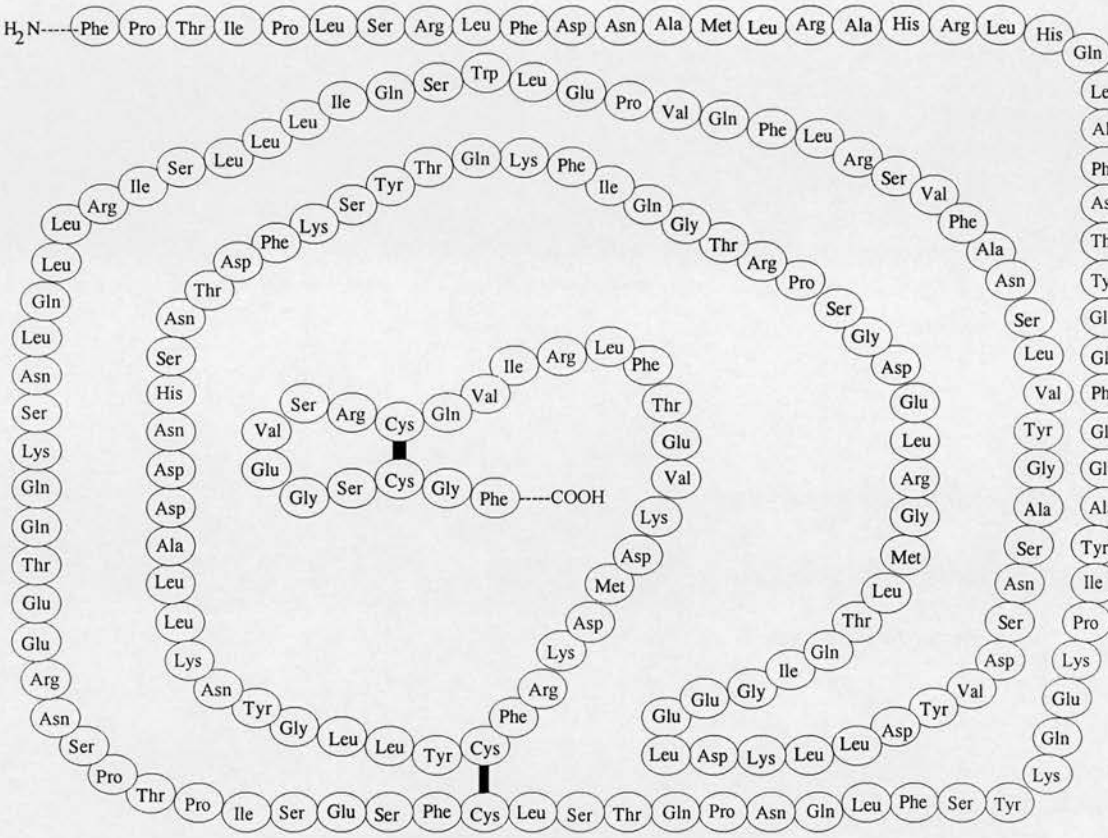

Figure 3

The amino acid sequence and structure of growth hormone

GROWTH HORMONE BIOLOGY

Growth hormone is a single chain, 191 amino acid in man, peptide with a molecular weight of 22,000 daltons. It has two intramolecular disulphide bonds, but in contrast to LH, FSH and TSH, is not glycosylated (Figure 3). In addition to the 22 KDa form, a 20 KDa species as well as larger aberrant forms have been isolated. GH is a member of a group of peptides with intramolecular disulphide bridges, including human chorionic somatomammotrophin,

a placental peptide secreted in large quantities (84 % homology) and prolactin (16% homology). The primate GH molecule and receptor have diverged from other species, with the consequence that until recombinant GH became available only human pituitary GH could be used in man.

As its name implies, GH promotes linear growth. However, the actions of GH are more complex and varied than might be gathered from its name and continue after the cessation of linear growth. The recognition of clinical sequelae of GH deficiency in adults has resulted in an appreciation of the role of GH in adult life (Salomon *et al.* 1989; Cuneo *et al.* 1992). Growth hormone promotes protein anabolism and the diversion of amino acid from oxidative to protein synthetic pathways (Griffin & Miller, 1974; Fain *et al.* 1965). Lipolysis and fat oxidation increase in response to GH, with a concomitant reduction in subcutaneous fat in children with GH deficiency (Clemmons *et al.* 1981). GH reduces insulin sensitivity, possibly secondary to changes in fatty acid and muscle metabolism. Longitudinal growth is the result of proliferation of chondrocytes in the epiphyseal growth plate. GH acts on the germinal zone of the growth plate to stimulate proliferation and differentiation of prechondrocytes. Clonal expansion and maturation of chondrocytes is IGF-I dependent (Isgaard *et al.* 1986). GH also promotes protein synthesis and mineral retention in bone and calcium absorption from the gut.

INTRODUCTION

GROWTH HORMONE-BINDING PROTEINS

Two discrete GH-binding proteins (GH-BP) have been described in human plasma (Baumann *et al.* 1986; Herington *et al.* 1986). The predominant GH-BP is a high affinity 60 kD single-chain glycoprotein structurally identical to the extracellular domain of the GH receptor (Baumann, 1991). One molecule of GH can bind two of GHBP however, due to the low concentration of GHBP the 1:1 complex is more prevalent. Plasma concentrations of GHBP are low in the fetus and neonate and rise gradually to a plateau by the late teens and remain constant throughout adult life (Daughaday *et al.* 1987; Silbergeld *et al.* 1989). Levels are slightly higher in women. The relationship between GH and GHBP is still under investigation. GHBP levels are reported to be unaltered in adult GH deficiency but increased in children with GHD, while acromegalics have low levels (Dempsher *et al.* 1990; Baumann *et al.* 1989a). GH therapy in children with GHD is reported not to alter plasma GHBP levels (Martha *et al.* 1992).

Forty to 50% of circulating GH is bound to GHBP, increasing to 80% following a pulse of GH secretion (Veldhuis *et al.* 1993; Baumann *et al.* 1988a). The half-life of bound GH is approximately 10 times greater than free hormone (Veldhuis *et al.* 1993). The pulsatile nature of GH secretion appears to be important for its action (see below), it is therefore a paradox of GHBP function that it smooths out the fluctuations in plasma GH caused by pulses of pituitary GH secretion but appears to enhance the effect of GH, presumably by lengthening the half-life.

The clinical significance of and usefulness of GHBP measurement is still being assessed. GHBP is low in patients with GH receptor abnormalities, eg Laron's syndrome and Pygmies (Rosenbloom *et al.* 1992; Baumann *et al.* 1991). Low plasma GHBP levels are also seen in systemic disorders associated with growth failure: malnutrition, diabetes, cirrhosis, renal failure, hypothyroidism and intensive care unit patients (Hochberg *et al.* 1992; Baumann *et al.* 1988b; Mercado *et al.* 1992; Hattori *et al.* 1992; Postel-Vinay *et al.* 1991; Amit *et al.* 1991).

INSULIN-LIKE GROWTH FACTOR-I

Daughaday observed that cartilage from hypophysectomised rats was unresponsive to GH added *in vitro*, but incorporated sulphate in response to normal rat serum and serum from GH treated hypophysectomised rats (Salmon & Daughaday, 1957). This "somatomedin theory" resulted in the appreciation that many of the actions of GH are not direct but rather the consequence of an intermediary. The term somatomedin is now regard as a generic designation for the GH-dependent growth factors. Insulin-like growth factor-I (IGF-I) has been shown to be the mediator of many of the metabolic actions of growth hormone. It is a peptide with a molecular weight of 7649 daltons and has a high degree of sequence homology with human proinsulin (Daughaday *et al.* 1972; Rinderknecht & Humbel, 1978). Growth hormone is the principal determinant of circulating IGF-I levels, with concentrations being high in acromegaly and low in growth hormone deficiency and Laron's syndrome (Hall & Sara, 1984). Fasting and malnutrition result in low plasma IGF-I levels,

although GH levels rise with fasting (Isley *et al.* 1983; Ho *et al.* 1988). The metabolic effects of IGF-I can be subdivided into insulin-like and growth-promoting (Froesch & Zapf, 1985). Although, insulin and IGF-I have their own receptors, some cross-affinity exists which possibly accounts for the mechanism by which IGF-I can induce hypoglycaemia (Humbel, 1990). Circulating IGF-I avidly binds in serum to specific binding proteins (IGF-BPs), of which six so far have been identified, such that under normal circumstances less than 1% is unbound and physiologically active (Ballard *et al.* 1989)(vide infra). Circulating IGF-II is largely hepatic in origin, but the physiological role of this 7471 dalton peptide remains obscure, as do the regulators of its secretion.

As alluded to above, IGF-I has direct effects on rat epiphyseal chondrocytes *in vitro*, but appears to act via different mechanisms on different cell populations (Lindahl *et al.* 1987c; Lindahl *et al.* 1987a; Lindahl *et al.* 1987b; Madsen *et al.* 1983). IGF-I is a potent stimulator of every anabolic process measured in muscle (Florini, 1987). The ability of GH to increase renal plasma flow and glomerular filtration rate is mediated by IGF-I (Guler *et al.* 1989). Studies in man suggest that IGF-I has a minimal effect on plasma free fatty acid concentrations and the regulation of lipolysis (Guler *et al.* 1987). One week of subcutaneous IGF-I did not effect plasma free fatty acid levels (Takano *et al.* 1991). It is probable that any effects that IGF-I has on adipose tissue is via activation of insulin receptors.

INTRODUCTION

IGF-BINDING PROTEINS (IGF-BPs)

IGF-I and -II bind avidly to specific binding proteins that are to be found in serum and all extracellular fluids. The amino acid sequence of six IGF-BPs have been determined (Ooi & Herington, 1990; Zapf *et al.* 1990; Shimasaki *et al.* 1990). The function of the IGF-BPs appear to be diverse and complex. IGF-BPs variously act as delivery molecules, a reservoir of circulating IGFs and, in different tissues and under distinct conditions, both to attenuate and potentiate the actions of IGF-BPs.

IGF-BP1 is a single chain peptide with a molecular weight of 25700 daltons. Circulating levels display a circadian pattern with high levels at night and are inversely related to and controlled by plasma insulin levels (Cotterill *et al.* 1993b). IGF-BP3 is the most abundant of the binding proteins and hence the main carrier of circulating IGF-I. The peptide core has a molecular weight of approximately 42000 but circulates as a ternary complex (molecular weight approximately 150,000) in association with an acid-labile subunit (Baxter, 1988). It has similar affinities for both IGF-I and -II. In parallel with IGF-I and -II, the liver is the main source of circulating IGF-BP3 (Schwander *et al.* 1983). The principal determinant of circulating levels is GH. IGF-BP3 levels do not change in response to a pulse of GH or to a single bolus of exogenous GH but rather fluctuate more slowly without circadian variation. Plasma IGF-BP3 levels correlate closely with integrated GH secretion (Blum *et al.* 1990). An example of the importance of IGF-BP3 in modulating the activity of IGF-I is discussed on page 45. Circulating IGF-BP4 is probably hepatic in origin, but

is expressed in many organs, ranging from brain to ovaries. It binds IGF-I and -II with equal high affinity and may modulate IGF-I activity, particularly in the brain (Brar & Chernausek, 1993).

IGF-BP2 is the major binding protein of the central nervous system. It was first identified from cerebrospinal fluid with subsequent studies demonstrating its wide distribution throughout the central nervous system (Rosenfeld *et al.* 1989). Similarly, IGF-BP6 is a predominately CNS binding protein and, in common with IGF-BP2, has high affinity for IGF-II, but is unique in having only 16 cysteine residues compared to the 18 found in the other IGF-BPs. IGF-BP5 distinguishes itself by binding tightly to extracellular matrix and may serve as a reservoir for IGFs.

THE PHYSIOLOGY OF GH SECRETION

Growth hormone secretion is pulsatile, as the result of a dynamic equilibrium between somatostatin and GHRH, with pulses occurring every 3 - 4 hours. The regulation of hypothalamic somatostatin and GHRH secretion is controlled through a complex network of neurons and neurotransmitters (vide infra).

Many pieces of evidence demonstrate the importance of pulsatile, as opposed to continuous GH secretion. Pulsatile GH administration to GH-deficient dwarf mice had a greater effect on the hepatic enzyme carbonic anhydrase III than continuous GH exposure (Jeffery *et al.*

1990). Similarly, a daily dose of GH divided into nine pulses stimulates greater growth of GH deficient rats than the same dose administered as either one or three pulses (Clark *et al.* 1985). Further, pulsatile GH exposure is necessary for optimal stimulation of IGF-I mRNA in growth plate and muscle (Isgaard *et al.* 1988).

A pulse of GH is initiated by GHRH; however, the size of pulse is determined by the condition of the somatotrophs. Studies in rats, given GHRH after pre-treatment with anti-somatostatin antiserum, suggest that there is tonic secretion of both GHRH and somatostatin into hypophyseal portal blood; superimposed onto this there is a 3 to 4 hourly pulse of GHRH, associated with trough levels of somatostatin, resulting in a pulse of GH (Tannenbaum & Ling, 1984). Similarly anti-GHRH antiserum blocks pulsatile GH release in the rat (Wehrenberg *et al.* 1982a). Hindmarsh et al. (1991), in a series of studies on normal volunteers entailing combinations of somatostatin or saline infusions paired with boluses of either saline or GHRH or somatostatin, demonstrated that the greatest pulse of GH secretion occurs after a bolus of GHRH at the termination of a somatostatin infusion. Similarly, Devesa et al. (1989) subdivided GH responses to GHRH by the status of endogenous somatostatin secretion. Further support for the importance of the endogenous somatostatin tone in the aetiology of GH pulses comes from the observation that a continuous infusion of GHRH can restore normal pulsatile GH secretion. This model is in accord with the known actions of GHRH and somatostatin on the somatotroph; the former stimulates GH synthesis and release, while the latter only inhibits secretion. GH feedback on the hypothalamus and GHRH secretion stimulates somatostatin secretion, which in turn

terminates the pulse (discussed below).

The magnitude and frequency of GH pulses is dependent on age, being greatest during puberty and declining thereafter (Finkelstein *et al.* 1972). The greatest release of GH occurs during deep sleep (Quabbe *et al.* 1966), but other factors such as stress, exercise, and postprandial glucose decline also induce GH secretion (Martin, 1976). Fasting increases GH secretion and might explain the nocturnal predominance of GH secretion (Hartman *et al.* 1992; Ho *et al.* 1988). However, this cannot be the full explanation as the majority of GH secretion occurs in the first three hours of sleep rather than progressively increasing and peaking before the morning meal (Takahashi *et al.* 1968). GH secretion frequently coincides with the first episode of slow-wave sleep. There has been considerable debate as to whether this nocturnal GH release and slow-wave sleep are interdependent or only temporally related. There is clear evidence that GH release is a sleep related event, in that delaying sleep delays GH release (Takahashi *et al.* 1968). Slow-wave sleep is frequently associated with GH release; in the jet-lag model the major nocturnal GH secretion remains associated with slow wave sleep (Golstein *et al.* 1983). However, this is not invariably so, and GH release frequently occurs before the onset of slow-wave sleep.

Pyridostigmine fails to enhance spontaneous or GHRH-induced GH secretion at night indicative that cholinergic tone is greater at night and hence somatostatin secretion less (Ghigo *et al.* 1990a). This phenomenon may account for the predominance of GH secretion at night.

INTRODUCTION

GROWTH HORMONE AUTOREGULATION

GH regulates its own secretion by two negative feedback loops: a direct effect exerted by the hormone itself, and an indirect one by IGF-I.

The secretion of GH can be inhibited by exogenous GH *in vivo* both in man and animal models (Abrams *et al.* 1971; Hagen *et al.* 1972; Sakuma & Knobil, 1970; Abe *et al.* 1983). Ross et al. (1987a) showed that pretreatment with GH attenuated the GH response to a subsequent bolus of GHRH, before any rise in plasma IGF-I had time to occur. Further, they demonstrated that inhibition of hypothalamic somatostatin secretion restored the GHRH induced rise in GH, suggesting that the GH autofeedback is mediated by hypothalamic somatostatin secretion (Ross *et al.* 1987d); this was subsequently confirmed by others (Kelijman & Frohman, 1991). Similarly, repeated doses of GHRH induce somatotroph refractoriness, an effect reversed by pyridostigmine (Hulse *et al.* 1986; Massara *et al.* 1986b).

Another level of GH autoregulation is mediated by IGF-I. A decade ago, Rosenfeld et al. (1984) identified IGF-I receptors, and even greater numbers of IGF-II receptors, on cultured rat anterior pituitary cells, and similarly both IGF-I and -II receptors have also been demonstrated in the hypothalamus (Goodyer *et al.* 1984). Lesniak et al. (1988) used an autoradiographic technique to study the distribution of IGF-I and IGF-II receptors (now better called IGF type I and 2 receptors) and showed the greatest concentration in the rat

brain to be in the median eminence of the hypothalamus, the site of secretion of GHRH and somatostatin into the portal venous circulation of the stalk. Equally high concentrations of both receptors were noted in the rat pituitary.

Early evidence for an action of IGF-I on GH secretion came from the studies of Berelowitz et al. (1981). Isolated rat hypothalami incubated in a highly purified somatomedin-C preparation released somatostatin in a dose-dependent manner. Similar studies on the effect of IGF-I on hypothalamic GHRH secretion have been contradictory (Aquila, 1991; Shibasaki *et al.* 1986). Berelowitz and colleagues also demonstrated a direct effect of somatomedin-C on growth hormone secretion from dispersed rat anterior pituitary cells. No effect of somatomedin-C on basal or stimulated GH secretion was seen after four hours incubation, but at 24 hours stimulated GH secretion was completely abolished. Similarly, incubation of pituitary cells with somatomedin-C reduces GH mRNA (Yamashita & Melmed, 1986).

In free-moving conscious rats with chronic indwelling cerebroventricular cannulae, a single intracerebroventricular bolus of highly purified somatomedin-C resulted in inhibition of GH secretion (Tannenbaum *et al.* 1983). The studies of both Berelowitz and Tannenbaum indicated an important role for the hypothalamus, and possibly somatostatin, in the feedback regulation of IGF-I on GH secretion, with a probable additional delayed effect at the pituitary. However, the interpretation of many of these early studies is complicated by the suspicion that the highly purified somatomedin-C preparations contained both IGF-I and

-II.

With the availability of recombinant preparations of both IGF-I and -II, Tannenbaum repeated her earlier studies using the same experimental paradigm. Neither intracerebroventricular recombinant IGF-I (in doses up to 10 μg) nor IGF-II (at doses up to 1 μg) affected GH secretion. However, intriguingly a dose of 1 μg IGF-I administered in conjunction with 1 μg IGF-II completely inhibited GH secretion (Harel & Tannenbaum, 1992b; Harel & Tannenbaum, 1992a). The synergy of centrally administered IGF-I and -II in inhibiting GH secretion in rats supports the belief that the somatomedin-C preparations contained both IGF-I and -II and underlines the importance of the hypothalamus in the regulation of GH secretion, possibly via somatostatin.

Initial studies in man with rhIGF-I involved the administration of single intravenous boluses at doses that induced hypoglycaemia. The consequential stimulation of growth hormone and cortisol secretion, in a manner analogous to insulin-induced hypoglycaemia, suggests an effect of hypoglycaemia rather than a direct effect of rhIGF-I on the hypothalamo-pituitary axis (Laron *et al.* 1988; Guler *et al.* 1987).

Detailed studies of the effects of rhIGF-I on GH secretion in euglycaemic normal volunteers have been limited. A subcutaneous infusion of 20 μg/kg/hr rhIGF-I inhibited nocturnal GH secretion in a single subject, and the GH response to GHRH in one of two subjects (Guler *et al.* 1989). Hartman and colleagues (1993) studied GH dynamics in a group of fasted

normal volunteers during an infusion of rhIGF-I. It was reasoned that feeding, in particular certain amino-acids and glucose, alter GH secretion, and hence subjects were studied fasting during euglycaemic "clamps". A clear and rapid inhibition of growth hormone secretion was found when rhIGF-I was infused intravenously at a dose of 10 μg/kg/hr. These data clearly indicate that in man rhIGF-1 can inhibit GH secretion, although it must be remembered that fasting itself increases GH secretion and causes changes in the IGF-binding proteins (IGF-BPs), in particular a rise in IGF-BP1 (Cotterill *et al.* 1993b).

Cotterill et al. (1993a) studied the effects of IGF-I therapy on GH secretion in two subjects with the GH-insensitivity syndrome. Pre-treatment overnight GH profiles proved levels to be grossly elevated. After six months of IGF-I therapy (120 μg twice daily), repeat profiles showed GH secretion to be inhibited following the evening dose of rhIGF-I, but to rise again gradually in the early hours of the morning. The return of GH secretion corresponded to falling plasma IGF-I levels. The shortened plasma half-life of exogenous IGF-I in the GH-insensitivity syndrome compared to normal volunteers can be explained by differences in IGF-binding proteins. IGF-BP3 is the principal plasma binding protein and the low levels associated with the GH-insensitivity syndrome allow rapid clearance of IGF-I from the circulation. This is an excellent example of the importance of IGF-BPs in modulating IGF-I action.

INTRODUCTION

INFLUENCE OF THYROID HORMONE

Normal linear growth is dependent upon complex interactions of many metabolic and endocrine systems; disturbance of any element may result in growth failure.

The interaction of GH and thyroid hormones is pivotal to normal growth and development. Hypothyroidism is associated with growth failure and diminished GH secretion in response to hypoglycaemia, arginine, GHRH and sleep; the changes are all reversible with treatment (Williams *et al.* 1985; Valcavi *et al.* 1986; Iwatsubo *et al.* 1967). Similarly, childhood thyrotoxicosis results in an increased number and size of spontaneous GH pulses and accelerated growth (Schlesinger *et al.* 1973; Iranmanesh *et al.* 1991). Thyroid hormones act directly on the pituitary to influence somatotroph numbers and the expression of the GH gene (Nyborg *et al.* 1984; Franklyn *et al.* 1986).

INFLUENCE OF GONADAL HORMONES

Oestrogens have a significant influence on GH secretion and account for the differences in GH release between men and women, and the changes in GH secretion seen during puberty. Oestrogens, both endogenous and exogenous, are known to enhance basal and stimulated GH release. Women have higher basal levels of GH than men, especially during the high oestrogenic phase of the menstrual cycle, and oestrogen pretreatment in men increases basal and arginine-stimulated GH secretion (Unger *et al.* 1965; Frantz & Rabkin, 1965; Merimee *et al.* 1966). Oestrogen priming is known to increase the GH response to hypoglycaemia;

however, pretreatment with the synthetic oestrogen stilboestrol does not effect the GH response to GHRH in children with short stature (Ross *et al.* 1987b). These data suggest that oestrogens act on the hypothalamus to increase GH secretion, possibly by increasing endogenous GHRH secretion.

INFLUENCE OF METABOLIC FACTORS

An apparent paradox exists in the effect of hyperglycaemia on GH secretion. In non-diabetic subjects, acute hyperglycaemia reduces basal and stimulated GH levels, while hypoglycaemia stimulates GH secretion (Roth *et al.* 1963; Penalva *et al.* 1989). The mechanism of hypoglycaemia provoked GH release is not fully established. It is independent of hypothalamic GHRH, as GHRH and hypoglycaemia have additive effects on GH secretion and GHRH pretreatment does not attenuate the GH response to hypoglycaemia (Shibasaki *et al.* 1985b; Page *et al.* 1987; Schulte *et al.* 1986; Shibasaki *et al.* 1985a). Somatostatin appears to play little part, as cholinergic blockade with atropine abolishes the GH response to GHRH but only slightly reduces the response to hypoglycaemia (Evans *et al.* 1985).

The regulation of growth hormone secretion is disturbed in diabetics. In contrast to normal volunteers, poorly controlled type-one diabetics have higher basal GH levels, larger and more frequent pulses and an exaggerated response to GHRH and exercise (Penalva *et al.* 1989; Asplin *et al.* 1989). Improved glycaemic control results in normalisation of GH

dynamics (Press *et al.* 1992).

The apparent paradox of poor linear growth despite increased growth hormone secretion, in diabetics, is indicative of end-organ resistance to the actions of GH and a compensatory rise in GH secretion. The low serum IGF-I levels seen in poorly controlled diabetics support this hypothesis (Tan & Baxter, 1986; Schaper *et al.* 1990).

Growth hormone is an important counter-regulatory hormone to the hypoglycaemic effect of insulin and in excess can cause insulin resistance. Diabetes mellitus occurs in twenty-five percent of acromegalics (Davidoff, 1926).

Free fatty acid (FFA) administration to normal subjects reduces the GH response to a variety of stimuli including hypoglycaemia and GHRH. The mechanism in man is unclear. Evidence in rats, pretreated with anti-somatostatin antibodies, suggests a role for somatostatin (Imaki *et al.* 1986). However, in man inhibition of somatostatin secretion with pyridostigmine does not restore the GH response to GHRH after FFA administration, indicative of a possible direct effect on the somatotroph (Penalva *et al.* 1990a).

NEUROTRANSMITTERS

The hypothalamic peptides GHRH and somatostatin are released from the nerve terminals in the median eminence into the portal circulation of the stalk and hence act on the

somatotrophs to regulate GH secretion. The release of GHRH and somatostatin are themselves under the regulation of a complex network of neurones and neurotransmitters. The amine neurotransmitters eg catecholamines (dopamine, adrenaline, noradrenaline), indoleamines (serotonin and melatonin) and histamine are secreted from neurones that arise in the brain stem (or pineal) and ascend in ventral and dorsal pathways to innervate limbic and hypothalamic structures, including the internal (noradrenaline, serotonin) and external (dopamine) layers of the median eminence. The tuberoinfundibular-dopamine pathway, which projects from the arcuate nucleus to the median eminence, releases dopamine directly into the hypophyseal portal pathways. Acetylcholine-containing neurones are to be found in the supraoptic nucleus and in the lateral pre-optic area, and it is believed that their neurones project to the median eminence. The mediobasal, posterior and median hypothalamus all contain γ-amino-butyric acid (GABA) neurones that project into the external layer of the median eminence. Great potential exists within the hypothalamus for interactions between neurotransmitters and between neurotransmitters and hypothalamic peptides. The ability of various drugs to act as agonists and antagonists for the neurotransmitters has been exploited in efforts to understand the role of the various neurotransmitters in the hypothalamic regulation of pituitary function.

ADRENERGIC PATHWAYS

It has been known for a long time that central adrenergic pathways play an important role in GH secretion. The pharmacological depletion of brain catecholamines by α-methyl-p-

tyrosine leads to abolition of pulsatile GH secretion in the rat, an effect negated by the administration of an α_2-adrenoceptor agonist, such as clonidine (Durand *et al.* 1977). The non-selective α-adrenergic antagonist phentolamine, and selective α_2-antagonist yohimbine, partially inhibit GH secretion during insulin-induced hypoglycaemia in healthy young men, while the α_1-antagonist prazosin had no effect (Tatar & Vigas, 1984). The results suggest that GH release during insulin hypoglycaemia is, in part, mediated by α_2-adrenoceptors. Clonidine stimulates GH release in man, an effect that can be blunted by yohimbine (Lanes & Hurtado, 1982; Lal *et al.* 1975; Krulich *et al.* 1982). Contradictory evidence exists as to whether α_2-adrenergic stimulation of GH release is via stimulation of GHRH or inhibition of somatostatin. Studies using specific antisera to somatostatin (anti-somatostatin) and GHRH (anti-GHRH) have suggested that α_2-adrenoceptor activation acts by stimulating GHRH secretion. In freely moving rats, clonidine stimulated GH secretion in anti-somatostatin treated and control rats, but failed to in anti-GHRH treated rats (Eden *et al.* 1981; Miki *et al.* 1984; Katakami *et al.* 1984). Clonidine was also able to stimulate GHRH secretion from perfused rat hypothalami (Kabayama *et al.* 1986). Studies in humans and rabbits indicate that clonidine acts via inhibition of somatostatin secretion. In 1990, two papers were published in the Journal of Clinical Endocrinology and Metabolism from different groups in Santiago de Compostela, Spain: Cordido et al. (1990) studied clonidine in combination with pyridostigmine, arginine and GHRH and concluded that clonidine acted on the hypothalamus to stimulate GHRH secretion, while Devesa et al. (1990) demonstrated potentiation of GHRH-induced GH secretion by clonidine, and surmised an action via somatostatin. A similar conclusion to the latter was reached in an independently

performed study of short children (Reiter *et al.* 1988). Further, Cordido's study is open to alternative interpretation. An additive effect of clonidine plus pyridostigmine compared to clonidine alone was taken as evidence that they act by different mechanisms: this assumption is only valid if supramaximal doses of clonidine and pyridostigmine are used and may not have been the case as the doses of both drugs are limited by side-effects. Devesa et al. (1991) have since published additional data confirming and extending their original observations. Minamitani et al. (1989) demonstrated in rabbits and Arce et al. (1990) in dogs that clonidine acts via inhibition of somatostatin rather than stimulation of GHRH. Although controversial, the balance of evidence indicates that α_2-adrenoceptor stimulation results in inhibition of somatostatin secretion in man, rabbits and dogs, but in rats via GHRH secretion.

β-adrenergic antagonists, such as atenolol, enhance the GH response to GHRH and insulin-induced hypoglycaemia (Chihara *et al.* 1985; Kelijman & Frohman, 1989; Martha *et al.* 1990). Similarly, β-adrenergic agonists, for example salbutamol, inhibit GH secretion and attenuate the GH response to GHRH, indicative that activation of β-adrenergic pathways inhibit GH secretion by stimulation of somatostatin release (Mazza *et al.* 1990; Martina *et al.* 1992).

The dopaminergic regulation of GH secretion in man has acquired particular significance because of the utilisation of the therapeutic use of dopamine agonists and the disparity of effect in physiological and pathological states. Under normal circumstances, dopamine and

the dopamine agonists, eg bromocriptine, potentiate the GH response to GHRH and increase spontaneous GH secretion, implying a somatostatin-mediated effect (Delitala *et al.* 1987a; Vance *et al.* 1987; Miell *et al.* 1991). In contrast, in acromegaly bromocriptine and other dopaminergic agonists inhibit GH secretion by a direct action via D2 dopamine receptors on tumourous somatotrophs (Besser *et al.* 1978).

CHOLINERGIC PATHWAYS

The availability of drugs capable of modulating the cholinergic nervous system has enabled extensive investigation of its role in the regulation of GH secretion. The cholinergic muscarinic antagonists atropine and pirenzepine block nocturnal and GHRH-induced GH secretion (Casanueva *et al.* 1986; Jordan *et al.* 1986; Peters *et al.* 1986; Delitala *et al.* 1987b). The anticholinesterase drug, pyridostigmine, increases cholinergic tone and thereby increases GH secretion and augments the GH response to a supramaximal dose of GHRH (Leveston & Cryer, 1980; Massara *et al.* 1986a). The ability of pyridostigmine to augment a supramaximal dose of GHRH is indicative that cholinergic regulation of GH secretion is independent of hypothalamic GHRH release. Acetylcholine and neostigmine inhibit somatostatin secretion from rat hypothalami in culture, an effect blocked by atropine (Richardson *et al.* 1980). Further evidence that the cholinergic tone on GH secretion is via somatostatin comes from the use of cysteamine, a somatostatin-depleting agent. In rats, cysteamine blocks GH secretion induced by the cholinergic agonist pilocarpine and the ability of atropine and pirenzepine to inhibit GHRH-induced GH secretion (Locatelli *et al.*

1986). Cholinergic nicotinic receptors are not involved in the regulation of GH secretion (Betti *et al.* 1985; Richardson *et al.* 1980).

GAMMA-AMINO-BUTYRIC ACID (GABA) PATHWAYS

In the human, basal GH secretion is stimulated by GABA and by a number of GABAergic drugs such as muscimol, baclofen and diazepam (Fioretti *et al.* 1978; Tamminga *et al.* 1978; Cavagnini *et al.* 1977). In contrast, GABA is able to reduce the GH response to insulin-induced hypoglycaemia and arginine (Cavagnini *et al.* 1980). The major tranquilliser and dopamine antagonist, pimozide, reduces the effect of GABA on GH secretion (Delitala *et al.* 1984), and a dualistic role of GABA on dopamine secretion and GH release has been suggested by Bercu & Diamond (1986).

OPIATE PATHWAYS

In man, morphine and β-endorphin do not stimulate GH secretion, whereas the long-acting analogue of met-enkephalin DAMME (D-Ala2,MePhe4-Met-enkephalin-(0)-l) stimulate GH secretion through naloxone-sensitive mechanisms (Tolis *et al.* 1975; Stubbs *et al.* 1978; Delitala *et al.* 1983). Naloxone fails to alter basal GH secretion but inhibits exercise-induced GH secretion in athletes. DAMME potentiates the GH response to GHRH, suggesting that opiates stimulate GH secretion by inhibiting somatostatin (Delitala *et al.* 1989). The role of opiates in the neuroregulation of GH secretion appears to be particularly

important at times of stress and during exertion.

CURRENT INVESTIGATION

IGF-I is a potent metabolic regulator with a diversity of actions. Recombinant IGF-I has therapeutic potential in states of acquired or inherited GH resistance, as well as an insulin-sparing effect in diabetes. As already discussed, plasma GH is the primary regulator of circulating IGF-I levels, while IGF-I is an important component in the feedback regulation of GH secretion. The influence of recombinant human IGF-I administered, by the therapeutic route, on pituitary function, in particular GH secretion, is studied here.

Much is known about the regulation of growth hormone secretion and its influence on linear growth; however, the relationship of quantitative GH secretion to final height has never been established. Previous investigations have related GH secretion to linear growth in children. For ethical reasons it is difficult to study truly normal children, which has resulted in a predominance of short children in studies. Pubertal status, age and growth velocity are all important variables which can be eliminated be studying young adults. The intention of this thesis is to relate final height to growth hormone secretion under various circumstances in young adults who have recently stopped growing and achieved their final height.

Studies on the influence of gender on the GH response to GHRH have reached

contradictory conclusions. The GH response to GHRH has been variously reported to be greater, the same and smaller in men than women. Any gender difference in GHRH responsiveness is clearly an important factor in recruiting volunteers for studies and in the interpretation of results. To clarify the situation, the GH response to GHRH has been compared between the sexes. In light of the existing confusion only male volunteers were used in the other studies included in this thesis.

Growth hormone secretion decreases with age. Many of the features of aging are comparable with the effects of GH deficiency in adults. With potentially unlimited supplies of GH, therapeutic trials are being undertaken of GH in the elderly. The mechanism of the age-related decline, and in particular the influence of the cholinergic nervous system in GH secretion has been studied in men.

Elevated plasma glucocorticoids are catabolic and inhibit the secretion of GH, an important anabolic hormone. A study of the mechanism of glucocorticoid-induced inhibition of GH secretion and a means of reversing it are included.

GHRH has been successfully used in the treatment of short stature and as the indications for GH therapy expand so the therapeutic potential for GHRH increases. GHRH has the advantage over recombinant GH, of inducing pulses of GH secretion rather than chronically elevated GH levels seen with GH therapy. Currently, it has the disadvantage compared to GH therapy of the necessity of at least twice-daily administration. Hence, cheaper to

synthesis and longer acting or orally active analogues of GHRH are of potential therapeutic interest. DC-21-346 is a new analogue of GHRH which in rats has been demonstrated to be 50 times more potent than conventional GHRH in stimulating GH release. The ability of DC-21-346 to stimulate GH secretion in man has therefore been studied.

SUBJECTS AND METHODS

VOLUNTEERS

Normal volunteers were recruited from medical, non-medical students and medical graduates at St. Bartholomew's Hospital and The City University. Great difficulty was experienced in the recruitment of certain groups of volunteers. It proved to be extremely difficult to recruit "short" and to a lesser extent "tall" subjects, and in addition "elderly" subjects. The necessary tall and short subjects were therefore recruited and studied in collaboration with Professor G. Delitala in Sassari, Sardinia, Italy. The elderly subjects were recruited and studied in collaboration with Professor M. Giusti in Genoa, Italy. For fear that oestrogen status may alter plasma GH, excepting one study which specifically looked at GH secretion in women, adult male volunteers were used throughout. All volunteers were healthy and non-obese (body mass index < 27 kg/m^2) as obesity is known to influence GH secretion (Castro *et al.* 1990). The female volunteers had a regular menstrual cycle of 28 days $\pm$ 2. All volunteers answered a basic questionnaire concerning their past health, incidental or therapeutic medication, alcohol and tobacco, and had a full medical examination to exclude disease. Any form of concurrent medication was an exclusion criteria. Prior to participation a full blood count, urea and electrolytes and liver function tests, ECG and urinalysis were performed: any abnormality resulted in exclusion of the volunteer. In the case of students, the Dean of the relevant college was informed of their entry into a study.

SUBJECTS AND METHODS

CLASSIFICATION OF VOLUNTEERS

Normal volunteers	Age > 18 years, male
"Tall" subjects	Age range 18-27 years, male Height ≥ 183 cm (> 90th centile, Tanner and Whitehouse 1975)
"Short" subjects	Age range 18-26 years, male Height ≤ 166 cm (< 10th centile, Tanner and Whitehouse 1975)
Female subjects	Not on oral contraceptive Regular menstrual cycle (28 days ± 2)
"Elderly" subjects	6 age ranges: 21-30 years 31-40 " 41-50 " 51-60 " 61-70 " > 70 "

SUBJECTS AND METHODS

DESIGN OF STUDIES

Where possible, and as stated in the relevant section, studies were performed in a randomised, double-blind and placebo-controlled design. Randomisation was performed using a Latin square.

BLOOD SAMPLES AND TESTS

All subjects, unless otherwise stated, were studied within the investigation unit on Francis Fraser ward at St. Bartholomew's Hospital. Subjects arrived on the ward between 08.00 h and 08.30 h following an overnight fast. They remained supine on a bed for the duration of the study; a cannula was placed in a forearm vein at least 30 minutes before starting each study. Insulin tolerance tests (ITT) and GHRH tests were started between 08.00 h and 09.00 h and blood was obtained through the in-dwelling cannula.

As already indicated, some of the "tall" and "short" subjects were studied in the Department of Endocrinology, University of Sassari, Sardinia, Italy. All the "elderly" subjects were studied in the Department of Endocrinology, University of Genoa, Italy under conditions similar to those detailed above.

SUBJECTS AND METHODS

PROTOCOLS

HANDLING OF SPECIMENS

For all studies, except the recombinant IGF-I study, blood was collected in a similar manner for the following hormone assays: GH, IGF-I, cortisol, testosterone, oestradiol and SHBG. Whole blood was collected into plastic tubes and centrifuged at 5000 rpm for 5 minutes and the serum separated and stored at -20° C. Samples from individual subjects were measured in the same assay.

For the IGF-I study, blood for measurement of GH, LH, FSH, TSH, cortisol, ACTH, prolactin, IGF-I, IGF-II, IGF bioactivity, glucose, insulin, C-peptide and IGF-binding protein was collected into lithium-heparin tubes. The tubes were then immediately placed in a centrifuge at 4°C and spun at 2500 rpm for 15 minutes. Plasma was immediately separated into plastic tubes and stored at -20°C.

Insulin Tolerance Test (ITT): Subjects remained supine and in a quiet environment for the duration of the test. Insulin (Actrapid, Novo Nordisk, Crawley) 0.15 U/kg was administered as an acute iv bolus, and blood taken basally and at 30 minute intervals (plus at 45 minutes) for 2 h for cortisol, GH, and blood sugar. At the termination of the test, a glucose drink (Lucozade, 380 mls containing 75 gms glucose) was given, followed by a substantial meal.

SUBJECTS AND METHODS

GHRH TESTS: Subjects remained supine and in a quiet environment for the duration of the test. The acute intravenous administration of GHRH provides a test of the readily releasable pool of pituitary GH. One hundred μg of intravenous GHRH(1-29)NH_2 has been demonstrated to induce a maximal GH response (Grossman *et al.* 1984a).

Oral pyridostigmine 120 mg or placebo was administered (time -60 min) one hour prior to an iv bolus of 100 μg GHRH(1-29)NH_2 (time 0 min). Blood was taken for measurement of GH at -75, -60, -15, 0, 15, 30, 45, 60, 75, 90, 105, 120 minutes.

DC-21-346 TESTS: Subjects remained supine and in a quiet environment for the duration of the test. DC-21-346 was administered as a iv bolus at time 0 min and blood was taken at -15, 0, 15, 30, 45, 60, 75, 90, 105, 120 minutes.

GH, LH and FSH PROFILES: 24 hour and overnight GH profiles were performed in a similar manner, starting at either 09.00 h or 21.00 h. Samples for GH, and when appropriate LH and FSH, measurement were obtained at 20 minute intervals. At the start of the study a 22 gauge "Quik-Cath" cannula was placed in a forearm vein, connected to a 30 cm extension (Lectro-Cath, volume 0.3 ml), and a 3 way tap. The cannula was kept patent with a 1 ml flush after each blood sample (50 units heparin/ml). Before each blood sample 0.6 ml was withdrawn from the 3 way tap and discarded, then a 1 ml sample for GH was taken.

SUBJECTS AND METHODS

IGF-I ADMINISTRATION

Subjects attended the investigation ward fasted at 08.00 h. They were given a bed and an antecubital vein was cannulated. At 09.00 h a subcutaneous injection of either recombinant IGF-I (rhIGF-I) (40 μg/kg) or placebo (diluent only) was administered.

Blood was obtained at 20 minute intervals for measurement of plasma GH, LH, FSH; at 60 minutes intervals for plasma prolactin, TSH, cortisol, ACTH, glucose assay and at 6 hourly intervals for plasma IGF bioactivity, IGF-I, IGF-II, insulin, C-peptide, and IGF binding proteins measurements over 24 hours. Twenty-four hours after rhIGF-I or placebo administration, an intravenous bolus of 100 μg of GHRH was administered and blood obtained for plasma GH measurement at 15 minute intervals for a further two hours. Bedside glucose monitoring (BM stix, Reflolux S, Boehringer Mannheim, Brighton, UK) was also performed at hourly intervals throughout the study.

One hour after the subcutaneous injection, breakfast was provided and the subjects were thereafter allowed to move freely within the ward. Meals were served at standardised times and bedtime was 22.30 h.

DEXAMETHASONE ADMINISTRATION

Subjects received 2 mg oral dexamethasone or placebo at precisely 6-hourly intervals

(09.00, 15.00, 21.00, 03.00, 09.00, 15.00, 21.00, 03.00 h) for 48 h before reporting to our investigation ward at 08.00 h, having eaten and drunk nothing since midnight, for a GHRH test.

ORIGINS OF DRUGS

For all the studies except the IGF-I study, the GHRH tests were performed using synthetic GHRH(1-29)NH_2, which was donated by Serono Laboratories (UK) Ltd (Welwyn Garden City).

For the IGF-I study, synthetic GHRH(1-29)NH_2 and IGF-I were donated by Pharmacia (Milton Keynes).

2 mg dexamethasone tablets were purchased from Organon Laboratories Ltd (Cambridge).

Synthetic DC-21-346 was a gift from Professor David H Coy of Tulane University School of Medicine, New Orleans, USA.

Identical 60 mg pyridostigmine and placebo capsules were manufactured by the pharmacy of St Bartholomew's Hospital.

SUBJECTS AND METHODS

HORMONE ASSAYS

GH, ACTH, TSH, LH, FSH and prolactin were measured by specific and sensitive immuno-radiometric assays (IRMA) developed by the North East Thames Regional Immunoassay unit (NETRIA). All assays were performed in duplicate and radioactivity was counted using NE1600 multihead γ-counters. The reagents for all the IRMAs were standardised.

IRMA reagents

Stock Phosphate Buffer

15.3 g $NaH_2PO_4.2H_20$

57.1 g Na_2HPO_4

to 1 litre with distilled water

Assay Buffer (prepared fresh for each assay)

10 ml stock phosphate buffer

1 ml 10% sodium azide

1 g bovine serum albumin - Fraction V

5 ml 10 % Tween 20

to 100 ml distilled water

Wash Buffer

100 ml stock 0.5 M phosphate buffer

10 ml 10% sodium azide

50 ml 10% Tween 20

to 1 litre distilled water

GROWTH HORMONE

A sheep anti-GH antiserum and a mouse monoclonal anti-GH antibody are used in this assay. The reaction is carried out at room temperature overnight with all reagents added simultaneously. Separation was achieved using low g centrifugation. Two simple washing steps are used to minimise the non-specific binding (blank). The sensitivity of the assay was 0.5 mU/l and the coefficient of variation 5.3% at 5.3 mU/l and 1.9% at 32 mU/l.

Procedure:

100 µl sample/standard

300 µl assay buffer

50 µl ^{125}I-labelled antibody (~100,000 counts per minute)

50 µl solid phase first antibody

vortex and placed on rotary mixer overnight

add 2 ml wash buffer

centrifuge for 5 minutes at 1000 g.

decant supernatant and repeat washing step

decant and count for 300 seconds

SUBJECTS AND METHODS

TSH, LH and FSH

A sheep anti-LH or FSH anti-serum and a purified anti-LH antibody (monoclonal for FSH) were the basis for a specific and sensitive IRMA for human LH and FSH. For the TSH assay, a sheep anti-TSH antiserum and a mouse monoclonal anti-TSH antibody were used. The reaction is carried out at room temperature overnight with all reagents added simultaneously. Separation was achieved using low g centrifugation. Two simple washing steps are used to minimise the non-specific binding (blank). The sensitivity of the TSH assay was 0.04 mU/l; LH and FSH 0.2 mU/l. The coefficient of variation for TSH was 4.1% at 1.1 mU/l and 2.3% at 10 mU/l, for LH 3.5% at 2.2 m U/l and 2.3% at 9.6 mU/l, and for FSH 7.0% at 2.6 mU/l and 4.4% at 9.6 mU/l.

Procedure:

50 µl sample/standard

300 µl assay buffer

50 µl ^{125}I-labelled antibody (~60,000 counts per minute)

50 µl solid phase first antibody

vortex and placed on rotary mixer overnight

add 2 ml wash buffer

centrifuge for 5 minutes at 1000 g.

decant supernatant and repeat washing step

decant and count for 300 seconds

SUBJECTS AND METHODS

PROLACTIN

The two antibodies were a sheep anti-prolactin and mouse monoclonal anti-prolactin antibody. The solid phase was reacted with the sample/standard at room temperature for approximately 3 hours. Following a wash step, the radioiodinated antibody was added and the reaction continued overnight. All reagents were added simultaneously. Separation was achieved using low g centrifugation. Two simple washing steps were used to minimise the non-specific binding (blank). The assay was sensitive down to 10 mU/l and the coefficient of variation was 5.3% at 151 mU/l and 2.9% at 460 mU/l.

Procedure:

50 µl sample/standard

400 µl assay buffer

50 µl solid phase antibody

vortex and placed on rotary mixer overnight

add 2 ml wash buffer

centrifuge for 5 minutes at 1000 g.

decant supernatant and repeat washing step

decant and add:

400 µl assay buffer

50 µl ^{125}I-labelled antibody (~60,000 counts per minute)

vortex tubes and place on rotary mixer overnight

add 2 ml wash buffer

centrifuge for 5 minutes at 1000 g.

decant supernatant and repeat washing step

count for 100 seconds

ADRENOCORTICOTROPHIN (ACTH)

Antibodies raised in rabbits against the two ends of the ACTH peptide (N-terminal and C-terminal) anti-(1-24)ACTH and anti-(18-39)ACTH antibody. The solid phase is reacted with the sample/standard at room temperature overnight. Following a wash step, the radioiodinated antibody is added and the reaction continued for 4 hours. The limit of detection was 10 ng/l and the coefficient of variation 9.5% at 58 ng/ml.

Procedure:

200 µl sample standard

50 µl solid phase First antibody (anti-(1-24)ACTH)

vortex tubes, then place on rotary mixer overnight

add 2 ml wash buffer

centrifuge for 5 min at 1000 g.

decant supernatant and repeat washing step

decant and add:

200 µl assay buffer

50 µl ^{125}I-labelled antibody (anti-(18-39)ACTH) (25,000 cpm)

vortex tubes and place on a rotary mixer for 4 hours

add 2 ml wash buffer

centrifuge for 5 min at 1000 g

decant supernatant and repeat washing step

decant and count for 300 seconds

INSULIN-LIKE GROWTH FACTOR-I

IGF-I was measured by radioimmunoassay.

Reagents

Assay buffer 0.03 M phosphate, with added protamine sulphate (0.04% w/v), sodium azide (0.04% w/v), EDTA (0.74% w/v) and Tween 20 (0.05% w/v); the buffer was titrated to pH 7.5 using sodium hydroxide. The first antibody, UBK 487, was raised in rabbit against IGF-I (Dr L Underwood and Dr JJ Van Wyk, University of North Carolina, USA). UBK 487 was stored at -20°C as 40 µl aliquots of 1/10 diluted serum until use, and then diluted to 1/1800 with reagent buffer. The final working dilution of UBK 487 was 1/18,000 (see below). The standard used in the assay was extracted pooled normal human serum. Normal human serum was defined as containing 1.0 U IGF-I/ml and equivalent to 159 ng/ml of a highly purified preparation of IGF-I. The extracted standard was stored as assay aliquots for each assay to give a standard curve of 71.4, 35.7, 17.9, 8.93, 4.46, 2.23, 1.12 mU/l (see below). Aliquots of ^{125}I-labelled IGF-I (prepared from recombinant human IGF-I (Pharmacia batch no. 2107) were diluted with reagent buffer to give 10,000 cpm/100 µl. The second antibody was donkey anti-rabbit Sac-cell.

SUBJECTS AND METHODS

Method of extraction

Before RIA, samples were first extracted using a standard acid ethanol method. 100 µl of sample was mixed with 400 µl of the acid ethanol mixture (12.5% 2 M hydrochloric acid and 87.5% absolute ethanol). After vortexing and standing for 5 minutes, the extraction mixture was centrifuged at 10,000 rpm for 5 minutes. 200 µl of the supernatant was removed and neutralised with 80 µl of Tris base (hydroxymethyl-amino-methane)(0.86 M). This neutralised extraction mixture, after vortexing and standing for 30 minutes at 4°C, was centrifuged as before. The supernatant was then used in the assay and will be termed the extracted sample. An extracted buffer was prepared using 100 µl of reagent buffer to which was added 400 µl, this mixture was then diluted with 3.6 ml of reagent buffer. The final preparation was used in the standard curve and will be termed extracted buffer. The extracted sample were diluted a further 32 times before assay.

Procedure:

To 50 µl aliquots of duplicate extracted samples, standard or buffer (zero and NSB tubes) was added 350 µl of reagent buffer followed by 50 µl of antibody and 50 µl of ^{125}I-labelled IGF-I. Label alone was added to empty total count tubes. UBK 487 was not added to NSB tubes, being replaced by a further 100 µl of reagent buffer. After vortexing, the assay tubes were incubated overnight at 4°C. 50 µl of Sac-cell was added to all tubes except to total count tubes. After vortexing, the assay tubes stood for 1 hour at 4°C, and then 1 ml of distilled water was added to all tubes except to total count tubes. After vortexing the assay tubes were centrifuged at 2000 rpm at 4°C for 30 minutes. The supernate was aspirated to waste. The pellet was then counted for 120 seconds. The assay working range was 0.25 -

1.6 U/ml (40 - 254 μg/l). Within assay coefficient of variation was less than 5% and the between assay coefficient of variation was less than 10%.

INSULIN-LIKE GROWTH FACTOR-II

IGF-II was measured by radioimmunoassay after acid-ethanol extraction using the same methodology and procedure as for IGF-I. An IGF-II monoclonal antibody (MAS 324, clone S1-F2;Seralab, Crawley Down, Sussex, UK) was used at a dilution of 1 in 54 000. Recombinant IGF-II (Pharmacia, Stockholm, Sweden) was used as standard to give an analytical range of 5 - 1250 μg/l. Bound and free ^{125}I-labelled IGF-II were separated using a solid-phase second antibody. Within assay coefficient of variation was 12.9% and between assay coefficient of variation was 4.7%. The cross-reactivity of IGF-I was 1.5% and insulin did not interfere in the assay.

IGF BIOASSAY

IGF bioactivity was measured by an Eluted Stain Assay (ESTA) using the Fischer rat thyroid cell line FRTL-5. This assay is based on the colour change seen in tetrazolium salts on mild reduction. One of these salts, MTT when reduced turns to its purple formazan. The formazan reaction, on addition to cell cultures, is only possible in the presence of viable and metabolically active cells.

Procedure:

SUBJECTS AND METHODS

FRTL-5 cells were routinely cultured in 6H medium (contains cortisol, insulin, TSH, transferrin, somatostatin and glycol-L-histidyl-L-lysine acetate) and plated out into 96-well microtitre plates. After 4 days 0H medium (no hormones), supplemented with 5% serum, was substituted and the cells grown for a further 9 days. For a further 48 hours, cells were grown in 100 µl OH medium with 0.1% bovine serum albumin (BSA). Samples (100 µl) were added to the microtitre wells and incubated for a further 48 hours. Cell activity was calculated by adding 10 µl of the tetrazolium salt MTT to each well and incubating for 30 minutes at 37°C. The formazan reaction product was then eluted from the cells into the medium by addition of acidified Triton X-100 (50 µl/well). The microtitre plates were agitated for a further 20 minutes at room temperature. The optical density of each well was measured using a Bio-Rad microtitre plate reader at test wavelength 595 nm and reference wavelength 655 nm. Within assay variation was 14% and between assay variation 13% (Claffey *et al.* 1994).

INSULIN

Insulin was measured by radioimmunoassay.

Reagents

Assay buffer was 0.05 M phosphate saline, with added bovine serum albumin (0.05% w/v), EDTA (0.37% w/v) and thiomersal (0.1% w/v); the buffer was titrated to pH 7.4 using sodium hydroxide. Antiserum, raised in guinea-pig against bovine insulin, was diluted to 1/32,000 with assay buffer to give a final dilution of 1/48,000. The standard used in this

assay was recombinant insulin, double diluted with reagent buffer to give a standard curve 200, 100, 50, 20, 10, 5, 2, 1 mU/l. ^{125}I-labelled insulin was obtained from Amersham International (Amersham, UK), stored as 2 μCi aliquots which were then diluted with reagent buffer to give 30,000 cpm/100 μl. The second antibody was sheep anti-guinea-pig gamma globulin prepared for each assay by dilution to 1/500 with reagent buffer, to which was added guinea-pig (carrier) serum at a dilution of 1/4000. This was mixed with the precipitating agent polyethylene glycol (PEG) (3% w/v), sodium chloride (0.09% w/v) and triton X-100 (50 μl/100 ml distilled water).

Procedure:

To 100 μl of duplicate sample, standard or buffer (zero and NSB tubes) was added 100 μl of ^{125}I-labelled insulin and 400 μl of anti-insulin antibody. Anti-insulin antibody was not added to NSB tubes being replaced by a further 400 μl of reagent buffer. Label alone was added to empty tubes (total count tubes). After vortexing, the assay tubes were incubated overnight at 4°C. 400 μl of second antibody/precipitating solution was added to each tube except total count tubes. After vortexing, the assay tubes were stood for 2 hours at 4°C, and then centrifuged at 2500 rpm for 30 minutes at 4°C. The supernate was aspirated and discarded, and the pellet washed with a further 1.5 ml of wash buffer. After vortexing, the assay tubes were centrifuged at 2500 rpm at 4°C for 30 minutes. The supernate was aspirated and discarded. The radioactivity in the solid phase pellet was then counted for 180 seconds. The working range of the assay was 1 - 200 mU/l. The within assay coefficient variation ranged from 4 - 6% and the between assay coefficient variation ranged from 8 - 10%.

SUBJECTS AND METHODS

C-PEPTIDE

Blood collected into trasylol was rapidly separated and the plasma frozen immediately at -20°C. Plasma C-peptide was measured in duplicate in using with second antibody separation. Sensitivity was 0.05 nmol/l and the within assay coefficient of variation was less than 4% and the between coefficient of variation was less than 15%.

CORTISOL

Cortisol was measured by an in-house radioimmunoassay.

Reagents

The assay buffer was 0.05 M phosphate; the buffer was titrated to pH 7.4 using sodium hydroxide. Antiserum to cortisol-3-carboxy-methyl-oxime (3CMO) attached to Keyhole limpet haemocyanin was raised in a sheep. Antiserum was diluted to 1/5,000 with assay buffer to which was added second antibody (donkey anti-sheep serum) to give a dilution of 1 in 20. Standards were prepared in human "charcoal stripped" serum to give a standard curve of 2000, 1000, 500, 250, 100, 50 nmol/l. An aliquot of ^{125}I-labelled cortisol-3CMO was diluted with reagent buffer containing ANS (12 mg/ml) to give 10,000 cpm/100 µl. The precipitating agent was polyethylene glycol 6000 (5% w/v).

Procedure:

To 50 µl of duplicate sample or standard was added 100 µl of ^{125}I-labelled cortisol 3CMO (with 12 mg/ml ANS) and 100 µl of antiserum. Label only was added to empty tubes for

the total count tubes. After vortexing the assay tubes were incubated for 90 minutes at 21°C. 1 ml of precipitating solution was added to each tube except total count tubes. After vortexing, the assay tubes were centrifuged at 3000 rpm for 30 minutes at 21°C. The supernate was aspirated to waste. Pellet radioactivity was counted for 100 seconds. The working range for this assay was 50 - 2000 nmol/l. The within assay coefficient variation for this assay was less than 5% and the between assay coefficient variation was < 7%.

TESTOSTERONE

Total testosterone in serum was determined, after ether extraction, by RIA.

Reagents

Standards, ^{125}I-Testosterone (10,000 cpm/100 µl; purchased from Dr M Wheeler, St Thomas' Hospital, London) and sheep antiserum (1:60,000 initial dilution; raised in-house against testosterone-3-CMO-KLH) were all in assay buffer (0.05 M phosphate).

Procedure:

Patient serum (0.1 ml for males / 0.5 ml for females) was extracted with 5 ml diethyl ether. The aqueous layer was "flash frozen" and the organic layer decanted into a clean glass tube. The ether was evaporated to dryness and the residue reconstituted in 500 µl 50 mmol phosphate buffer (pH 7.5). To 100 µl standard or reconstituted sample was added 100 µl ^{125}I-testosterone (10,000 cpm) and 100 µl antiserum. Separation of antibody-bound and free hormone was achieved using 500 µl dextran coated charcoal suspension (1% w/v). Thereafter, radioactivity in the control pellet was determined. The assay had three quality

control values at approximately 2.0, 5.0 and 12.0 nmol/l. Between assay variability at these concentrations was less than 10 % (Samaras *et al.* 1992).

OESTRADIOL

Oestradiol was measured with a commercial kit purchased from Diagnostic Products Limited (Llanberis, Wales). It is a double antibody kit that uses a simple late addition of tracer.

Procedure:

Patient serum was incubated with antiserum for 1 hour, after which ^{125}I-oestradiol was added and the mixture incubated for a further 2 hours. A PEG-assisted second antibody solution was used to stop the RIA. After centrifugation (3000 rpm for 30 min at 18^{0}C) and aspiration of the supernate, the radioactivity in the pellet was determined, and, by extrapolation from serum standards, the concentration in the patient's serum was derived. The assay had three quality control samples at approximately 150, 600 and 1100 pmol/l. The between assay variability at these concentrations was less than 8%.

PROGESTERONE

Progesterone was measured by direct RIA, using in-house reagents.

Procedure:

The assay used a phosphate/citrate buffer at pH 4.0 in order to obviate the necessity of an

extraction step. Patient serum/standards and QC's were incubated with ^{125}I-progesterone and a goat raised anti-progesterone antibody. After incubation for two and a half hours, separation was achieved by adding 20% polyethylene glycol. Three QC samples were used at 6, 30 and 60 nmol/l. Between assay variability was less than 10% at these concentrations (Wathen *et al.* 1984).

SEX HORMONE BINDING GLOBULIN

SHBG was measured by a competitive protein binding assay, based upon the binding of ^{3}H-DHT to SHBG.

Procedure:

The assay was performed in duplicate with four tubes for each patient sample. To each tube 400 µl serum diluted 8 fold with 50 mmol phosphate buffer was added. To two tubes 100 µl of a mixture of 0.75 ng DHT, including 15 000 dpm ^{3}H-DHT (D tubes), in buffer was added and to two further "quenching" tubes 100 µl of a mixture of 100 ng DHT, including 15 000 dpm ^{3}H-DHT (T tubes). All tubes were incubated for 10 minutes at 4°C. The SHBG was precipitated by addition of an equal volume of saturated ammonium sulphate (4°C) mixed and incubated for a further 10 minutes. This was followed by centrifugation (3000 rpm, 20 min., 4°C). The supernate (free fraction) was decanted into a vial containing scintillation fluid (4 ml). Percentage of DHT-bound was calculated by: (counts in T - D tubes/ counts in T tubes) x 100

This figure was then used to read the value for SHBG from the standard curve. The assay employs three QC samples at 20, 40 and 80 nmol/l. Between assay variability at these concentrations was less than 6% (Fattah and Chard, 1981).

GLUCOSE

Plasma glucose was measured by the hexokinase/glucose dehydrogenase method using the Coulter CPA analyzer.

SUBJECTS AND METHODS

STATISTICS

Samples below the level of assay sensitivities were assigned a value of zero. "Area under the curve" (AUC) was calculated by the trapezoidal method. Data with a Gaussian distribution were analysed by paired or unpaired t-tests. Non-parametric paired data were analysed by the Wilcoxon rank sum test and unpaired data with the Mann-Whitney U test (Siegel, 1956). One-way analysis of variance was used as indicated. For ease of viewing in this thesis, however, the raw data have been presented graphically. Error bars have not been included in figures if both the data lack statistical significance and inclusion of error bars would detract from the clarity of the figure. In every case, statistical significance was taken as $P \leq 0.05$. Two-tailed probability tables were used except where indicated.

GH pulsatility was analysed using the "*Pulsar*" programme of Merriam and Wachter. This software is fundamentally a "threshold' method, scaled to the assay standard deviation, as in the modified Santen and Bardin method (1973). Essentially, Santen and Bardin defined a peak as a rise of 20% or more from a nadir. The criterion of 20% represented approximately 3 coefficients of variation of the assay. As the CV of an assay varies at different points on the standard curve, this method was modified such that a peak was defined in terms of "n" times the CV for the appropriate point on the standard curve.

Pulsar calculates a "baseline" to represent the contribution of circadian rhythms and long-term trends purged of ultradian rhythms i.e. trends with time constants less than 6 - 12

hours. Pulses are judged against a baseline rather than a single point nadir, the width of peaks is considered in setting the threshold for a peak, ie wide peaks need not be as high as narrow ones, and the method iterates to attempt to distinguish clusters of peaks from trends. The programme was originally developed for gonadotrophin analysis, and has been adapted for analysis of GH data. This involved assessing default characteristics within *pulsar* for the analysis of GH (see below).

This method has the advantage over alternative pulse analysis algorithms that peaks are measured from smoothed baselines, which are less vulnerable to single bad datum points or "notches" in peaks. Peak heights are scaled in terms of assay noise, and amplitude of the noise can vary depending upon the dose; this is most useful when the data lie towards one end of the standard curve. Peaks do not have to be as high if they are sustained, which random variations are less likely to be.

A large number of pooled samples were analysed, and means and standards deviation were calculated at the various different GH levels (see below). Assay noise was then represented by a linear equation describing the assay standard deviation (SD) at any GH concentration (y):.

$$\text{standard deviation} = (0.89y + 14.5)/100$$

Using the linear equation the G-values (the number of SD's a rise of GH must exceed for 1, 2, 3, 4 and 5 times points respectively in order to represent a GH pulse) were assessed

empirically.

Ten representative 9-hour GH profiles were plotted, and visual assessment for peaks was performed blind by ten observers. The G-value were then set by eye to minimise the false-negative and false-positive results of the visually assessed peaks. Various G-values produced from probability theory were assessed, but found to produce large numbers of false positive GH pulses. The higher G values that have been derived by Hindmarsh et al. (1987) were found to be the most suitable.

When a smoothing time of 540 minutes was used (the automatic default value in interactive mode) there was a tendency for peaks in certain profiles to be missed, with a concomitant increase in the baseline. Reduction of the smoothing time to 6 hours (360 minutes) appeared to resolve this problem.

The remaining default values for the GH

Number of iterations:	6
Weight assigned to peak points:	0.10
Peak splitting (SD):	3.0
G(1):	6.50
G(2):	5.20
G(3):	3.80
G(4):	3.00
G(5):	2.40

TABLE 1

Within and between assay coefficient of variation for the GH IRMA

WITHIN ASSAY		**BETWEEN ASSAY**	
Mean GH (mU/l)	**CV(%)**	**Mean GH (mU/l)**	**CV(%)**
0.4	22.7	2.7	8.5
5.3	5.3	11.0	4.8
12.7	2.0	30.3	6.9
32.5	1.9	74.4	9.7
82.8	2.4		
153.3	4.4		

RESULTS

THE EFFECT OF SUBCUTANEOUS RECOMBINANT IGF-I (rhIGF-1 40 μg/kg) ON ANTERIOR PITUITARY FUNCTION

Twelve healthy non-obese male volunteers were studied on two occasions in random order. The two limbs of the study were rhIGF-I (40 μg/kg subcutaneously) followed by GHRH (100 μg intravenously) at 24 hours and placebo followed by GHRH. The rhIGF-I was administered and GHRH tests were performed in the manner described in the previous chapter. All studies were carried out at a minimum interval of two weeks in a randomized double-blind manner. The details of the subject are given in Table 2.

Analysis for a treatment-period interaction indicated that there was neither an order nor carry-over effect between the two limbs (Jones & Kenward, 1989).

There was a significant increase of 80% in AUC for plasma IGF-I measured by radioimmunoassay following rhIGF-I administration compared with placebo (Table 3). Mean plasma IGF-I immunoactivity peaked at six hours and thereafter gradually declined but was still above baseline at 24 hours, although within the normal range (Figure 4). A significant reduction of approximately 15% in AUC for plasma IGF-II was seen following rhIGF-I administration (Figure 5). AUC for IGF bioactivity was significantly increased by 30% following rhIGF-I administration. As with IGF-I immunoreactivity, the peak occurred

at 6 hours and thereafter decreased (Figure 6).

There was no biochemical or clinical evidence of hypoglycaemia, the lowest laboratory blood glucose recorded was 3.9 mmol/l, and no difference in mean blood glucose between the two limbs of the study (rhIGF-I mean 5.43 mmol/l SEM ± 0.06 v placebo 5.55 ± 0.06)(Figure 7).

No difference existed in AUC, pulse number or pulse amplitude for plasma GH, LH or FSH between the two limbs of the study (Tables 4, 5, Figures 8, 9, 10). The AUC for GH following intravenous GHRH (100 μg) was not significantly different, but the peak GH response was greater following rhIGF-I (mean 97.5 ± SEM 14.6 v 56.3 mU/l ± 9.6, p = 0.05, Figure 11). Administration of rhIGF-I resulted in a significant fall in AUC for plasma TSH and at each individual time-point mean plasma TSH was lower following rhIGF-I than placebo. The circadian rhythm of TSH remained intact (Figure 12). There was no change in free thyroxine and free triiodothyronine 24 hours after rhIGF-I administration. No change was seen in AUC for plasma ACTH, cortisol or prolactin (Table 4, Figures 13, 14, 15).

Both mean plasma C-peptide (0.91 nmol/l ± 0.05 v 0.73 ± 0.06, p = 0.03, Wilcoxon sign rank test) and insulin (15.36 mU/l ± 1.18 v 10.81 ± 1.02, p = 0.03, Wilcoxon sign ranked test) were lower following rhIGF-I. As C-peptide and insulin were measured only at 6 hourly intervals, it is not possible to be precise about the time scale of the changes; however

plasma levels had returned to baseline 18 hours after rhIGF-I (Figures 16, 17).

IGF-BP3 was the most abundant binding protein identified on the Western ligand blots and did not alter with administration of rhIGF-I. Evaluation of IGF-BP1 levels showed no discernible change with rhIGF-I (Table 6, Figure 18).

All subjects experienced transient discomfort at the injection site, probably due to the pH of the diluent. No side-effects were encountered with IGF-I, specifically no symptoms of hypoglycaemia were reported. Short-lived facial flushing following GHRH was uniform.

In summary, a single subcutaneous dose of 40 μg/kg of recombinant human IGF-I increased AUC for plasma IGF-I by 80%, measured by radioimmunoassay, and IGF bioactivity by 60%. Blood glucose did not change but plasma insulin and C-peptide fell by 20%. The primary object of this study was to examine the effect of IGF-I, by the therapeutic route, on anterior pituitary function. No change was seen in physiological GH secretion but pretreatment with IGF-I augmented the GH response to an intravenous bolus of 100 μg GHRH 24 hours later. Area under the curve for TSH fell by 30% following IGF-I but no change occurred in ACTH (or cortisol), LH, FSH or prolactin secretion.

RESULTS

Table 2

The age, weight, height and body mass index of the 12 subjects receiving IGF-I

SUBJECT	AGE (years)	WEIGHT (kg)	HEIGHT (m)	BMI (kg/m^2)
1	22	75.0	1.70	24.2
2	23	66.9	1.76	23.1
3	23	61.2	1.69	21.4
4	26	82.3	1.88	23.1
5	22	84.9	1.90	23.5
6	22	66.6	1.77	21.2
7	24	64.2	1.81	19.6
8	21	84.5	1.84	25.0
9	27	67.5	1.81	20.6
10	23	89.5	1.91	24.5
11	23	71.6	1.68	25.3
12	23	66.5	1.71	22.7
MEAN	**23.4**	**73.4**	**1.79**	**22.9**
RANGE	**21-27**	**61.2-89.5**	**1.68-1.91**	**19.6-25.4**

Table 3

The 24 hour mean "area under the curve" for IGF-I and IGF-II measured by radioimmunoassay and IGF bioactivity (mean ± SEM shown)

Drug	IGF-I(RIA)	IGF-II(RIA)	IGF(bio)
	(ng.h/ml)	(ng.h/ml)	(U.h/l)
rhIGF-I	7065 ± 33[1]	9308 ± 403	22.5 ± 3.4[2]
Placebo	3895 ± 204	11052 ± 451[1]	14.2 ± 1.8

[1] $p < 0.0001$, [2] $p = 0.001$ vs placebo

Table 4

The 24 hour mean "area under the curve" for each of the anterior pituitary hormones (mean ± SEM shown)

	Area under curve	
	placebo	**IGF-I**
GH (mU.h/l)	86.6 ± 14.7	79.2 ± 14.6
LH (mU.h/l)	123 ± 13.2	122 ± 10.5
FSH (mU.h/l)	85.0 ± 26.5	83.9 ± 23.4
TSH (mU.h/l)	42.5 ± 5.98	33.0 ± 3.36[1]
ACTH (ng.h/l)	279.8 ± 18.0	272.9 ± 22.8
Cortisol (nmol.h/l)	4986 ± 290	4849 ± 314
Prolactin (mU.h/l)	7201 ± 2336	7152 ± 2250

[1] $p = 0.01$ vs placebo

Table 5

The results of "*pulsar*" analysis of GH and LH secretion

	number of pulses		**mean pulse amplitude** (mU/l)	
	placebo	**IGF-I**	**placebo**	**IGF-I**
GH	4.1 ± 0.9	2.7 ± 0.4	22.3 ± 4.5	28.5 ±6.7
LH	10.8 ± 0.6	11.2 ± 0.8	2.8 ± 0.2	2.8 ± 0.2

Table 6

Plasma IGF-BPs, measured by Western Ligand Blot and quantified by densitometry, following recombinant human IGF-I or placebo in 12 healthy volunteers. Mean values expressed in arbitory units

TREATMENT			**rhIGF-I**			
TIME	**08.40**	**09.00**	**15.00**	**21.00**	**03.00**	**09.00**
BP4	1103	980	846	1029	1094	1094
BP1	1974	1639	1856	1826	1640	1625
BP2	4449	5396	6778	5951	4810	5164
BP3	12913	13428	15085	14430	13175	13916
			Placebo			
BP4	1069	1134	991	1082	1158	999
BP1	1887	1868	1534	1632	1619	1561
BP2	4803	4429	6162	6200	3788	5178
BP3	13300	12597	12773	12721	12729	14282

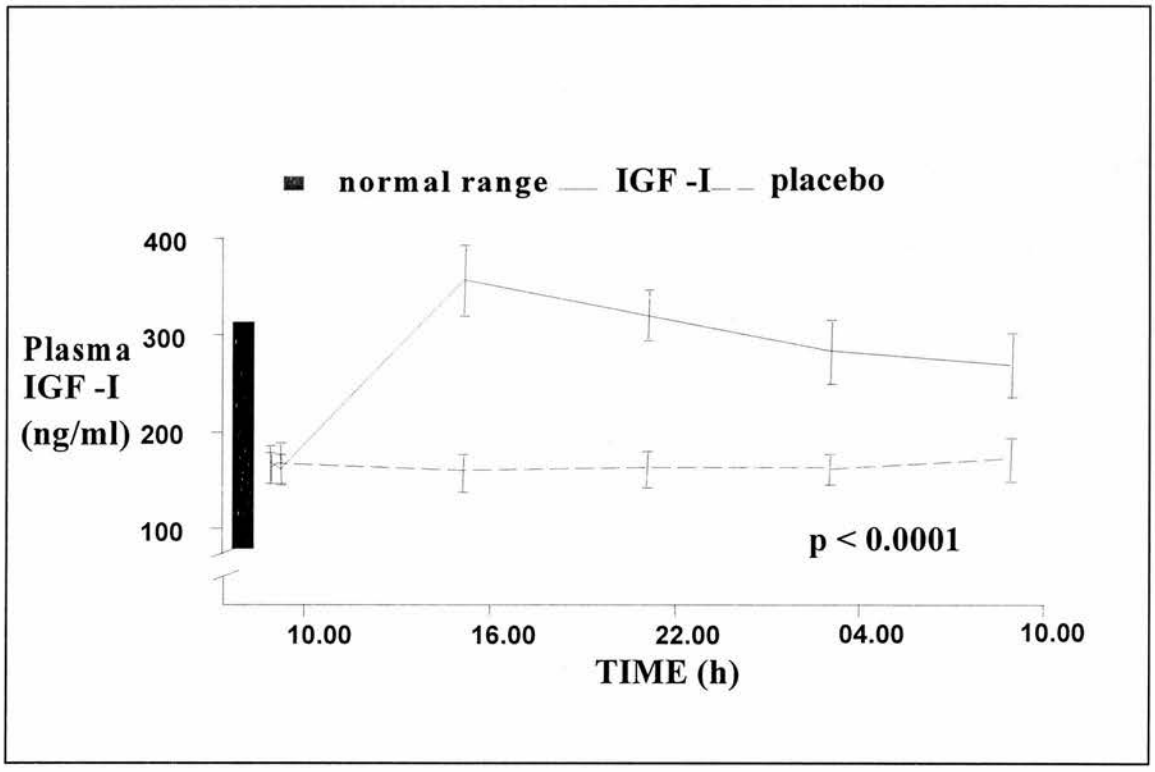

Figure 4

24 hour profile for mean plasma IGF-I measured by radioimmunoassay in 12 healthy subjects following 40 µg/kg rhIGF-I or placebo at 09.00 h given subcutaneously (mean ± SEM shown)

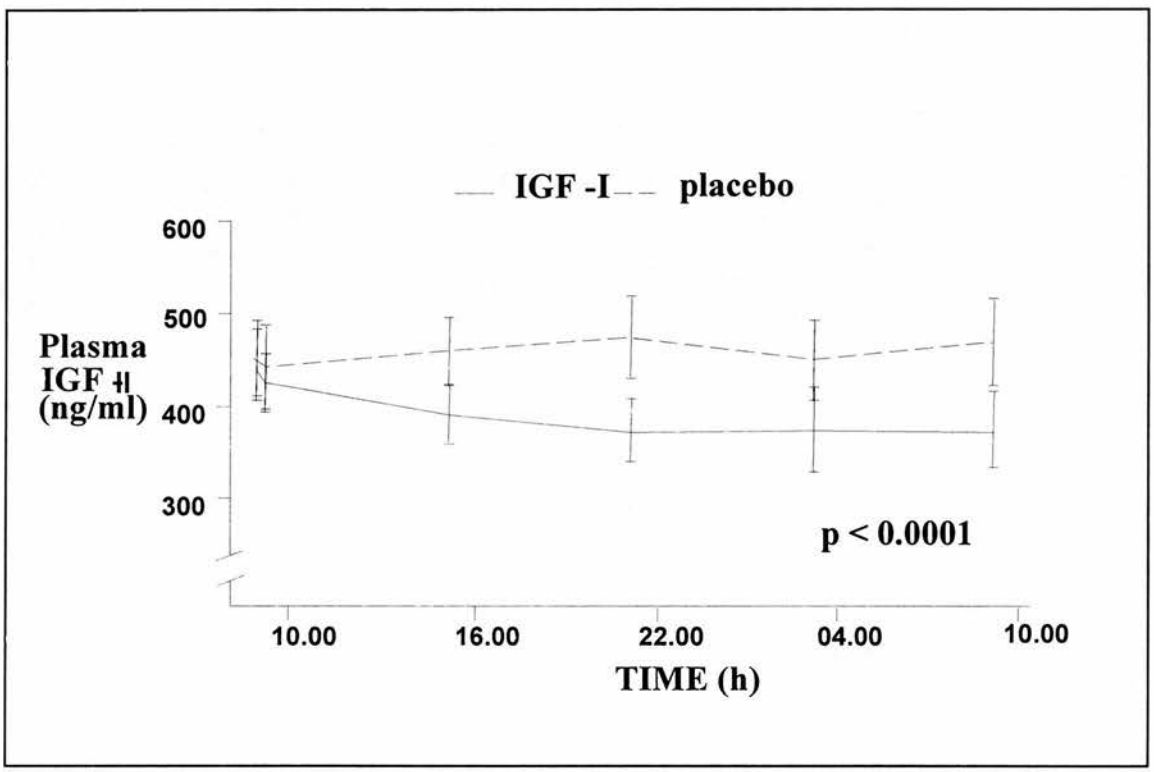

Figure 5

24 hour profile for mean plasma IGF-II measured by radioimmunoassay in 12 healthy subjects following 40 μg/kg rhIGF-I or placebo given subcutaneously at 09.00 h (mean ± SEM shown) .

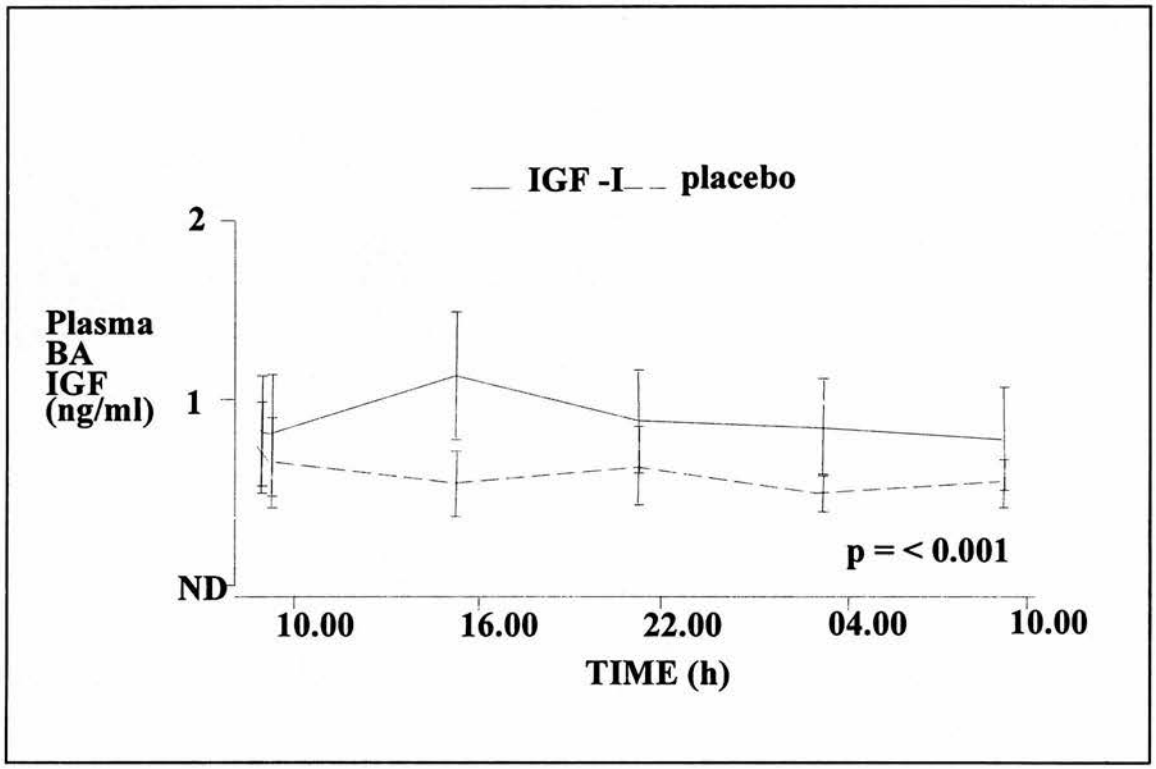

Figure 6

24 hour profile of mean plasma IGF bioactivity in 12 healthy subjects following 40 μg/kg rhIGF-I or placebo given subcutaneously at 09.00 h (mean ± SEM shown).

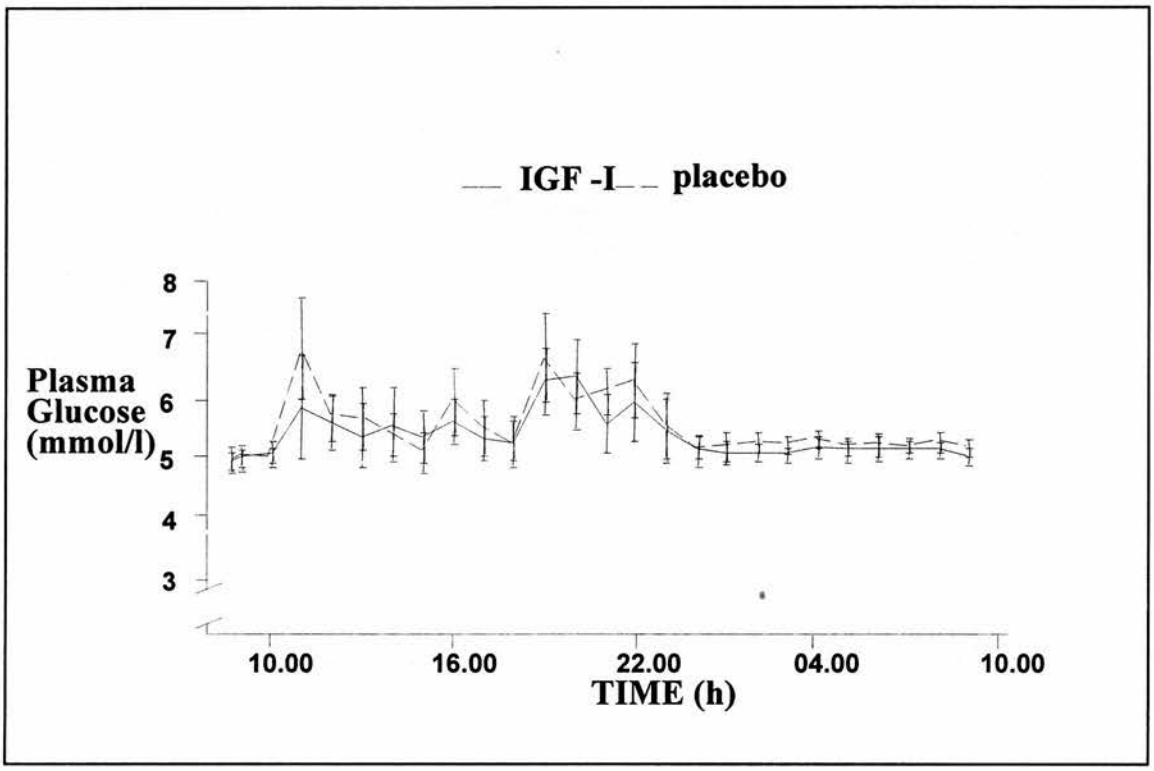

Figure 7

24 hour profile for mean plasma glucose in 12 healthy subjects following 40 μg/kg rhIGF-I or placebo given subcutaneously at 09.00 h (mean ± SEM shown).

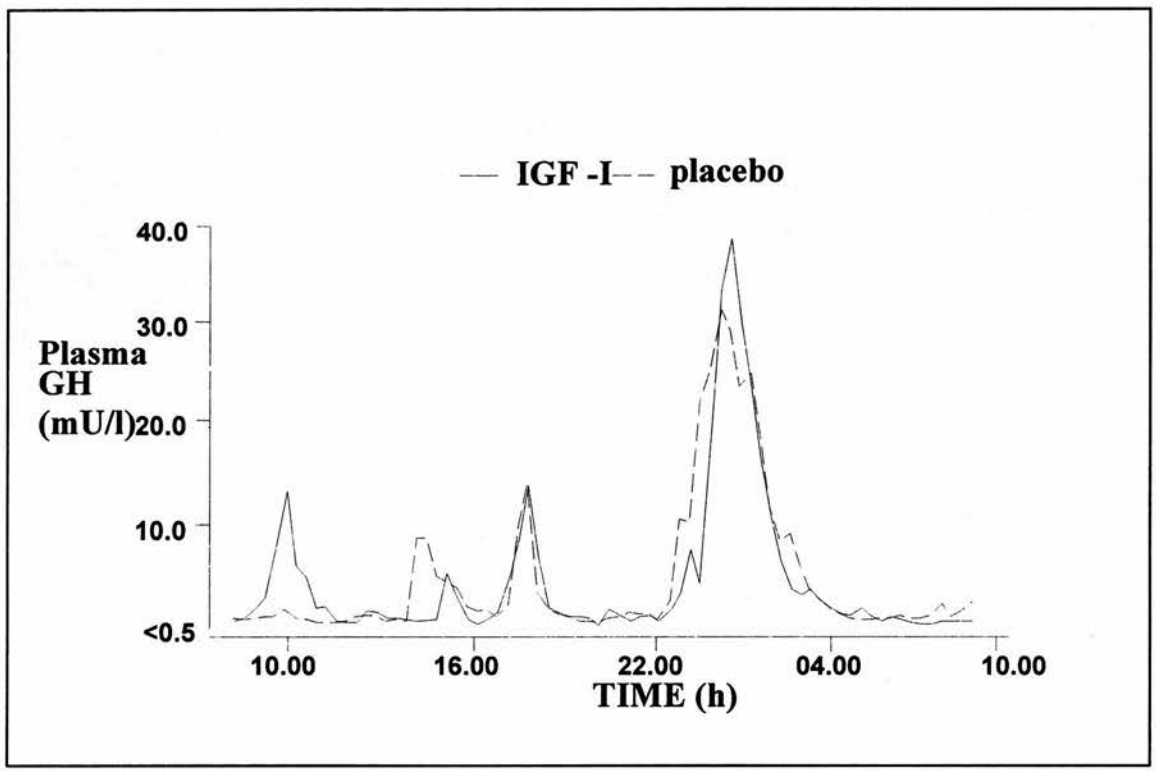

Figure 8

24 hour profile for mean plasma growth hormone in 12 healthy subjects following 40 μg/kg rhIGF-I or placebo given subcutaneously at 09.00 h (error bars not shown).

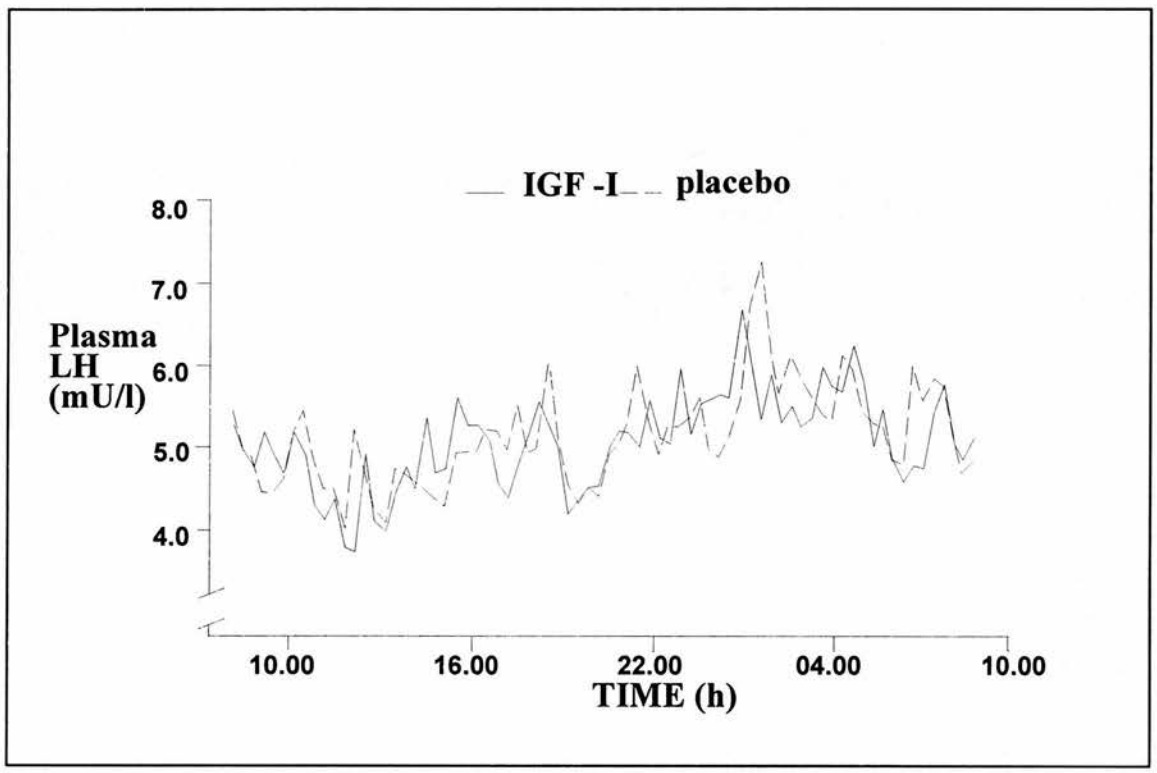

Figure 9

24 hour profile for mean plasma LH in 12 healthy subjects following 40 μg/kg rhIGF-I or placebo given subcutaneously at 09.00 h (error bars not shown).

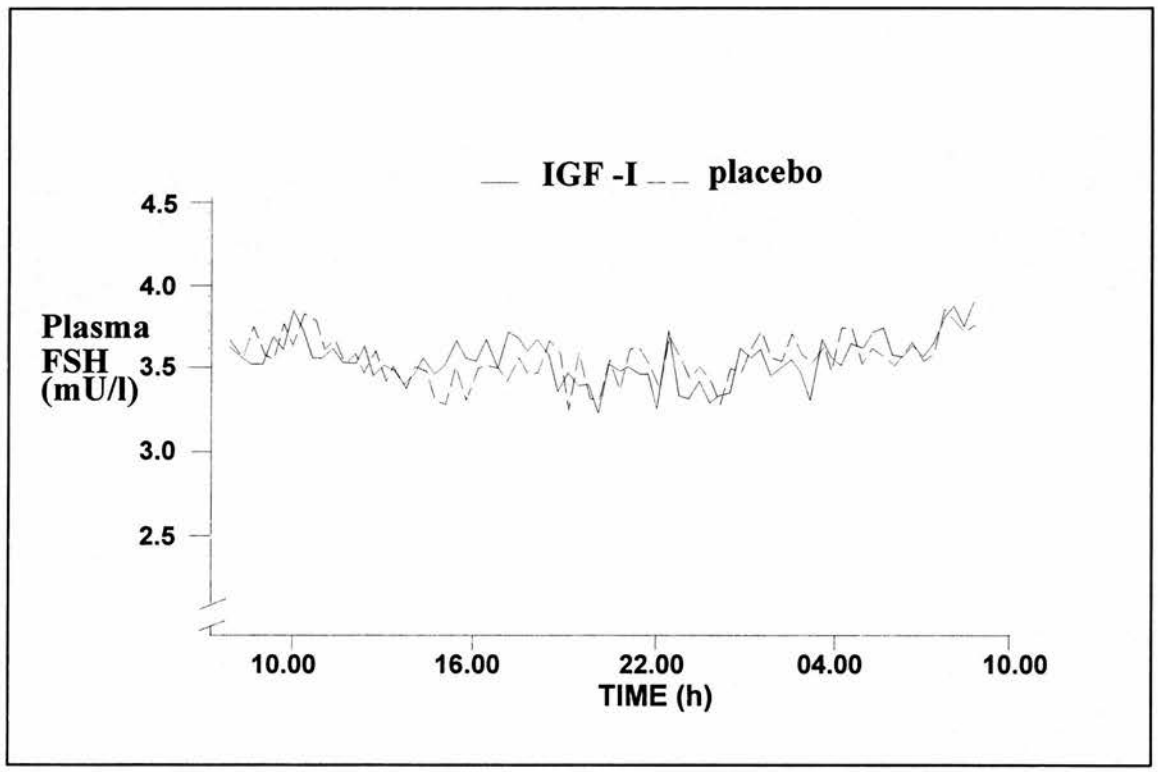

Figure 10

24 hour profile for mean plasma FSH in 12 healthy subjects following 40 μg/kg rhIGF-I or placebo given subcutaneously at 09.00 h (error bars not shown).

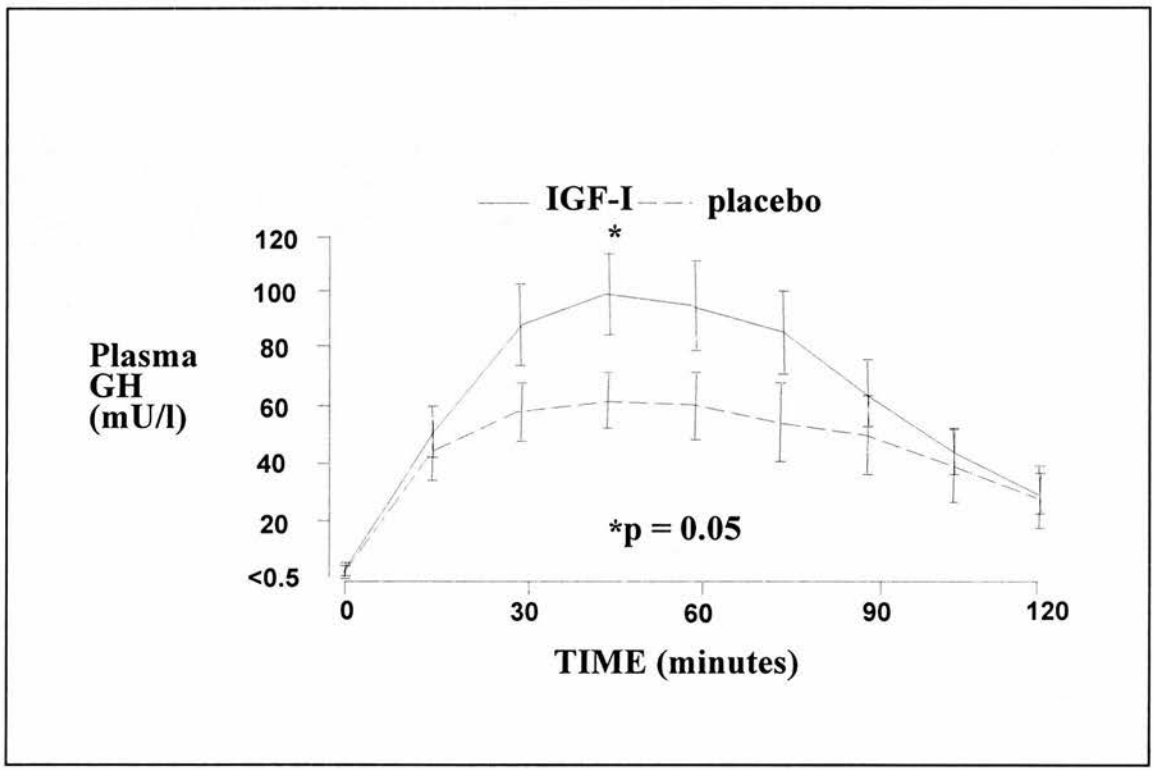

Figure 11

Mean GH values in 12 healthy subjects following intravenous GHRH (100 μg) at time 0 (09.00 h day 2), 24 hours after either rhIGF-I (40 μg/kg) or placebo (mean ± SEM shown).

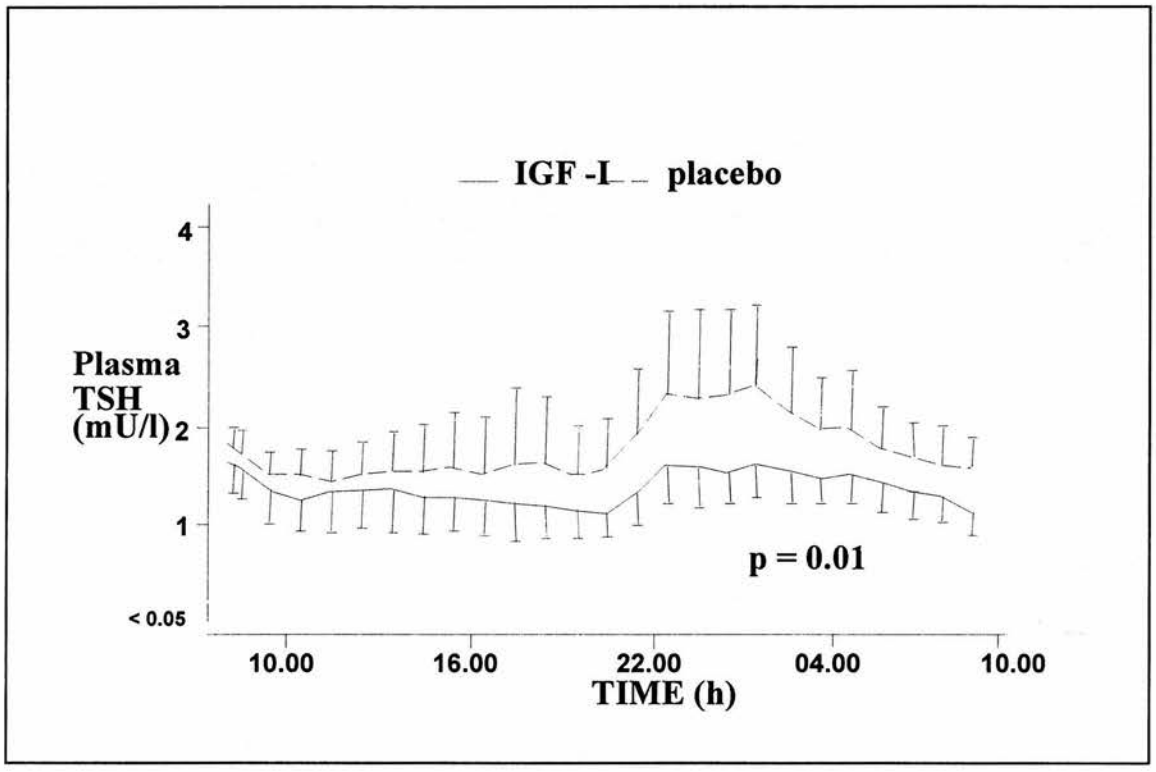

Figure 12

24 hour profile for mean plasma TSH in 12 healthy subjects following 40 μg/kg rhIGF-I or placebo given subcutaneously at 09.00 h (mean ± SEM shown).

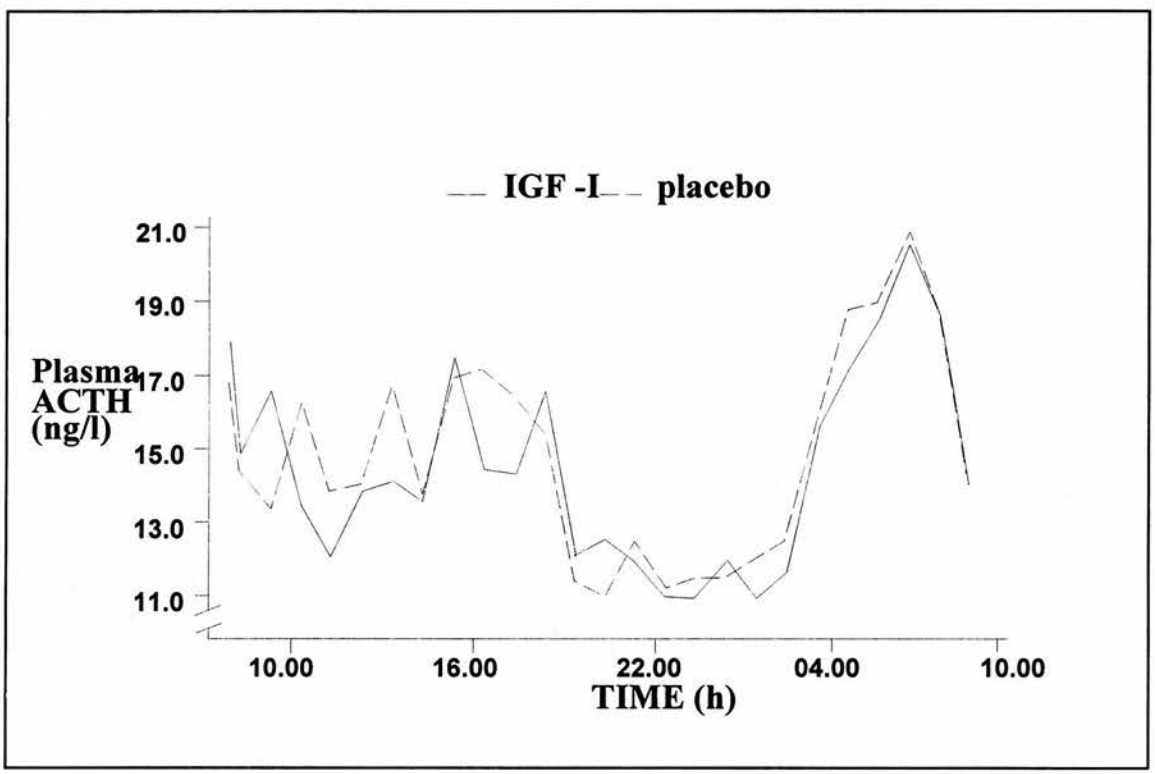

Figure 13

24 hour profile for mean plasma ACTH in 12 healthy subjects following 40 μg/kg rhIGF-I or placebo given subcutaneously at 09.00 h (SEM not shown).

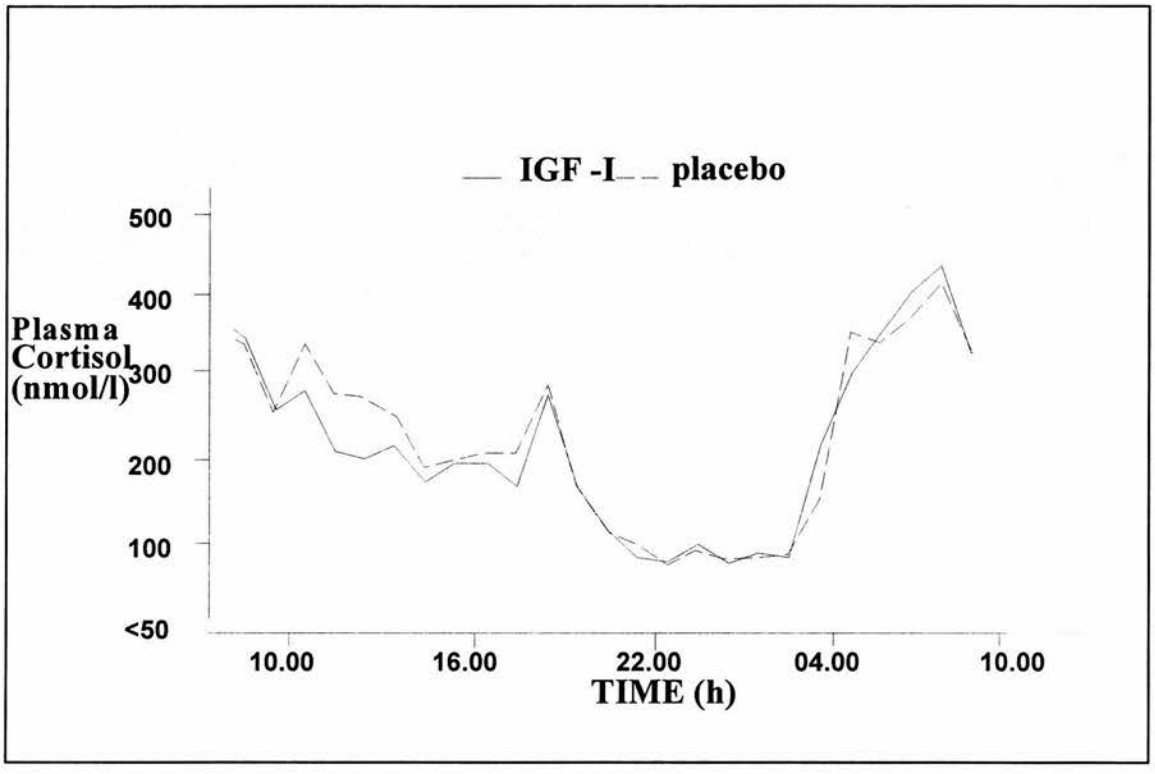

Figure 14

24 hour profile for mean plasma cortisol in 12 healthy subjects following 40 μg/kg rhIGF-I or placebo given subcutaneously at 09.00 h (SEM not shown).

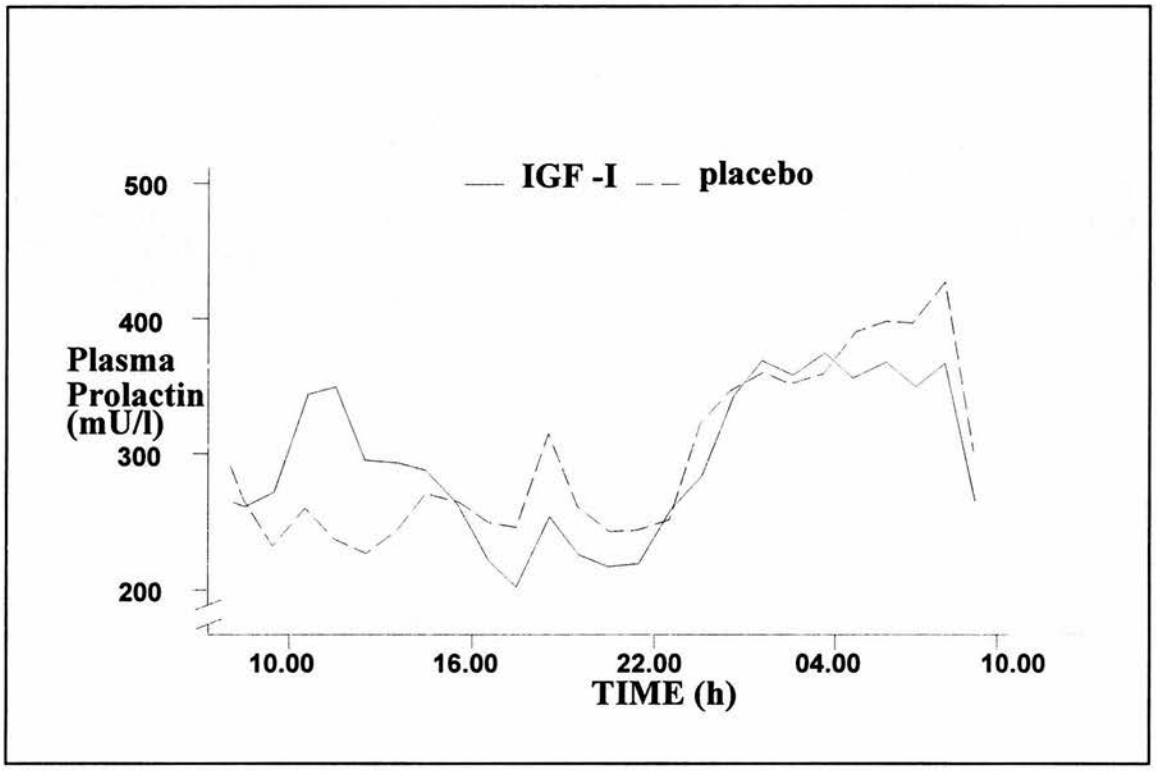

Figure 15

24 hour profile for mean plasma prolactin in 12 healthy subjects following 40 μg/kg rhIGF-I or placebo given subcutaneously at 09.00 h (SEM not shown).

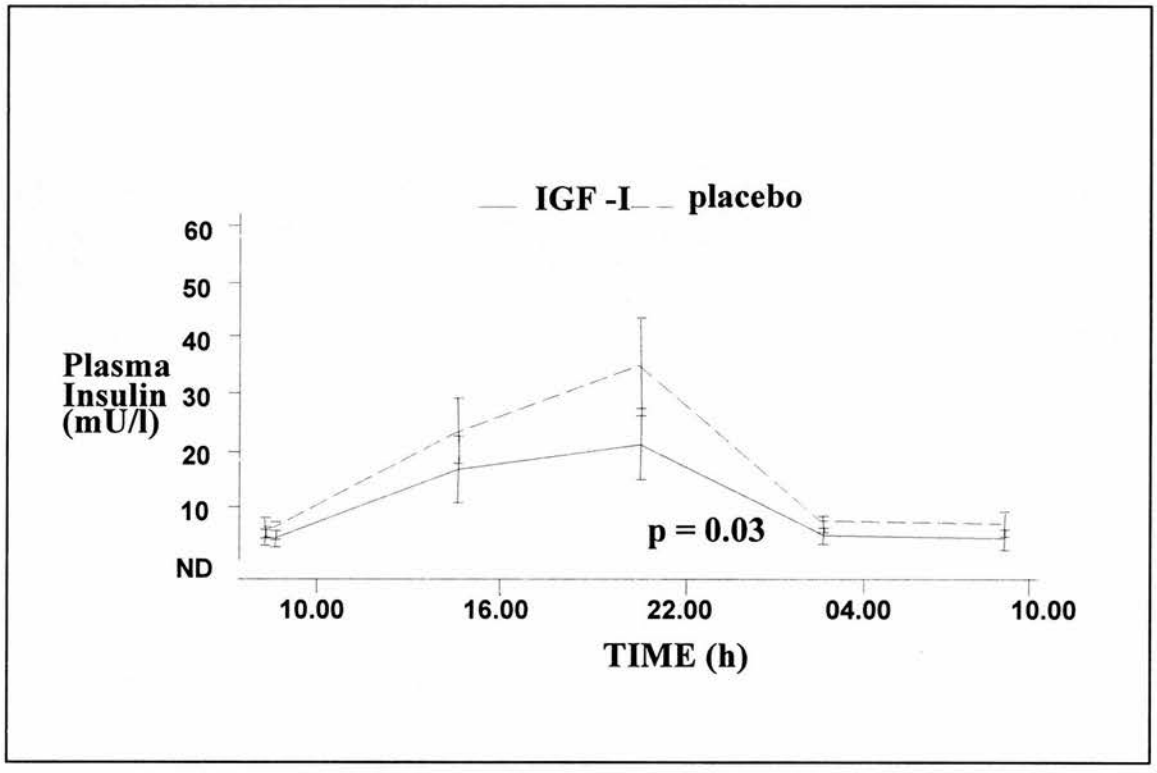

Figure 16

24 hour profile for mean plasma insulin in 12 healthy subjects following 40 μg/kg rhIGF-I or placebo given subcutaneously at 09.00 h (mean ± SEM shown).

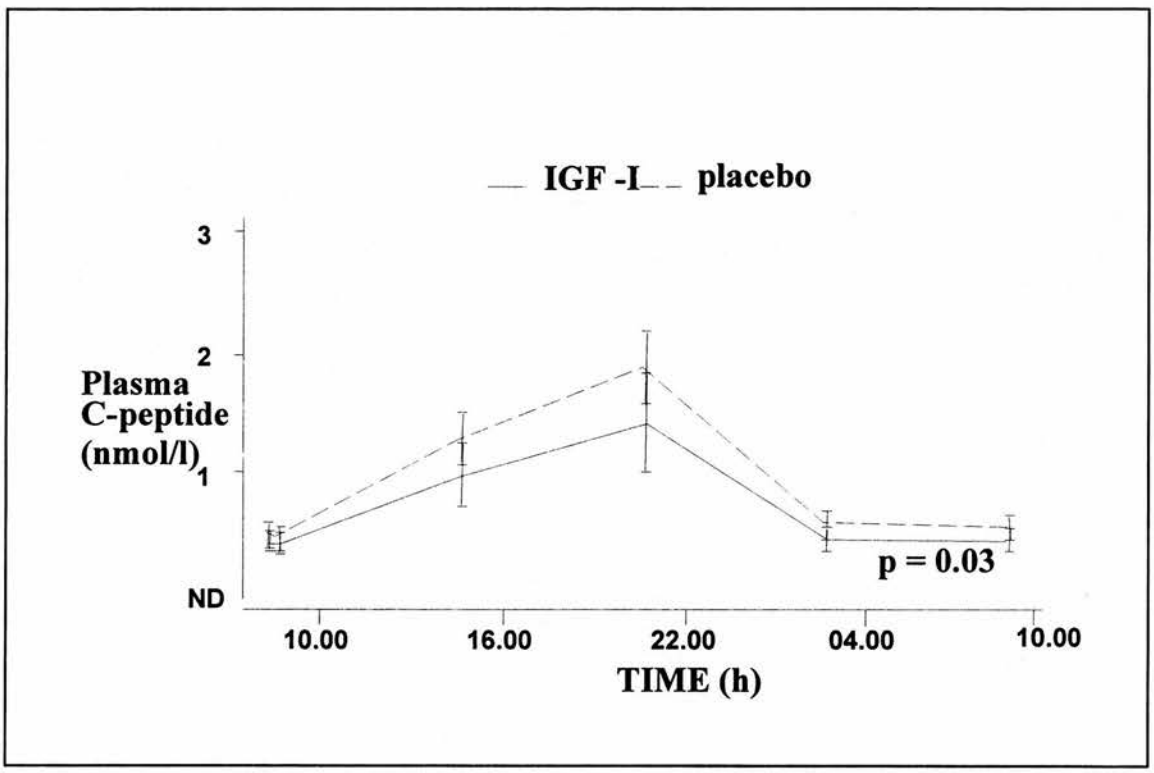

Figure 17

24 hour profile for mean plasma C-peptide in 12 healthy subjects following 40 μg/kg rhIGF-I or placebo given subcutaneously at 09.00 h (mean ± SEM shown).

RESULTS

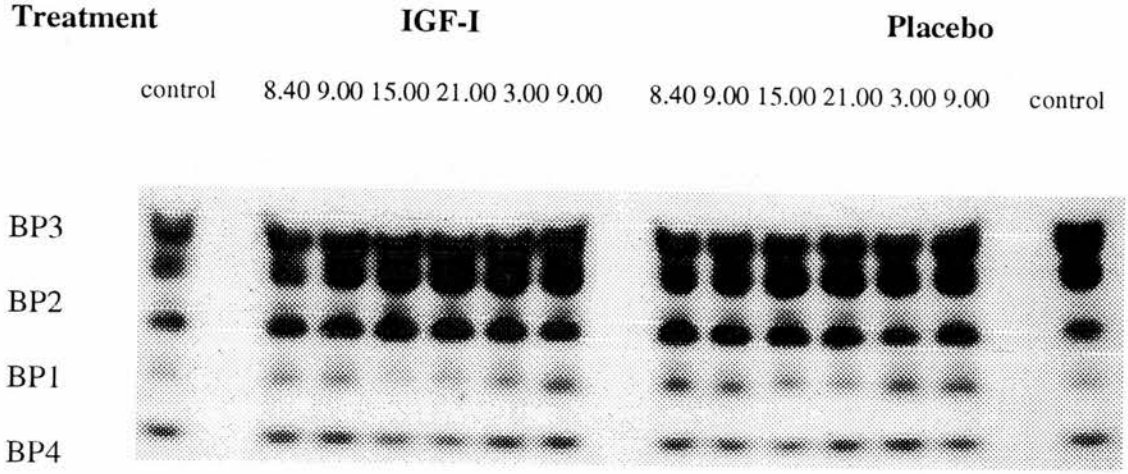

Figure 18

The effect of subcutaneous rhIGF-I (40 μg/kg) on IGF-BPs measured by Western Ligand Blot in one subject.

RESULTS

A COMPARISON OF QUANTITATIVE GROWTH HORMONE SECRETION BETWEEN TALL (≥ 183 cm) AND SHORT (≤ 166 cm) YOUNG MEN

Twenty tall and twenty short endocrinologically-normal young men (see previous chapter) were studied on four occasions in random order. An insulin tolerance test, overnight sampling and GHRH tests, with and without pyridostigmine, were performed in the manner described in the previous chapter. All studies were performed at a minimum of weekly intervals. The details of the subjects are given in table 7.

The tall (mean 187.7 cm ± SEM 1.0) subjects were significantly older (mean 22.8 years ± SEM 0.5 v 20.9 ± 0.6, $p < 0.001$) and heavier (81.1 kg ± 2.0 v 60.1 ± 1.1, $p < 0.001$) than the shorter (mean 163.5 cm ± 0.4) subjects, but no difference existed in body mass index (23.0 kg/m^2 ± 5.4 v 22.5 ± 3.7, NS).

No difference existed between the two groups in either the peak or "area under the curve" for the GH response to GHRH with and without pyridostigmine (Figures 19, 20). Similarly, the GH response to hypoglycaemia was the same in both groups (Figures 21).

Analysis of spontaneous GH secretion between 21.00 h and 06.00 h was performed by calculation of the "area under the curve" and using the pulse analysis software *pulsar* (see previous chapter). The AUC for the 9 hour sampling period did not differ between the two groups, but examination of GH secretion during the time of maximal secretion 00.00 -

03.00 suggested secretion rates to be greater in the tall subjects (Figure 22, Table 10). *Pulsar* analysis failed to reveal differences in pulse number, length or amplitude of the largest pulse between the two groups, but a trend towards mean pulse amplitude being greater in the tall subjects was detected although this did not reach statistical significance (Table 9).

No relationship was found between serum IGF-I and the GH response to hypoglycaemia or GHRH or spontaneous nocturnal secretion. Likewise, there was no relationship between serum IGF-I and height. No difference existed in serum IGF-I, testosterone or thyroxine values between the two groups (Table 8).

In summary, the GH response to pharmacological stimuli, hypoglycaemia and GHRH, did not differ between the tall and short subjects. No statistically significant difference in spontaneous GH secretion was detected, although AUC for the period of maximal secretion, namely midnight to 03.00 h, and mean pulse amplitude tended towards being greater in the tall subjects (Tables 9 and 10).

RESULTS

Table 7

Age, weight , height and body mass index in 20 "tall" (≥ 183 cm) and 20 "short" (≤ 166 cm) subjects

SUBJECT	AGE (years)	WEIGHT (kg)	HEIGHT (m)	BODY MASS INDEX (kg/m^2)
TALL				
1	26	80	1.89	22.4
2	21	81	1.94	21.5
3	22	86	1.91	23.6
4	24	74	1.81	22.6
5	21	87	1.87	24.9
6	21	83	1.89	23.2
7	21	80	1.97	20.9
8	22	91	1.89	25.4
9	22	86	1.84	25.4
10	20	70	1.94	18.6
11	26	77	1.84	22.7
12	18	80	1.85	23.4
13	23	89	1.90	24.6
14	23	75	1.83	22.4
15	24	73	1.84	21.6
16	26	110	1.90	30.4
17	27	84	1.90	23.2
18	23	76	1.86	22.0
19	22	72	1.84	21.3
20	24	68	1.83	20.3
MEAN	**22.8**	**81.1**	**187.7**	**23.0**
RANGE	**18-27**	**68-110**	**183-197**	**18.6-30.4**
SHORT				
21	20	53	1.63	20.0
22	20	60	1.60	23.4
23	26	60	1.66	21.8
24	24	60	1.65	22.0
25	21	65	1.66	23.6
26	22	65	1.66	23.6
27	19	55	1.64	20.5
28	18	66	1.64	24.6
29	22	67	1.64	25.0
30	23	65	1.63	24.5
31	18	53	1.60	20.7
32	24	63	1.63	23.8
33	18	60	1.64	22.3
34	18	53	1.61	20.4
35	18	53	1.64	19.7
36	25	59	1.64	22.0

RESULTS

SUBJECT	AGE (years)	WEIGHT (kg)	HEIGHT (m)	BODY MASS INDEX (kg/m^2)
37	19	58	1.63	21.8
38	25	66	1.64	24.6
39	20	60	1.63	22.6
40	19	62	1.63	23.4
MEAN	**20.9**	**60.1**	**163.5**	**22.5**
RANGE	**18-25**	**53-67**	**160-166**	**19.7-25.0**

Table 8

Serum IGF-I, testosterone and thyroxine values in 20 "tall" (≥ 183 cm) and 20 "short" (≤ 166 cm) subjects

SUBJECT	IGF-I (ng/ml)	Testosterone (nmol/l)	T4 (nmol/l)
TALL			
1.	179	14.0	105
2.	210	17.0	46
3.	195	24.4	74
4.	291	26.3	87
5.	230	19.6	74
6.	377	28.0	70
7.	301	21.0	66
8.	172	21.5	68
9.	321	23.3	85
10.	57	18.9	78
11.	186	26.0	115
12.	227	17.5	113
13.	232	25.0	91
14.	175	20.5	125
15.	272	14.5	116
16.	188	9.5	132
17.	230	11.5	93
18.	238	20.0	99
19.	301	10.5	110
20.	228	21.0	91
MEAN	**230.5**	**19.5**	**91.9**
SEM	**15.37**	**1.2**	**5.0**

RESULTS

SUBJECT	IGF-I (ng/ml)	Testosterone (nmol/l)	T4 (nmol/l)
SHORT			
21.	228	21.0	100
22.	298	21.1	62
23.	221	19.1	85
24.	162	23.6	87
25.	203	18.9	97
26.	125	25.0	93
27.	248	20.0	98
28.	323	18.0	85
29.	115	13.0	115
30.	70	14.5	89
31.	281	27.0	116
32.	216	9.0	106
33.	351	15.5	85
34.	233	15.5	145
35.	442	47.5	110
36.	169	20.5	84
37.	242	22.5	103
38.	234	16.5	120
39.	200	17.5	115
40.	251	14.0	94
MEAN	**230.6**	**19.9**	**99.9**
SEM	**18.9**	**1.7**	**3.9**

Table 9

The results of pulse analysis of overnight spontaneous GH (mU/l) secretion by "*pulsar*"

	Subjects	
	TALL	**SHORT**
number of pulses	2.45 ± 0.24	3.0 ± 0.24
mean amplitude	20.3 ± 3.4	13.6 ± 2.8
largest peak	32.1 ± 5.9	26.3 ± 2.8
mean length (min)	113.9 ± 10.4	90.4 ± 8.0

Table 10

"Area under the curve" for overnight spontaneous GH secretion

	Subjects	
Time	**TALL**	**SHORT**
21.00 h - 06.00 h	198 ± 38.8	151 ± 21.6 (mU.min/l)
00.00 h - 03.00 h	124.8 ± 29.0	68.1 ± 10.4

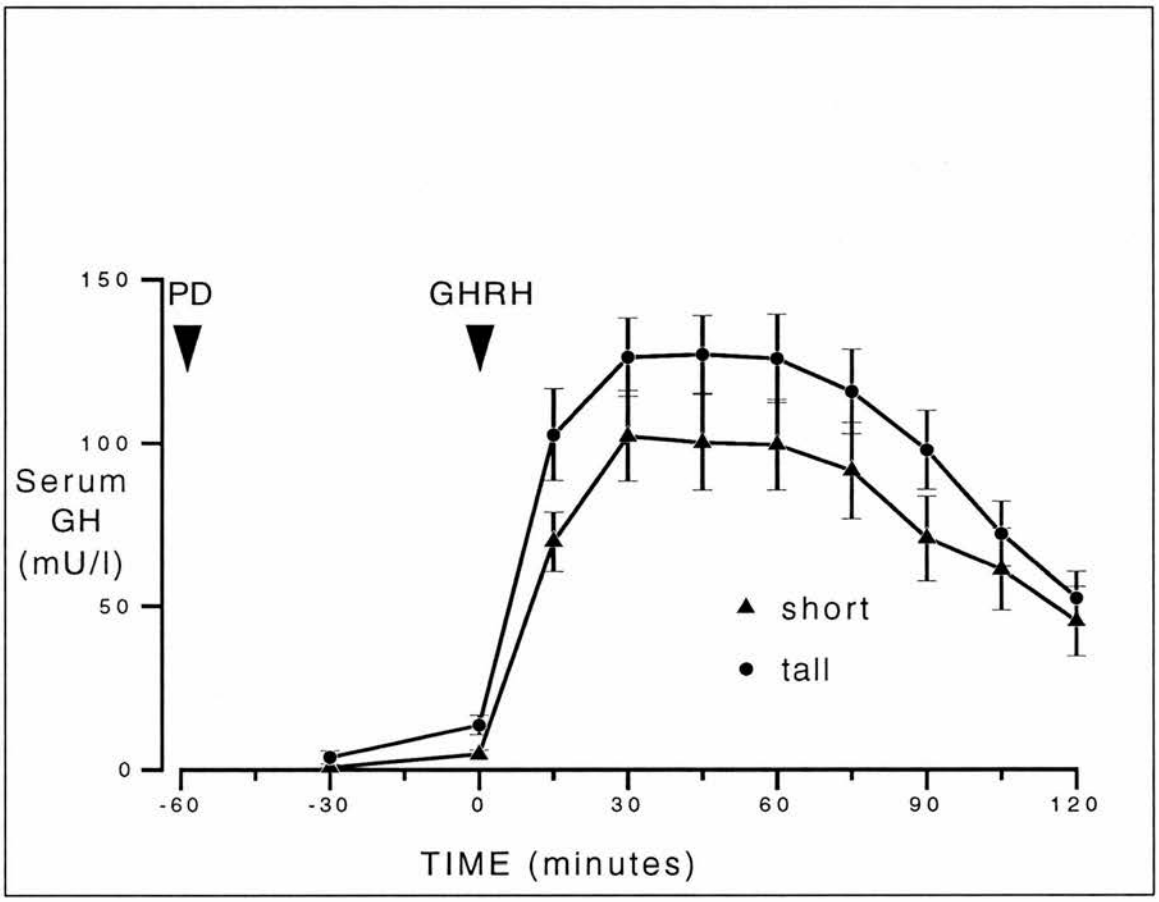

Figure 19

The mean GH response to GHRH (100 μg iv) and pyridostigmine (120 mg, oral) in 20 "tall" (≥ 183 cm) and "short" (≤ 166 cm) healthy volunteers. Mean ± SEM shown.

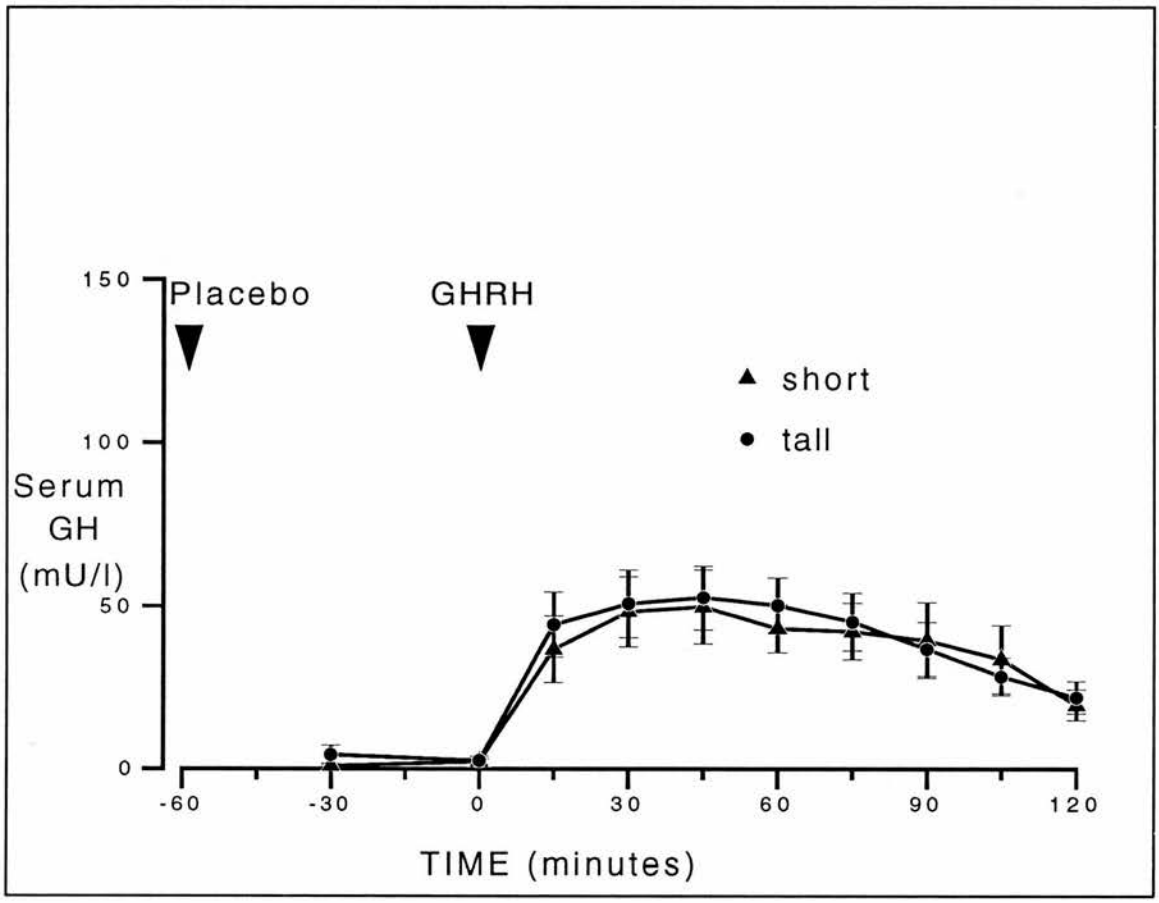

Figure 20

The mean GH response to GHRH (100 μg iv) and placebo in 20 "tall" (≥ 183 cm) and 20 "short" (≤ 166 cm) healthy volunteers. Mean ± SEM shown.

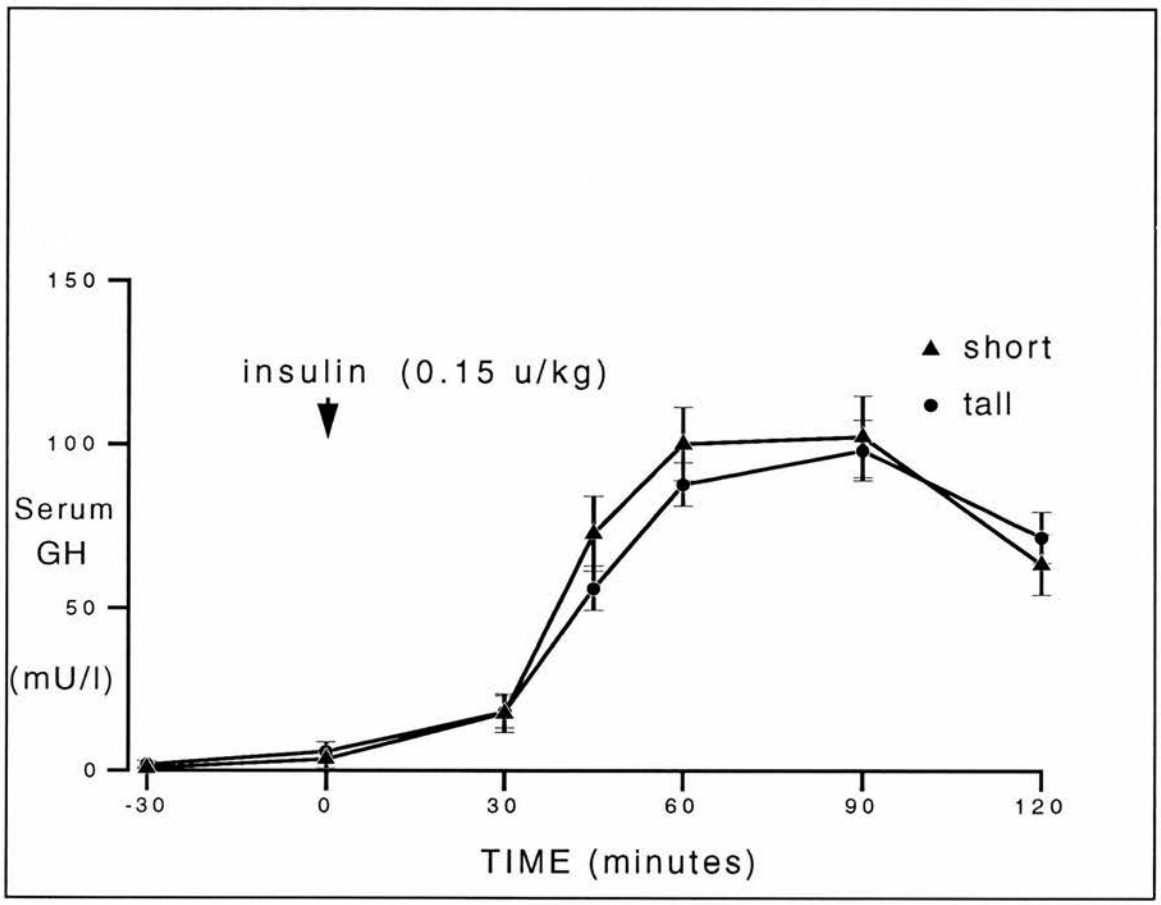

Figure 21

The serum GH response to hypoglycaemia (blood sugar < 2.2 mmol/l) in 20 "tall" (≥ 183 cm) and 20 "short" (≤ 166 cm). Mean ± SEM shown.

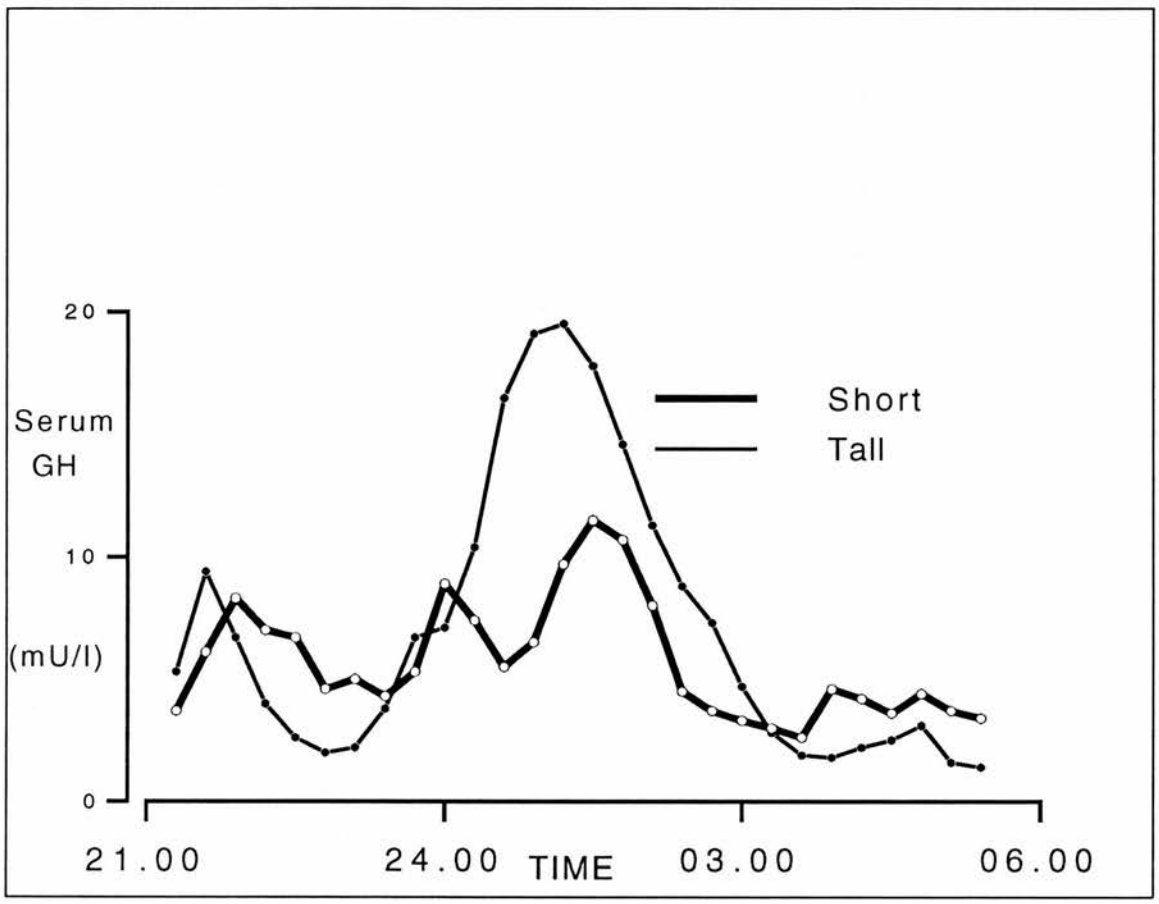

Figure 22

Mean serum GH nocturnal profiles in 20 "tall" (≥ 183 cm) and 20 "short" (≤ 166 cm) (error bars not shown).

RESULTS

THE GH RESPONSE TO GHRH IN MEN AND WOMEN, AND IN WOMEN AT DIFFERENT STAGES IN THE MENSTRUAL CYCLE

Eight healthy non-obese male and female volunteers were studied. The men underwent two GHRH tests, with and without pyridostigmine. The female volunteers were not on the oral contraceptive pill and had regular menstrual cycles, 28 days ± 2. The women were studied on four occasions; two GHRH tests were performed, with and without pyridostigmine, between days 1-3 of the menstrual cycle and again between days 14-16, a total of four GHRH tests. The GHRH tests were performed in the manner described in the Methods chapter. Studies were carried out at a minimum of two day intervals in a randomized double-blind manner. The details of the subjects are provided in Table 11.

The male subjects were significantly older (mean 36.6 year ± SEM 3.2 v 26.7 ± 1.8, unpaired t-test $p = 0.02$), heavier (72.2 kg ± 4.5 v 61.3 ± 1.6, unpaired t-test $p = 0.04$) and taller (mean 1.77 m ± 0.03 v 1.62 ± 0.02, unpaired t-test $p = 0.002$) but no difference existed in body mass index (22.7 kg/m^2 ± 0.87 v 23.7 ± 0.98, NS). Mean serum oestradiol levels were appropriate for the stages of the menstrual cycle and serum testosterone in the reference range in all subjects (Table 13). Two subjects (# 1, 2) had a mid-cycle serum progesterone indicative of ovulation.

No difference existed in the mean AUC for GH following GHRH between men and women, or women at different stages of the menstrual cycle. Similarly, although pre-

treatment with pyridostigmine increased the GH response to GHRH, again no difference existed between the three groups (Table 12, Figures 23, 24). The increment and peak GH response were very similar in each group (Figures 25, 26). Serum IGF-I levels did not differ either between women at different stages of the menstrual cycle (mean 165.0 ng/ml ± SEM 16.5 v 156.8 ± 15.3, NS) or between men and women (mean 165.0 ng/ml ± SEM 16.5 or 156.8 ± 15.3 v 165.6 ± 16.7, NS) (Table 13).

In summary, no difference was found in the GH response to GHRH between men and women or in women at different stages of the menstrual cycle.

RESULTS

Table 11

The age, weight, height and body mass index of the 8 males and 8 females studied

SUBJECT	AGE (years)	WEIGHT (kg)	HEIGHT (m)	BMI (kg/m^2)
FEMALE				
1	27	64.8	1.63	24.4
2	30	54.8	1.67	21.1
3	30	57.5	1.53	24.5
4	26	60.2	1.51	26.4
5	36	67.1	1.59	26.6
6	22	57.2	1.74	18.9
7	21	67.2	1.61	25.9
8	22	62.3	1.68	22.1
MEAN	**26.7**	**61.3**	**1.62**	**23.7**
RANGE	**21-36**	**54.8-67.2**	**1.51-1.74**	**18.9-26.6**
MALE				
9	46	72.7	1.77	23.2
10	25	64.2	1.69	22.5
11	35	99.2	1.91	27.2
12	34	75.4	1.76	24.4
13	44	54.4	1.66	19.7
14	26	67.2	1.79	21.0
15	33	73.0	1.76	23.6
16	50	72.0	1.89	20.1
MEAN	**36.6**	**72.2**	**1.77**	**22.7**
RANGE	**25-50**	**54.4-99.2**	**1.66-1.91**	**19.7-27.2**

Table 12

"Area under the curve" (mU.min/l) for serum GH following GHRH (100 μg, iv) in combination with either pyridostigmine (PD) or placebo (PL) (mean ± SEM shown)

Limb	Early-luteal	Mid-cycle	Male
GHRH + PL	151.5 ± 68.3	149.7 ± 42.7	165.1 ± 65.2
GHRH + PD	499.8 ± 80.7	439.4 ± 106.6	554.7 ± 108.3

Table 13

Serum oestradiol, progesterone, testosterone and IGF-I of subjects sub-divided into early luteal, mid-cycle and male

SUBJECT	E_2 (pmol/l)	Prog. (nmol/l)	Test. (nmol/l)	IGF-I (ng/ml)
FEMALE - EARLY CYCLE				
1	132	7.8	2.7	102
2	84	1.7	1.8	175
3	122	<4	1.2	150
4	92	<4	1.9	126
5	187	13.4	1.4	132
6	59	<4	1.4	245
7	79	4.5	1.3	183
8	93	<4	1.9	207
MEAN	**106**	**4.5**	**1.7**	**165.0**
RANGE	**59-187**	**<4-13.4**	**1.2-1.9**	**102-245**
FEMALE - MID-CYCLE				
1	328	20.5	2.3	108
2	839	54	2.4	172
3	804	<4	1.7	144
4	170	<4	1.4	171
5	677	<4	1.7	107
6	381	<4	2.3	230
7	784	<4	2.2	195
8	360	<4	2.3	128
MEAN	**542.9**	**12.4**	**2.1**	**156.8**
RANGE	**170-839**	**<4-54**	**1.4-2.3**	**107-230**
MALE				
9	83	N/A	22.3	180
10	84	N/A	25.5	104
11	97	N/A	19.3	218
12	108	N/A	22.3	153
13	106	N/A	35.8	135
14	127	N/A	30.5	247
15	60	N/A	20.8	155
16	129	N/A	22.5	133
MEAN	**99.2**	**N/A**	**24.8**	**165.6**
RANGE	**60-129**	**N/A**	**19.3-35.8**	**104-247**

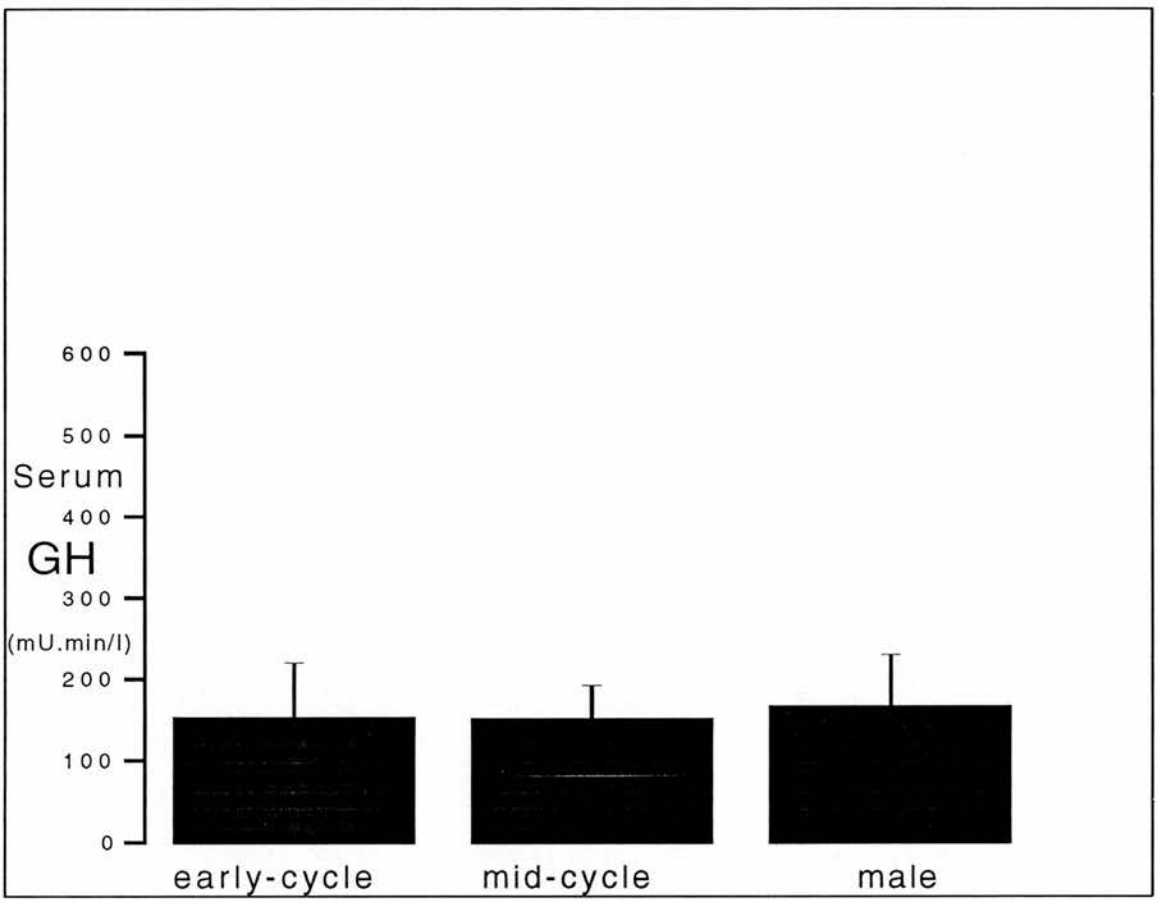

Figure 23

The "area under the curve" for serum GH following GHRH (100 μg, iv) and oral placebo in eight males and females. Mean ± SEM shown.

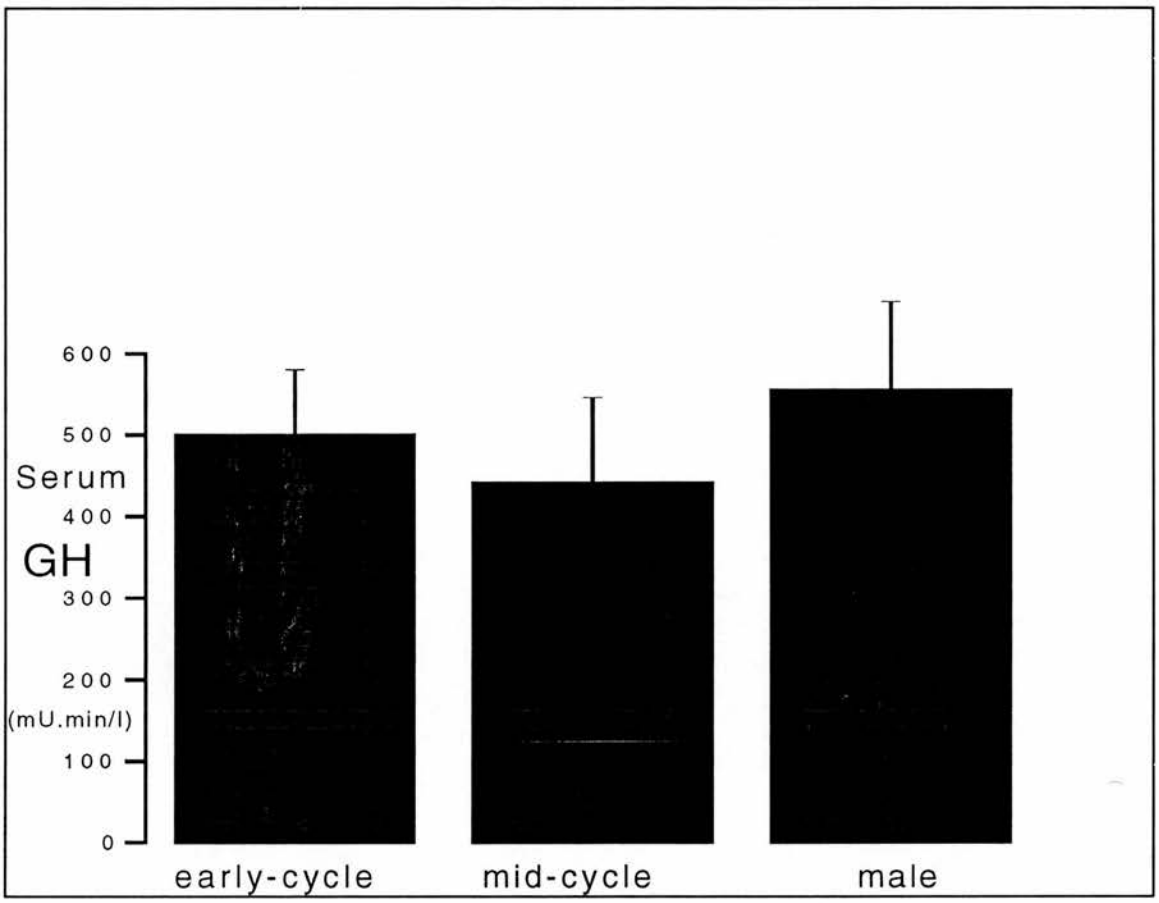

Figure 24

The "area under the curve" for serum GH following GHRH (100 μg, iv) and 120 mg oral pyridostigmine in eight males and females. Mean ± SEM shown.

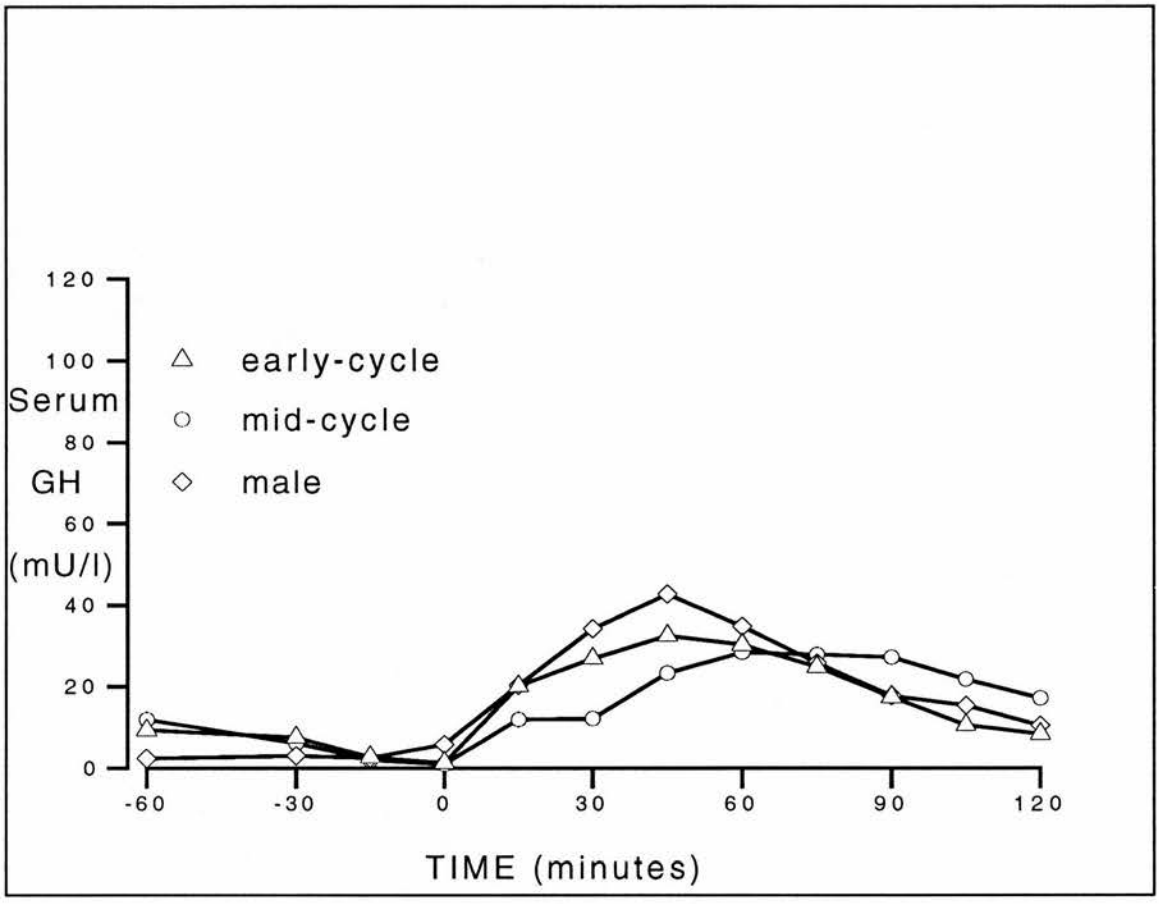

Figure 25

The serum GH response to GHRH (100 μg, iv) plus oral placebo in eight males and females (error bars not shown).

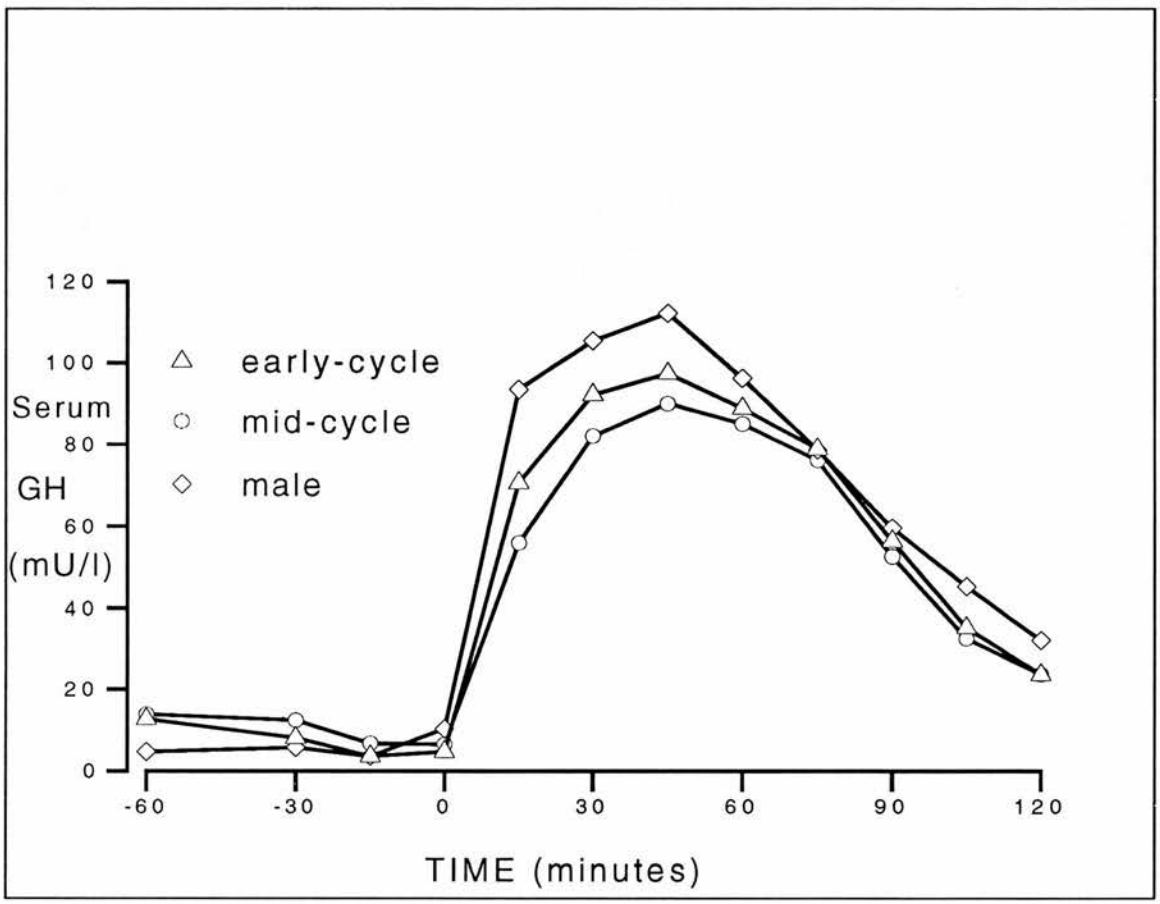

Figure 26

The AUC for serum GH following GHRH (100 μg, iv) plus pyridostigmine (120 mg, oral) or placebo in eight males and females (error bars not shown).

RESULTS

THE EFFECT OF AGE ON THE GH RESPONSE TO GHRH IN MEN

Six healthy non-obese male volunteers in each of the six decades from the twenties to the seventies were studied. Each subject underwent two GHRH tests, with and without pyridostigmine. The GHRH tests were performed in the manner described in the previous chapter. Studies were carried out at a minimum of seven day intervals in a randomized double-blind manner. The mean age of each group of volunteers was in the middle of their decade and there was no significant difference in body mass index between the ages (Table 14).

The mean GH response and AUC following GHRH preceded by an oral placebo for each of the age groups are given in Figures 27, 29, and for GHRH combined with pyridostigmine in Figures 28, 30. One-way analysis of variance demonstrates that the GH response to GHRH falls with age (ANOVA, $p < 0.05$). At all ages pyridostigmine potentiates the GH response to GHRH. To allow a comparison of the changes in the relative influence of pyridostigmine on GH secretion with age, the proportional effect of pyridostigmine on GH secretion in each subject was calculated by the formula:
(AUC for GHRH plus pyridostigmine - GHRH plus placebo)/ GHRH plus placebo.

One-way analysis of variance demonstrates that, as with exogenous GHRH, the ability of pyridostigmine to stimulate GH secretion declines with age (ANOVA, $p < 0.05$). Age was negatively correlated with AUC for GH ($r = -0.4$, $p = 0.016$).

RESULTS

An additional analysis of the data, comparing the youngest 12 subjects the oldest 12 subjects, demonstrated that the younger subjects had a significantly greater AUC for GH following GHRH (162.7 mU.min/l ± 40.4 v 86.4 ± 15.6, unpaired t-test $p < 0.01$) and although the relative effect of pyridostigmine declined it was not significantly different between the young and elderly subjects (2.5 ± 0.5 v 1.3 ± 0.4, NS). The mean serum IGF-I levels were lower in the over 70 age group compared to the 20 - 30 year olds (224.8 ng/ml ± 10.1 v 161.2 ± 37.4, unpaired t-test $p < 0.01$)(Table 15).

Table 14

Age, weight, height and body mass index of subjects sub-divided by age

SUBJECTS	AGE (years)	WEIGHT (kg)	HEIGHT (m)	BMI (kg/m^2)
20s				
1	24	77	1.71	26.3
2	21	119	1.95	31.3
3	20	76	1.76	24.6
4	25	70	1.65	25.7
5	30	82	1.80	25.3
6	27	65	1.72	22.0
MEAN	**24.5**	**81.5**	**1.76**	**25.8**
RANGE	**20-30**	**65-119**	**1.71-1.95**	**22.0-31.3**
30s				
7	34	57	1.66	20.7
8	35	63	1.85	18.4
9	38	85	1.91	23.3
10	35	88	1.85	25.7
11	36	70	1.69	24.5
12	31	80	1.80	24.6
MEAN	**34.8**	**73.8**	**1.79**	**22.8**
RANGE	**31-38**	**57-88**	**1.66-1.91**	**18.4-25.7**
40s				
13	41	66	1.80	20.3
14	47	65	1.74	22.0
15	48	64	1.68	22.6
16	46	70	1.71	23.9
17	42	56	1.66	20.3
18	46	72	1.77	23.2
MEAN	**45.0**	**65.5**	**1.72**	**22.0**
RANGE	**41-48**	**56-72**	**1.66-1.80**	**20.3-23.9**
50s				
19	59	77	1.80	23.7
20	58	67	1.69	23.5
21	51	75	1.77	23.9
22	59	60	1.70	20.7
23	58	72	1.60	28.1
24	50	72	1.89	20.1
MEAN	**55.8**	**70.5**	**1.74**	**23.3**
RANGE	**50-59**	**60-77**	**1.60-1.89**	**20.1-23.9**

RESULTS

SUBJECTS	AGE (years)	WEIGHT (kg)	HEIGHT (m)	BMI (kg/m^2)
60s				
25	69	80	1.70	27.6
26	68	75	1.70	25.9
27	62	60	1.75	19.6
28	61	88	1.91	24.1
29	61	65	1.69	22.8
MEAN	**64.2**	**73.6**	**1.75**	**24.0**
RANGE	**61-69**	**60-88**	**1.69-1.91**	**19.6-27.6**
70s				
30	79	53	1.68	18.7
31	74	74	1.75	24.1
32	74	62	1.73	20.7
33	71	70	1.72	23.7
34	88	77	1.80	23.7
35	71	70	1.69	24.5
MEAN	**76.1**	**67.6**	**1.72**	**22.6**
RANGE	**71-88**	**53-77**	**1.68-1.75**	**18.7-24.5**

Table 15

Serum IGF-I and testosterone of subjects sub-divided by age

SUBJECTS	IGF-I (ng/ml)	Testosterone (nmol/l)
20s		
1	199	16.0
2	210	10.5
3	241	14.5
4	200	10.0
5	244	13.0
6	255	18.0
MEAN	**224**	**13.66**
RANGE	**199-255**	**10-18**
30s		
7	161	14.5
8	161	20.0
9	154	14.5
10	283	10.0
11	158	15.0
12	211	9.0
MEAN	**188.0**	**13.8**
RANGE	**154-283**	**9-20**

RESULTS

SUBJECTS	IGF-I (ng/ml)	Testosterone (nmol/l)
40s		
13	129	9.0
14	116	14.0
15	171	12.5
16	248	10.5
17	115	15.5
18	190	26.0
MEAN	**161.5**	**14.5**
RANGE	**115-248**	**9-26**
50s		
19	155	13.5
20	157	14.0
21	287	21.2
22	164	22.8
23	120	11.8
24	129	30.5
MEAN	**168.7**	**18.9**
RANGE	**155-287**	**11.8-30.5**
60s		
25	428	14.5
26	139	8.0
27	119	11.5
28	91	15.0
29	209	13.0
MEAN	**197.2**	**12.4**
RANGES	**91-428**	**8-15**
70s		
30	155	15.0
31	155	5.0
32	129	12.5
33	109	12.0
34	339	15.0
35	80	12.5
MEAN	**161.2**	**12.0**
RANGES	**80-339**	**5-15**

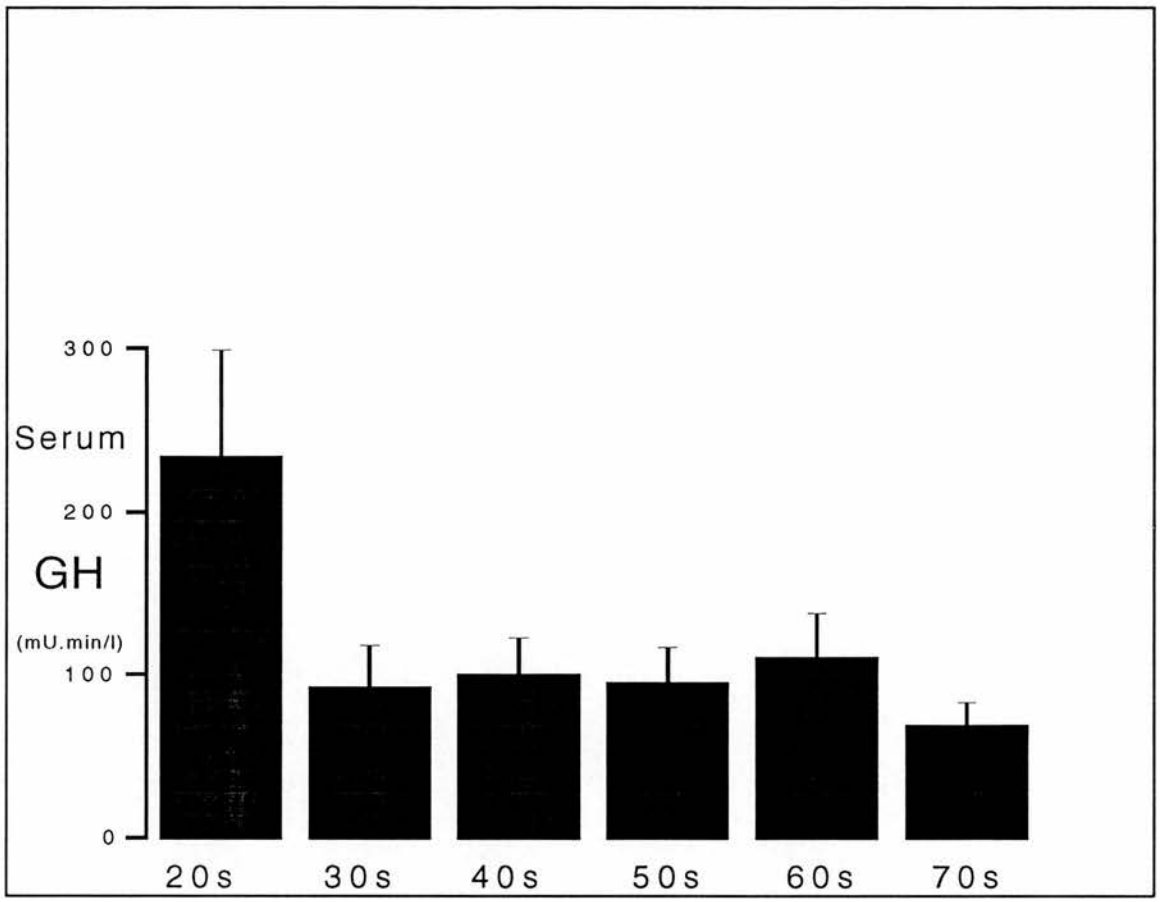

Figure 27

The AUC for serum GH following GHRH (100 μg, iv) plus placebo in six male volunteers from each of six decades (20 - 29, 30 - 39, 40 - 49, 50 - 59, 60 - 69, > 70 yr). Values are means ± SEM.

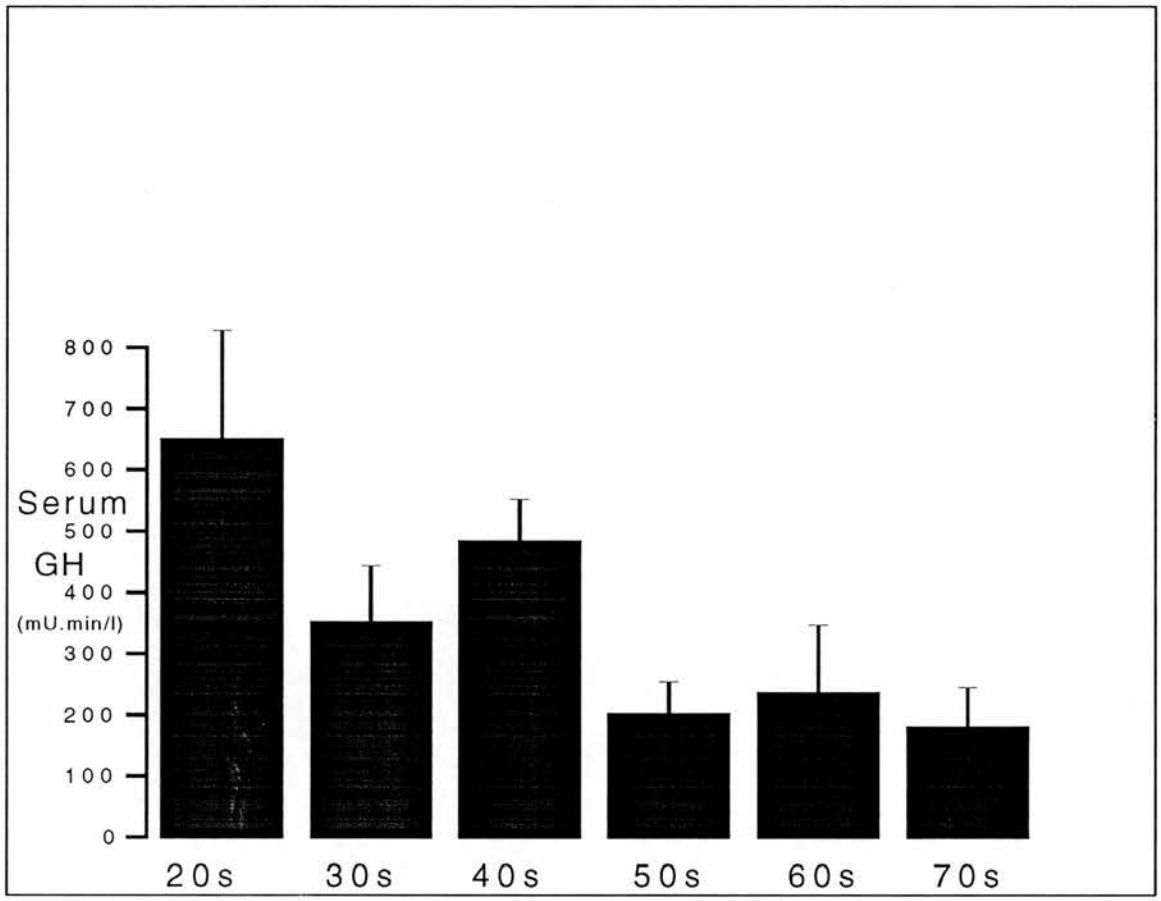

Figure 28

The AUC for serum GH following GHRH (100 μg, iv) plus pyridostigmine (120 mg, oral) in six male volunteers from each of six decades (20 - 29, 30 - 39, 40 - 49, 50 - 59, 60 - 69, > 70 yr). Values are means ± SEM.

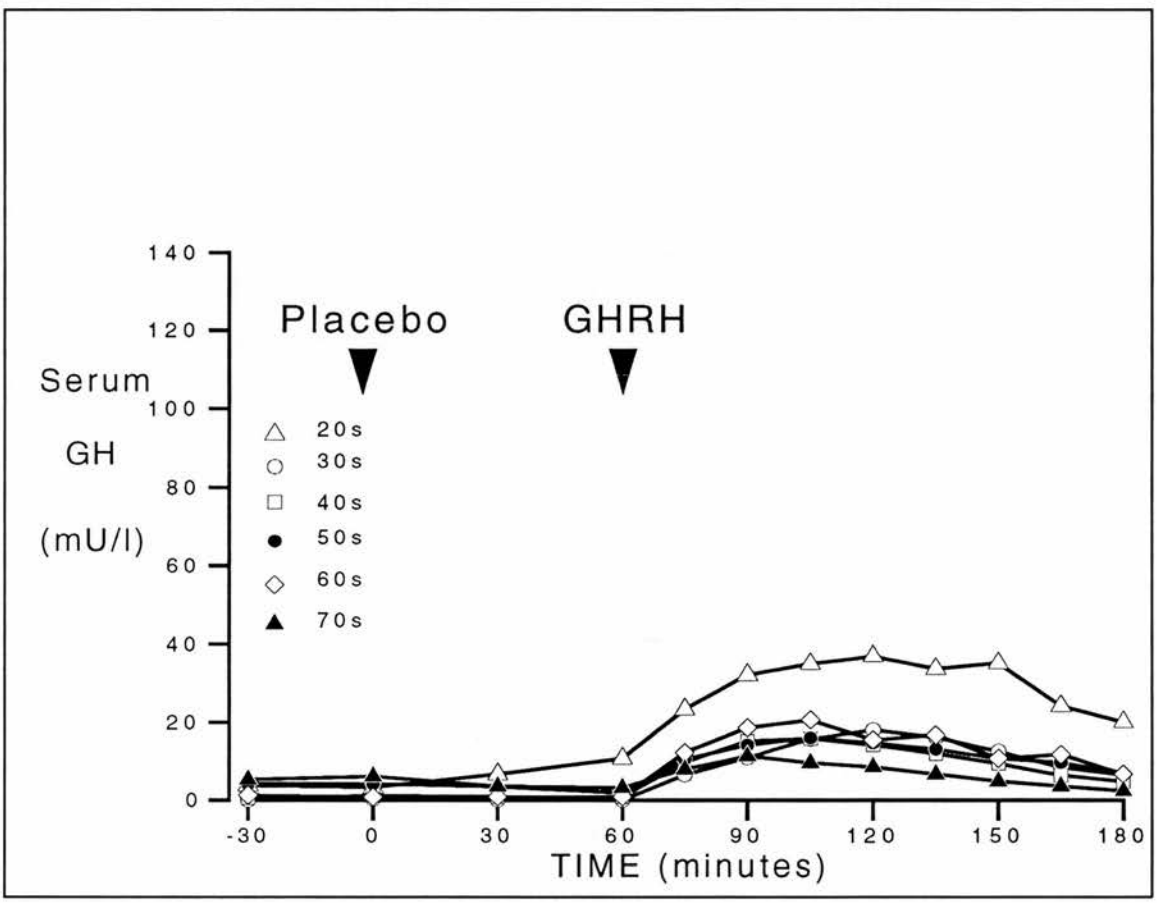

Figure 29

The mean serum GH response to GHRH (100 μg, iv) plus pyridostigmine (120 mg, oral) or placebo in six male volunteers from each of six decades (20 - 29, 30 - 39, 40 - 49, 50 - 59, 60 - 69, > 70 yr). Error bars not shown.

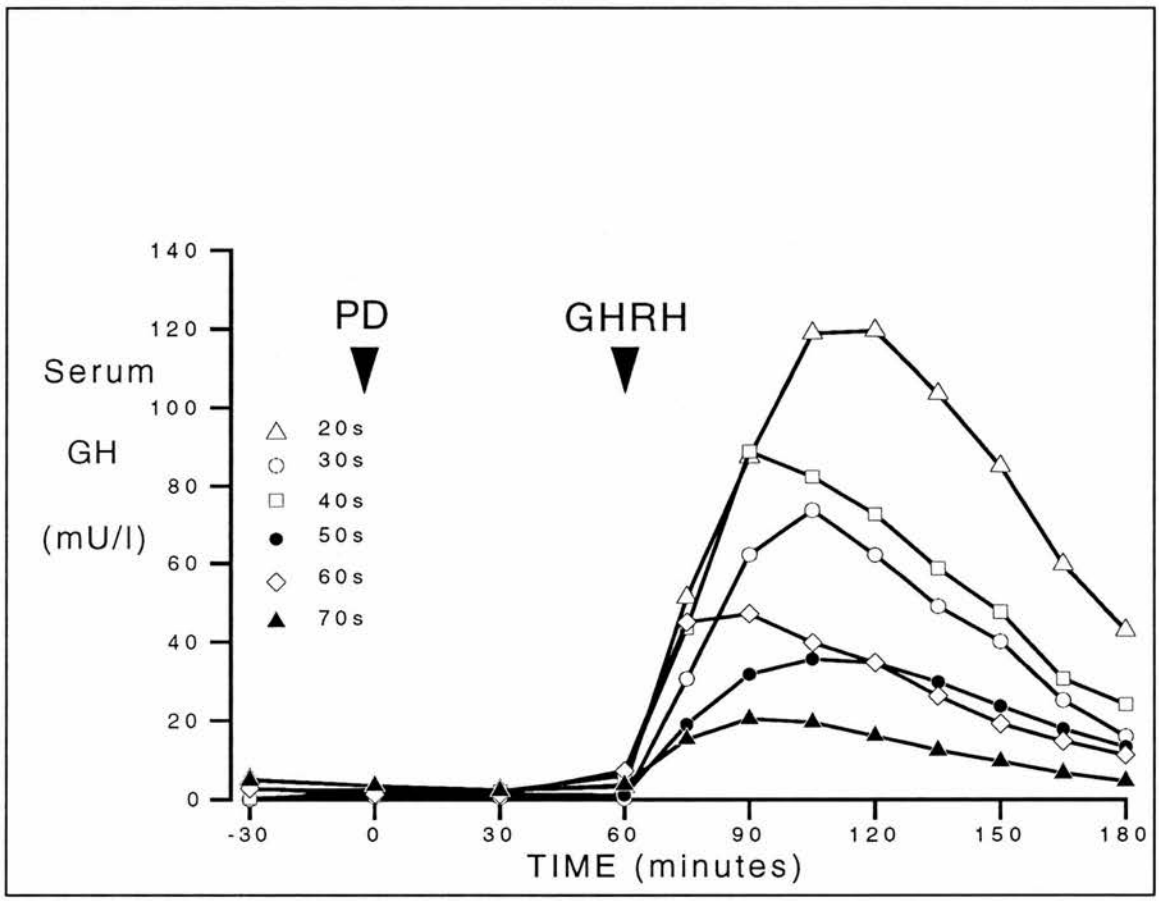

Figure 30

The mean serum GH response to GHRH (100 μg, iv) plus pyridostigmine (120 mg, oral) or placebo in six male volunteers from each of six decades (20 - 29, 30 - 39, 40 - 49, 50 - 59, 60 - 69, > 70 yr). Error bars not shown.

RESULTS

THE EFFECT OF PRE-TREATMENT WITH DEXAMETHASONE ON THE GH RESPONSE TO INTRAVENOUS GHRH

Eight healthy non-obese male volunteers were studied on three occasions in random order. The three limbs of the study were placebo-placebo-GHRH [experiment 1], dexamethasone-pyridostigmine-GHRH [experiment 2] and dexamethasone-placebo-GHRH [experiment 3]. The dexamethasone administration and GHRH tests were performed in the manner described in the previous chapter. All studies were carried out at a minimum of weekly intervals in a randomized double-blind manner. The details of the subjects are given in Table 16.

Pretreatment with 2 mg dexamethasone at 6-hourly intervals for 48 h (experiment 3) produced a clear and significant attenuation in the AUC for GH response to GHRH compared with placebo treatment (634 ± 211 vs 4267 ± 1183 mU.min/l, Wilcoxon test $p < 0.02$). The AUC for the GH response to GHRH after dexamethasone was significantly greater when preceded by 120 mg pyridostigmine rather than placebo (1938 ± 631 vs 634 ± 211 mU.min/l, Wilcoxon test $p < 0.02$). However, the response to GHRH was still significantly less after dexamethasone and pyridostigmine than in the placebo control study (1938 ± 631 to 4267 mU.min/l ± 1183, $p < 0.02$). The effect of 48 h of dexamethasone or placebo and pyridostigmine or placebo on the GH response to GHRH for each of the experiments is shown in Figures 31 and 32.

RESULTS

All subjects experienced transient facial flushing with GHRH and two subjects complained of nausea with pyridostigmine, but no side-effects were encountered with dexamethasone.

In summary, in normal volunteers 8 mg per day of oral dexamethasone inhibits the GH response of GHRH. 120 mg pyridostigmine partially reverses the inhibitory defect of dexamethasone on GH secretion, indicative that dexamethasone-induced inhibition of GH may be at least in part somatostatin mediated. The dose of pyridostigmine which can be used is restricted by side-effects. It is possible that the effect of dexamethasone might be fully reversed by a larger dose of pyridostigmine.

Table 16

The age, weight, height and body mass index of the 8 subjects receiving dexamethasone

SUBJECT	AGE (years)	WEIGHT (kg)	HEIGHT (m)	BMI (kg/m^2)
1	35	66	1.78	20.8
2	24	60	1.66	21.8
3	25	80	1.89	22.4
4	23	71	1.74	23.6
5	31	60	1.73	20.0
6	21	84	1.84	25.0
7	22	74	1.79	23.1
8	30	82	1.94	21.8
mean	**26.4**	**72.1**	**1.79**	**22.3**
range	**21-35**	**60-84**	**1.66-1.94**	**20.0-25.0**

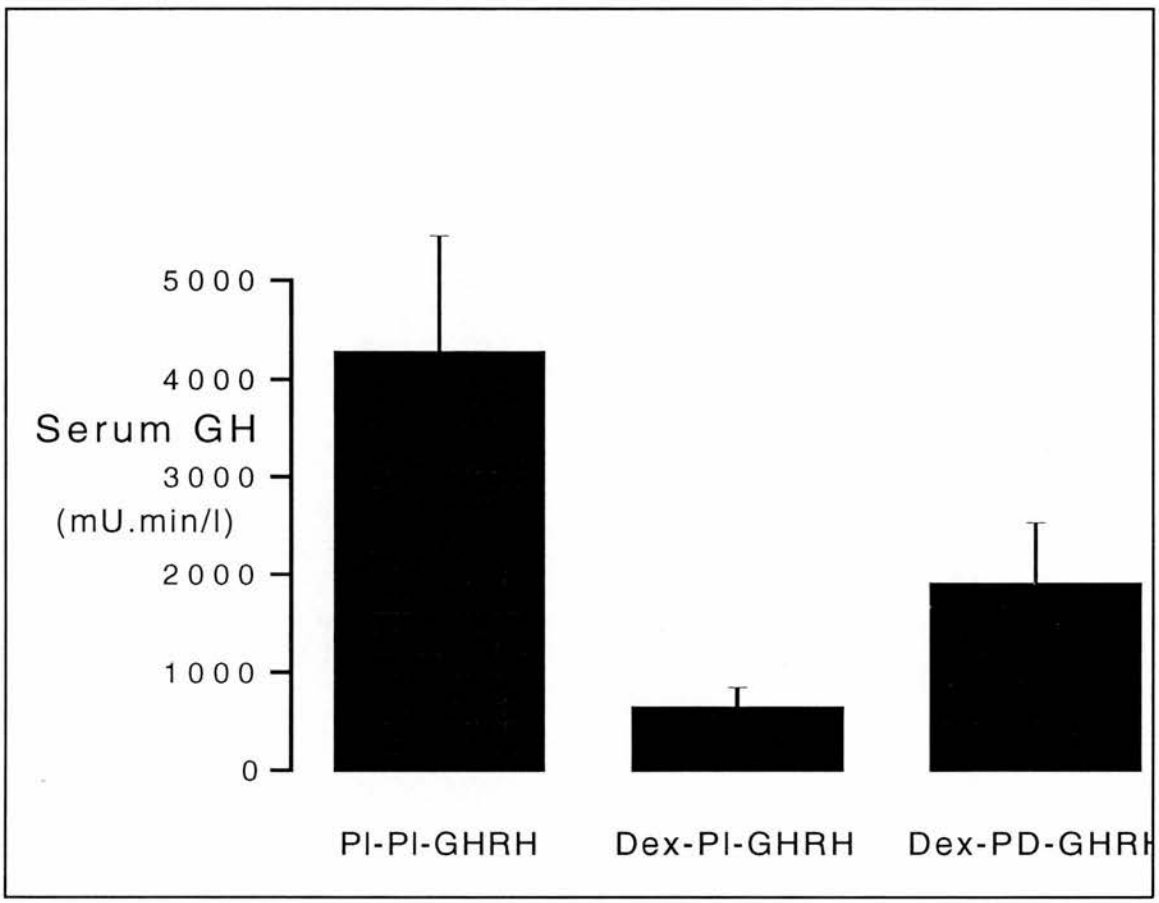

Figure 31

The "area under curve" for serum GH following GHRH administration in eight healthy subjects given dexamethasone (Dex) or placebo (Pl) for 48 hours with 120 mg pyridostigmine (PD) or placebo 60 min before GHRH. Values are means ± SEM.

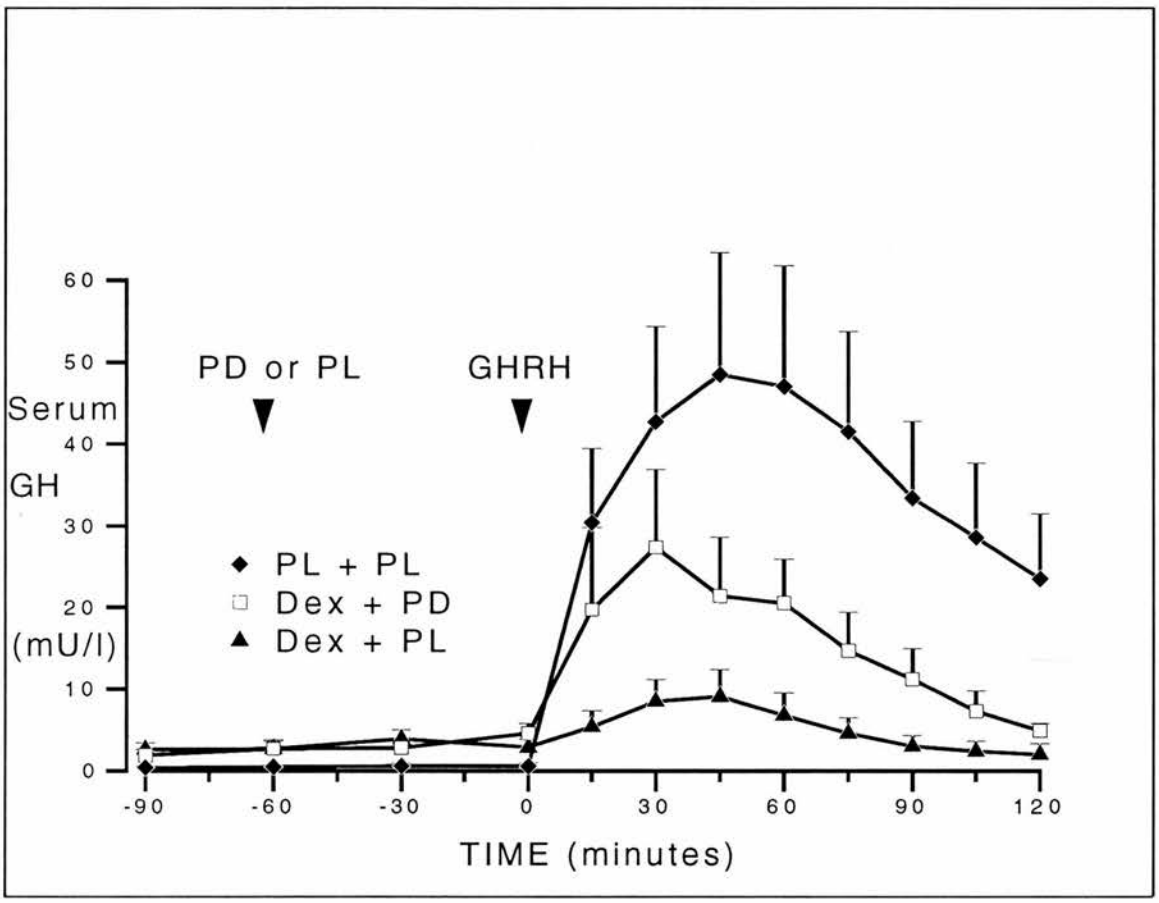

Figure 32

The serum GH response to GHRH (100 μg) in eight subjects given dexamethasone (Dex) or placebo (PL) for 48 hours with 120 mg pyridostigmine (PD) or placebo 60 min (first arrow) before the GHRH (second arrow). Means ± SEM are shown.

RESULTS

A COMPARISON OF THE GH RESPONSE TO GHRH(1-29)NH_2 AND A SYNTHETIC GHRH ANALOGUE (DC-21-346) IN HEALTHY VOLUNTEERS

The GHRH analogue DC-21-346 had not previously been administered to a human. Therefore, it was necessary to perform a preliminary dose-finding study. Three subjects received intravenous doses of 1, 5, 10, 100 and 200 μg and subcutaneous doses of 50 and 100 μg DC-21-346 (Figures 33, 34). The lack of GH response to DC-21-346 at doses of 1, 5 and 10 μg established the need for larger doses to be used and given intravenously for the principal study, hence doses of 50, 100 and 200 μg of DC-21-346 were adopted.

Eight healthy non-obese male volunteers were studied on four occasions. Each subject received DC-21-346 at three doses 50, 100 and 200 μg plus on a further occasion 100 μg GHRH(1-29)NH_2. The DC-21-346 and GHRH tests were performed in the manner described in the Methods chapter. Studies were carried out at a minimum of seven day intervals in a randomized double blind manner determined by a Latin square. The details of the subject are given in Table 17.

The GH response to DC-21-346 was very variable and consequently no statistically significant difference existed in the AUC or peak GH response between 100 μg GHRH and 50, 100, 200 μg DC-21-346 (Table 18; Figures 35 and 36). There was a trend towards a greater response to 100 μg than 50 μg DC-21-346, the former response being comparable with that seen with 100 μg GHRH. However, the AUC for 200 μg was less than the

response to 100 μg DC-21-346. Similarly, in the pilot study, the greatest response was seen in the two subjects when they received 100 μg DC-21-346 and both had a more modest GH response to 200 μg (Figure 33).

The only side-effects encountered with DC-21-346 were short-lived flushing and tachycardia. Subjectively, the flushing experienced with DC-21-346 was more severe than with GHRH.

In summary, in healthy volunteers an intravenous bolus of 100 μg DC-21-346 was approximately equipotent with 100 μg of $GHRH_{(1-29)}NH_2$.

RESULTS

Table 17

Age, weight, height and body mass index of subjects receiving DC-21-346

SUBJECTS	AGE (years)	WEIGHT (kg)	HEIGHT (m)	BMI (kg/m^2)
1	30	78.9	1.74	26.1
2	26	70.1	1.76	22.6
3	29	75.6	1.83	22.6
4	24	60.0	1.66	21.8
5	26	68.1	1.79	21.2
6	24	73.5	1.77	20.7
7	22	76.8	1.86	22.2
8	21	82.0	1.89	22.9
MEAN	**25.3**	**73.1**	**1.78**	**22.5**
RANGE	**21-30**	**60.0-82.0**	**1.66-1.89**	**20.7-26.1**

Table 18

The mean "area under the curve" for serum GH following either 50, 100, 200 μg of DC-21-346 and 100 μg GHRH (mean ± range shown)

	GHRH	**DC-21-346**		
	100 μg	**50 μg**	**100 μg**	**200 μg**
MEAN (mU.min/l)	403.3	157.7	481.1	282.8
RANGE	90 - 885	65 - 309	96 - 1552	145 - 462

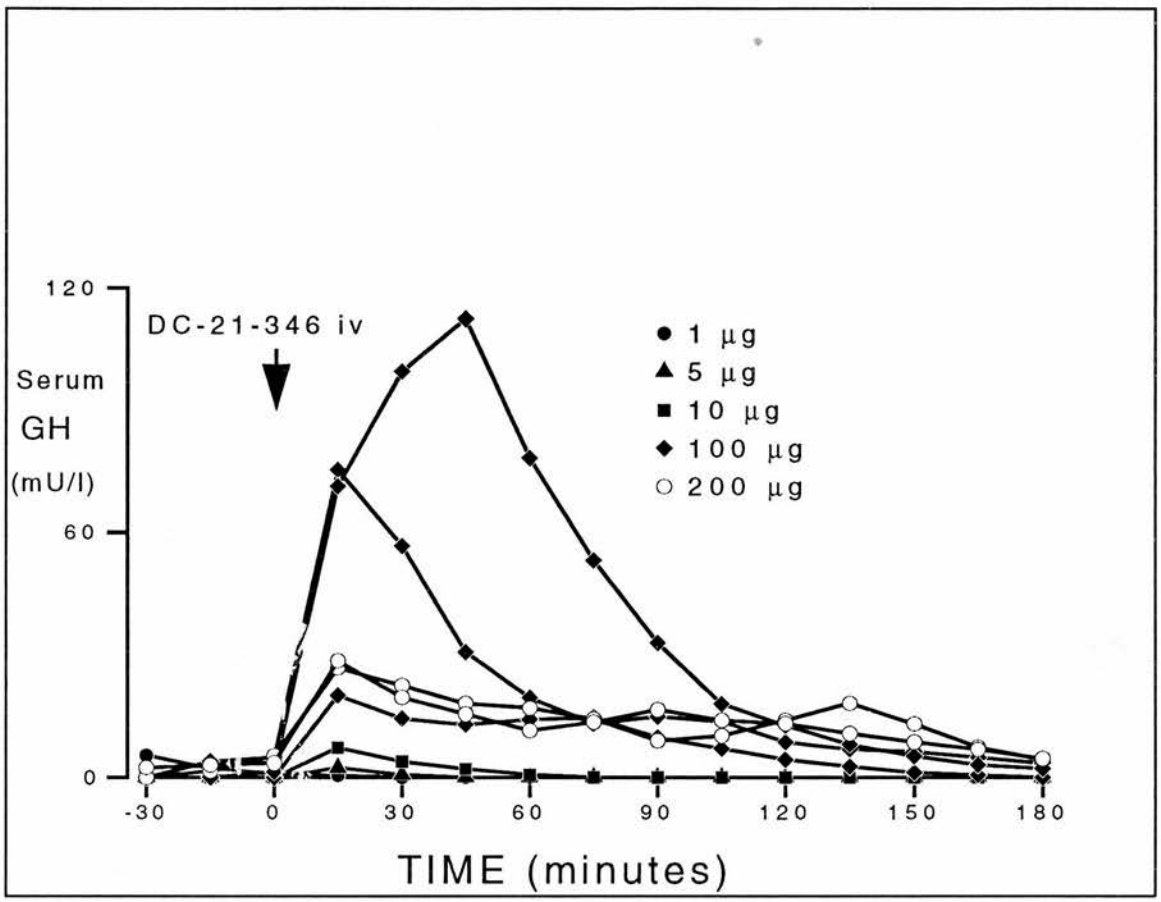

Figure 33

Serum GH responses, in individual subjects, to intravenously administered 1, 5, 10, 100 and 200 µg DC-21-346.

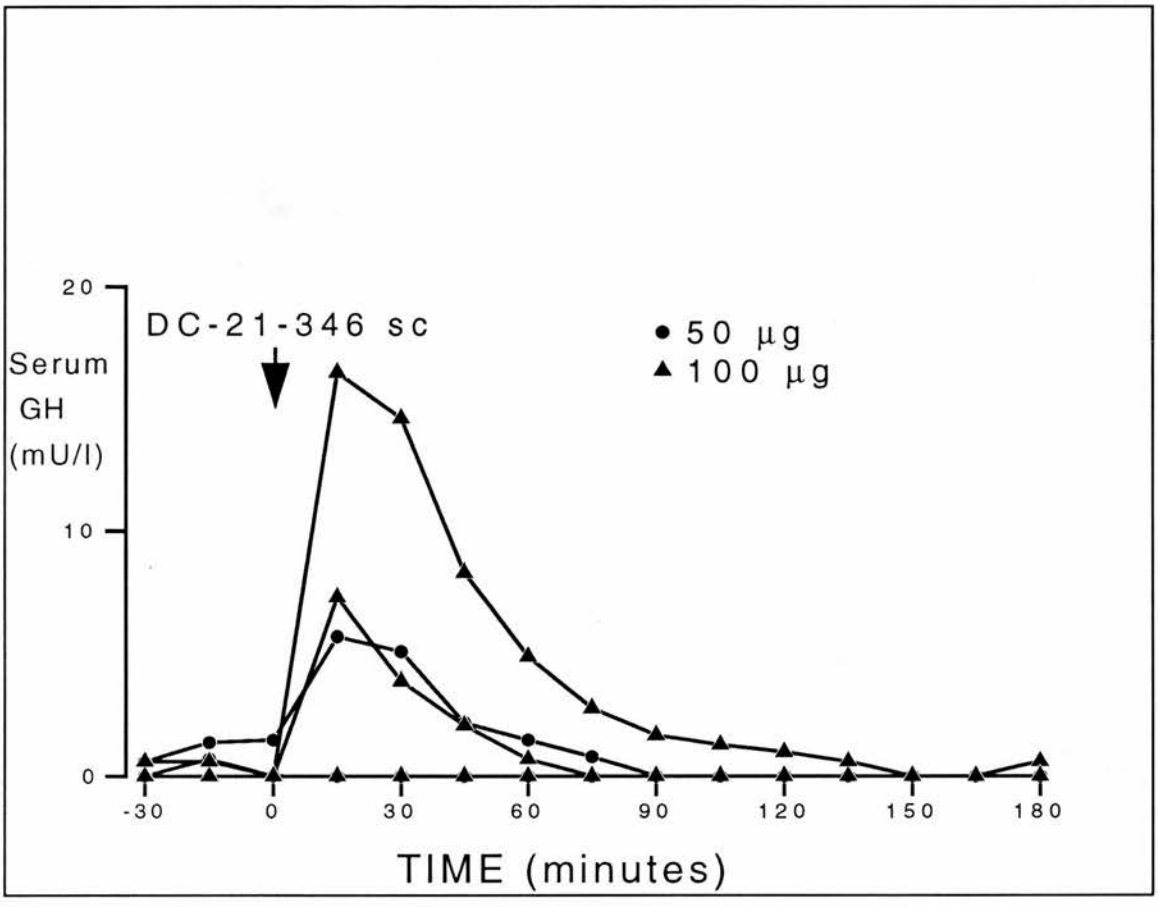

Figure 34

Serum GH responses, in individual subjects, to subcutaneously administered 50 and 100 µg DC-21-346.

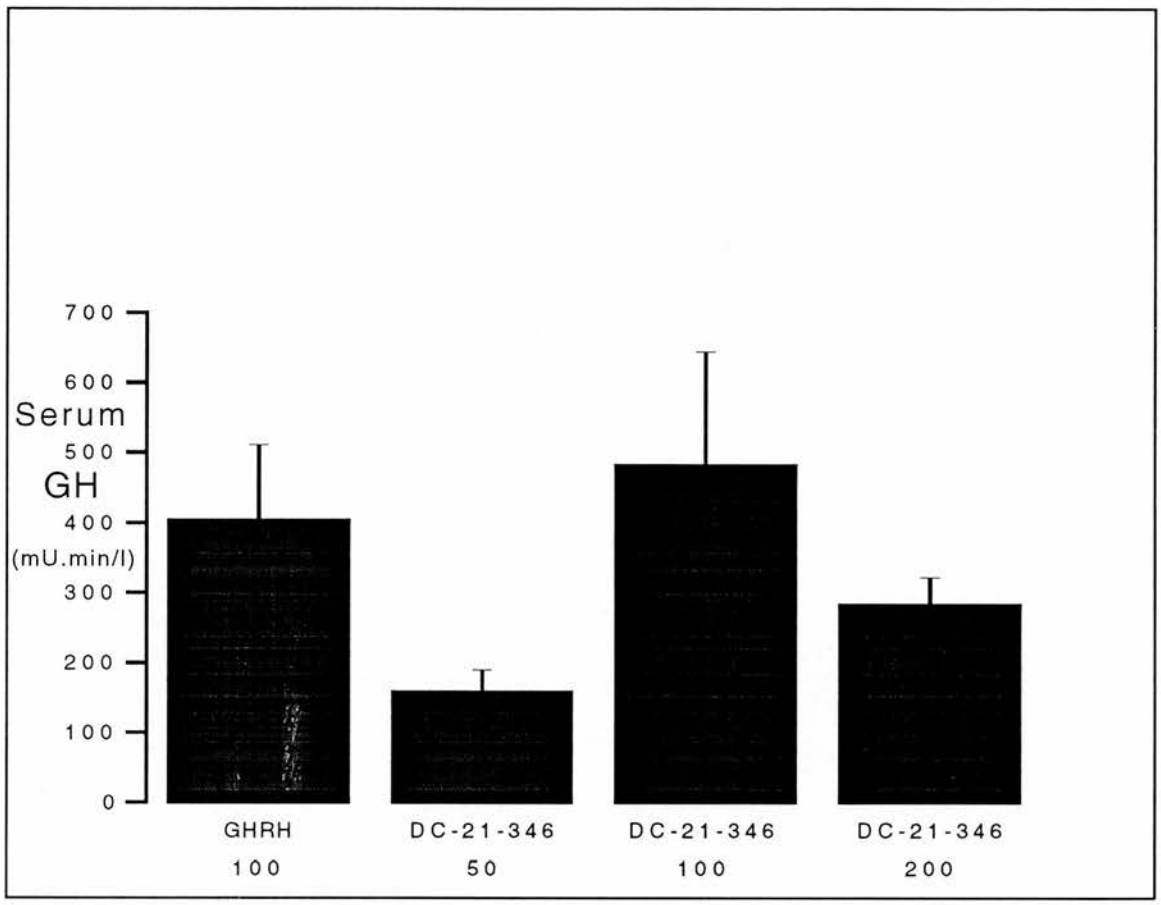

Figure 35

The mean "area under the curve" for serum GH following either 50, 100, 200 μg of DC-21-346 and 100 μg GHRH (mean ± SEM shown).

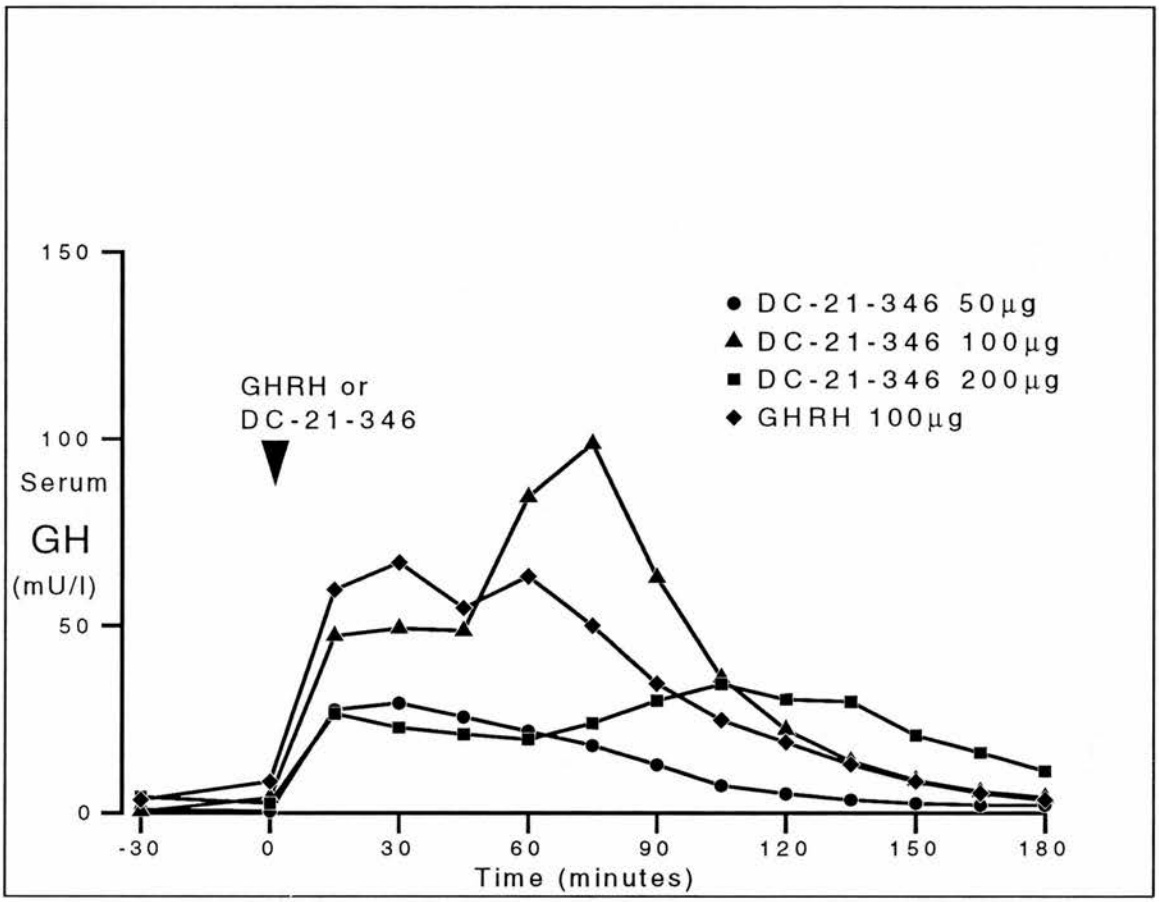

Figure 36

The mean serum GH response to intravenously administered GHRH (100 µg) or DC-21-346 (50, 100 and 200 µg) in eight healthy subjects (error bars not shown).

DISCUSSION

THE EFFECT OF SUBCUTANEOUS RECOMBINANT IGF-I (rhIGF-I 40 μg/kg) ON ANTERIOR PITUITARY FUNCTION

This study was designed to investigate the effect of rhIGF-I on basal anterior pituitary function using a dose (40 μg/kg) which was unlikely to cause hypoglycaemia. This dose was sufficient to raise the circulating levels of bioactive IGF by 60%, and resulted in changes in plasma insulin, C-peptide and TSH.

The fall seen in plasma IGF-II following rhIGF-I administration has been reported previously (Walker *et al.* 1991; Guler *et al.* 1989). IGF-I and -II have similar affinities to IGFBP-3 and the fall in IGF-II is probably due to the displacement of IGF-II by IGF-I from the IGFBPs, principally IGFBP-3 (Martin & Baxter, 1986), with the dislodged non protein-bound IGF-II then subject to rapid clearance from the circulation.

As discussed in the Introduction, IGF-I can autoregulate its own secretion by acting on the hypothalamo-pituitary axis to modulate GH secretion. In vitro, IGF-I is involved in the feedback regulation of GH secretion, with reported effects on both the hypothalamus and pituitary. Berelowitz et al. (1981) demonstrated that isolated rat hypothalami incubated with highly purified natural somatomedin-C released somatostatin in a dose-dependent manner. Studies on the effect of IGF-I on GHRH secretion have produced contradictory

results (Aquila, 1991; Shibasaki *et al.* 1986). Both GH mRNA and secretion are reduced by incubation with highly purified somatomedin-C (Berelowitz *et al.* 1981; Yamashita & Melmed, 1986). Further, when given intracerebroventricularly (icv), highly purified somatomedin-C has been shown to inhibit GH secretion in conscious rats (Tannenbaum *et al.* 1983; Abe *et al.* 1983). However, it is now believed that the preparations of highly purified somatomedin-C used in many of these early experiments, contained both IGF-I and -II. In contrast, icv recombinant IGF-I, in doses up to 10 μg, failed to alter GH secretion in rats. Likewise, icv recombinant IGF-II did not inhibit GH secretion. However, 1 μg IGF-I administered icv with 1 μg IGF-II resulted in a significant inhibition of GH secretion (Harel & Tannenbaum, 1992b; Harel & Tannenbaum, 1992a). The synergy of centrally administered IGF-I and -II in inhibiting GH secretion in rats underlines the importance of the hypothalamus in the regulation of GH secretion, possibly via modulating somatostatin secretion.

In a study of two euglycaemic men, a subcutaneous infusion of rhIGF-I at 20 μg/hr/kg inhibited nocturnal and GHRH-induced GH secretion in one of the subjects (Guler *et al.* 1989). Likewise, plasma GH levels were inhibited in a single subject with Laron's syndrome by an infusion of up to 24 μg/kg/hr of rhIGF-I (Walker *et al.* 1991). Cotterill et al. (1993a) demonstrated, in two children with Laron's syndrome, inhibition of GH secretion in the hours immediately after 120 μg/kg rhIGF-I subcutaneously. The inhibition was short-lived as the low IGFBP-3 levels associated with Laron's syndrome resulted in rapid clearance of rhIGF-I from the plasma. An intravenous infusion of 10 μg/kg/hr to

fasted normal volunteers, during euglycaemic clamps, resulted in suppression of GH secretion rates within 60 minutes, by which time total plasma IGF-I levels were increased 1.6 fold (Hartman *et al.* 1993). However, the interpretation is complicated by the increase in GH secretion and IGFBP-1 associated with fasting (Cotterill *et al.* 1993b). The lack of effect of IGF-I on GH secretion in this study in normal volunteers is in accordance with the results of Miell et al. (1992): using the same dose of subcutaneous rhIGF-I, they failed to demonstrate a change in GH secretion in patients catabolic after surgery.

It is probable that rhIGF-I, in larger doses than used here (Hartman *et al.* 1993), may inhibit GH secretion, but the route of administration may also be important. The slower entry of subcutaneously administered IGF-I may avoid saturating the binding capacity of the IGF-BPs although ultimately more IGF-I is available for release to target tissues. The increase in IGF bioactivity, without concurrent hypoglycaemia, supports this contention and contrasts with the uniformly hypoglycaemic effect seen in studies using intravenously administered IGF-I. The potentiation by rhIGF-I of the GH response following GHRH has not previously been reported. The potentiation of the GHRH response may be the result of either IGF-I induced inhibition of hypothalamic GHRH secretion or increased hypothalamic somatostatin secretion with a consequential accumulation of GH within somatotrophs that is then released in response to synthetic GHRH. The effect of the recombinant IGF-I on the secretion of these hypothalamic peptides must be subtle as no change in spontaneous GH secretion could be discerned. This area requires further exploration.

DISCUSSION

While GH, prolactin, LH and FSH levels or patterns of pulsatility did not change, there was a significant reduction in plasma TSH of the order of 30% (Figures 8, 9, 10, 12 and 15). This has previously been reported in adults with Laron's syndrome as well as in healthy volunteers (Laron *et al.* 1990; Lieberman *et al.* 1992).

Reduced TSH secretion might have been a consequence of IGF-I acting on the hypothalamus to stimulate secretion of somatostatin, an important regulator of TSH secretion (Hall *et al.* 1973). This seems unlikely however because no associated change in GH was seen. A more plausible possibility is a peripheral action of IGF-I to increase conversion of T_4 to T_3, with T_3 being the more active at the hypothalamus and pituitary to inhibit TSH secretion. In support of the latter hypothesis, Salomon and colleagues (1989) observed that in GH-deficient adults treated with GH, several subjects on fixed doses of thyroxine replacement therapy developed symptoms of thyrotoxicosis associated with a rise in plasma T_3. In a group of normal subjects given 0.125 mg GH daily for 4 days, a fall in plasma TSH and T_4 and a rise in T_3 was seen (Grunfeld *et al.* 1988), a finding confirmed by Jorgensen et al. (1994). Similar changes in thyroid function plus an increase in metabolic rate have been reported with one week of rhIGF-I therapy in normal volunteers (Zenobi, 1993). No change was seen in our study, however, in plasma free T_4 and free T_3. This may be indicative that any change in the values of plasma thyroid hormones, with the limitation of free thyroid hormone assays, may be too small to detect by 24 hours. Tachycardia on commencing rhIGF-I therapy has been reported and may be indicative of mild transient thyrotoxicosis (Vasconez *et al.* 1994). The effects of IGF-I on thyroid

function need to be further studied. For example, changes in thyroid function may account for alterations in lipid profiles with IGF-I therapy and thyroid dysfunction itself alters IGFBPs (Miell *et al.* 1993).

Recombinant IGF-I did not alter plasma ACTH or cortisol. All subjects had a plasma cortisol of < 50 nmol/l at some point between 24.00 h and 02.00 h (Figures 13, 14).

Plasma insulin and C-peptide levels fell in parallel following rhIGF-I and in the absence of any change in blood glucose (Figures 7, 16 and 17). Suppression of insulin secretion in the absence of hypoglycaemia has been reported previously (Guler *et al.* 1989; Walker *et al.* 1991; Lieberman *et al.* 1992) and Boulware et al. (1992) demonstrated insulin suppression during a 5 mmol/l euglycaemic clamp in subjects receiving an intravenous infusion of 20 μg/kg/hr rhIGF-I. It is difficult to differentiate between the relative influences of increased glucose clearance caused by IGF-I (Moxley *et al.* 1990; Jacob *et al.* 1989) and a secondary fall in plasma insulin from a direct effect of IGF-I on pancreatic islet cells to inhibit insulin secretion *in vitro*, as has been shown by Leahy and Vanderkerkhove (1990). Fifty μg/kg rhIGF-I eight hourly in lambs resulted in a small but significant rise in blood sugar presumably secondary to the documented suppression of insulin secretion (Cottam *et al.* 1992), and similarly Walker et al. (1991) noted post-prandial hyperglycaemia in a single patient with Laron's syndrome, treated by a continuous intravenous infusion of IGF-I. Hyperglycaemia in combination with low plasma insulin levels suggests that IGF-I inhibits insulin secretion in vivo. In our study, there was no

evidence of hyperglycaemia.

Circulating IGFBP-3 is principally hepatic in origin and is quantitatively the most important of the IGF-I binding proteins (Blum *et al.* 1990). Plasma levels are regulated by GH and it is therefore not surprising that, in the absence of an effect of rhIGF-I on GH secretion, IGFBP-3 levels do not change. Plasma IGFBP-1 levels are inversely related to circulating insulin levels and therefore an increase would be expected secondary to the inhibition of insulin secretion by rhIGF-I seen in this study (Darendeliler *et al.* 1990; Holly *et al.* 1988). That no change was observed in IGFBP-1 may reflect the fact that insulin levels only fell by 30%, and a corresponding increase in IGFBP-1 is unlikely to be detected on Western ligand blotting (Table 6, Figure 18).

In conclusion, our data confirm 40 μg/kg of rhIGF-I subcutaneously does not cause hypoglycaemia, but increases IGF bioactivity. The data support the belief that IGF-I acts directly to inhibit insulin secretion. No changes were seen in anterior pituitary function other than a fall in plasma TSH. The influence of rhIGF-I on thyroid function requires to be studied further. The GH response to GHRH was enhanced 24 hours after the IGF-I administration. There was no evidence of GH suppression at any time during the study.

DISCUSSION

THE INFLUENCE OF PYRIDOSTIGMINE ON GROWTH HORMONE SECRETION

Pyridostigmine has been extensively used in this research to modulate GH secretion. As alluded to in the introduction, pyridostigmine is thought to act on cholinergic neurones to inhibit somatostatin secretion and hence stimulate GH secretion. Appreciation of the importance of the cholinergic nervous system in the regulation of GH secretion was hindered by the observation that the cholinergic antagonist, atropine, did not block the GH response to hypoglycaemia (Blackard & Waddell, 1969). However, the significance of cholinergic neurones in the regulation of GH secretion was established by subsequent *in vitro* and *vivo* experiments. The addition of acetylcholine to rat hypothalamic segments inhibits somatostatin release (Richardson *et al.* 1980). *In vitro* in rats, the ability of cholinergic drugs to influence GH secretion is lost following pretreatment with the somatostatin-depleting drug, cysteamine, or anterolateral deafferentation of the mediobasal hypothalamus (Locatelli *et al.* 1986). In man, pyridostigmine augments and atropine abolishes the GH response to GHRH. However, atropine applied directly to cultured anterior pituitary cells is unable to inhibit the effect of GHRH, indicative that the cholinergic effect on GH secretion is not at the level of the pituitary (Massara *et al.* 1986a; Casanueva *et al.* 1986). The median eminence is the probable site of the cholinergic-somatostatinergic neuronal interaction. Both somatostatin nerve terminals and muscarinic cholinergic receptors are present in the median eminence which lies outwith the blood brain barrier, allowing systemically administered drugs, such as pyridostigmine , to demonstrate

their pharmacological properties on the neurones of the median eminence (Epelbaum *et al.* 1981; Morley *et al.* 1977).

Physostigmine, an extract of the calabar bean, was the first reversible acetylcholinesterase inhibitor to be identified. Kleinwachter (1864) described its use as an antidote to atropine poisoning, and the initial report of its value in mysthaenia gravis was published in 1934 (Walker, 1934). Therapeutically, physostigmine has been superseded by the synthetic muscarinic cholinergic agonists pyridostigmine and neostigmine. They are both quaternary ammonium compounds and as such unlikely to cross the blood-brain barrier (Borland *et al.* 1985). The peak plasma concentration of pyridostigmine occurs 1 - 2 hours after oral administration (Aquilonius *et al.* 1980). Side-effects encountered with pyridostigmine are dose related: gastro-intestinal cramps or diarrhoea, nausea, sweating and excess salivation. In patients with mysthaenia gravis overdosage results in increased muscle weakness. The side-effects encountered in the subjects reported here were limited to minimal gastro-intestinal disturbance.

The standard dose of pyridostigmine used in studies of GH secretion in adults is 120 mg (Ghigo *et al.* 1990c; Ghigo *et al.* 1990e; Ross *et al.* 1987d; Penalva *et al.* 1989; Corsello *et al.* 1992). Barbarino et al. (1991) demonstrated in men that the effect of pyridostigmine on GH secretion is dose-dependent between 30 and 120 mg, without any evidence of plateauing at the top dose studied. Castro et al. (1990) showed a greater peak GH response following 180 mg pyridostigmine than with 120 mg in obese subjects, although this did not

reach statistical significance because of the limited numbers tested. Evidence exists that 150 and 180 mg pyridostigmine stimulate greater secretion of GH than 120 mg (G. Delitala, personal communication). However, more detailed investigation of this, and adoption of a larger dose in clinical studies, has been limited by the side-effects. Hence, the dose of pyridostigmine discussed here, 120 mg, is not at the top of the dose-response curve but is rather the maximum tolerable dose. This is important in considering the results presented, as the influence of somatostatin on GH secretion is only partially modulated by 120 mg pyridostigmine.

DISCUSSION

THE RELATIONSHIP OF GROWTH HORMONE TO LINEAR GROWTH

The regulation of growth is complex and many factors determine growth velocity and final height. The rudimentary observation that children with GH-secreting pituitary adenomas become "giants", demonstrates a quantitative relationship between growth velocity and GH secretion. However, on more detailed scrutiny the relationship is more complex and non-linear. In children with GHD, doubling the dose of GH (10 to 20 mIU/l or 30 to 60 mIU/l) increases linear growth velocity only 1.3 fold (Preece *et al.* 1976; Frasier *et al.* 1981). Detailed study of the relationship of growth velocity and GH dose indicates a log-linear response, ie growth velocity increases in a linear fashion as a function of the logarithm of the dose (Frasier *et al.* 1981). The contribution of GH to final height accounts for less than 33% of adult height. Children with either absolute GHD or GH resistance, eg Laron's syndrome, attain a final height of approximately 110 - 140 cm; the mean male adult height in Britain is 175 cm. Many factors, in addition to GH, play a role in determining growth velocity and final height, some being clearly defined and understood, while others are just starting to be considered.

The influence of sex steroids on growth has long been known. Growth rate is maximal at puberty and syndromes of sex steroid deficiency and excess are associated with aberrant growth patterns. Sex steroids regulate growth by two means; a quantitative effect on GH secretion and a direct effect on bone. Mean adult height is 13 cm greater in men than women. Therefore, it might appear paradoxical that young women secrete more GH than

men. Thompson (1972a) was the first to demonstrate integrated GH secretion to be greater in young women than men of corresponding age. More recently, Ho et al. (1987) confirmed these findings and revealed a greater decline in total and pulsatile GH in older women than men. GH secretion correlated with plasma oestradiol levels. Similarly, the GH response to arginine and insulin-induced hypoglycaemia is larger in women, and oestrogens, but not androgens, result in an augmented response to these stimuli (Merimee *et al.* 1969; Merimee & Fineberg, 1971). Likewise, oestrogen therapy increases the GH response to exercise, arginine and hypoglycaemia (Frantz & Rabkin, 1965; Wiedemann *et al.* 1976; Merimee *et al.* 1969).

In men, no relationship exists between spontaneous or stimulated GH secretion and plasma testosterone levels (Butenandt *et al.* 1976; Thompson *et al.* 1972b; Ho *et al.* 1987). However, pretreatment with testosterone has been variously reported to increase and alternatively to have no effect on GH secretion in response to arginine and hypoglycaemia (Illig & Prader, 1970; Martin *et al.* 1968; Merimee *et al.* 1969). A possible explanation of these apparently contradictory results comes from studies using oxandrolone, a synthetic derivative of testosterone which has a higher anabolic to androgenic ratio compared to that of testosterone (Fox *et al.* 1962). Link et al. (1986) compared, in peripubertal boys, the effect of testosterone and oxandrolone on 24 hour GH profiles. GH secretion increased 4.3 fold from baseline in the testosterone-treated boys but no change was seen in the oxandrolone-treated group. Bierich (1983) also failed to demonstrate an increase in mean 24 hour GH secretion with oxandrolone therapy. Oxandrolone, in contrast to testosterone,

cannot be aromatised to 17β-oestradiol. It is probable that the increased GH release following testosterone therapy is the result of a secondary increase in plasma 17β-oestradiol levels. A physiological role for oestrogens in the male pubertal growth spurt is supported by the correlation between integrated GH concentrations and plasma oestradiol levels, while no correlation exists with testosterone levels (Ho *et al.* 1987; Thompson *et al.* 1972b; Butenandt *et al.* 1976). Further, evidence for the importance of the oestrogens in contrast to androgens is from the androgen resistance syndrome. These phenotypic females, but with a XY karyotype, have normal physiological male levels of oestradiol, secondary to aromisation, and a normal pubertal growth spurt again indicating the role of oestrogens rather than androgens in regulating growth in males (Zachman *et al.* 1986).

The elevated plasma androgen levels of untreated or inadequately treated congenital adrenal hyperplasia result in premature epiphyseal fusion and consequent short stature; mean adult height is 153 cm in men and 150 cm in women in untreated patients (Klingensmith *et al.* 1977; Urban *et al.* 1978). Children with syndromes associated with hypogonadism, such as Kallman's and Klinefelter's syndrome, have normal prepubertal growth but lack a pubertal growth spurt. However, final height is increased secondary to delayed epiphyseal fusion. These examples do not clarify whether it is androgens per se or aromatisation-derived oestrogens that regulate epiphyseal fusion. Insight can be gained from the report of a man with end-organ resistance to the effects of oestrogens, secondary to a mutation of the oestrogen receptor, with elevated plasma oestradiol levels and normal androgen levels. At 28 years of age he was 204 cm tall with a bone age of 15. His upper to lower body

segment ratio was 0.88 (normal 0.96) and the limited auxological data available suggests that he did not have a pubertal growth spurt (Smith *et al.* 1994). Many lessons can be learned from this case. The delayed epiphyseal fusion in this male with normal plasma androgens is indicative that, in males as well as females, epiphyseal fusion is an oestrogen-dependent process. The abnormal body proportion is comparable with that seen in syndromes of hypogonadism, such as Klinefelter's and Kallman's. The pubertal spurt in sitting height is predominantly sex steroid dependent and GH independent, while the increase in leg length is GH dependent (Copeland *et al.* 1977). The lack of a pubertal growth spurt is further evidence of the importance of oestrogens in the pubertal growth spurt in males (discussed above).

Evidence for a GH independent action of androgens on linear growth is limited. In the studies of oxandrolone, described above, an increase in growth velocity was documented in the absence of increased GH secretion (Link *et al.* 1986; Bierich, 1983). There is no evidence that androgens are involved in the regulation of growth in females.

Adequate nutrition is paramount in ensuring optimal linear growth. The best studied example of the influence of nutrition on growth of a population group is the Japanese experience since the Second World War. Improved nutrition between 1948 and 1978 resulted in a mean increase in the height of a Japanese six year old of 6.7 cm. At times of calorie shortage, nutrition is the most important factor in promoting the linear growth. Evidence indicates that the Japanese have reached their maximal height but remain on

average approximately 7 cm shorter than Europeans and Americans (Murata & Hibi, 1992). The difference is probably the consequence of as yet unidentified genetic factors.

Genetic factors underlying racial differences in height are important determinants of final height, although the relative contributions of genes and environment can be difficult to determine. The Pygmy race is synonymous with short stature. Pygmies have a normal plasma GH response to hypoglycaemia but the plasma IGF-I response to a fixed dose of GH is impaired; plasma IGF-I and GHBP levels are low, indicative of an abnormality of the GH receptor or related second messenger pathways (Merimee *et al.* 1989; Merimee *et al.* 1990; Rimoin *et al.* 1967; Baumann *et al.* 1989b). The Mountain Ok people of Papua-New Guinea are almost as short as the Pygmies but have normal plasma IGF-I levels and low GHBP, indicative of a different defect in the growth axis (Baumann *et al.* 1991). Each level of the GH axis; the GH receptor, GHBP, the ability to generate IGF-I, the IGF-BP, the IGF-I receptor, is a potential site for racial variation and by extrapolation may contribute to the variation in the general population. Gross abnormalities, eg Laron's syndrome, result in a clear phenotype; more subtle defects are less readily identified. The IGF-I generate test, which measures the IGF-I response to a standard dose of GH, is an attempt to recognise more elusive genetic anomalies (Cotterill *et al.* 1994). The persistence, after correction of dietary deficiencies, of a difference in final height between Japanese and Europeans may be a consequence of the adolescent growth spurt occurring younger age in the Japanese (Murata & Hibi, 1992). The underlying trigger to this phenomenon is not understood but probably is genetic rather than environmental. The importance of non-GH

axis genetic factors are even more difficult to quantitate. Ogata and Matsuo (1993) showed that the final height of patients with sex chromosome aberrations is defined by the dosage of pseudoautosomal and Y-specific growth genes. It must be envisaged that genetic studies such as these will ultimately cast light on the non-GH cellular events regulating growth.

DISCUSSION

A COMPARISON OF QUANTITATIVE GROWTH HORMONE SECRETION BETWEEN TALL (≥ 183 cm) AND SHORT (≤ 166 cm) YOUNG MEN

In the study reported in this thesis, the relationship of GH secretion to final height was explored by comparing quantitative GH in a group of tall (> 90th centile) and short (> 10th centile) young men. The subjects were healthy, post-pubertal, endocrinologically normal men between the ages of 18 and 27. Adults were studied in preference to children/juveniles for three reasons. Firstly, it is extremely difficult to gain access to a group of truly normal healthy children, particularly a group of tall and short children. Secondly, by studying a younger group defined by height, growth velocity, age and pubertal stage would be variables. Such a design would also fail to answer the intrinsic question of the relationship of GH secretion to final height. In addition, as others have noted, it is extremely difficult for ethical reasons to study normal children (Hindmarsh *et al.* 1987), particularly, as insulin-induced hypoglycaemia, in inexperienced hands, has resulted in children's deaths (Shah *et al.* 1992).

A potential drawback in studying adults, would exist if there is any change in GH secretion between puberty and maturity. Linear growth does not stop due to reduced GH secretion, but rather as a result of fusion of the long bone epiphyses. No apparent difference exists in the GH response to pharmacological stimuli between puberty and the age range of this study. As demonstrated in this thesis, the GH response to GHRH falls with age, but is unlikely to be significant by the age of 27. The GH response to insulin-induced

hypoglycaemia is unaltered in middle-aged men compared to younger subjects (Wakabayashi *et al.* 1986; Kalk *et al.* 1973).

Studies of spontaneous GH secretion have, with near uniformity, indicated an age-related decline. Alone, Dudl et al. failed to demonstrate a decline in GH secretion with age, a result at odds with all other groups (Prinz *et al.* 1988; Dudl *et al.* 1973; Carlson *et al.* 1972; Finkelstein *et al.* 1972; Rudman *et al.* 1981; Ho *et al.* 1987). Data on changes in spontaneous GH secretion between the end of puberty and the age of subjects in this study are very limited. Zadik and colleagues in two studies of integrated concentration of GH (IC-GH) secretion studied 292 subjects between 7 and 65 years of age. No decline was noted in IC-GH before 18 years of age (Zadik *et al.* 1990b). Although IC-GH fell with age, normal stature men and women may retain GH levels typical of puberty into the third decade of life, despite completing puberty and achieving final height (Zadik *et al.* 1985). In conclusion, although IC-GH has its limitations, it seems reasonable to believe no significant age related decrease in spontaneous GH secretion will have occurred yet in the subjects studied here.

The optimal manner of assessing GH secretion is controversial. Prior to the availability of recombinant GH, pharmacological tests of GH secretion were used to determine the eligibility of short children for treatment with cadaveric GH. The eligibility criteria related "supply and demand" rather than any biological markers of growth. Historically, a "normal response" meant GH therapy was not indicated. Clonidine, levodopa, arginine, exercise,

DISCUSSION

GHRH and insulin-induced hypoglycaemia all have their proponents as tests of GH reserve. All these tests are pharmacological rather than physiological tests of GH secretion, and although capable of identifying children with short stature due to severe GHD, are less good at discriminating between normal and children with short stature (Donaldson *et al.* 1991; Gelato *et al.* 1986; Hindmarsh *et al.* 1987).

A more physiological approach to the assessment of GH secretion is to measure spontaneous secretion over time. Two strategies exist for measuring 24 hour growth hormone levels: the measurement of GH on discrete samples collected at fixed intervals, eg 20 minutes as in this study, or measurement on continuously drawn samples, eg each sample being drawn over thirty minutes and aliquots of each being pooled to calculate an integrated concentration of GH. IC-GH measurement is performed with a continuous withdrawal pump and hence is less laborious for the investigator and less disruptive for the subject, but has the major disadvantage of not allowing GH pulsatility to be studied. The optimal frequency of discrete blood samples for GH is controversial. Evans and colleagues demonstrated that five-minute sampling identified more pulses than either fifteen or twenty minute samples (Evans *et al.* 1987), while Holl et al. (1991) showed that a sampling frequency of thirty seconds further enhanced pulse identification. The biological significance of micropulses remains to be determined.

The results of two 24 hour integrated concentrations of GH from different occasions correlate better than the results of repeated provocative pharmacological stimulation tests

(Zadik *et al.* 1990a). However, reproducibility of results is also a problem with secretion profiles; pooled overnight GH samples in children with short stature showed a between-night variation of -62 to +162% (Donaldson *et al.* 1989). Albertsson-Wikland and colleagues (1992) obtained two 24 hour GH profiles in nine prepubertal children with a mean interval of 1.5 years between samples. As a group no differences existed between the initial and repeat sampling; however the number and amplitude of peaks varied in individuals by ± 30% and it was concluded that one must have "a sound scepticism relating biological phenomena to a single profile of an individual child".

Although GH profiles are a more physiological test of GH secretion than provocative stimuli, they offer little diagnostic advantage when evaluating a short child. Rose et al. (1988) identified a group of short children with low stimulated GH values and normal mean 24 hour GH concentrations and concluded that GH profiles were inferior to pharmacological tests. Alternatively, it has been argued that stimulated GH values may underestimate spontaneous GH secretion (Donaldson *et al.* 1991; Siegel *et al.* 1984; Plotnick *et al.* 1979). This controversy largely revolves around relating biological phenomena to "normal ranges". It is extremely difficult, for ethical reasons, to obtain reference ranges from a truly normal cross-section of children. The situation is further complicated by differences in response depending on pubertal stage. The difficulty in selecting children, particularly those with milder GH insufficiency, for GH therapy based on pharmacological criteria, is reflected in the trend towards greater reliance on height and growth velocity (Brook, 1992).

DISCUSSION

For the purposes of this study, to maximise information growth hormone secretion was assessed under four conditions. All subjects underwent testing with insulin-induced hypoglycaemia as this is both a potent stimulus and a widely used test of GH reserve. The GH response to GHRH was used as a measure of the readily releasable pool of GH. GHRH was combined with pyridostigmine to gauge the contribution of cholinergic tone to GH secretion. Spontaneous overnight (9 hour) GH profiles, with a sampling interval of 20 minutes, were performed as a measure of spontaneous GH secretion. A sample interval of 20 minutes was chosen as it allows identification of major GH pulses.

In this study, no difference existed in the GH response to hypoglycaemia or GHRH with and without pyridostigmine between the tall and the short groups (Figures 19, 20 and 21), consistent with the observations, discussed above, that pharmacological tests are poor at discriminating between short and normal individuals. No consensus exists as to the minimum stimulated GH response in a short child needed to exclude GH insufficiency, but 20 to 30 mU/l are commonly suggested cut-offs. It is noteworthy that the mean GH response, in this study, to hypoglycemia was 106 mU/l (range 30 - 195) and to GHRH without pyridostigmine 59.6 mU/l (range 2.6 to 233) and 126 mU/l (range 23 - 233) with pyridostigmine.

The only previous study to relate the GH response to GHRH to final height reported a greater response in tall subjects compared to controls of average height. Batrinos and colleagues (1989) documented a greater response in a group of 20 tall (height > 187 cm)

members of the Greek Presidential Guard compared to 17 civilians of normal stature (height range 171 - 177 cm). The data contained in this thesis are at odds with Batrinos' results. Differences in variables such as diet, body fat and physical fitness between civilian volunteers and members of the Presidential guard may explain the discrepancy. Percentage body fat and physical fitness are known to influence GH secretion (Weltman *et al.* 1994) and Hagberg et al. (1988) improved the GH response of an elderly cohort with 12 weeks of treadmill training. In keeping with the data presented here, Gelato et al. (1986) performed GHRH tests in normal children and failed to document a relationship between GH response and height.

The results of both insulin tolerance and GHRH tests support the data from children that, excluding those with severe growth hormone deficiency, pharmacological tests are poor at discriminating between short and tall normal individuals.

This is the first study to relate final height to spontaneous GH secretion. Previous studies have related quantitative GH secretion, growth velocity and pubertal status before final height has been achieved. In a group of 119 children of normal stature, ranging between Tanner stages I and V, no correlation existed between growth rate and 24-hour IC-GH; however, pulses cannot be analysed by this technique (Zadik *et al.* 1990b). This is consistent with the lack of a difference in area under the curve for the nine hour overnight sampling period in this study. In both children and adults, a large proportion of GH secretion occurs within 2 hours of the onset of sleep (Quabbe *et al.* 1966; Takahashi *et al.*

1968; Honda *et al.* 1969; Eastman & Lazarus, 1973; Parker *et al.* 1969). The lights-out time, for subjects in this study, was 22.30 h, although the subjects did not instantly fall sleep. The area under the curve for GH secretion during the period of maximal GH secretion (24.00 h - 03.00 h) appears greater in the tall subjects (Table 10, Figure 22), although failing to reach statistical significance. Hindmarsh and colleagues (1987), in a study of poorly growing prepubertal children, noted an asymptotic relationship between height velocity (expressed in terms of a standard deviation score) and the sum of GH pulse amplitudes. Stated a different way, faster growing subjects secreted more GH than slower growing subjects but the range is a continuum with no dividing point between a short and normal child. The population studied was very different from ours, the fast growing child had an SDS of + 0.4 and the subjects were subdivided on the basis of the growth hormone response to hypoglycaemia, < 7 mU/l, < 15 mU/l and > 15 mU/l. This contrasts with the mean response in our subjects of 106 mU/l. At the opposite end of the growth spectrum, Albertsson-Wikland (1983) noted children growing at +2 SDS secreted more GH than short children. Mean pulse amplitude data from this study were interesting, with tall subjects secreting more (mean 20.3 mU/l ± SEM 3.4 v 13.6 ± 2.8) although the difference did not reach statistical significance.

In conclusion, this study was designed to explore the relationship of quantitative GH secretion to final height. Dynamic stimulation tests were unable to discriminate between the tall and short groups of subjects, indicative of the limitations of the diagnostic value of these tests. Assessment of nocturnal GH secretion was interesting. Although no difference

existed in the area under the curve for GH secretion for the total sampling period, there was a suggestion that in the tall subjects the area under the curve for the period midnight and 03.00 h and the mean pulse amplitude for the nine hour sampling period were greater, although neither reached significance. However, it must be concluded that no significant difference in GH secretion dynamics could be detected between the tall and short healthy young men in this study.

DISCUSSION

THE GH RESPONSE TO GHRH IN MEN AND WOMEN, AND IN WOMEN AT DIFFERENT STAGES IN THE MENSTRUAL CYCLE

Publications are contradictory as to whether there is a difference in the GH response to GHRH between men and women. For this reason, the studies included in this thesis, other than this one, were confined to men. As discussed above, it is undisputed that spontaneous and insulin-induced GH secretion is greater in young women than young men. In both sexes, the GH response correlates with plasma oestrogen levels, in which case it might be anticipated that if a difference exists between the sexes in the GH response to GHRH, then variation should be seen in the menstrual cycle.

This study was designed to clarify three points not previously addressed in a single cohort, viz., does a difference exist between men and women in the response GH to GHRH, is there a difference in cholinergic tone between the sexes and finally, does the response to GHRH vary during the menstrual cycle ? Eight healthy young women with regular menstrual cycles (28 ± 2 days) and eight young men were studied. The men underwent two GHRH tests, with and without GHRH, while the women were studied in a similar manner in early and mid-menstrual cycle.

Several groups have previously compared the GH response to GHRH between men and women but there has been a lack of consensus in the results. Smals et al. (1986b) reported a greater response in men while conversely both Lang et al. (1987) and Benito et al. (1991)

found a greater response in women. Gelato and various colleagues, in a study of peripubertal children and in a separate study in adults, failed to demonstrate a difference between the sexes, a finding corroborated by Arvat et al. (1993)(Gelato *et al.* 1984; Gelato *et al.* 1986).

In this study, no difference existed in AUC or peak GH response to GHRH either between the sexes, or in the women, at different stages of the menstrual cycle. The effect of pyridostigmine was the same on all limbs of the study (Table 12, Figures 23, 24, 25 and 26). No relationship existed between oestradiol levels and the GH response to GHRH (Table 13).

It is difficult to explain the contradictory results of other studies. The remarkable feature of the Benito study is that the mean peak GH response to GHRH in the men was approximately a quarter of that found in the women, much greater than the differences between the sexes in the GH response to other stimuli (Merimee *et al.* 1969; Merimee & Fineberg, 1971). If circulating oestrogen levels accounted for the difference, then a variation with the menstrual cycle might be anticipated, but this was not seen (male 9.26 ± 4.62 nmol/l, female day 1, 80.34 ± 23.8 nmol/l and, day 12, 74.68 ± 20.74 nmol/l [in original paper stated in ng/ml, conversion factor 2]). Smals et al. alone found a greater GH response in men. It is interesting to note that the peak GH response in the men was 82 ± 22 nmol/l, compared with 30 ± 8 nmol/l in women (Smals *et al.* 1986b). The GH response to GHRH is known to be very variable, in part due to fluctuating cholinergic and

somatostatinergic tone and this may explain the disparate results reported (Penalva *et al.* 1990b).

Two previous studies have used pyridostigmine to circumvent the influence of variable cholinergic tone on the GH response to GHRH, and reached very different conclusions. Uniquely, Barbarino et al. (1991) reported that pyridostigmine enhanced the GH response to GHRH in men, but had no effect in women at doses of 30, 60 and 120 mg. Another atypical feature of this study is the authors' comment that "women taking 120 mg pyridostigmine had more severe side-effects then men", and hence only five of the eight female subjects received 120 mg of pyridostigmine. The data provided are generally difficult to interpret, the AUC for the GH response to GHRH alone, for both men and women, is presented in the form of a bar chart, but no analysis is offered. The results presented here clearly demonstrate that pyridostigmine potentiates the GH response to GHRH in both sexes equally (Table 12, Figures 23, 24, 25 and 26), a conclusion supported by Arvat et al. (1993).

Merimee and Fineberg (1971) noted that the GH response to hypoglycaemia is greater pre- and post-ovulation than during menstruation. No difference existed in the GH response in the women studied here nor in any of the other studies that have compared the GH response to GHRH during the menstrual cycle (Evans *et al.* 1984; Benito *et al.* 1991; Gelato *et al.* 1984).

DISCUSSION

The ability of oestrogens to augment the GH response to hypoglycaemia and arginine, but not GHRH, requires examination. Ross et al. (1987b) treated a group of 14 prepubertal children with stilboestrol or placebo for 48 hours on two occasions, prior to performing insulin tolerance and GHRH tests. Stilboestrol enhanced the GH response to hypoglycaemia, but the response to GHRH was unaltered. This implies that oestrogens act on the hypothalamus rather than on the pituitary. Support for this conclusion comes from rodent experiments. Data from rats indicate that oestrogen replacement therapy reverses the decrease in hypothalamic somatostatin mRNA following oophorectomy, although the effect on somatostatin may be indirect and mediated via oestrogen-induced changes in catecholamine metabolism (Werner *et al.* 1988; McEwen, 1980).

In conclusion, the GH response to GHRH does not differ between the beginning and middle of the menstrual cycle or between the sexes. Taken in conjunction with studies of spontaneous and insulin-induced hypoglycaemia, oestrogens appear to exert their influence on GH secretion at a hypothalamic level, rather than directly on the pituitary. The results of this study imply that it is not necessary to exclude women from studies involving the administration of exogenous GHRH and indeed that they may be studied throughout the first 14 days of the cycle and possibly at any stage of the menstrual cycle.

DISCUSSION

THE EFFECT OF AGE ON THE GH RESPONSE TO GHRH IN MEN

Growth hormone, as its name implies, is intimately involved in the regulation of linear growth. However, as discussed in the introduction, GH has a multitude of actions in addition to its effects on epiphyseal growth plates. An appreciation of the importance of the metabolic effects of GH has come with recognition of the consequences of growth hormone deficiency (GHD) in adults.

Patients with hypopituitarism on full conventional replacement therapy, but not GH, are reported to have increased morbidity and mortality, specifically from cardiovascular problems (Rosen & Bengtsson, 1990). This observation and detailed investigation of adults with GHD has led to the description of a syndrome of adult GH deficiency (Cuneo *et al.* 1992). Adults with GHD are said to have depression, anxiety, reduced vitality, increased social isolation, reduced cardiac function, increased abdominal adiposity, reduced strength and exercise capacity and cold intolerance (Rosen *et al.* 1993a; Rosen *et al.* 1993b; Salomon *et al.* 1992; Bengtsson *et al.* 1992; Salomon *et al.* 1993; Cittadini *et al.* 1994; Merola *et al.* 1993). Growth hormone therapy in such adults is associated with improved well-being and plasma lipid profile, increased strength, lean body mass, muscle mass and bone density (Salomon *et al.* 1989; Orme *et al.* 1992; O'Halloran *et al.* 1993; Whitehead *et al.* 1992; Russell-Jones *et al.* 1993; Eden *et al.* 1993; Vandeweghe *et al.* 1993; Beshyah *et al.* 1994; Thuesen *et al.* 1994; Sartorio & Narici, 1994), indicative that GH is a potent metabolic regulator in addition to a stimulator of linear growth in children. Many of the

traits of adult GHD are characteristic of the "normal aging process". Aging is associated with reduced protein synthesis, a decrease in lean body mass and bone density, increase in body fat and weight gain (Forbes & Reina, 1970; Forbes & Halloran, 1976; Chon *et al.* 1980; Riggs *et al.* 1981; Sherman *et al.* 1990). In particular, the distribution of age-related adipose tissue is typically intra-abdominal rather than gluteal. Excess abdominal fat results in insulin resistance, raised lipids and increased risk of diabetes mellitus, hypertension and coronary heart disease (Kalkhoff *et al.* 1983). Twenty-four GH profiles have confirmed that spontaneous GH secretion is reduced by between 15 and 70% (Finkelstein *et al.* 1972; Zadik *et al.* 1985; Ho *et al.* 1987; Vermeulen, 1987; Corpas *et al.* 1992a), with a reduction in the frequency and amplitude of nocturnal pulses of GH (Carlson *et al.* 1972; Prinz *et al.* 1988; Rudman *et al.* 1981). It has been proposed that the decline in GH secretion with age is contributory to many of the deleterious metabolic consequences of aging, and might be reversed with GH therapy (Hoffman *et al.* 1992; Zachwieja *et al.* 1994; Rudman *et al.* 1990). In both adults with GHD and the elderly, large therapeutic studies are on-going to determine whether long-term benefits accrue.

This study was designed to explore the aetiology of the age-related decline in GH secretion. To determine whether the reduced GH secretion was hypothalamic or pituitary in origin, a combination of intravenous GHRH and pyridostigmine or placebo was used to stimulate GH secretion in men aged 20 through 88 years.

Consistent with previously published data, there was a progressive fall in the GH response

to GHRH with age (Figure 27). The majority of publications on the GH response to GHRH have found an age-related fall in GH secretion (Ghigo *et al.* 1992; Iovino *et al.* 1989; Giusti *et al.* 1992; Coiro *et al.* 1991), although two groups failed to demonstrate a difference (Corpas *et al.* 1992b; Pavlov *et al.* 1986). Pavlov et al. (1986) explained the decrease in GH response with age on the basis of increased body fat. The absence of a difference in body mass index between the groups excludes the possibility of body fat as an explanation of the data presented here.

In this study, pyridostigmine administration, in combination with GHRH, resulted in an augmented GH response at all ages but did not eliminate the age-dependent decline in GH secretion (Figure 28). Previous studies with pyridostigmine have found similar results (Giusti *et al.* 1992; Ghigo *et al.* 1992). Analysis for the relative effect of pyridostigmine demonstrated that, as with exogenous GHRH, its ability to stimulate GH secretion diminished with age. In other words, the reduced GH response to GHRH cannot be explained purely in terms of increased cholinergic-regulated somatostatin secretion.

In contrast to pyridostigmine, arginine, in combination with GHRH, is able to fully restore the GH response in the elderly (Ghigo *et al.* 1990d). Arginine is believed to inhibit somatostatin secretion by an alternative pathway to pyridostigmine, possibly via nitric oxide. This implies, contrary to the results with pyridostigmine, that the effect of somatostatin increases with age. Several explanations are possible to explain this discrepancy. The cholinergic regulation of somatostatin secretion may be less important

in the elderly than arginine related mechanisms, or the aging hypothalamus may be less sensitive to pyridostigmine. These explanations are improbable as arginine in clinical studies of obesity, hypoglycaemia, anorexia nervosa, hyperthyroidism, obesity and Cushing's syndrome consistently induces greater GH secretion than pyridostigmine (Ghigo *et al.* 1990b; Giustina *et al.* 1992; Cappa *et al.* 1993; Ghigo *et al.* 1993; Procopio *et al.* 1995). As discussed, 120 mg of pyridostigmine is the maximum tolerated dose of pyridostigmine, but is not at the top of the dose-response curve. Thirty grammes of arginine intravenously have a maximal effect on GH secretion. Hence, the ability of arginine to stimulate greater GH secretion may be a phenomenon of dosage rather than a reflection of any true difference in inhibition of somatostatin secretion. No data exist to indicate an age-related difference in the clearance of pyridostigmine.

The reduced GH response with age to GHRH is indicative of a pituitary responsiveness which may be primary or secondary to hypothalamic changes. The ability of arginine to restore GH secretion implies a role for hypothalamic somatostatin. The ability of repetitive administration of GHRH to elderly men to stimulate GH secretion, such that IGF-I levels return to normal, implies down-regulation of somatotroph function in the elderly secondary to reduced GHRH secretion (Muller *et al.* 1988; Corpas *et al.* 1992b). Likewise, the health of the somatotroph is indicated by the lack of age-related difference in the response to hypoglycaemia (Ghigo *et al.* 1990d; Kalk *et al.* 1973; Wakabayashi *et al.* 1986). Data from rats indicate reduced GHRH and increased somatostatin secretion in elderly animals (Ge *et al.* 1989; De Gennaro Colonna *et al.* 1989; Morimoto *et al.* 1988). The increase in

somatostatin secretion is in keeping with the observation of reduced acetylcholine secretion in the brains of elderly rats (Gibson *et al.* 1981). *In vitro* data from rats also imply changes in the elderly pituitary. The *in vivo* data from man indicating reduced GH secretion in response to GHRH are paralleled by *in vitro* studies in elderly rats, where somatostatin secretion is not a factor (Ceda *et al.* 1986). Contradictory evidence exists on the viability of second messenger pathways in aging somatotrophes. Initial reports suggested reduced activity of the GHRH-cyclic adenosine-3',5'-monophosphate (cAMP) signal pathway, but a recent report has suggested that the second messenger pathway is intact but the population of functioning somatotrophs is only 50-60% of that in young rats (Ceda *et al.* 1986; Robberecht *et al.* 1986; Shimokawa *et al.* 1994).

In conclusion, the serum GH response to GHRH decreases with age. Modulation of somatostatin secretion with pyridostigmine augments the GH response at all ages. The influence of pyridostigmine is not greater in the elderly, indicating that the age-related decline cannot entirely be attributed to an age-related increase in cholinergic nervous-system regulated somatostatin secretion. Studies with arginine have demonstrated that GH secretion can be fully restored, suggesting that increased somatostatin is the principal cause of diminished GH secretion in the elderly, although alteration in somatotroph function may also contribute.

DISCUSSION

THE EFFECT OF PRE-TREATMENT WITH DEXAMETHASONE ON THE GH RESPONSE TO INTRAVENOUS GHRH

The inhibitory effects of supraphysiological levels of glucocorticoids on growth have long been known, both when they have been used for the suppression of inflammation, as well as in management of Cushing's syndrome (Friedman & Strang, 1966; McArthur *et al.* 1979; Preece, 1976). Basal levels of growth hormone (GH) and the response to insulin-induced hypoglycaemia and GH-releasing hormone (GHRH) are impaired in Cushing's syndrome (Demura *et al.* 1972; Smals *et al.* 1986a; Burguera *et al.* 1990) and in healthy volunteers given oral glucocorticoids to simulate Cushing's syndrome (Hartog *et al.* 1964; Nakagawa *et al.* 1969; von Werder *et al.* 1971; Burguera *et al.* 1990). However, the site of action of corticosteroids in this situation remains uncertain. Previous studies have indicated that the failure of GHRH to stimulate GH secretion in the presence of exogenous GH, glucose, free fatty acids and obesity can be reversed, at least partially, with pyridostigmine (Ross *et al.* 1987d; Penalva *et al.* 1989; Penalva *et al.* 1990a; Castro *et al.* 1990), implying that the reduction in GH under these conditions, without pyridostigmine, is due to increased somatostatinergic tone. This study was designed to investigate the mechanism of the inhibitory effect of short-term sustained increased circulating corticosteroid levels on stimulated GH secretion.

The inhibitory effects of sustained elevated plasma glucocorticoid levels on linear growth and GH secretion are well known. However, confusion exists as to the acute effects of

glucocorticoids on GH secretion. Casanueva and colleagues (1990) have shown that dexamethasone, given at 4 mg intravenously or 8 mg orally, stimulates secretion of GH. The same workers have, in addition, shown that 4 mg dexamethasone iv potentiates the GH response to GHRH administered 3 h later (Burguera *et al.* 1990). In contrast, 25 mg of oral cortisone acetate administered 60 min before GHRH has been reported to ablate the GH response (Giustina *et al.* 1990a). Dexamethasone (8 mg) given orally 12 h before GHRH or 22 mg given over 48 h have both been shown to inhibit the GH response to GHRH, although 1 mg 9 h earlier had no effect (Burguera *et al.* 1990; Del Balzo *et al.* 1990; Rupprecht *et al.* 1990). The results of this study demonstrate a loss of the GH response to GHRH in healthy volunteers after 48 h of elevated plasma glucocorticoid levels, a result in accordance with the findings of Burguera et al. (1990). The failure of a supraphysiological dose of GHRH(1-29)NH_2 to restore GH secretion makes it unlikely that the glucocorticoid-induced inhibition of GH secretion is due simply to suppression of hypothalamic GHRH. In this study the response to GHRH is significantly, although only partially, restored by 120 mg oral pyridostigmine administered 60 min before the GHRH, a result in accordance with those of others (Del Balzo *et al.* 1990; Giustina *et al.* 1991). Giustina et al. (1990b) demonstrated that 50 mg oral cortisone acetate 60 min before GHRH could inhibit the subsequent GH response, and that the addition of pyridostigmine fully restored the GH response. It therefore seems probable that dexamethasone acts acutely to inhibit GH secretion by increasing hypothalamic somatostatin secretion. The failure of pyridostigmine to reverse the dexamethasone-induced inhibition of GH secretion fully is in accord with the finding that 120 mg pyridostigmine also failed to restore the GH response to GHRH in subjects pretreated with glucose and GH (Penalva *et al.* 1989; Ross

et al. 1987d). However, as discussed above, the adoption of a larger dose in clinical studies has been limited by the side-effects. The inability of pyridostigmine to reverse the dexamethasone-induced inhibition of GH release completely may be dose-related, 120 mg being insufficient to inhibit hypothalamic somatostatin secretion entirely. The partial reversibility of the GH response to GHRH seen in this study after 48 hours of dexamethasone administration, contrasts with the full reversibility reported by Giustina et al. (1990b) 60 minutes after cortisone acetate. It seems possible that after 2 days of elevated plasma glucocorticoid levels, the exposure to increased somatostatin may have substantially depleted the readily releasable pool of GH. In patients with Cushing's syndrome, pyridostigmine was unable to restore, even partially, the GH response to GHRH (Leal-Cerro *et al.* 1990), indicating that somatostatin secretion is less important in inducing inhibition of GH secretion in subjects with chronically elevated circulating glucocorticoids. A direct effect of dexamethasone on somatotrophs in the present studies cannot be excluded.

In conclusion, this study has demonstrated that 2 mg of dexamethasone administered every 6 hours for 48 hours inhibits the GH response to GHRH. The effect of GHRH can in part be restored by 120 mg pyridostigmine, indicating that the mechanism of the inhibition may be increased hypothalamic somatostatin secretion. The failure of pyridostigmine to restore the GH response fully may be due to the use of a submaximal dose of pyridostigmine being used, or alternatively to the fact that other mechanisms are also involved, such as reduced pituitary stores of readily releasable GH.

DISCUSSION

A COMPARISON OF THE GH RESPONSE TO GHRH(1-29)NH_2 AND A SYNTHETIC GHRH ANALOGUE (DC-21-346) IN HEALTHY VOLUNTEERS

The potential therapeutic indications for GH are expanding beyond children with classical GH insufficiency. In children, GH therapy increases growth velocity in children with Turner's syndrome and renal failure, and is under investigation in inflammatory bowel disease and other disorders associated with growth failure (Rosenfeld *et al.* 1992; Fine *et al.* 1994). In adults, as already discussed, evidence is accumulating for the benefits of GH therapy in adults with growth hormone deficiency, and the elderly. The value of GH therapy is being studied in patients on long-term glucocorticoid therapy (Giustina *et al.* 1995).

Growth hormone treatment is limited by the necessity of parental administration, expense and, at least theoretically, by the resultant unphysiological chronically elevated plasma GH levels. GHRH has emerged as a potential alternative to GH therapy in many clinical settings. The majority of children with isolated GH deficiency have a hypothalamic defect of synthesis or release of GHRH (Grossman *et al.* 1984b). Continuous infusion of GHRH has been demonstrated to result in augmentation of pulsatile GH secretion in normal men and GH-insufficient children (Tannenbaum & Ling, 1984; Vance *et al.* 1985; Sassolas *et al.* 1986; Rochiccioli *et al.* 1986; Kirk *et al.* 1994). GHRH is a simpler molecule to synthesise and, therefore, has the potential to be cheaper than GH therapy. The first demonstration of the therapeutic long-term efficacy of GHRH came from Ross et al.

(1987c). They demonstrated that twice daily subcutaneously-administered $GHRH_{(1-29)}NH_2$ could stimulate linear growth in approximately 50% of children with severe GH deficiency, a finding subsequently GHRH confirmed and extended to children without classical GH insufficiency (Thorner *et al.* 1988; Brain *et al.* 1990; Duck *et al.* 1992; Kirk *et al.* 1994). The demonstration that repetitive administration of GHRH to elderly men and patients with Cushing's syndrome augments GH secretion suggests that GHRH therapy may have a therapeutic role in these situations and other conditions of reduced GH secretion with an intact pituitary (Muller *et al.* 1988; Corpas *et al.* 1992b; Leal-Cerro *et al.* 1993). The current disadvantage of GHRH therapy is the need for parental administration at least twice daily, compared to once a day for GH, or else the necessity for a child to wear an infusion pump continuously.

The recognition of the value of GHRH therapy has spurred research in two areas, viz. the development of a depot preparation of slow release GHRH and superpotent GHRH analogues. No depot preparation of GHRH is currently available.

In order to be a viable proposition, an analogue of GHRH would either have to be a superagonist at the GHRH receptor and/or resistant to degradation. The native forms of GHRH are shown in Figure 1, with the GH releasing activity of GHRH residing in the N-terminal 29 amino acids (Lance *et al.* 1984; Ling *et al.* 1984).

Studies on the tertiary structure of GHRH underlie attempts to develop a superpotent

GHRH analogue. GHRH has considerable sequence homology with glucagon, vasoactive intestinal peptide (VIP) and secretin, and can thus be classified as a member of the secretin-glucagon family of peptides. Physicochemical studies suggest that the C-terminal two thirds of the human $GHRH_{(1-29)}NH_2$ (residues 13 - 29) possess a distinct amphiphilic α-helical structure both in solution and when receptor-bound (Velicelebi *et al.* 1986). GHRH, in common with other peptide hormones acting at the membrane, eg calcitonin, has a characteristic amphiphilic secondary structure: one face of the molecule has preferentially hydrophobic residues, whereas the hydrophilic domain is on the opposite side (DeGrado *et al.* 1981).

The preferred confirmation of the N-terminal decapeptide region is less well established. Coy et al. (Coy *et al.* 1985) demonstrated the favoured structure is a β-bend (residues 8 - 12), formed around the Asn in position 8 (Garnier *et al.* 1978). The presence of an aromatic residue at position-1 has also been shown to be a requirement for full receptor-ligand interaction (Ling *et al.* 1984). Moreover, the residues in positions-1&2 are important in the metabolism of GHRH. *In vivo* plasma studies have shown that enzymatic degradation of GHRH is initiated by a dipeptidylpeptidase which recognises the NH_2-terminal Tyr^1-Ala^2-sequence (Frohman *et al.* 1986).

Many strategies have been used to enhance the GH releasing activity of GHRH. Enhancement of the amphiphilic α-helical properties of the central and C-terminal regions of GHRH by substitution with helix-favouring amino acids, particularly Ala, can result in

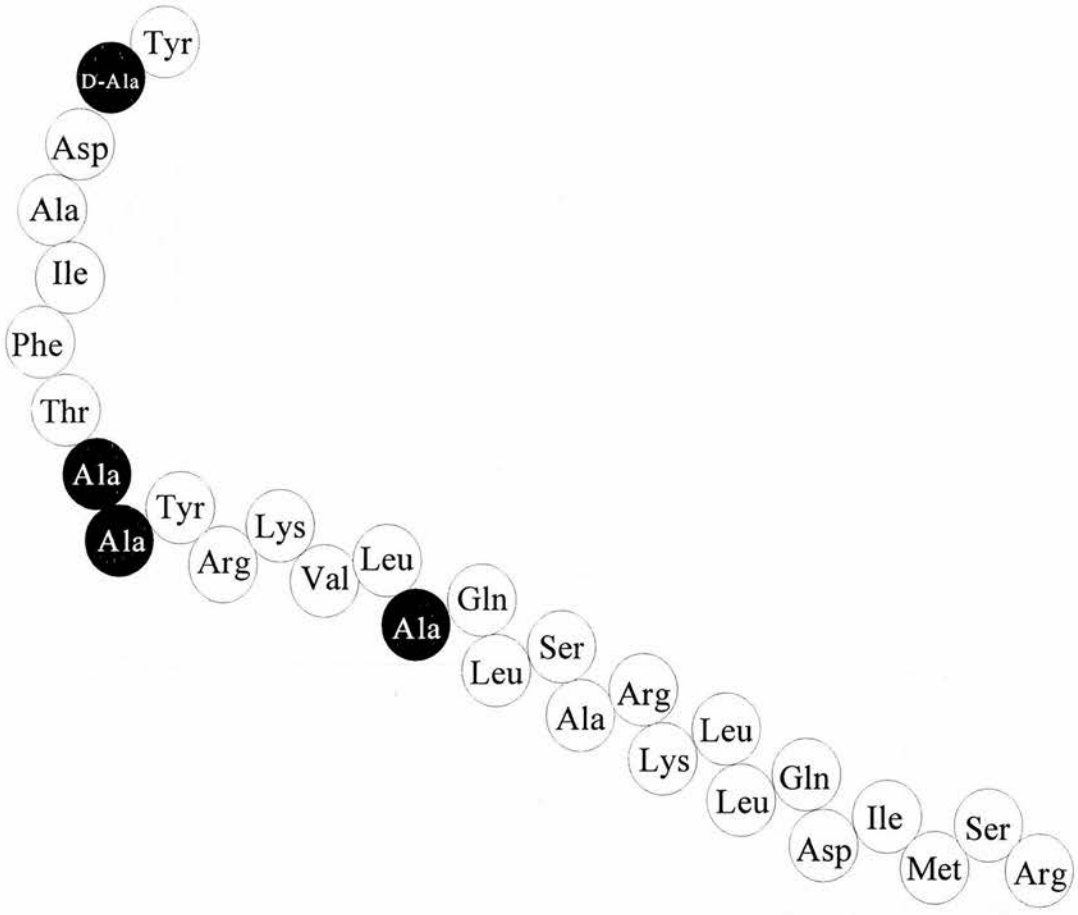

Figure 37

The amino acid sequence and structure of DC-21-346, with substitutions

significant improvements in GH-releasing potencies *in vitro* (Velicelebi *et al.* 1986; Tou *et al.* 1986). Substitution of the Asn in position in 8 with D-Asn has been shown to stabilise the β-bend. Lance et al. (1984) demonstrated that a substitution of D-Ala in position 2 resulted in a 50-fold increase in GH secretion in anaesthetised rats and as D-amino acids are known to favour β-bend formation, this adds further support to the belief that the N-terminal adopts a β-bend configuration. Modifications, such as D-Tyr in position-1, result in large increases in *in vivo* potency by blocking dipeptidylpeptidase

activity (Felix *et al.* 1988).

DC-21-346 was selected for clinical trials from a series of GHRH analogues tested for GH releasing activity on a monolayer of dispersed rat anterior pituitary (Coy *et al.* 1991). DC-21-346 is a 29 amino acid peptide with four substitutions intended to increase the GH releasing potency of $GHRH_{(1-29)}NH_2$ (Figure 37). The D-Ala substitution in position-2 has been shown to increase activity many fold possibly due to improved biological stability, although *in vitro* data and time course experiments indicate that the high activity may be the result of increased receptor affinity. Alanine is the best amino acid α-helix-inducing residue (Lyu *et al.* 1990; O'Neil & DeGrado, 1990). The Ala substitutions for Asn in position-8 and Ser in position-9 increase the probability of α-helix formation at the expense of β-turn formation from positions-5 through -10. Similarly, the Ala substitution for Gly^{15} enhances α-helix structure. DC-21-346 stimulated 49 times more GH release than $GHRH_{(1-29)}NH_2$ from monolayers of rat anterior pituitary cells (Coy *et al.* 1991).

Disappointingly, as the data presented here indicate, DC-21-346 has no advantage over human sequence GHRH(1-29)NH_2 and possibly has antagonistic properties at high doses (200 μg) (Table 18, Figures 35, 36).

Human and rat GHRH have 30 % non-homology; 13 amino acids in 43, 8 in the C-terminal 29. Rat GHRH is two to three times more potent in stimulating GH secretion from rat pituitary than human (Speiss *et al.* 1983). Similarly, rat and mouse GHRH are equipotent

in inducing GH secretion from mouse somatotrophs, both being more potent than human. However, the encouraging data from the cultured rat pituitary cells justified a clinical study, in the hope that DC-21-346 would be a more potent alternative to GHRH and hence cheaper therapeutic option. Unfortunately, DC-21-346 has no obvious therapeutic advantage. No human somatotroph cell line exists to test analogues prior to clinical studies.

In conclusion, the expanding indications for GH therapy, and the emergence of GHRH as alternative, has spawned development of GH secretagogues. DC-21-346 is a 29 amino acid synthetic analogue of GHRH $_{(1\text{-}29)}NH_2$ designed to have greater affinity at the GHRH receptor and resistance to degradation. *In vitro* studies demonstrated DC-21-346 to be 49 times more potent than GHRH $_{(1\text{-}29)}NH_2$ in stimulating GH secretion from rat anterior pituitary cells. Unfortunately, the data presented here indicates DC-21-346 to be no more effective in stimulating GH, in man, than GHRH $_{(1\text{-}29)}NH_2$. Differences in the GHRH receptor between man and rat probably account for the discrepancy in potency. In the absence of a primate somatotroph cell line, it remains difficult to screen GHRH analogues meaningfully and studies on a second non-rodent species prior to human trials are indicated.

CONCLUSIONS

The work reported explored the neuroregulation of growth hormone secretion in man. In particular, the consequences for pituitary function of IGF-I were studied. In previous studies, hypoglycaemia has complicated IGF-I administration, but, at the dose and by the route of administration used here, hypoglycaemia was not a problem. The only change observed in spontaneous pituitary hormone secretion was a reduction in TSH secretion. Although no change was detected in spontaneous GH secretion over the 24 hours following IGF-I, the GH response to GHRH, administered at 24 hours, was potentiated by prior IGF-I administration.

In a unique comparison of quantitative GH secretion in tall and short normal young adults, provocative tests were unable to distinguish between the two cohorts. Assessment of spontaneous nocturnal of GH secretion suggested that mean GH pulse amplitude was greater in the tall subjects, although the difference did not reach significance.

Previous studies have drawn contradictory conclusions on the influence of gender and oestrogens on the GH response to GHRH. It has been variously reported that the response is greater, smaller or no different in females compared to males. The data included show that no difference exists between the sexes, nor at different stages of the menstrual cycle. As plasma GH is known to correlate with plasma oestrogen levels, these results are indicative that oestrogens exert their leverage on GH secretion at a hypothalamic, rather

than pituitary, site.

Many clinical and biochemical stigmata of aging are found in adults with growth hormone deficiency, suggesting an aetiological role for diminished GH secretion in the aging process. Improved understanding of the mechanism of the age-related decline in GH secretion may allow endogenous GH secretion to be pharmacologically manipulated. Data presented here confirm previous reports of a progressive fall in the GH response to GHRH with age in the subjects, who ranged between 20 and 88 years. Modulation of somatostatin secretion, with the anticholinesterase pyridostigmine, augmented GH release at all ages but, as with GHRH, the effect of pyridostigmine declined with age. This implies that diminished GH secretion cannot be attributed entirely to increased cholinergic-regulated somatostatin secretion, but that other factors, such as changes in endogenous GHRH or reduced somatotroph sensitivity, may play an important role.

Elevated circulating glucocorticoid levels inhibit spontaneous and pharmacologically provoked GH secretion. The catabolic properties of glucocorticoids may, in part, be due to inhibition of, the anabolic hormone, GH. The results proffered show that in the short-term, pyridostigmine is able partially to restore the GH response to GHRH, suggesting that glucocorticoids act on the hypothalamus to stimulate somatostatin secretion. High doses of pyridostigmine result in uncomfortable gastrointestinal side-effects which makes 120 mg the largest acceptable dose, although not the maximal for GH secretion. The inability of pyridostigmine fully to restore the GH response to GHRH may signify additional

mechanisms by which glucocorticoids inhibit GH secretion, but more probably a dose of 120 mg of pyridostigmine had a submaximal effect. Better tolerated inhibitors of somatostatin secretion may be better able to restore GH secretion in the presence of elevated plasma glucocorticoid levels.

GHRH therapy has successfully stimulated linear growth in both short children with severe GH deficiency and normal growth hormone dynamics. The therapeutic potential of GHRH is limited by the necessity for twice daily injection. DC-21-346, a GHRH analogue, proved to be a potent stimulus for GH release in rats, fifty times more potent than conventional GHRH. Unfortunately, in normal volunteers, DC-21-346 offered no advantage over $GHRH_{(1-29)}NH_2$. The human and rat GHRH receptors differ; future GHRH analogues will need to be tested on primate rather than rodent somatotrophs.

It is hoped that the findings presented in this thesis will be used to advance our knowledge of this important subject, and that further research will enable the new questions that have emerged during these studies to be addressed.

REFERENCES

ABE, H., MOLITCH, M.E., VAN WYK, J.J. & UNDERWOOD, L.E. (1983) Human growth hormone and somatomedin C suppress the spontaneous release of growth hormone in unanesthetized rats. *Endocrinology*, **113**, 1319-1324.

ABRAMS, R.L., GRUMBACH, M.M. & KAPLAN, S.L. (1971) The effect of administration of human growth hormone on the plasma growth hormone, cortisol, glucose, and free fatty acid response to insulin: evidence for growth hormone autoregulation in man. *Journal of Clinical Investigation*, **50**, 940-950.

ALBERTSSON-WIKLAND, K., ROSBERG, S., ISAKSSON, O. & WESTPHAL, O. (1983) Secretory pattern of growth hormone in children of different growth rates. *Acta Endocrinologica,Supplement*, **256**, 72(Abstract)

ALBERTSSON-WIKLAND, K. & ROSBERG, S. (1992) Reproducibility of 24-h growth hormone profiles in children. *Acta Endocrinologica*, **126**, 109-112.

AMIT, T., HERTZ, P., ISHSHALOM, S., LOTAN, R., LUBOSHITZKI, R., YOUDIM, M.B. & HOCHBERG, Z. (1991) Effects of hypo-thyroidism or hyper-thyroidism on growth hormone-binding protein. *Clinical Endocrinology*, **35**, 159-162.

AQUILA, M.C. (1991) Insulin-like growth factor I regulates somatostatin (SRIF) release and messenger ribonucleic acid levels in rat hypothalamus. *74th American Endocrine Society (San Antonio)*, 1283(Abstract)

AQUILONIUS, S.-M., ECKERNAS, S.-A., HARTVIG, P., LINDSTROM, B. & OSTERMAN, P.O. (1980) Pharmacokinetics and oral bioavailability of pyridostigmine. *European Journal of Clinical Pharmacology*, **18**, 423-428.

ARCE, V., CELLA, S.G., LOCHE, S., GHIGO, E., DEVESA, J. & MULLER, E.E. (1990) Synergistic effect of growth hormone-releasing hormone (GHRH) and clonidine in stimulating GH release in young and old dogs. *Brain Research*, **537**, 359-362.

ARIMURA, A., SMITH, W.D. & SCHALLY, A.V. (1976) Blockade of the stress-induced decrease in blood GH by anti-somatostatin serum in rats. *Endocrinology*, **98**, 540-543.

ARVAT, E., CAPPA, M., CASANUEVA, F.F., DIEGUEZ, C., GHIGO, E., NICOLOSI, M., VALCAVI, R. & ZINI, M. (1993) Pyridostigmine potentiates growth hormone (GH)-releasing hormone-induced GH release in both men and women. *Journal of Clinical Endocrinology and Metabolism*, **76**, 374-377.

REFERENCES

ASPLIN, C.M., FARIA, A.C., CARLSEN, E.C., VACCARO, V.A., BARR, R.E., IRANMANESH, A., LEE, M.M., VELDHUIS, J.D. & EVANS, W.S. (1989) Alterations in the pulsatile mode of growth hormone release in men and women with insulin-dependent diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism*, **69**, 239-245.

BALLARD, J., BAXTER, R., BINOUX, M., CLEMMONS, D., DROP, S., HALL, K., HINTZ, R., RECHLER, M., RUTANEN, E. & SCHWANDER, J. (1989) On the nomenclature of the IGF binding proteins. *Acta Endocrinologica*, **121**, 751-752.

BARBARINO, A., CORSELLO, S.M., TOFANI, A., SCIUTO, R., DELLACASA, S., ROTA, C.A. & BARINI, A. (1991) Sexual dimorphism of pyridostigmine potentiation of growth hormone (GH)-releasing hormone-induced GH release in humans. *Journal of Clinical Endocrinology and Metabolism*, **73**, 75-78.

BARINAGA, M., BILEZIKJIAN, L.M., VALE, W.W., ROSENFELD, M.G. & EVANS, R.M. (1985) Independent effects of growth hormone releasing factor on growth hormone release and gene transcription. *Nature*, **314**, 279-281.

BATRINOS, M., GEORGIADIS, E., PANITSA-FAFLIA, C. & STRATIGOPOULOS, S. (1989) Increased GH response to GHRH in normal tall men. *Clinical Endocrinology*, **30**, 13-17.

BAUMANN, G., STOLAR, M.W., AMBURN, K., BARSANO, C.P. & DEVRIES, B.C. (1986) A specific growth hormone-binding protein in human plasma: initial characterization. *Journal of Clinical Endocrinology and Metabolism*, **62**, 134-141.

BAUMANN, G., AMBURN, K. & SHAW, M.A. (1988a) The circulating growth hormone (GH)-binding protein complex: a major constituent of plasma GH in man. *Endocrinology*, **122**, 976-984.

BAUMANN, G., SHAW, M.A., MERIMEE, T.J. & CLEMMONS, D.R. (1988b) Growth hormone-binding protein in human plasma: downregulation by prolonged fasting in lean but not obese subjects. *Clinical Research*, **36**, 477A(Abstract)

BAUMANN, G., SHAW, M.A. & MERIMEE, T.J. (1989b) Low levels of high-affinity growth hormone-binding protein in African pygmies. *New England Journal of Medicine*, **320**, 1705-1709.

BAUMANN, G. (1991) Growth hormone heterogeneity: genes, isohormones, variants, and binding proteins. [Review]. *Endocrine Reviews*, **12**, 424-449.

BAUMANN, G., SHAW, M.A., BRUMBAUGH, R.C. & SCHWARTZ, J. (1991) Short stature and decreased serum growth hormone-binding protein in the Mountain Ok People of Papua New Guinea. *Journal of Clinical Endocrinology and Metabolism*, **72**, 1346-1349.

REFERENCES

BAXTER, R.C. (1988) Characterization of the acid-labile subunit of the growth hormone-dependent insulin-like growth factor binding protein complex. *Journal of Clinical Endocrinology and Metabolism*, **67**, 265-272.

BENGTSSON, B.A., BRUMMER, R.J.M., EDEN, S., ROSEN, T. & SJOSTROM, L. (1992) Effects of growth hormone on fat mass and fat distribution. *Acta Paediatrica*, **81**, 62-65.

BENITO, P., AVILA, L., CORPAS, M.S., JIMENEZ, J.A., CACICEDO, L. & FRANCO, F.S. (1991) Sex differences in growth hormone response to growth hormone-releasing hormone. *Journal of Endocrinological Investigation*, **14**, 265-268.

BERCU, B. & DIAMOND, F. (1986) Growth hormone neurosecretory dysfunction. In *Clinics in Endocrinology and Metabolism* (eds M. Savage & R. Randall), pp. 537-590. WB Saunders, London.

BERELOWITZ, M., SZABO, M., FROHMAN, L.A., FIRESTONE, S., CHU, L. & HINTZ, R.L. (1981) Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. *Science*, **212**, 1279-1281.

BESHYAH, S.A., THOMAS, E., KYD, P., SHARP, P., FAIRNEY, A. & JOHNSTON, D.G. (1994) The effect of growth hormone replacement therapy in hypopituitary adults on calcium and bone metabolism. *Clinical Endocrinology*, **40**, 383-391.

BESSER, G.M., WASS, J.A. & THORNER, M.O. (1978) Acromegaly--results of long term treatment with bromocriptine. *Acta Endocrinologica,Supplement*, **216**, 187-198.

BETTI, R., CASANUEVA, F.F., CELLA, S.G. & MULLER, E.E. (1985) Activation of the cholinergic system and growth hormone release in the dog: functional interactions with other neurotransmitters. *Acta Endocrinologica*, **108**, 36-43.

BIERICH, J.R. (1983) Treatment of constitutional delay of growth and adolescence with human growth hormone. *Klinische Padiatrie*, **195**, 309-316.

BLACKARD, W.G. & WADDELL, C.C. (1969) Cholinergic blockade and growth hormone responsiveness to insulin hypoglycaemia. *Proceedings of the Society for Experimental Biology and Medicine*, **131**, 192-196.

BLOCH, B., BRAZEAU, P., LING, N., BOHLEN, P., ESCH, F., WEHRENBERG, W.B., BENOIT, R., BLOOM, F. & GUILLEMIN, R. (1983) Immunohistochemical detection of growth hormone-releasing factor in brain. *Nature*, **301**, 607-608.

REFERENCES

BLUM, W.F., RANKE, M.B., KIETZMANN, K., GAUGGEL, E., ZEISEL, H.J. & BIERICH, J.R. (1990) A specific radioimmunoassay for the growth hormone (GH)-dependent somatomedin-binding protein: its use for diagnosis of GH deficiency. *Journal of Clinical Endocrinology and Metabolism*, **70**, 1292-1298.

BODEN, G., SIVITZ, M.C., OWEN, O.E., ESSA-KOUMAR, N. & LANDOR, L.H. (1975) Somatostatin suppresses secretin and pancreatic exocrine secretion. *Science*, **190**, 163

BOLER, J., ENZMANN, F., FOLKERS, K., BOWERS, C.Y. & SCHALLY, A.V. (1969) The identity of chemical and hormonal properties of the thyrotropin-releasing hormone and pyroglutamyl-histidyl-proline amide. *Biochemical and Biophysical Research Communications*, **37**, 705-710.

BORLAND, R.G., BRENNAN, D.H., NICHOLSON, D.H. & SMITH, P.A. (1985) Studies on the possible central and peripheral effects in man of a cholinesterase inhibitor (pyridostigmine). *Human Toxicology*, **4**, 293-300.

BOULWARE, S.D., TAMBORLANE, W.V., MATTHEWS, L.S. & SHERWIN, R.S. (1992) Diverse effects of insulin-like growth factor I on glucose, lipid, and amino acid metabolism. *American Journal of Physiology*, **262**, 130-133.

BRAIN, C.E., HINDMARSH, P.C. & BROOK, C.G.D. (1990) Continuous subcutaneous $GHRH_{(1-29)}NH_2$ promotes growth over 1 year in short, slowly growing children. *Clinical Endocrinology*, **32**, 153-163.

BRAR, A.K. & CHERNAUSEK, S.D. (1993) Localization of IGF binding protein-4 expression in the developing and adult rat brain: analysis by in situ hybridization. *Journal of Neuroscience Research*, **35**, 103-114.

BRAZEAU, P., VALE, W., BURGUS, R., LING, N., BUTCHER, M., RIVIER, J. & GUILLEMIN, R. (1973) Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science*, **179**, 77-79.

BROOK, C.G.D. (1992) Who's for growth hormone ?. *British Medical Journal*, **304**, 131-132.

BROWN, M., LING, N. & RIVIER, J. (1981) Somatostatin-28, somatostatin-14 and somatostatin analogs: effects on thermoregulation. *Brain Research*, **214**, 127-135

BROWN, M.R. (1983) Central nervous system sites of action of bombesin and somatostatin to influence plasma epinephrine levels. *Brain Research*, **276**, 253-277.

REFERENCES

BURGUERA, B., MURUAIS, C., PENALVA, A., DIEGUEZ, C. & CASANUEVA, F.F. (1990) Dual and selective actions of glucocorticoids upon basal and stimulated growth hormone release in man. *Neuroendocrinology*, **51**, 51-58.

BURGUS, R., DUNN, T. & DESIDERIO, D. (1970) Characterisation of the hypothalamic hypophysiotropic TSH-releasing factor (TRF) of ovine origin. *Nature*, **226**, 321-325.

BUTENANDT, O., EDER, R., BIDLINGMAIER, F. & KNORR, D. (1976) Mean 24-hour growth hormone and testosterone concentrations in relation to pubertal growth spurt in boys with normal or delayed puberty. *European Journal of Pediatrics*, **122**, 85-92.

CAPPA, M., GROSSI, A., BENEDETTI, S., DRAGO, F., LOCHE, S. & GHIGO, E. (1993) Effect of the enhancement of the cholinergic tone by pyridostigmine on the exercise-induced growth hormone release in man. *Journal of Endocrinological Investigation*, **16**, 421-424.

CARLSON, H.E., GILLIN, J.C., GORDEN, P. & SNYDER, F. (1972) Absence of sleep-related growth hormone peaks in aged normal subjects and in acromegaly. *Journal of Clinical Endocrinology and Metabolism*, **34**, 1102-1105.

CASANUEVA, F.F., VILLANUEVA, L., DIEGUEZ, C., CABRANES, J.A., DIAZ, Y., SZOKE, B., SCANLON, M.F., SCHALLY, A.V. & FERNANDEZ CRUZ, A. (1986) Atropine blockade of growth hormone (GH)-releasing hormone-induced GH secretion in man is not exerted at pituitary level. *Journal of Clinical Endocrinology and Metabolism*, **62**, 186-191.

CASANUEVA, F.F., BURGUERA, B., MURUAIS, C. & DIEGUEZ, C. (1990) Acute administration of corticoids: a new and peculiar stimulus of growth hormone secretion in man. *Journal of Clinical Endocrinology and Metabolism*, **70**, 234-237.

CASTRO, R.C., VIEIRA, J.G., CHACRA, A.R., BESSER, G.M., GROSSMAN, A.B. & LENGYEL, A.M. (1990) Pyridostigmine enhances, but does not normalise, the GH response to GH-releasing hormone in obese subjects. *Acta Endocrinologica*, **122**, 385-390.

CAVAGNINI, F., INVITTI, C., DI LANDRO, A., TENCONI, L., MARASCHINI, C. & GIROTTI, G. (1977) Effects of a gamma aminobutyric acid (GABA) derivative, baclofen, on growth hormone and prolactin secretion in man. *Journal of Clinical Endocrinology and Metabolism*, **45**, 579-584.

CAVAGNINI, F., INVITTI, C., PINTO, M., MARASCHINI, C., DI LANDRO, A., DUBINI, A. & MARELLI, A. (1980) Effect of acute and repeated administration of gamma aminobutyric acid (GABA) on growth hormone and prolactin secretion in man. *Acta Endocrinologica*, **93**, 149-154.

REFERENCES

CEDA, G.P., VALENTI, G., BUTTURINI, U. & HOFFMAN, A.R. (1986) Diminshed pituitary responsiveness to growth hormone-releasing factor in aging male rats. *Endocrinology*, **118**, 2109-2114.

CHAO, C.C., HOEFFLER, J.P. & FRAWLEY, L.S. (1988) A cellular basis for functionally releasable pools in somatotropes. *Life Sciences*, **42**, 701-706.

CHIHARA, K., KODAMA, H., KAJI, H., KITA, T., KASHIO, Y., OKIMURA, Y., ABE, H. & FUJITA, T. (1985) Augmentation by propranolol of growth hormone-releasing hormone-(1-44)-NH2-induced growth hormone release in normal short and normal children. *Journal of Clinical Endocrinology and Metabolism*, **61**, 229-233.

CHON, S., VARTSKY, D. & YASAMURA, S. (1980) Compartmental body composition based on total body nitrogen, potassium and calcium. *American Journal of Physiology*, **239**, E5524-E5530.

CITTADINI, A., CUOCOLO, A., MEROLA, B., FAZIO, S., SABATINI, D., NICOLAI, E., COLAO, A., LONGOBARDI, S., LOMBARDI, G. & SACCA, L. (1994) Impaired cardiac performance in GH-deficient adults and its improvement after GH replacement. *American Journal of Physiology*, **267**, E219-E225.

CLAFFEY, D., YATEMAN, M., LANE, P., EALEY, P., WASS, J., MARSHALL, N. & HOLLY, J. (1994) The development and characterization of an eluted stain assay (ESTA) for insulin-like growth factors. *Growth Regulation*, **3**, 215-225.

CLARK, R.G., JANSSON, J.O., ISAKSSON, O. & ROBINSON, I.C.A.F. (1985) Intravenous growth hormone: growth responses to patterned infusions. *Journal of Endocrinology*, **104**, 53-61.

CLEMMONS, D.R., UNDERWOOD, L.E. & VAN WYK, J.J. (1981) Hormonal control of immunoreactive somatomedin production by human cultured fibroblasts. *Journal of Clinical Investigation*, **67**, 10-19.

COIRO, V., VOLPI, R., CAVAZZINI, U., BERTONI, P., CORRADI, A., BIANCONI, L., DAVOLI, C., ROSSI, G. & CHIODERA, P. (1991) Restoration of Normal Growth Hormone Responsiveness to GHRH in Normal Aged Men by Infusion of Low Amounts of Theophylline. *Journal of Gerontology*, **46**, M155-M158.

COPELAND, K.C., PAUNIER, L. & SIZONENKO, P.C. (1977) The secretion of adrenal androgens and growth hormone patterns of patients with hypogonadotrophic hypogonadism and idiopathic delayed puberty. *Journal of Pediatrics*, **91**, 985-990.

REFERENCES

CORDIDO, F., DIEGUEZ, C. & CASANUEVA, F.F. (1990) Effect of central cholinergic neurotransmission enhancement by pyridostigmine on the growth hormone secretion elicited by clonidine, arginine, or hypoglycemia in normal and obese subjects. *Journal of Clinical Endocrinology and Metabolism*, **70**, 1361-1370.

CORPAS, E., HARMAN, S.M., PINEYRO, M.A., ROBERSON, R. & BLACKMAN, M.R. (1992a) Growth hormone (GH)-releasing hormone-(1-29) twice daily reverses the decreased GH and insulin-like growth factor-i levels in old men. *Journal of Clinical Endocrinology and Metabolism*, **75**, 530-535.

CORPAS, E., HARMAN, S.M., PINEYRO, M.A., ROBERSON, R. & BLACKMAN, M.R. (1992b) GHRH 1-29 twice daily reverses the decreased GH and IGF-I levels in old men. *Journal of Clinical Endocrinology and Metabolism*, **75**, 530-535.

CORSELLO, S.M., TOFANI, A., DELLACASA, S., SCIUTO, R., ROTA, C.A., COLASANTI, S., BINI, A., BARINI, A. & BARBARINO, A. (1992) Activation of cholinergic tone by pyridostigmine reverses the inhibitory effect of corticotropin-releasing hormone on the growth hormone-releasing hormone-induced growth hormone secretion. *Acta Endocrinologica*, **126**, 113-116.

COTTAM, Y.H., BLAIR, H.T., GALLAHER, B.W., PURCHAS, R.W., BREIER, B.H., MCCUTCHEON, S.N. & GLUCKMAN, P.D. (1992) Body growth, carcass composition and endocrine changes in lambs chronically treated with recombinantly derived insulin-like growth factor-I. *Endocrinology*, **130**, 2924-2930.

COTTERILL, A.M., CAMACHOHUBNER, C., HOLLY, J.M.P. & SAVAGE, M.O. (1993a) Rapid communication - the effect of recombinant human insulin-like growth factor-I treatment on growth hormone secretion in two subjects with growth hormone insensitivity (Laron syndrome). *Clinical Endocrinology*, **39**, 119-122.

COTTERILL, A.M., HOLLY, J.M.P., AMIEL, S. & WASS, J.A.H. (1993b) Suppression of endogenous insulin secretion regulates the rapid rise of insulin-like growth factor binding protein (IGFBP)-1 levels following acute hypoglycaemia. *Clinical Endocrinology*, **38**, 633-639.

COTTERILL, A.M., CAMACHO-HUBNER, C., WOODS, K., MARTINELLI, C., DUQUESNOY, P. & SAVAGE, M.O. (1994) The insulin-like growth factor I generation test in the investigation of short stature.. *Acta Paediatrica (Supplement)*, **399**, 128-130.

COY, D.C., MURPHY, W.A., SUEIRAS-DIAZ, J., COY, E.J. & LANCE, V.A. (1985) Structure-activity studies on the N-terminal region of growth hormone releasing factor. *Journal of Medicinal Chemistry*, **28**, 181-185.

REFERENCES

COY, D.C., HOCART, S.J. & MURPHY, W.A. (1991) Human growth hormone-releasing hormone analogues with much improved in vitro growth hormone-releasing potencies in rat pituitary cells. *European Journal of Pharmacology*, **204**, 179-185.

CUNEO, R.C., SALOMON, F., MCGAULEY, G.A. & SONKSEN, P.H. (1992) Review - the growth hormone deficiency syndrome in adults. *Clinical Endocrinology*, **37**, 387-397.

CUSHING, H. (1910) *The Pituitary Body and its Disorders.* Lippincott, Philadelphia.

DARENDELILER, F., HINDMARSH, P.C., PREECE, M.A., COX, L. & BROOK, C.G. (1990) Growth hormone increases rate of pubertal maturation. *Acta Endocrinologica*, **122**, 414-416.

DAUGHADAY, W.H., HALL, K., RABEN, M.S., SALMON, W.D.J., VAN DEN BRANDE, J.L. & VAN WYKE, J.J. (1972) Somatomedin: Proposed designation for sulphation factor. *Nature*, **235**, 107

DAUGHADAY, W.H., TRIVEDI, B. & ANDREWS, B.A. (1987) The ontogeny of serum GH binding protein in man: a possible indicator of hepatic GH receptor development. *Journal of Clinical Endocrinology and Metabolism*, **65**, 1072-1074.

DAVIDOFF, L.M. (1926) Studies in acromegaly.III. The anamnesis and symptomatology in one hundred cases. *Endocrinology*, **10**, 461-483.

DAVIES, R.R., TURNER, S.J., ORSKOV, H. & JOHNSTON, D.G. (1985) The interaction of human pancreatic growth hormone releasing factor 1-44 with somatostatin in vivo in normal man. *Clinical Endocrinology*, **23**, 271-276.

DE GENNARO COLONNA, V., ZOLI, M., COCCHI, D., MAGGI, A., MARRAMA, P., AGNATI, L.F. & MULLER, E.E. (1989) Reduced growth hormone releasing factor (GHRF)-like immunoreactivity and GHRF gene expression in the hypothalamus of aged rats. *Peptides*, **10**, 705-708.

DEGRADO, W.F., KEZDY, F.J. & KAISER, E.T. (1981) Design, synthesis and characterization of a cytotoxic peptide with melittin-like activity. *Journal of American Chemical Society*, **103**, 679-681.

DEL BALZO, P., SALVATORI, R., CAPPA, M. & GERTNER, J.M. (1990) Pyridostigmine does not reverse dexamethasone-induced growth hormone inhibition. *Clinical Endocrinology*, **33**, 605-612.

DELITALA, G., GROSSMAN, A. & BESSER, G.M. (1983) Opiate peptides control growth hormone through a cholinergic mechanism in man. *Clinical Endocrinology*, **18**, 401-405.

REFERENCES

DELITALA, G., MANNELLI, M. & GIUSTI, M. (1984) Relation of opiates to hormonal secretion in man. In *Opiod modulation of endocrine function* (eds G. Delitala, M. Motta & M. Serio), pp. 65-79. Raven Press, New York.

DELITALA, G., PALERMO, M., ROSS, R., COY, D., BESSER, G.M. & GROSSMAN, A. (1987a) Dopaminergic and cholinergic influences on the growth hormone response to growth hormone-releasing hormone in man. *Neuroendocrinology*, **45**, 243-247.

DELITALA, G., PALERMO, M., ROSS, R., COY, D., BESSER, G.M. & GROSSMAN, A. (1987b) Dopaminergic and cholinergic influences on the growth hormone response to growth hormone-releasing hormone in man. *Neuroendocrinology*, **45**, 243-247.

DELITALA, G., TOMASI, P.A., PALERMO, M., ROSS, R.J., GROSSMAN, A. & BESSER, G.M. (1989) Opioids stimulate growth hormone (GH) release in man independently of GH-releasing hormone. *Journal of Clinical Endocrinology and Metabolism*, **69**, 356-358.

DEMPSHER, D.P., BIER, D.M., TOLLEFSEN, S.E., ROTWEIN, P.S., DAUGHADAY, W.H., JENSEN, M.C., GALGANI, J.P., HEATH-MONNIG, E. & TRIVEDI, B. (1990) Whole body nitrogen kinetics and their relationship to growth in short children treated with recombinant human growth hormone. *Pediatric Research*, **28**, 394-400.

DEMURA, R., DEMURA, H., NUNOKAWA, T., BABA, H. & MIURA, K. (1972) Responses of plasma ACTH, GH, LH, and 11-hydroxysteroids to various stimuli in patients with Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism*, **34**, 852-859.

DEUBEN, R. & MEITES, J. (1964) Stimulation of pituitary growth hormone release by a hypothalamic extractin vivo. *Endocrinology*, **74**, 408-414.

DEVESA, J., LIMA, L., LOIS, N., LECHJGA, M.T., ARCHIE, V. & TRESGUERRES, J.A.F. (1989) Reasons for the variability in growth hormone (GH) responses to GHRH challange: The endogenous hypothalamic-somatostatin rhythm (HRS). *Clinical Endocrinology*, **30**, 367-377.

DEVESA, J., ARCE, V., LOIS, N., TRESGUERRES, J. & LIMA., L. (1990) Alpha-2 adrenergic agonism enhances the growth hormone response to GH-releasing hormone through an inhibition of hypothalamic somatostatin release in normal men. *Journal of Clinical Endocrinology and Metabolism*, **71**, 1581-1588.

REFERENCES

DEVESA, J., DIAZ, M.J., TRESGUERRES, J.A.F., ARCE, V. & LIMA, L. (1991) Evidence that alpha2-adrenergic pathways play a major role in growth hormone (GH) neuroregulation - alpha2-adrenergic agonism counteracts the inhibitory effect of muscarinic cholinergic receptor blockade on the GH response to GH-releasing hormone, while alpha2-adrenergic blockade diminishes the potentiating effect of increased cholinergic tone on such stimulation in normal men. *Journal of Clinical Endocrinology and Metabolism*, **73**, 251-256.

DONALDSON, D.L., HOLLOWELL, J.G., PAN, F., GIFFORD, R.A. & MOORE, W.V. (1989) Growth hormone secretory profiles: variation on consecutive nights. *Journal of Pediatrics*, **115**, 51-56.

DONALDSON, D.L., PAN, F., HOLLOWELL, J.G., STEVENSON, J.L., GIFFORD, R.A. & MOORE, W.V. (1991) Reliability of stimulated and spontaneous growth hormone (GH) levels for identifying the child with low GH secretion. *Journal of Clinical Endocrinology and Metabolism*, **72**, 647-652.

DUCK, S.C., SCHWARZ, H.P., COSTIN, G., RAPAPORT, R., ARSLANIAN, S., HAYEK, A., CONNORS, M. & JARAMILLO, J. (1992) Subcutaneous growth hormone-releasing hormone therapy in growth hormone-deficient children - first year of therapy. *Journal of Clinical Endocrinology and Metabolism*, **75**, 1115-1120.

DUDL, R.J., ENSINCK, J.W., PALMER, H.E. & WILLIAMS, R.H. (1973) Effect of age on growth hormone secretion in man. *Journal of Clinical Endocrinology and Metabolism*, **37**, 11-16.

DURAND, D., MARTIN, J.B. & BRAZEAU, P. (1977) Evidence for a role of alpha-adrenergic mechanisms in regulation of episodic growth hormone secretion in the rat. *Endocrinology*, **100**, 722-728.

EASTMAN, C.J. & LAZARUS, L. (1973) Growth hormone release during sleep in growth retarded children. *Archives of Disease In Childhood*, **48**, 502-507.

EDEN, S., ERIKSSON, E., MARTIN, J.B. & MODIGH, K. (1981) Evidence for a growth hormone releasing factor mediating alpha-adrenergic influence on growth hormone secretion in the rat. *Neuroendocrinology*, **33**, 24-27.

EDEN, S., WIKLUND, O., OSCARSSON, J., ROSEN, T. & BENGTSSON, B.A. (1993) Growth hormone treatment of growth hormone-deficient adults results in a marked increase in lp(a) and HDL cholesterol concentrations. *Arteriosclerosis and Thrombosis*, **13**, 296-301.

REFERENCES

EPELBAUM, J., ARANCIBIA, L.T., HERMAN, J.P., KORDON, C. & PALKOVITS, M. (1981) Topography of median eminence somatostatinergic innervation. *Brain Research*, **230**, 412-416.

ESCH, F.S., BOHLEN, P., LING, N.C., BRAZEAU, P.E., WEHRENBERG, W.B., THORNER, M.O., CRONIN, M.J. & GUILLEMIN, R. (1982) Characterization of a 40 residue peptide from a human pancreatic tumor with growth hormone releasing activity. *Biochemical and Biophysical Research Communications*, **109**, 152-158.

EVANS, H. & LONG, J. (1921) The effect of the anterior lobe administered intraperitoneally upon growth, maturity, and oestrous cycles of the rat. *Anatomy Record*, **21**, 62-63.

EVANS, P., DIEGUEZ, C., FOORD, S., PETERS, J., HALL, R. & SCANLON, M. (1985) The effect of cholinergic blockade on the growth hormone and prolactin response to insulin hypoglycaemia. *Clinical Endocrinology*, **22**, 733-737.

EVANS, W.S., BORGES, J.L., VANCE, M.L., KAISER, D.L., ROGOL, A.D., FURLANETTO, R., RIVIER, J., VALE, W. & THORNER, M.O. (1984) Effects of human pancreatic growth hormone-releasing factor-40 on serum growth hormone, prolactin, luteinizing hormone, follicle-stimulating hormone, and somatomedin-C concentrations in normal women throughout the menstrual cycle. *Journal of Clinical Endocrinology and Metabolism*, **59**, 1006-1010.

EVANS, W.S., FARIA, A.C., CHRISTIANSEN, E., HO, K.Y., WEISS, J., ROGOL, A.D., JOHNSON, M.L., BLIZZARD, R.M., VELDHUIS, J.D. & THORNER, M.O. (1987) Impact of intensive venous sampling on characterization of pulsatile GH release. *American Journal of Physiology*, **252**, E549-E556.

FAIN, J.N., KOVACEV, V.P. & SCOW, R.O. (1965) Effect of growth hormone and dexamethasone on lipolysis and metabolism in isolated fat cells of the rat. *Journal of Biological Chemistry*, **240**, 3522-3529.

FATTAH,D.I. & CHARD,T. (1978) Simplified method for measuring sex-hormone binding globulin. *Clinical Chemistry*, **27**, 1277-1279.

FELIX, A.M., HEIMER, E.P., WANG, C., LAMBROS, T.J., FOURNIER, A., MOWLES, T.F., MAINES, S., CAMPBELL, R.M., WEGRZYNSKI, B.B., TOOME, V., FRY, D. & MADISON, V.S. (1988) Synthesis, biological activity and conformational analysis of cyclic GRF analogs. *International Journal of Peptide and Protein Research*, **32**, 441-454.

REFERENCES

FINE, R.N., KOHAUT, E.C., BROWN, D. & PERLMAN, A.J. (1994) Growth after recombinant human growth hormone treatment in children with chronic renal failure - report of a multicenter randomized double-blind placebo-controlled study. *Journal of Pediatrics*, **124**, 374-382.

FINKELSTEIN, J.W., BOYAR, R.M., ROFFWARG, H.P., KREAM, J. & HELLMAN, L. (1972) Age-related change in the twenty-four-hour spontaneous secretion of growth hormone. *Journal of Clinical Endocrinology and Metabolism*, **35**, 665-670.

FIORETTI, P., MELIS, G.B., PAOLETTI, A.M., PARODO, G., CAMINITI, F., CORSINI, G.U. & MARTINI, L. (1978) Gamma-amino-beta-hydroxy butyric acid stimulates prolactin and growth hormone release in normal women. *Journal of Clinical Endocrinology and Metabolism*, **47**, 1336-1340.

FLORINI, J. (1987) Hormonal control of muscle growth. *Muscle and Nerve*, **10**, 577-598.

FORBES, G.B. & HALLORAN, E. (1976) The adult decline in lean body mass. *Human Biology*, **48**, 162-173.

FORBES, G.B. & REINA, J.C. (1970) Adult lean body mass declines with age: some longitudinal observations. *Metabolism: Clinical and Experimental*, **19**, 653-663.

FOX, M., MINOT, A.S. & LIDDLE, G.W. (1962) Oxandrolone: a potent anabolic steroid of novel chemical configuration. *Journal of Clinical Endocrinology and Metabolism*, **22**, 921-924.

FRANKLYN, J.A., LYNAM, T., DOCHERTY, K., RAMSDEN, D.B. & SHEPPARD, M.C. (1986) Effect of hypothyroidism on pituitary cytoplasmic concentrations of messenger RNA encoding thyrotrophin beta and alpha subunits, prolactin and growth hormone. *Journal of Endocrinology*, **108**, 43-47.

FRANTZ, A. & RABKIN, M. (1965) Effects of estrogen and sex difference on secretion of human growth hormone. *Journal of Endocrinology*, **25**, 1470-1480.

FRASIER, S.D., COSTIN, G., LIPPE, B.M., ACETO, T. & BUNGER, P.F. (1981) A dose-response curve for human growth hormone. *Journal of Clinical Endocrinology and Metabolism*, **53**, 1213-1217.

FRIEDMAN, M. & STRANG, L.B. (1966) Effect of long-term corticosteroids and corticotrophin on growth in children. *Lancet*, **ii**, 569-572.

FROESCH, E.R. & ZAPF, J. (1985) Insulin-like growth factors and insulin: comparative aspects. *Diabetologia*, **28**, 485-493.

REFERENCES

FROHMAN, L.A., DOWNS, T.R., WILLIAMS, T., HEIMER, E.P., PAN, Y.E. & FELIX, A.M. (1986) Rapid enzymatic degradation of growth hormone-releasing hormone by plasma in vitro and in vivo to a biologically inactive product cleaved at the NH_2 terminus. *Journal of Clinical Investigation*, **78**, 906-913.

GARNIER, J., OSGUTHORPE, D. & ROBSON, B. (1978) Analysis of the accuracy and the implications of simple methods for predicting the secondary structure of globular proteins. *Journal of Molecular Biology*, **120**, 97-120.

GE, F., TSAGARAKIS, S., REES, L.H., BESSER, G.M. & GROSSMAN, A. (1989) Relationship between growth hormone-releasing hormone and somatostatin in the rat: effects of age and sex on content and in-vitro release from hypothalamic explants. *Journal of Endocrinology*, **123**, 53-58.

GELATO, M.C., PESCOVITZ, O., CASSORLA, F., LORIAUX, D.L. & MERRIAM, G.R. (1983) Effects of a growth hormone releasing factor in man. *Journal of Clinical Endocrinology and Metabolism*, **57**, 674-676.

GELATO, M.C., PESCOVITZ, O.H., CASSORLA, F., LORIAUX, D.L. & MERRIAM, G.R. (1984) Dose-response relationship for the effects of growth hormone-releasing factor-(1-44)-NH_2 in young adult men and women. *Journal of Clinical Endocrinology and Metabolism*, **59**, 197-201.

GELATO, M.C., MALOZOWSKI, S., CARUSO-NICOLETTI, M., LEVINE-ROSS, J., PESCOVITZ, O.H., ROSE, S., LORIAUX, D.L., CASSORLA, F. & MERRIAM, G.R. (1986) Growth hormone (GH) responses to GH-releasing hormone during the pubertal development in normal boys and girls: comparison to idiopathic short stature and GH deficiency. *Journal of Clinical Endocrinology and Metabolism*, **63**, 174-179.

GHIGO, E., ARVAT, E., MAZZA, E., MONDARDINI, A., CAPPA, M., MULLER, E.E. & CAMMANI, F. (1990a) Failure of pyridostigmine to increase both basal and GHRH-induced GH secretion in the night. *Acta Endocrinologica*, **122**, 37-40.

GHIGO, E., BELLONE, J., MAZZA, E., IMPERIALE, E., PROCOPIO, M., VALENTE, F., LALA, R., DE SANCTIS, C. & CAMANNI, F. (1990b) Arginine potentiates the GHRH- but not the pyridostigmine-induced GH secretion in normal short children. Further evidence for a somatostatin suppressing effect of arginine. *Clinical Endocrinology*, **32**, 763-767.

GHIGO, E., GOFFI, S., ARVAT, E., NICOLOSI, M., PROCOPIO, M., BELLONE, J., IMPERIALE, E., MAZZA, E., BARACCHI, G. & CAMANNI, F. (1990c) Pyridostigmine partially restores the GH responsiveness to GHRH in normal aging. *Acta Endocrinologica*, **123**, 169-173.

REFERENCES

GHIGO, E., GOFFI, S., NICOLOSI, M., ARVAT, E., VALENTE, F., MAZZA, E., GHIGO, M.C. & CAMANNI, F. (1990d) Growth hormone (GH) responsiveness to combined administration of arginine and GH-releasing hormone does not vary with age in man. *Journal of Clinical Endocrinology and Metabolism*, **71**, 1481-1485.

GHIGO, E., IMPERIALE, E., BOFFANO, G.M., MAZZA, E., BELLONE, J., ARVAT, E., PROCOPIO, M., GOFFI, S., BARRECA, A., CHIABOTTO, P. & ET AL (1990e) A new test for the diagnosis of growth hormone deficiency due to primary pituitary impairment: combined administration of pyridostigmine and growth hormone-releasing hormone. *Journal of Endocrinological Investigation*, **13**, 307-316.

GHIGO, E., GOFFI, S., ARVAT, E., IMPERIALE, E., BOFFANO, G.M., VALETTO, M.R., MAZZA, E., SANTI, I., MAGLIONA, A., BOGHEN, M.F., BOCCUZZI, G. & CAMANNI, F. (1992) A neuroendocrinological approach to evidence an impairment of central cholinergic function in aging. *Journal of Endocrinological Investigation*, **15**, 665-670.

GHIGO, E., NICOLOSI, M., ARVAT, E., MARCONE, A., DANELON, F., MUCCI, M., FRANCESCHI, M., SMIRNE, S. & CAMANNI, F. (1993) Growth hormone secretion in alzheimers disease - studies with growth hormone-releasing hormone alone and combined with pyridostigmine or arginine. *Dementia*, **4**, 315-320.

GIBSON, G.E., PETERSON, C. & JENDEN, D.J. (1981) Brain acetylcholine synthesis decline with senescence. *Science*, **213**, 674-676.

GIUSTI, M., MARINI, G., PELUFFO, F., VALENTI, S., CARATTI, C. & GIORDANO, G. (1992) Effect of cholinergic tone on growth hormone-releasing hormone-induced secretion of growth hormone in normal aging. *Aging Clinical and Experimental Research*, **4**, 231-237.

GIUSTINA, A., DOGA, M., BODINI, C., GIRELLI, A., LEGATI, F., BOSSONI, S. & ROMANELLI, G. (1990a) Acute effects of cortisone on growth hormone response to growth hormone-releasing hormone in normal adult subjects. *Acta Endocrinologica*, **122**, 206-210.

GIUSTINA, A., GIRELLI, A., DOGA, M., BODINI, C., BOSSONI, S., ROMANELLI, G. & WEHRENBERG, W.B. (1990b) Pyridostigmine blocks the inhibitory effect of glucocorticoids on growth hormone-releasing hormone stimulated growth hormone secretion in normal man. *Journal of Clinical Endocrinology and Metabolism*, **70**, 580-584.

REFERENCES

GIUSTINA, A., GIRELLI, A., ALBERTI, D., BOSSONI, S., BUZI, F., DOGA, M., SCHETTINO, M. & WEHRENBERG, W.B. (1991) Effects of pyridostigmine on spontaneous and growth hormone-releasing hormone stimulated growth hormone secretion in children on daily glucocorticoid therapy after liver transplantation. *Clinical Endocrinology*, **35**, 491-498.

GIUSTINA, A., SCHETTINO, M., BUSSI, A.R., LEGATI, F., LICINI, M., ZUCCATO, F. & WEHRENBERG, W.B. (1992) Effect of arginine on the GHRH-stimulated GH secretion in patients with hyperthyroidism. *Hormone Research*, **38**, 256-259.

GIUSTINA, A., BUSSI, A.R., JACOBELLO, C. & WEHRENBERG, W.B. (1995) Effects of recombinant human growth hormone (GH) on bone and intermediary metabolism in patients receiving chronic glucocorticoid treatment with suppressed endogenous GH response to GH-releasing hormone. *Journal of Clinical Endocrinology and Metabolism*, **80**, 122-129.

GOLSTEIN, J., VAN CAUTER, E., DESIR, D., NOEL, P., SPIRE, J.P., REFETOFF, S. & COPINSCHI, G. (1983) Effects of "jet lag" on hormonal patterns. IV. Time shifts increase growth hormone release. *Journal of Clinical Endocrinology and Metabolism*, **56**, 433-440.

GOODYER, C.G., DE STEPHANO, L., LAI, W.H., GUYDA, H.J. & POSNER, B.I. (1984) Characterization of insulin-like growth factor receptors in rat anterior pituitary, hypothalamus, and brain. *Endocrinology*, **114**, 1187-1195.

GREEN, J. & HARRIS, G.W. (1947) The neurovascular link between the neurohypophysis and adenohypophesis. *Journal of Endocrinology*, **5**, 136-146.

GRIFFIN, E.E. & MILLER, L.L. (1974) Effects of hypophysectomy of liver donor on net synthesis of specific plasma proteins by the isolated perfused rat liver. Modulation of synthesis of albumin, fibrinogen, alpha 1-acid glycoprotein, alpha 2-(acute phase)-globulin, and haptoglobin by insulin, cortisol, triiodothyronine, and growth hormone. *Journal of Biological Chemistry*, **249**, 5062-5069.

GROSSMAN, A., SAVAGE, M.O., WASS, J.A., LYTRAS, N., SUEIRAS DIAZ, J., COY, D.H. & BESSER, G.M. (1983) Growth-hormone-releasing factor in growth hormone deficiency: demonstration of a hypothalamic defect in growth hormone release. *Lancet*, **2**, 137-138.

GROSSMAN, A., LYTRAS, N., SAVAGE, M.O., WASS, J.A., COY, D.H., REES, L.H., JONES, A.E. & BESSER, G.M. (1984a) Growth hormone releasing factor: comparison of two analogues and demonstration of hypothalamic defect in growth hormone release after radiotherapy. *British Medical Journal*, **288**, 1785-1787.

REFERENCES

GROSSMAN, A., SAVAGE, M.O., LYTRAS, N., PREECE, M.A., SUEIRAS DIAZ, J., COY, D.H., REES, L.H. & BESSER, G.M. (1984b) Responses to analogues of growth hormone-releasing hormone in normal subjects, and in growth-hormone deficient children and young adults. *Clinical Endocrinology*, **21**, 321-330.

GRUNFELD, C., SHERMAN, B.M. & CAVALIERI, R.R. (1988) The acute effects of human growth hormone administration on thyroid function in normal men. *Journal of Clinical Endocrinology and Metabolism*, **67**, 1111-1114.

GUILLEMIN, R., BRAZEAU, P., BOHLEN, P., ESCH, F., LING, N. & WEHRENBERG, W.B. (1982) Growth hormones-releasing factor from a human pancreatic tumor that caused acromegaly. *Science*, **218**, 585-587.

GULER, H.P., ZAPF, J. & FROESCH, E.R. (1987) Short-term metabolic effects of recombinant human insulin-like growth factor I in healthy adults. *New England Journal of Medicine*, **317**, 137-140.

GULER, H.P., SCHMID, C., ZAPF, J. & FROESCH, E.R. (1989) Effects of recombinant insulin-like growth factor I on insulin secretion and renal function in normal human subjects. *Proceedings of the National Academy of Sciences of the United States of America*, **86**, 2868-2872.

HAGBERG, J.M., SEALS, D.R., YERG, J.E., GAVIN, J., GINGERICH, R., BHARTUR, P. & HOLLOSZY, J. (1988) Metabolic responses to exercise in young and old athletes and sedentary men. *Journal of Applied Physiology*, **65**, 900-908.

HAGEN, T.C., LAWRENCE, A.M. & KIRSTEINS, L. (1972) Autoregulation of growth hormone secretion in normal subjects. *Metabolism: Clinical and Experimental*, **21**, 603-610.

HALL, K. & SARA, V.R. (1984) Somatomedin levels in childhood, adolescence and adult life. *Journal of Clinical Endocrinology and Metabolism*, **13**, 91-112.

HALL, R., SCHALLY, A.V., EVERED, D., KASTIN, A.J., MORTIMER, C.H., TUNBRIDGE, W.M.G., WEIGHTMAN, D., PHENEKOS, C., MCNEILLY, A.S., GOLDIE, D.J., COY, D.H. & BESSER, G.M. (1973) Action of Growth-hormone-release inhibitory hormone in healthy men and in acromegaly. *Lancet*, **2**, 581-584.

HAREL, Z. & TANNENBAUM, G.S. (1992a) Centrally administered insulin-like growth II fails to alter pulsatile growth hormone secretion or food intake. *Neuroendocrinology*, **56**, 161-168.

REFERENCES

HAREL, Z. & TANNENBAUM, G.S. (1992b) Synergistic interaction between insulin-like growth factors-I and -II in central regulation of pulsatile growth hormone secretion. *Endocrinology*, **131**, 758-764.

HARTMAN, M.L., VELDHUIS, J.D., JOHNSON, M.L., LEE, M.M., ALBERTI, K.G., SAMOJLIK, E. & THORNER, M.O. (1992) Augmented growth hormone (GH) secretory burst frequency and amplitude mediate enhanced GH secretion during a two-day fast in normal men. *Journal of Clinical Endocrinology and Metabolism*, **74**, 757-765.

HARTMAN, M.L., CLAYTON, P.E., JOHNSON, M.L., CELNIKER, A., PERLMAN, A.J., ALBERTI, K.G.M.M. & THORNER, M.O. (1993) A low dose euglycemic infusion of recombinant human Insulin-like Growth Factor-I rapidly suppresses fasting-enhanced pulsatile growth hormone secretion in humans. *Journal of Clinical Investigation*, **91**, 2453-2462.

HARTOG, M., GAAFAR, M.A. & FRASER, R. (1964) Effects of corticosteroids on serum growth hormone. *Lancet*, **ii**, 376-378.

HATTORI, N., KURAHACHI, H., IKEKUBO, K., ISHIHARA, T., MORIDERA, K., HINO, M., SAIKI, Y. & IMURA, H. (1992) Serum growth hormone-binding protein, insulin-like growth factor-I, and growth hormone in patients with liver cirrhosis. *Metabolism: Clinical and Experimental*, **41**, 377-381.

HELLMAN, B. & LERNMARK, A. (1969a) Inhibition of the in vitro secretion of insulin by an extract of pancreatic alpha-1 cells. *Endocrinology*, **84**, 1484-1488.

HELLMAN, B. & LERNMARK, A. (1969b) Evidence for an inhibitor of insulin release in the pancreatic islets. *Diabetologia*, **5**, 22-24.

HERINGTON, A.C., YMER, S. & STEVENSON, J. (1986) Identification and characterization of specific binding proteins for growth hormone in normal human sera. *Journal of Clinical Investigation*, **77**, 1817-1823.

HINDMARSH, P., SMITH, P.J., BROOK, C.G. & MATTHEWS, D.R. (1987) The relationship between height velocity and growth hormone secretion in short prepubertal children. *Clinical Endocrinology*, **27**, 581-591.

HINDMARSH, P.C., BRAIN, C.E., ROBINSON, I.C.A.F., MATTHEWS, D.R. & BROOK, C.G.D. (1991) The interaction of growth hormone releasing hormone and somatostatin in the generation of a GH pulse in man. *Clinical Endocrinology*, **35**, 353-360.

REFERENCES

HO, K.Y., EVANS, W.S., BLIZZARD, R.M., VELDHUIS, J.D., MERRIAM, G.R., SAMOJLIK, E., FURLANETTO, R., ROGOL, A.D., KAISER, D.L. & THORNER, M.O. (1987) Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. *Journal of Clinical Endocrinology and Metabolism*, **64**, 51-58.

HO, K.Y., VELDHUIS, J.D., JOHNSON, M.L., FURLANETTO, R., EVANS, W.S., ALBERTI, K.G. & THORNER, M.O. (1988) Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. *Journal of Clinical Investigation*, **81**, 968-975.

HOCHBERG, Z., HERTZ, P., COLIN, V., ISHSHALOM, S., YESHURUN, D., YOUDIM, M.B.H. & AMIT, T. (1992) The distal axis of growth hormone (GH) in nutritional disorders - GH-binding protein, Insulin-like Growth Factor-I (IGF-I), and IGF-I receptors in obesity and anorexia nervosa. *Metabolism: Clinical and Experimental*, **41**, 106-112.

HOFFMAN, A.R., LIEBERMAN, S.A. & CEDA, G.P. (1992) Growth hormone therapy in the elderly - implications for the aging brain. *Psychoneuroendocrinology.*, **17**, 327-333.

HOLL, R.W., HARTMAN, M.L., VELDHUIS, J.D., TAYLOR, W.M. & THORNER, M.O. (1991) Thirty-second sampling of plasma growth hormone in man: correlation with sleep stages. *Journal of Clinical Endocrinology and Metabolism*, **72**, 854-861.

HOLLY, J.M., BIDDLECOMBE, R.A., DUNGER, D.B., EDGE, J.A., AMIEL, S.A., HOWELL, R., CHARD, T., REES, L.H. & WASS, J.A. (1988) Circadian variation of GH-independent IGF-binding protein in diabetes mellitus and its relationship to insulin. A new role for insulin?. *Clinical Endocrinology*, **29**, 667-675.

HONDA, Y., TAKAHASHI, K., TAKAHASHI, S., AZUMI, K., IRIE, M., SAKUMA, M., TSUSHIMA, T. & SHIZUME, K. (1969) Growth hormone secretion during nocturnal sleep in normal subjects. *Journal of Clinical Endocrinology and Metabolism*, **29**, 20-29.

HULSE, J.A., ROSENTHAL, M.S., CUTTLER, L., KAPLAN, S.L. & GRUMBACH, M.M. (1986) The effect of pulsatile administration, continuous infusion, and diurnal variation on the growth hormone (GH) response to GH-releasing hormone in normal men. *Journal of Clinical Endocrinology and Metabolism*, **63**, 872-878.

HUMBEL, R.E. (1990) Insulin-like growth factors I and II. *Eur.J.Biochem.*, **190**, 445-462.

ILLIG, R. & PRADER, A. (1970) Effect of testosterone on growth hormone secretion in patients with anorchia and delayed puberty. *Journal of Clinical Endocrinology and Metabolism*, **30**, 615-618.

REFERENCES

IMAKI, T., SHIBASAKI, T., MASUDA, A., HOTTA, M., YAMAUCHI, N., DEMURA, H., SHIZUME, K., WAKABAYASHI, I. & LING, N. (1986) The effect of glucose and free fatty acids on growth hormone (GH)-releasing factor-mediated GH secretion in rats. *Endocrinology*, **118**, 2390-2394.

IOVINO, M., MONTELEONE, P. & STEARDO, L. (1989) Repetitive growth hormone-releasing hormone administration restores the attenuated growth hormone (GH) response to GH releasing hormone testing in normal aging. *Journal of Clinical Endocrinology and Metabolism*, **69**, 910-913.

IRANMANESH, A., LIZARRALDE, G., JOHNSON, M.L. & VELDHUIS, J.D. (1991) Nature of altered growth hormone secretion in hyperthyroidism. *Journal of Clinical Endocrinology and Metabolism*, **72**, 108-115.

ISGAARD, J., NILSSON, A., LINDAHL, A., JANSSON, J.O. & ISAKSSON, O.G. (1986) Effects of local administration of GH and IGF-I on longitudinal bone growth in rats. *American Journal of Physiology*, **250**, E367-E372.

ISGAARD, J., CARLSSON, L.M.S., ISAKSSON, O.G.P. & JANSSON, J.O. (1988) Pulsatile intravenous growth (GH) infusion to hypophysectomised rats increase insulin-like growth factor 1 messanger ribonucleic acid in skeletal tissues more effectively than continuous GH infusion. *Endocrinology*, **123**, 2605-2610.

ISLEY, W.L., UNDERWOOD, L.E. & CLEMMONS, D.R. (1983) Dietary components that regulate serum somatomedin-C concentrations in humans. *Journal of Clinical Investigation*, **71**, 175-182.

IWATSUBO, H., OMORI, K., OKADA, Y., FUKUCHI, M., MIYAI, K., ABE, H. & KUMAHARA, Y. (1967) Human growth hormone secretion in primary hypothyroidism before and after treatment. *Journal of Clinical Endocrinology and Metabolism*, **27**, 1751-1754.

JACOB, R., BARRETT, E., PLEWE, G., FAGIN, K.D. & SHERWIN, R.S. (1989) Acute effects of insulin-like growth factor I on glucose and amino acid metabolism in the awake fasted rat. Comparison with insulin. *Journal of Clinical Investigation*, **83**, 1717-1723.

JEFFERY, S., CARTER, N.D., CLARK, R.G. & ROBINSON, I.C.A.F. (1990) The episodic secretory pattern of growth hormone regulates liver carbonic anhydrase secretion. III Studies in normal and mutant GH-deficient dwarf mice. *Biochemical Journal*, **266**, 69-74.

JOHANSSON, C., EFENDIC, S., WISEN, O., UVNAS-WALLENSTEN, K. & LUFT, R. (1978) Effects of short-term somatostatin infusion on the gastric and intestinal propulsion in humans. *Scandanavian Journal of Gastroenterology*, **13**, 481-483.

REFERENCES

JORDAN, V., DIEGUEZ, C., VALCAVI, R., ARTIOLI, C., PORTIOLI, I., RODRIGUEZ ARNAO, M.D., GOMEZ PAN, A., HALL, R. & SCANLON, M.F. (1986) Lack of effect of muscarinic cholinergic blockade on the GH responses to GRF 1-29 and TRH in acromegalic subjects. *Clinical Endocrinology*, **24**, 415-420.

JORGENSEN, J.O.L., MOLLER, J., LAURSEN, T., ORSKOV, H., CHRISTIANSEN, J.S. & WEEKE, J. (1994) Growth hormone administration stimulates energy expenditure and extrathyroidal conversion of thyroxine to triiodothyronine in a dose-dependent manner and suppresses circadian thyrotrophin levels: studies in GH-deficient adults. *Clinical Endocrinology*, **41**, 609-614.

KABAYAMA, Y., KATO, Y., MURAKAMI, Y., TANAKA, H. & IMURA, H. (1986) Stimulation by alpha-adrenergic mechanisms of the secretion of growth hormone-releasing factor (GRF) from perifused rat hypothalamus. *Endocrinology*, **119**, 432-434.

KALK, W.J., VINIK, A.I., PIMSTONE, B.L. & JACKSON, W.P. (1973) Growth hormone responses to insulin hypoglycaemia in the elderly. *Journal of Gerontology*, **30**, 431-433.

KALKHOFF, R.K., HARTZ, A.H., RUPLEY, D., KISSEBAH, A.H. & KELBER, S. (1983) Relationship of fat distribution to blood pressure, carbohydarate tolerance, and plasma lipids in healthy obese women. *Journal of Laboratory and Clinical Medicine*, **102**, 621-627.

KATAKAMI, H., KATO, Y., MATSUSHITA, N. & IMURA, H. (1984) Effects of neonatal treatment with monosodium glutamate on growth hormone release induced by clonidine and prostaglandin E1 in conscious male rats. *Neuroendocrinology*, **38**, 1-5.

KELIJMAN, M. & FROHMAN, L.A. (1989) Beta-adrenergic modulation of growth hormone (GH) autofeedback on sleep-associated and pharmacologically induced GH secretion. *Journal of Clinical Endocrinology and Metabolism*, **69**, 1187-1194.

KELIJMAN, M. & FROHMAN, L.A. (1991) The Role of the Cholinergic Pathway in Growth Hormone Feedback. *Journal of Clinical Endocrinology and Metabolism*, **72**, 1081-1087.

KIRK, J.M.W., TRAINER, P.J., MURPHY, J., MAJROWSKI, W., SAVAGE, M.O. & BESSER, G.M. (1994) Treatment with GHRH(1-29)NH_2 in children with idiopathic short stature induces a sustained increase in linear growth velocity. *Clinical Endocrinology*, **41**, 487-494.

KLEINWACHTER, F. (1864) Beobachtung uber die Wirkung des Calabar-Extracts gegen Atropinvergiftung. *Berliner Klinische Wochenschrift*, **50**, 369-371.

REFERENCES

KLINGENSMITH, G.J., GARCIA, S.C., JONES, H.W., MIGEON, C.J. & BLIZZARD, R.M. (1977) Glucocorticoid treatment of girls with congenital adrenal hyperplasia: effects on height, sexual maturation, and fertility. *Journal of Pediatrics*, **90**, 996-1004.

KRULICH, L., DHARIWAL, A.P. & MCCANN, S.M. (1968) Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. *Endocrinology*, **83**, 783-790.

KRULICH, L., MAYFIELD, M.A., STEELE, M.K., MCMILLEN, B.A., MCCANN, S.M. & KOENIG, J.I. (1982) Differential effects of pharmacological manipulations of central alpha 1- and alpha 2-adrenergic receptors on the secretion of thyrotropin and growth hormone in male rats. *Endocrinology*, **110**, 796-804.

LAL, S., TOLIS, G., MARTIN, S.B., BROWN, G.M. & GUYDA, H. (1975) Effect of clonidine on growth hormone, prolactin, luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone in the serum of normal men. *Journal of Clinical Endocrinology and Metabolism*, **41**, 827-832.

LANCE, V.A., MURPHY, W.A., SUEIRAS DIAZ, J. & COY, D.H. (1984) Super-active analogs of growth hormone-releasing factor (1-29)-amide. *Biochemical and Biophysical Research Communications*, **119**, 265-272.

LANES, R. & HURTADO, E. (1982) Oral clonidine-an effective growth hormone-releasing agent in prepubertal subjects. *Journal of Pediatrics*, **100**, 710-714.

LANG, I., SCHERNTHANER, G., PIETSCHMANN, P., KURZ, R., STEPHENSON, J. & TEMPL, H. (1987) Effects of sex and age on growth hormone response to growth hormone-releasing hormone in healthy individuals. *Journal of Clinical Endocrinology and Metabolism*, **65**, 535-540.

LARON, Z., KLINGER, B., ERSTER, B. & ANIN, S. (1988) Effect of acute administration of insulin-like growth factor-I in patients with Laron-type dwarfism. *Lancet*, **2**, 1170-1172.

LARON, Z., KLINGER, B., SILBERGELD, A., LEWIN, R., ERSTER, B. & GIL-AD, I. (1990) Intravenous administration of recombinant IGF-I lowers serum GHRH and TSH. *Acta Endocrinologica*, **123**, 378-382.

LEAHY, J.L. & VANDEKERKHOVE, K.M. (1990) Insulin-like growth factor-I at physiological concentrations is a potent inhibitor of insulin secretion. *Endocrinology*, **126**, 1593-1598.

REFERENCES

LEAL-CERRO, A., PEREIRA, J.L., GARCIA-LUNA, P.P., ASTORGA, R., CORDIDO, F., CASANUEVA, F.F. & DIEGUEZ, C. (1990) Effect of enhancement of endogenous cholinergic tone with pyridostigmine on growth hormone (GH) responses to GH-releasing hormone in patients with Cushing's syndrome. *Clinical Endocrinology*, **33**, 291-295.

LEALCERRO, A., PUMAR, A., VILLAMIL, F., ASTORGA, R., DIEGUEZ, C. & CASANUEVA, F.F. (1993) Growth hormone releasing hormone priming increases growth hormone secretion in patients with cushing's syndrome. *Clinical Endocrinology*, **38**, 399-403.

LESNIAK M A., HILL, J.M., KIESS, W., ROJESKI, M., PERT, C.B. & ROTH, J. (1988) Receptors for insulin-like growth factors I and II: autoradiographic localization in rat brain and comparison to receptors for insulin. *Endocrinology*, **123**, 2089-2099.

LEVESTON, S.A. & CRYER, P.E. (1980) Endogenous cholinergic modulation of growth-hormone secretion in normal and acromegalic humans. *Metabolism: Clinical and Experimental*, **29**, 703-706.

LIEBERMAN, S.A., BUKAR, J., CHEN, S.A., CELNIKER, A.C., COMPTON, P.G., COOK, J., ALBU, J., PERLMAN, A.J. & HOFFMAN, A.R. (1992) Effects of Recombinant Human Insulin-like growth factor-I (rhIGF-I) on total and free IGF-I concentrations, IGF-binding proteins, and glycaemic response in humans. *Journal of Clinical Endocrinology and Metabolism*, **75**, 30-36.

LIN, H., BOLLINGER, J., LING, N. & REICHLIN, S. (1984) Immunoreactive growth hormone-releasing factor in human stalk median emminence. *Journal of Clinical Endocrinology and Metabolism*, **58**, 1197-1199.

LINDAHL, A., ISGAARD, J., CARLSSON, L. & ISAKSSON, O.G. (1987a) Differential effects of growth hormone and insulin-like growth factor I on colony formation of epiphyseal chondrocytes in suspension culture in rats of different ages. *Endocrinology*, **121**, 1061-1069.

LINDAHL, A., ISGAARD, J. & ISAKSSON, O.G. (1987b) Growth hormone in vivo potentiates the stimulatory effect of insulin-like growth factor-1 in vitro on colony formation of epiphyseal chondrocytes isolated from hypophysectomized rats. *Endocrinology*, **121**, 1070-1075.

LINDAHL, A., NILSSON, A. & ISAKSSON, O.G. (1987c) Effects of growth hormone and insulin-like growth factor-I on colony formation of rabbit epiphyseal chondrocytes at different stages of maturation. *Journal of Endocrinology*, **115**, 263-271.

REFERENCES

LING, N., BAIRD, A., WEHRENBERG, W., UENO, N., MUNEGUMI, T., CHIANG, T., REGNO, M. & BRAZEAU, P. (1984) Synthesis and in vitro bioactivity of human response to growth hormone-releasing hormone in the rat: evidence for mediation by somatostatin. *Biochemical and Biophysical Research Communications*, **122**, 304-310.

LINK, K., BLIZZARD, R.M., EVANS, W.S., KAISER, D.L., PARKER, M.W. & ROGOL, A.D. (1986) The effect of androgens on the pulsatile release and the twenty-four-hour mean concentration of growth hormone in prepubertal males. *Journal of Clinical Endocrinology and Metabolism*, **62**, 159-164.

LOCATELLI, V., TORSELLO, A., REDAELLI, M., GHIGO, E., MASSARA, F. & MULLER, E. (1986) Cholinergic agonist and antagonist drugs modulate the growth hormone response to growth hormone-releasing hormone in the rat: evidence for mediation by somatostatin. *Journal of Endocrinology*, **111**, 271-278.

LOSA, M., SCHOPOHL, J., MULLER, O.A. & VON WERDER, K. (1984) Stimulation of growth hormone secretion with human growth hormone releasing factors (GRF1-44, GRF1-40, GRF1-29) in normal subjects. *Klinische Wochenschrift*, **62**, 1140-1143.

LYU, P.C., LIFF, M.I., MARKY, L.A. & KALLENBACH, N.R. (1990) Side chain contributions to the stability of alpha-helical structure in peptides. *Science*, **250**, 669-673.

MACLEOD, R.M., FONTHAM, E.H. & LEHMEYER, J.E. (1970) Prolactin and growth hormone production as influenced by catecholamines and agents that affect brain catecholamines. *Neuroendocrinology*, **6**, 283-294.

MACLEOD, R.M. & LEHMEYER, J.E. (1974) Studies on the mechanism of the dopamine-mediated inhibition of prolactin secretion. *Endocrinology*, **94**, 1077-1185.

MADSEN, K., FRIBERG, U., ROOS, P., EDEN, S. & ISAKSSON, O. (1983) Growth hormone stimulates the proliferation of cultured chondrocytes from rabbit ear and rat rib growth cartilage. *Nature*, **304**, 545-547.

MARIE, P. (1886) Sur deux cas d'acromegalie. Hypertrophie singuliere non congenitale des extremites superieures, inferierures et cephaliques. *Rev Med*, **6**, 297-333.

MARTHA, P.M., REITER, E.O., DAVILA, N., SHAW, M.A., HOLCOMBE, J.H. & BAUMANN, G. (1992) Serum growth hormone (GH)-binding protein/receptor - an important determinant of GH responsiveness. *Journal of Clinical Endocrinology and Metabolism*, **75**, 1464-1469.

REFERENCES

MARTHA, P.M.J., BLIZZARD, R.M., THORNER, M.O. & ROGOL, A.D. (1990) Atenolol enhances nocturnal growth hormone (GH) release in GH-deficient children during long term GH-releasing hormone therapy. *Journal of Clinical Endocrinology and Metabolism*, **70**, 56-61.

MARTIN, J.B. (1976) Brain regulation of growth hormone secretion. In *Frontiers in Neuroendocrinology* (eds L. Martin & W. F. Ganong), pp. 129-169. Raven Press, New York.

MARTIN, J.L. & BAXTER, R.C. (1986) Insulin-like growth factor-binding protein from human plasma. Purification and characterization. *Journal of Biological Chemistry*, **261**, 8754-8760.

MARTIN, L.G., CLARK, J.W. & CONNOR, T.B. (1968) Growth hormone secretion enhanced by androgens. *Journal of Clinical Endocrinology and Metabolism*, **28**, 425-428.

MARTINA, V., MIOLA, C., MACCARIO, M., TALLIANO, M., ARVAT, E., GHIGO, E. & CAMANNI, F. (1992) Inhibition by salbutamol of GHRH-induced GH release in type 1 diabetes mellitus. *Hormone and Metabolic Research*, **24**, 520-523.

MASSARA, F., GHIGO, E., DEMISLIS, K., TANGOLO, D., MAZZA, E., LOCATELLI, V., MULLER, E.E., MOLINATTI, G.M. & CAMANNI, F. (1986a) Cholinergic involvement in the growth hormone releasing hormone-induced growth hormone release: studies in normal and acromegalic subjects. *Neuroendocrinology*, **43**, 670-675.

MASSARA, F., GHIGO, E., MOLINATTI, P., MAZZA, E., LOCATELLI, V., MULLER, E.E. & CAMANNI, F. (1986b) Potentiation of cholinergic tone by pyridostigmine bromide re-instates and potentiates the growth hormone responsiveness to intermittent administration of growth hormone-releasing factor in man. *Acta Endocrinologica*, **113**, 12-16.

MAYO, K. (1992) Molecular cloning and expression of a pituitary-specific receptor for growth hormone-releasing hormone. *Molecular Endocrinology*, **6**, 1734-1744.

MAYO, K.E., CERELLI, G.M., LEBO, R.V., BRUCE, B.D., ROSENFELD, M.G. & EVANS, R.M. (1985) Gene encoding human growth hormone-releasing factor precursor: structure, sequence, and chromosomal assignment. *Proceedings of the National Academy of Sciences of the United States of America*, **82**, 63-67.

MAZZA, E., GHIGO, E., BELLONE, J., ARVAT, E., REVELLI, E., CELLA, S.G., BRAMBILLA, F., MULLER, E.E. & CAMANNI, F. (1990) Effects of alpha- and beta-adrenergic agonists and antagonists on growth hormone secretion in man. *Endocrinologia Experimentalis*, **24**, 211-219.

REFERENCES

MCARTHUR, R.G., HAYLES, A.B. & SALASSA, R.M. (1979) Childhood Cushing disease: results of bilateral adrenalectomy. *Journal of Pediatrics*, **95**, 214-219.

MCEWEN, B.S. (1980) Binding and Metabolism of sex steroids by the hypothalamic-pituitary unit: physiological implications. *Annual Review of Physiology*, **42**, 97-110.

MERCADO, M., MOLITCH, M.E. & BAUMANN, G. (1992) Low plasma growth hormone binding protein in IDDM. *Diabetes*, **41**, 605-609.

MERIMEE, T.J., BERGESS, J.A. & RABINOWITZ, D. (1966) Sex-determined variation in serum insulin and growth hormone response to amino acid stimulation. *Journal of Clinical Endocrinology and Metabolism*, **26**, 791-793.

MERIMEE, T.J., RABINOWITZ, D. & FINEBERG, S.E. (1969) Arginine-initiated release of human growth hormone. *New England Journal of Medicine*, **280**, 1434-1438.

MERIMEE, T.J., GRANT, M.B., BRODER, C.M. & CAVALLI-SFORZA, L.L. (1989) Insulin-like growth factor secretion by human B-lymphocytes: a comparison of cells from normal and pygmy subjects. *Journal of Clinical Endocrinology and Metabolism*, **68**, 978-984.

MERIMEE, T.J., BAUMANN, G. & DAUGHADAY, W.H. (1990) Growth hormone-binding protein: II. Studies in pygmies and normal statured subjects. *Journal of Clinical Endocrinology and Metabolism*, **70**, 1183-1188.

MERIMEE, T.J. & FINEBERG, S.E. (1971) Studies of the sex based variation of human growth hormone secretion. *Journal of Clinical Endocrinology and Metabolism*, **33**, 896-902.

MEROLA, B., CITTADINI, A., COLAO, A., LONGOBARDI, S., FAZIO, S., SABATINI, D., SACCA, L. & LOMBARDI, G. (1993) Cardiac structural and functional abnormalities in adult patients with growth hormone deficiency. *Journal of Clinical Endocrinology and Metabolism*, **77**, 1658-1661.

MERRIAM, G.R. & WACHTER, K.W. (1982) Algorithms for the study of episodic hormone secretion. *American Journal of Physiology*, **243**, E310-E318.

MIELL, J.P., CORDER, R., PRALONG, F.P. & GAILLARD, R.C. (1991) Effects of Dexamethasone on Growth Hormone (GH)-Releasing Hormone, Arginine and Dopaminergic Stimulated GH Secretion, and Total Plasma Insulin-Like Growth Factor-I Concentrations in Normal Male Volunteers. *Journal of Clinical Endocrinology and Metabolism*, **72**, 675-681.

REFERENCES

MIELL, J.P., TAYLOR, A.M., JONES, J., BUCHANAN, C.R., RENNIE, J., SHERWOOD, R., LEICESTER, R. & ROSS, R.J.M. (1992) Administration of human recombinant insulin-like growth factor-I to patients following major surgery. *Clinical Endocrinology*, **37**, 542-551.

MIELL, J.P., TAYLOR, A.M., ZINI, M., MAHESHWARI, H.G., ROSS, R.J.M. & VALCAVI, R. (1993) Effects of hypothyroidism and hyperthyroidism on insulin- like growth factors (IGFs) and growth hormone-binding and IGF-binding proteins. *Journal of Clinical Endocrinology and Metabolism*, **76**, 950-955.

MIKI, N., ONO, M. & SHIZUME, K. (1984) Evidence that opiatergic and alpha-adrenergic mechanisms stimulate rat growth hormone release via growth hormone-releasing factor (GRF). *Endocrinology*, **114**, 1950-1952.

MINAMITANI, N., CHIHARA, K., KAJI, H., KODAMA, H., KITA, T. & FUJITA, T. (1989) Alpha 2-adrenergic control of growth hormone (GH) secretion in conscious male rabbits: involvement of endogenous GH-releasing factor and somatostatin. *Endocrinology*, **125**, 2839-2845.

MORIMOTO, N., KAWAKAMI, F., MAKINO, S., CHIHARA, K., HASEGAWA, M. & IBATA, Y. (1988) Age-related changes in growth hormone releasing factor and somatostatin in the rat hypothalamus. *Neuroendocrinology*, **47**, 459-464.

MORLEY, B.J., LORDEN, J.F., BROWN, G.B., KEMP, G.E. & BRADLEY, R.J. (1977) Regional distribution of nicotinic acetylcholine receptor in rat brain. *Brain Research*, **134**, 161-166.

MORTIMER, C.H., BESSER, G.M., MCNEILLY, A.S., TUNBRIDGE, W.M., GOMEZ PAN, A. & HALL, R. (1973) Interaction between secretion of the gonadotrophins, prolactin, growth hormone, thyrotrophin and corticosteroids in man: the effects of LH FSH-RH, TRH and hypoglycaemia alone and in combination. *Clinical Endocrinology*, **2**, 317-326.

MOXLEY, R.T., ARNER, P., MOSS, A., SKOTTNER, A., FOX, M., JAMES, D. & LIVINGSTON, J.N. (1990) Acute effects of insulin-like growth factor I and insulin on glucose metabolism in vivo. *American Journal of Physiology*, **259**, E561-E567.

MULLER, E.E., COIRO, V., PIOLI, G., NAVA, C., COCCHI, D. & PASSERI, M. (1988) Short-term administration of GHRH in aged subjects: priming effect on the pituitary and increased plasma SM-C levels. *8th International Congress of Endocrinology*, 118(Abstract)

MURATA, M. & HIBI, I. (1992) Nutrition and the secular trend of growth. *Hormone Research (supplement)*, **38**, 89-96.

REFERENCES

NAKAGAWA, K., HORIUCHI, Y. & MASHIMO, K. (1969) Responses of plasma growth hormone and corticosteroids to insulin and arginine with or without prior administration of dexamethasone. *Journal of Clinical Endocrinology and Metabolism*, **29**, 35-40.

NYBORG, J.K., NGUYEN, A.P. & SPINDLER, S.R. (1984) Relationship between thyroid and glucocorticoid hormone receptor occupancy, growth hormone gene transcription, and mRNA accumulation. *Journal of Biological Chemistry*, **259**, 12377-12381.

O'NEIL, K.T. & DEGRADO, W.F. (1990) A thermodynamic scale for the helix-forming tendencies of the commonly occurring amino acids. *Science*, **250**, 646-650.

OGATA, T. & MATSUO, N. (1993) Sex chromosome aberrations and stature: deduction of the principal factors involved in the determination of adult height. *Human Genetics*, **91**, 551-562.

O'HALLORAN, D.J., TSATSOULIS, A., WHITEHOUSE, R.W., HOLMES, S.J., ADAMS, J.E. & SHALET, S.M. (1993) Increased bone density after recombinant human growth hormone (GH) therapy in adults with isolated GH deficiency. *Journal of Clinical Endocrinology and Metabolism*, **76**, 1344-1348.

OOI, G.T. & HERINGTON, A.C. (1990) Recognition of insulin-like-growth-factor-binding proteins in serum and amniotic fluid by an antiserum against a low-molecular- mass insulin-like-growth-factor-inhibitor/binding protein. *Biochemical Journal*, **267**, 615-620.

ORME, S.M., SEBASTIAN, J.P., OLDROYD, B., STEWART, S.P., GRANT, P.J., STICKLAND, M.H., SMITH, M.A. & BELCHETZ, P.E. (1992) Comparison of measures of body composition in a trial of low dose growth hormone replacement therapy. *Clinical Endocrinology*, **37**, 453-459.

PAGE, M.D., KOPPESCHAAR, H.P., EDWARDS, C.A., DIEGUEZ, C. & SCANLON, M.F. (1987) Additive effects of growth hormone releasing factor and insulin hypoglycaemia on growth hormone release in man. *Clinical Endocrinology*, **26**, 589-595.

PARKER, D.C., SASSIN, J.F., MACE, J.W., GOTLIN, R.W. & ROSSMAN, L.G. (1969) Human growth hormone release during sleep: electroencephalographic correlation. *Journal of Clinical Endocrinology and Metabolism*, **29**, 871-874.

PAVLOV, E.P., HARMAN, S.M., MERRIAM, G.R., GELATO, M.C. & BLACKMAN, M.R. (1986) Responses of growth hormone and somatomedin-C to GH-releasing hormone in healthy aging men. *Journal of Clinical Endocrinology and Metabolism*, **62**, 595-600.

REFERENCES

PENALVA, A., BURGUERA, B., CASABIELL, X., TRESGUERRES, J.A., DIEGUEZ, C. & CASANUEVA, F.F. (1989) Activation of cholinergic neurotransmission by pyridostigmine reverses the inhibitory effect of hyperglycemia on growth hormone (GH) releasing hormone-induced GH secretion in man: does acute hyperglycemia act through hypothalamic release of somatostatin?. *Neuroendocrinology*, **49**, 551-554.

PENALVA, A., GAZTAMBIDE, S., VAZQUEZ, J.A., DIEGUEZ, C. & CASANUEVA, F.F. (1990a) Role of cholinergic muscarinic pathways on the free fatty acid inhibition of GH responses to GHRH in normal men. *Clinical Endocrinology*, **33**, 171-176.

PENALVA, A., MURUAIS, C., CASANUEVA, F.F. & DIEGUEZ, C. (1990b) Effect of enhancement of endogenous cholinergic tone with pyridostigmine on the dose-response relationships of growth hormone (GH)-releasing hormone-induced GH secretion in normal subjects. *Journal of Clinical Endocrinology and Metabolism*, **70**, 324-327.

PETERS, J.R., EVANS, P.J., PAGE, M.D., HALL, R., GIBBS, J.T., DIEGUEZ, C. & SCANLON, M.F. (1986) Cholinergic muscarinic receptor blockade with pirenzepine abolishes slow wave sleep-related growth hormone release in normal adult males. *Clinical Endocrinology*, **25**, 213-217.

PLOTNICK, L.P., LEE, P.A., MIGEON, C.J. & KOWARSKI, A.A. (1979) Comparison of physiological and pharmacological tests of growth hormone function in children with short stature. *Journal of Clinical Endocrinology and Metabolism*, **48**, 811-815.

POPA, G. & FIELDING, U. (1930) A portal circulation from the pituitary to the hypothalamic region. *Journal of Anatomy*, **65**, 88-91.

POSTEL-VINAY, M.C., TAR, A., CROSNIER, H., BROYER, M., RAPPAPORT, R., TONSHOFF, B. & MEHLS, O. (1991) Plasma growth hormone-binding activity is low in uraemic children. *Pediatric Nephrology*, **5**, 545-547.

PREECE, M.A. (1976) The effect of administered corticosteroids on the growth of children. *Postgraduate Medical Journal*, **52**, 625-630.

PREECE, M.A., TANNER, J.M., WHITEHOUSE, R.H. & CAMERON, N. (1976) Dose dependence of growth response to human growth hormone in growth hormone deficiency. *Journal of Clinical Endocrinology and Metabolism*, **42**, 477-483.

PRESS, M., CAPRIO, S., TAMBORLANE, W.V., BHUSHAN, R., THORNER, M.O., VALE, W., RIVIER, J. & SHERWIN, R.S. (1992) Pituitary response to growth hormone-releasing hormone in IDDM. Abnormal responses to insulin and hyperglycaemia. *Diabetes*, **41**, 17-21.

REFERENCES

PRINZ, P.N., WEITZMAN, E.D., CUNNINGHAM, G.R. & KARACAN, I. (1988) Plasma growth hormone during sleep in young and aged men. *Gerontology*, **38**, 519-524.

PROCOPIO, M., INVITTI, C., MACCARIO, M., GROTTOLI, S., CAVAGNINI, F., CAMANNI, F. & GHIGO, E. (1995) Effect of arginine and pyridostigmine on the GHRH-induced GH rise in obesity and Cushing's syndrome. *International Journal of Obesity*, **19**, 108-112.

QUABBE, H.J., SCHILLING, E. & HELGE, H. (1966) Pattern of growth hormone secretion during a 24-hour fast in normal adults. *Journal of Clinical Endocrinology and Metabolism*, **26**, 1173-1177.

RAYNOR, K., MURPHY, W.A., COY, D.H., TAYLOR, J.E., MOREAU, J.P., YASUDA, K., BELL, G.I. & REISINE, T. (1993a) Cloned somatostatin receptors: identification of subtype- selective peptides and demonstration of high affinity binding of linear peptides. *Molecular Pharmacology*, **43**, 838-844.

RAYNOR, K., O'CARROLL, A.M., KONG, H., YASUDA, K., MAHAN, L.C., BELL, G.I. & REISINE, T. (1993b) Characterization of cloned somatostatin receptors SSTR4 and SSTR5. *Molecular Pharmacology*, **44**, 385-992.

REICHLIN, S. (1961) Growth hormone content of pituitaries from rats with hypothalamic lesions. *Endocrinology*, **69**, 225-230.

REITER, E.O., MORRIS, A.H., MACGILLIVRAY, M.H. & WEBER, D. (1988) Variable estimates of serum growth hormone concentrations by different radioassay systems. *Journal of Clinical Endocrinology and Metabolism*, **66**, 68-71.

REZEK, M., HAVLICEK, V., LEYBIN, L., LABELLA, F.S. & FRIESEN, H. (1978) Opiate-like naloxone-reversible actions of somatostatin given intracerebrally. *Canadian Journal of Physiology and Pharmacology*, **56**, 227-231.

REZEK, M. & NOVIN, D. (1977) Hepatic-portal nutrient infustion: effect on feeding in intact and vagotomized rabbits. *American Journal of Physiology*, **232**, E119-E130.

RICHARDSON, S.B., HOLLANDER, C.S., D'ELETTO, R., GREENLEAF, P.W. & THAW, C. (1980) Acetylcholine inhibits the release of somatostatin from rat hypothalamus in vitro. *Endocrinology*, **107**, 122-129.

RIGGS, B.L., WAHNER, H.W., DUNN, W.L., MAZESS, R.B., OFFORD, K.P. & MELTON, L.J. (1981) Differential changes in bone mineral density of the appendicular and axial skeleton with aging: relationship to spinal osteoporosis. *Journal of Clinical Investigation*, **67**, 328-335.

REFERENCES

RIMOIN, D.L., MERIMEE, T.J., RABINOWITZ, D., MCKUSICK, V.A. & CAVALLI-SFORZA, L.L. (1967) Growth hormone in African Pygmies. *Lancet*, **2**, 523-526.

RINDERKNECHT, E. & HUMBEL, R.E. (1978) The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *Journal of Biological Chemistry*, **253**, 2769-2776.

RIVIER, J., SPIESS, J., THORNER, M. & VALE, W. (1982) Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. *Nature*, **300**, 276-278.

ROBBERECHT, P., GILLARD, M., WAELBROECK, M., CAMUS, C., DE NEEF, P. & CHRISTOPHE, J. (1986) Decreased stimulation of adenylate cyclase by growth hormone-releasing factor in the anterior pituitary of old rats. *Neuroendocrinology*, **44**, 429-432.

ROBBINS, R. (1988) Somatostatin and the Cerebral Cortex. In *Advances in Experimental and Biology - Somatostatin* (eds Y. Patel & G. Tannenbaum), pp. 201-215. Plenum Press, New York.

ROCHICCIOLI, P.E., TAUBER, M., UBOLDI, F., COUDE, F. & MORRE, M. (1986) Effect of overnight constant infusion of growth hormone(GH)-releasing hormone-(1-44) on 24-hour GH secretion in children with partial GH deficiency. *Journal of Clinical Endocrinology and Metabolism*, **63**, 1100-1105.

ROSE, S.R., ROSS, J.L., URIARTE, M.M., BARNES, K.M., CASSORLA, F.G. & CUTLER, G.B. (1988) The advantage of measuring stimulated as compared with spontaneous growth hormone levels in the diagnosis of growth hormone deficiency. *New England Journal of Medicine*, **319**, 201-207.

ROSEN, T., BOSAEUS, I., TOLLI, J., LINDSTEDT, G. & BENGTSSON, B.A. (1993a) Increased body fat mass and decreased extracellular fluid volume in adults with growth hormone deficiency. *Clinical Endocrinology*, **38**, 63-71.

ROSEN, T., HANSSON, T., GRANHED, H., SZUCS, J. & BENGTSSON, B.A. (1993b) Reduced bone mineral content in adult patients with growth hormone deficiency. *Acta Endocrinologica*, **129**, 201-206.

ROSEN, T. & BENGTSSON, B.A. (1990) Premature mortality due to cardiovascular disease in hypopituitarism. *Lancet*, **336**, 285-288.

ROSENBLOOM, A.L., SAVAGE, M.O., BLUM, W.F., GUEVARAAGUIRRE, J. & ROSENFELD, R.G. (1992) Clinical and biochemical characteristics of growth hormone receptor deficiency (laron syndrome). *Acta Paediatrica*, **81**, 121-124.

REFERENCES

ROSENFELD, R.G., CEDA, G., WILSON, D.M., DOLLAR, L.A. & HOFFMAN, A.R. (1984) Characterization of high affinity receptors for insulin-like growth factors I and II on rat anterior pituitary cells. *Endocrinology*, **114**, 1571-1575.

ROSENFELD, R.G., PHAM, H., CONOVER, C.A., HINTZ, R.L. & BAXTER, R.C. (1989) Structural and immunological comparison of insulin-like growth factor binding proteins of cerebrospinal and amniotic fluids. *Journal of Clinical Endocrinology and Metabolism*, **68**, 638-646.

ROSENFELD, R.G., ATTIE, K.M., FRANE, J., JOHANSON, A.J., KUNTZE, J., BRASEL, J.A., BURSTEIN, S., CARA, J.F., CHERNAUSEK, S., GOTLIN, R.W., LIPPE, B.M., MAHONEY, P.C., MOORE, W.V. & SAENGER, P. (1992) Growth hormone therapy in turner's syndrome - an update on final height. *Acta Paediatrica*, **81**, 3-6.

ROSENTHAL, S.M., SCHRIOCK, E.A., KAPLAN, S.L., GUILLEMIN, R. & GRUMBACH, M.M. (1983) Synthetic human pancreas growth hormone-releasing factor (hpGRF1-44-NH2) stimulates growth hormone secretion in normal men. *Journal of Clinical Endocrinology and Metabolism*, **57**, 677-679.

ROSS, R.J., BORGES, F., GROSSMAN, A., SMITH, R., NGAHFOONG, L., REES, L.H., SAVAGE, M.O. & BESSER, G.M. (1987a) Growth hormone pretreatment in man blocks the response to growth hormone-releasing hormone; evidence for a direct effect of growth hormone. *Clinical Endocrinology*, **26**, 117-123.

ROSS, R.J., GROSSMAN, A., DAVIES, P.S., SAVAGE, M.O. & BESSER, G.M. (1987b) Stilboestrol pretreatment of children with short stature does not affect the growth hormone response to growth hormone-releasing hormone. *Clinical Endocrinology*, **27**, 155-161.

ROSS, R.J., RODDA, C., TSAGARAKIS, S., DAVIES, P.S., GROSSMAN, A., REES, L.H., PREECE, M.A., SAVAGE, M.O. & BESSER, G.M. (1987c) Treatment of growth-hormone deficiency with growth-hormone-releasing hormone. *Lancet*, **1**, 5-8.

ROSS, R.J., TSAGARAKIS, S., GROSSMAN, A., NHAGAFOONG, L., TOUZEL, R.J., REES, L.H. & BESSER, G.M. (1987d) GH feedback occurs through modulation of hypothalamic somatostatin under cholinergic control: studies with pyridostigmine and GHRH. *Clinical Endocrinology*, **27**, 727-733.

ROTH, J., GLICK, S.M., YALOW, S. & BERSON, S.A. (1963) Secretion of human growth hormone: physiologic and experimental modification. *Metabolism: Clinical and Experimental*, **XII**, 577-579.

RUDMAN, D., VINTNER, M.H., ROGERS, C.M., LUBIN, M.F., FLEMING, G.H. & RAYMOND, P.B. (1981) Impaired growth hormone secretion in the adult population. *Journal of Clinical Investigation*, **67**, 1361-1369.

REFERENCES

RUDMAN, D., FELLER, A.G., NAGRAJ, H.S., GERGENS, G.A., LALITHIA, P.Y., GOLDBERG, A.F., SCHLENCKER, R.A., COHN, L., RUDMAN, I.W. & MATTSON, D.E. (1990) Effect of human growth hormone in men over 60 years old. *New England Journal of Medicine*, **323**, 1-6.

RUPPRECHT, R., NIEHAUS, A. & LESCH, K.P. (1990) The influence of dexamthesone on growth hormone (GH). Response to GH-releasing hormone in normal man. *Journal of Neural Transmission*, **79**, 51-57.

RUSSELL-JONES, D.L., WEISSBERGER, A.J., BOWES, S.B., KELLY, J.M., THOMASON, M., UMPLEBY, A.M., JONES, R.H. & SONKSEN, P.H. (1993) Rapid communication - the effects of growth hormone on protein metabolism in adult growth hormone deficient patients. *Clinical Endocrinology*, **38**, 427-431.

SAEED UZ ZAFAR, M., MELLINGER, R.C., FINE, G., SZABO, M. & FROHMAN, L.A. (1979) Acromegaly associated with a bronchial carcinoid tumor: evidence for ectopic production of growth hormone-releasing activity. *Journal of Clinical Endocrinology and Metabolism*, **48**, 66-71.

SAKUMA, M. & KNOBIL, E. (1970) Inhibition of endogenous growth hormone secretion by exogenous growth hormone infusion in the rhesus monkey. *Endocrinology*, **86**, 890-894.

SALMON, W.D. & DAUGHADAY, W.H. (1957) A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. *The Journal of Laboratory and Clinical Medicine*, **49**, 825-836.

SALOMON, F., CUNEO, R.C., HESP, R. & SONKSEN, P.H. (1989) The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *New England Journal of Medicine*, **321**, 1797-1803.

SALOMON, F., CUNEO, R.C., HESP, R., MORRIS, J.F., POSTON, L. & SONKSEN, P.H. (1992) Basal metabolic rate in adults with growth hormone deficiency and in patients with acromegaly - relationship with lean body mass, plasma insulin level and leucocyte sodium pump activity. *Clinical Science*, **83**, 325-330.

SALOMON, F., CUNEO, R. & SONKSEN, P.H. (1993) Insulin sensitivity and insulin resistance in growth- hormone-deficient adults. *Hormone Research*, **40**, 34-36.

SAMARAS, S.E., HAGEN, D.R., SHIMASAKI, S., LING, N. & HAMMOND, J.M. (1992) Expression of insulin-like growth factor-binding protein-2 and - 3 messenger ribonucleic acid in the porcine ovary: localization and physiological changes. *Endocrinology*, **130**, 2739-2744.

REFERENCES

SANTEN, J.R. & BARDIN, C.W. (1973) Episodic luteininzing hormone secretion in man. *Journal of Clinical Investigation*, **52**, 2617-2628.

SARTORIO, A. & NARICI, M.V. (1994) Growth hormone (GH) treatment in GH-deficient adults: effects on muscle size, strength and neural activation. *Clinical Physiology*, **14**, 527-537.

SASSOLAS, G., CHAYVIALLE, J.A., PARTENSKY, C., BERGER, G., TROUILLAS, J., BERGER, F., CLAUSTRAT, B., COHEN, R., GIROD, C. & GUILLEMIN, R. (1983) Acromegaly, clinical expression of the production of growth hormone releasing factor in pancreatic tumors. *Annales D'Endocrinologie*, **44**, 347-354.

SASSOLAS, G., COHEN, J., BASTUJI, H., VERMEULEN, E., CABRERA, P., ROUSSEL, B. & JOUVET, M. (1986) Nocturnal continuous infusion of growth hormone (GH)-releasing hormone results in a dose-dependent accentuation of episodic GH secretion in normal men. *Journal of Clinical Endocrinology and Metabolism*, **63**, 1016-1022.

SCHALLY, A.V., ARIMURA, A., BABA, Y., NAIR, R.M., MATSUO, H., REDDING, T.W. & DEBELJUK, L. (1971) Isolation and properties of the FSH and LH-releasing hormone. *Biochemical and Biophysical Research Communications*, **43**, 393-399.

SCHAPER, N., VERHOEFF, A., SLUITER, W., ROELSE, H., REITSMA, W. & DOORENBOS, H. (1990) The growth hormone response to growth hormone-releasing hormone and its relationship to serum insulin-like growth factor I in Type I diabetes mellitus. *Acta Endocrinologica*, **122**, 13-18.

SCHARRER, E. & SCHARRER, B. (1954) Hormones produced by neurosecretory cells. *Progress in Brain Research.*, **10**, 183-232.

SCHLESINGER, S., MACGILLIVRAY, H. & MUNSCHAUSER, R. (1973) Acceleration of growth and bone maturation in childhood thyrotoxicosis. *Journal of Pediatrics*, **18**, 233-236.

SCHULTE, H.M., ALLOLIO, B., FROHWEIN, U.S., BENKER, G. & REINWEIN, D. (1986) Continuous infusion of growth hormone releasing hormone does not alter the growth hormone response to different stress stimuli. *Clinical Endocrinology*, **25**, 511-517.

SCHWANDER, J.C., HAURI, C., ZAPF, J. & FROESCH, E.R. (1983) Synthesis and secretion of insulin-like growth factor and its binding protein by the perfused rat liver: dependence on growth hormone status. *Endocrinology*, **113**, 297-305.

SEEBURG, P. & ADELMAN, J. (1984) Characterisation of cDNA for precursor of human luteinizing hormone-releasing hormone. *Nature*, **311**, 666-668.

REFERENCES

SHAH, A., STANHOPE, R. & MATTHEW, D. (1992) Lesson of the Week - Hazards of Pharmacological Tests of Growth Hormone Secretion in Childhood. *British Medical Journal*, **304**, 173-174.

SHALET, S.M., BEARDWELL, C.G., MACFARLANE, I.A., ELLISON, M.L., NORMAN, C.M., REES, L.H. & HUGHES, M. (1979) Acromegaly due to production of a growth hormone releasing factor by a bronchial carcinoid tumor. *Clinical Endocrinology*, **10**, 61-67.

SHERMAN, S.S., HOLLIS, B.W. & TOBIN, J.D. (1990) Vitamin D status and related parameters in a healthy population: the effects of age, sex and season. *Journal of Clinical Endocrinology and Metabolism*, **71**, 405-413.

SHIBASAKI, T., HOTTA, M., MASUDA, A., IMAKI, T., OBARA, N., DEMURA, H., LING, N. & SHIZUME, K. (1985a) Plasma GH responses to GHRH and insulin-induced hypoglycemia in man. *Journal of Clinical Endocrinology and Metabolism*, **60**, 1265-1267.

SHIBASAKI, T., HOTTA, M., MASUDA, A., IMAKI, T., OBARA, N., DEMURA, H., LING, N. & SHIZUME, K. (1985b) Plasma GH responses to GHRH and insulin-induced hypoglycemia in man. *Journal of Clinical Endocrinology and Metabolism*, **60**, 1265-1267.

SHIBASAKI, T., YAMACHI, N., HOTTA, M., MASUDA, A., IMAKI, T., DEMURA, H., LING, N. & SHIZUME, K. (1986) In vitro release of growth hormone-releasing factor from rat hypothalamus: effects of insulin-like growth factor-I. *Regulatory Peptides*, **15**, 47-53.

SHIMASAKI, S., UCHIYAMA, F., SHIMONAKA, M. & LING, N. (1990) Molecular cloning of the cDNAs encoding a novel insulin-like growth factor-binding protein from rat and human. *Molecular Endocrinology*, **4**, 1451-1458.

SHIMOKAWA, I., HIGAMI, Y., OKIMOTO, T. & IKEDA, T. (1994) The growth hormone-releasing hormone-cyclic adenosine-3,5-monophosphate signal pathway in somatotropes is practically intact during aging. *Neuroendocrinology*, **60**, 575-580.

SIEGEL, S. (1956) *Non-parametric statistics for the behavioral sciences* McGraw-Hill Book Company, New York.

SIEGEL, S.F., BECKER, D.J., LEE, P.A., GUTAI, J.P., FOLEY, T.P. & DRASH, A.L. (1984) Comparison of physiologic and pharmacologic assessment of growth hormone secretion. *American Journal of Diseases of Children*, **138**, 540-543.

SILBERGELD, A., LAZAR, L., ERSTER, B., KERET, R., TEPPER, R. & LARON, Z. (1989) Serum growth hormone binding protein activity in healthy neonates, children and young adults: correlation with age, height and weight. *Clinical Endocrinology*, **31**, 295-303.

REFERENCES

SMALS, A.E.M., PIETERS, G.F., BENRAAD, T.J. & KLOPPENBORG, P.W.C. (1986a) Human pancreatic growth hormone releasing hormone fails to stimulate growth hormone both in Cushing's disease and in Cushing's syndrome adrenocortical adenoma. *Clinical Endocrinology*, **24**, 401-407.

SMALS, A.E.M., PIETERS, G.F., SMALS, A.G.H., BENRAAD, T.J., VAN LAARHOVEN, J. & KLOPPENBORG, P.W.C. (1986b) Sex difference in human growth hormone (GH) response to intravenous human pancreatic GH-releasing hormone administration in young adults. *Journal of Clinical Endocrinology and Metabolism*, **62**, 336-341.

SMITH, E.P., BOYD, J., FRANK, G.R., TAKAHASHI, H., COHEN, R.M., SPECKER, B., WILLIAMS, T.C., LUBAHN, D.B. & KORACH, K.S. (1994) Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *New England Journal of Medicine*, **331**, 1056-1061.

SONKSEN, P.H., AYRES, A.B., BRAIMBRIDGE, M., CORRIN, B., DAVIES, D.R., JEREMIAH, G.M., OATEN, S.W., LOWY, C. & WEST, T.E. (1976) Acromegaly caused by pulmonary carcinoid tumours. *Clinical Endocrinology*, **5**, 503-513.

SOUTHREN, A.L. (1960) Functioning metastatic bronchial carcinoid with elevated levels of serum and cerebrospinal fluid serotonin and pituitary adenoma. *Journal of Clinical Endocrinology and Metabolism*, **20**, 298-307.

SPADA, A., VALLAR, L. & FAGLIA, G. (1993) G-proteins and hormonal signalling in human pituitary tumors: genetic mutations and functional alterations. *Frontiers in Neuroendocrinology*, **14**, 214-232.

SPEISS, J., RIVIER, J. & VALE, W. (1983) Characterization of rat hypothalamic growth hormone-releasing factor. *Nature*, **303**, 532-535.

SPIESS, J., RIVIER, J., THORNER, M. & VALE, W. (1982) Sequence analysis of a growth hormone releasing factor from a human pancreatic islet tumor. *Biochemistry*, **21**, 6037-6040.

STACHURA, M.E. & TYLER, J.M. (1987) Growth hormone-releasing factor-44 specificity for components of somatotroph and lactotroph immediate release pool substructures. *Endocrinology*, **120**, 1719-1726.

STUBBS, W.A., DELITALA, G., JONES, A., JEFFCOATE, W.J., EDWARDS, C.R., RATTER, S.J., BESSER, G.M., BLOOM, S.R. & ALBERTI, K.G. (1978) Hormonal and metabolic responses to an enkephalin analogue in normal man. *Lancet*, **2**, 1225-1227.

REFERENCES

TAKAHARA, J., ARIMURA, A. & SCHALLY, A.V. (1975) Assessment of GH releasing hormone activity in sephadex-separated fractions of porcine hypothalamic extracts by hypophyseal portal vessel infusion in the rat. *Acta Endocrinologica*, **78**, 428-434.

TAKAHASHI, Y., KIPNIS, D.M. & DAUGHADAY, W.H. (1968) Growth hormone secretion during sleep. *Journal of Clinical Investigation*, **47**, 2079-2090.

TAKANO, K., HIZUKA, N., SHIZUME, K., ASAKAWA, K., FUKUDA, I. & DEMURA, H. (1991) Repeated sc administration of recombinant human Insulin-like Growth Factor-I (igf-i) to human subjects for seven days. *Growth Regulation*, **1**, 23-28.

TAMMINGA, C.A., NEOPHYTIDES, A., CHASE, T.N. & FROHMAN, L.A. (1978) Stimulation of prolactin and growth hormone secretion by muscimol, a gamma-aminobutyric acid agonist. *Journal of Clinical Endocrinology and Metabolism*, **47**, 1348-1351.

TAN, K. & BAXTER, R. (1986) Serum insulin-like growth factor I levels in adult diabetic patients: the effect of age. *Journal of Clinical Endocrinology and Metabolism*, **63**, 651-655.

TANNENBAUM, G.S., GUYDA, H.J. & POSNER, B.I. (1983) Insulin-like growth factors: a role in growth hormone negative feedback and body weight regulation via brain. *Science*, **220**, 77-79.

TANNENBAUM, G.S. & LING, N. (1984) The interrelationship of growth hormone (GH)-releasing factor and somatostatin in generation of the ultradian rhythm of GH secretion. *Endocrinology*, **115**, 1952-1957.

TATAR, P. & VIGAS, M. (1984) Role of alpha 1- and alpha 2-adrenergic receptors in the growth hormone and prolactin response to insulin-induced hypoglycemia in man. *Neuroendocrinology*, **39**, 275-280.

TERRY, L.C. & MARTIN, J.B. (1981) The effects of lateral hypothalamic-medial forebrain stimulation and somatostatin antiserum on pulsatile growth hormone secretion in freely behaving rats: evidence for a dual regulatory mechanism. *Endocrinology*, **109**, 622-627.

THOMPSON, R.G., RODRIGUES, A., KOWARSKI, A. & BLIZZARD, R.M. (1972a) Growth hormone: metabolic clearance rates, integrated concentrations and production rates in normal adults and the effect of prednisolone. *Journal of Clinical Investigation*, **51**, 3193-3199.

REFERENCES

THOMPSON, R.G., RODRIGUEZ, A., KOWARSKI, A., MIGEON, C.J. & BLIZZARD, R.M. (1972b) Integrated concentrations of growth hormone correlated with plasma testosterone and bone age in preadolescent and adolescent males. *Journal of Clinical Endocrinology and Metabolism*, **35**, 334-337.

THORNER, M.O., PERRYMAN, R.L., CRONIN, M.J., DRAZNIN, M.B., JOHANSON, A.J., ROGOL, A.D., JANE, J.A., RUDOLF, L.E., HORVATH, E., KOVACS, K. & VALE, W. (1982) Somatotroph hyperplasia: successful treatment of acromegaly by removal of a pancreatic islet tumor secreting a growth hormone-releasing factor. *Transactions of the Association of American Physicians*, **95**, 177-187.

THORNER, M.O., ROGOL, A.D., BLIZZARD, R.M., KLINGENSMITH, G.J., NAJJAR, J., MIRSA, R., BURR, I., CHAO, G., MARTHA, P., MCDONALD, J., PEZZOLI, S., CHITWOOD, J., FURLANETTO, R., RIVER, J., VALE, W.W., SMITH, P. & BROOK, C.G.D. (1988) Acceleration of growth rate in growth hormone-deficient children treated with human growth hormone-releasing hormone. *Pediatric Research*, **24**, 145-151.

THUESEN, L., JORGENSEN, J.O.L., MULLER, J.R., KRISTENSEN, B.O., SKAKKEBAEK, N.E., VAHL, N. & CHRISTIANSEN, J.S. (1994) Short and long-term cardiovascular effects of growth hormone therapy in growth hormone deficient adults. *Clinical Endocrinology*, **41**, 615-620.

TOLIS, G., HICKEY, J. & GUYDA, H. (1975) Effects of morphine on serum growth hormone, cortisol, prolactin and thyroid stimulating hormone in man. *Journal of Clinical Endocrinology and Metabolism*, **41**, 797-800.

TOU, J.S., KAEMPFE, L.A., VINEYARD, B.D., BUONOMO, F.C., DELLA-FERA, M.A. & BAILE, C.A. (1986) Amphiphilic growth hormone releasing factor (GRF) analogs: peptide design and biological activity in vivo. *Biochemical and Biophysical Research Communications*, **139**, 763-770.

UNGER, R., EISENTRAUT, A., MADISON, L. & SIPERSTEIN, M. (1965) Fasting levels of growth hormone in men and women. *Nature*, **205**, 804-805.

UNGER, R.H. & ORCI, L. (1977) Possible roles of the pancreatic D-cell in the normal and diabetic states. *Diabetes*, **26**, 241-244.

URBAN, M.D., LEE, P.A. & MIGEON, C.J. (1978) Adult height and fertility in men with congenital virilizing adrnal hyperplasia. *New England Journal of Medicine*, **299**, 1392-1396.

REFERENCES

VALCAVI, R., JORDAN, V., DIEGUEZ, C., JOHN, R., MANICARDI, E., PORTIOLI, I., RODRIGUEZ ARNAO, M.D., GOMEZ PAN, A., HALL, R. & SCANLON, M.F. (1986) Growth hormone responses to GRF 1-29 in patients with primary hypothyroidism before and during replacement therapy with thyroxine. *Clinical Endocrinology*, **24**, 693-698.

VALE, W., SPIESS, J., RIVIER, C. & RIVIER, J. (1981) Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science*, **213**, 1394-1397.

VANCE, M.L., KAISER, D.L., EVANS, W.S., THORNER, M.O., FURLANETTO, R., RIVIER, J., VALE, W., PERISUTTI, G. & FROHMAN, L.A. (1985) Evidence for a limited growth hormone (GH)-releasing hormone (GHRH)-releasable quantity of GH: effects of 6-hour infusions of GHRH on GH secretion in normal man. *Journal of Clinical Endocrinology and Metabolism*, **60**, 370-375.

VANCE, M.L., KAISER, D.L., FROHMAN, L.A., RIVIER, J., VALE, W.W. & THORNER, M.O. (1987) Role of dopamine in the regulation of growth hormone secretion: dopamine and bromocriptine augment growth hormone (GH)-releasing hormone-stimulated GH secretion in normal man. *Journal of Clinical Endocrinology and Metabolism*, **64**, 1136-1141.

VANDEWEGHE, M., TAELMAN, P. & KAUFMAN, J.M. (1993) Short-term and long-term effects of growth hormone treatment on bone turnover and bone mineral content in adult growth hormone-deficient males. *Clinical Endocrinology*, **39**, 409-415.

VASCONEZ, O., MARTINEZ, V., MARTINEZ, A., HIDALGO, F., DIAMOND, F.B., ROSENBLOOM, A.L., ROSENFELD, R.G., GUEVARA-AGUIRRE J. (1994) Heart rate increases in patients with growth hormone receptor deficiency treated with insulin-like growth factor I. *Acta Paediatrica Scandanavia (Supplement)*, **399**, 137-139.

VEBER, D.F., HOLLY, F.W., PALEVEDA, W.J., NUTT, R.F., BERGSTRAND, S.J., TORCHIANA, M., GLITZER, M.S., SAPERSTEIN, R. & HIRSCHMANN, R. (1978) Conformationally restricted bicyclic analogs of somatostatin. *Proceedings of the National Academy of Sciences of the United States of America*, **75**, 2636-2640.

VEBER, D.F., FREIDLINGER, R.M., PERLOW, D.S., PALEVEDA, W.J.J., HOLLY, F.W., STRACHAN, R.G., NUTT, R.F., ARISON, B.H., HOMNICK, C., RANDALL, W.C., GLITZER, M.S., SAPERSTEIN, R. & HIRSCHMANN, R. (1981) A potent cyclic hexapeptide analogue of somatostatin. *Nature*, **292**, 55-58.

REFERENCES

VELDHUIS, J.D., JOHNSON, M.L., FAUNT, L.M., MERCADO, M. & BAUMANN, G. (1993) Influence of the high-affinity growth hormone (GH)-binding protein on plasma profiles of free and bound GH and on the apparent half-life of GH. Modeling analysis and clinical applications [published errata appear in J Clin Invest 1993 May; 91(5):following 2334 and 1993 Aug;92(2):following 1108]. *Journal of Clinical Investigation*, **91**, 629-641.

VELICELEBI, G., PATTHI, S. & KAISER, E.T. (1986) Design and biological activity of analogs of growth hormone releasing factor with potential amphiphilic helical carboxyl termini. *Proceedings of the National Academy of Sciences of the United States of America*, **83**, 5397-5399.

VERMEULEN, A. (1987) Nyctohemoral growth hormone profiles in young and aged men: correlations with somatomedin-C levels. *Journal of Clinical Endocrinology and Metabolism*, **64**, 884-888.

VON WERDER, K., HANE, S. & FORSHAM, P.H. (1971) Suppression of the hypothalamic-pituitary-adrenal axis and growth hormone release with dexamethasone. *Hormone and Metabolic Research*, **3**, 171-173.

WAKABAYASHI, I., SHIBASAKI, T. & LING, N. (1986) A divergence of plasma growth hormone response between growth hormone-releasing factor and insulin-induced hypoglycaemia among middle-aged healthy male subjects. *Clinical Endocrinology*, **24**, 279-283.

WALKER, J.L., GINALSKA-MALINOWSKA, M., ROMER, T.E., PUCILOWSKA, J.B. & UNDERWOOD, L.E. (1991) Effects of the infusion of insulin-like growth factor I in a child with growth hormone insensitivity syndrome (Laron dwarfism). *New England Journal of Medicine*, **324**, 1483-1488.

WALKER, M.B. (1934) Treatment of myasthenia gravis with physostigmine. *Lancet*, **226**, 1200-1201.

WATHEN, N.C., PERRY, L., LILFORD, R.J. & CHARD, T. (1984) Interpretation of single progesterone measurement in diagnosis of anovulation and defective luteal phase: observations on analysis of the normal range. *British Medical Journal*, **288**, 7-9.

WEHRENBERG, W.B., BRAZEAU, P., LUBEN, R., BOHLEN, P. & GUILLEMIN, R. (1982a) Inhibition of the pulsatile secretion of growth hormone by monoclonal antibodies to the hypothalamic growth hormone releasing factor (GRF). *Endocrinology*, **111**, 2147-2148.

REFERENCES

WEHRENBERG, W.B., LING, N., BOHLEN, P., ESCH, F., BRAZEAU, P. & GUILLEMIN, R. (1982b) Physiological roles of somatocrinin and somatostatin in the regulation of growth hormone secretion. *Biochemical and Biophysical Research Communications*, **109**, 562-567.

WELTMAN, A., WELTMAN, J.Y., HARTMAN, M.L., ABBOTT, R.D., ROGOL, A.D., EVANS, W.S. & VELDHUIS, J.D. (1994) Relationship between age, percentage body fat, fitness, and 24- hour growth hormone release in healthy young adults: effects of gender. *Journal of Clinical Endocrinology and Metabolism*, **78**, 543-548.

WERNER, H., KOCH, Y., BALDINO JR., F. & GOZES, I. (1988) Steroid regulation of somatostatin mRNA in the rat hypothalamus. *Journal of Biological Chemistry*, **263**, 7666-7671.

WHITEHEAD, H.M., BOREHAM, C., MCLLRATH, E.M., SHERIDAN, B., KENNEDY, L., ATKINSON, A.B. & HADDEN, D.R. (1992) Growth hormone treatment of adults with growth hormone deficiency - results of a 13-month placebo controlled cross-over study. *Clinical Endocrinology*, **36**, 45-52.

WIEDEMANN, E., SCHWARTZ, E. & FRANTZ, A.G. (1976) Acute and chronic estrogen effects upon serum somatomedin activity, growth hormone, and prolactin in man. *Journal of Clinical Endocrinology and Metabolism*, **42**, 942-952.

WILLIAMS, T., MAXON, H., THORNER, M.O. & FROHMAN, L.A. (1985) Blunted growth hormone (GH) response to GH-releasing hormone in hypothyroidism resolves in the euthyroid state. *Journal of Clinical Endocrinology and Metabolism*, **61**, 454-456.

WISLOCKI, G.B. & KING, L.S. (1936) Permeability of the hypophysis and hypothalamus to vital dyes, with study of hypophyseal blood supply. *American Journal of Anatomy*, **58**, 421-472.

YAMASHITA, S. & MELMED, S. (1986) Insulin-like growth factor I action on rat anterior pituitary cells: suppression of growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology*, **118**, 176-182.

ZACHMAN, M., PRADER, A., SOBEL, E.H., CRIGLER, J.F., RITZEN, E.M., ATARES, M. & FERRANDEZ, A. (1986) Pubertal growth in patients with androgen insensitivity: indirect evidence for the importance of oestrogens in pubertal growth of girls. *Journal of Pediatrics*, **108**, 694-697.

ZACHWIEJA, J.J., BIER, D.M. & YARASHESKI, K.E. (1994) Growth hormone administration in older adults: effects on albumin synthesis. *American Journal of Physiology*, **266**, E840-E844.

REFERENCES

ZADIK, Z., CHALEW, S., MCCARTER, R.J., MEISTAS, M. & KOWARSKI, A. (1985) The influence of age on the 24-hour integrated concentration of growth hormone in normal individuals. *Journal of Clinical Endocrinology and Metabolism*, **60**, 513-516.

ZADIK, Z., CHALEW, S.A., GILULA, Z. & KOWARSKI, A.A. (1990a) Reproducibility of growth hormone testing procedures: a comparison between 24-hour integrated concentrations and pharmacological stimulation. *Journal of Clinical Endocrinology and Metabolism*, **71**, 1127-1130.

ZADIK, Z., CHALEW, S.A. & KOWARSKI, A. (1990b) Assessment of growth hormone secretion in normal stature children using 24-hour integrated concentration of GH and pharmacological stimulation. *Journal of Clinical Endocrinology and Metabolism*, **71**, 932-936.

ZAPF, J., KIEFER, M., MERRYWEATHER, J., MUSIARZ, F., BAUER, D., BORN, W., FISCHER, J.A. & FROESCH, E.R. (1990) Isolation from adult human serum of four insulin-like growth factor (IGF) binding proteins and molecular cloning of one of them that is increased by IGF I administration and in extrapancreatic tumor hypoglycemia. *Journal of Biological Chemistry*, **265**, 14892-14898.

ZENOBI, P.D. (1993) Insulin-like Growth Factor (IGF)-I alters the profile of thyroid hormones in vivo. *75th American Endocrine Society (Las Vegas)*, 1317(Abstract)

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The effect of recombinant IGF-I on anterior pituitary function in healthy volunteers

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Summary

OBJECTIVE Insulin-like growth factor-I is the mediator of many of the actions of GH and is a potent metabolic regulator. Recombinant IGF-I (rhIGF-I) is of potential value in the treatment of syndromes associated with either GH or insulin resistance. This study was designed to assess the effects of subcutaneous (s.c.) rhIGF-I on anterior pituitary function.

DESIGN Double-blind, placebo controlled, randomized cross-over study. The interval between investigations was 2 weeks.

SUBJECTS Twelve normal volunteers received on one occasion a single s.c. dose of 40 μg/kg rhIGF-I and on the other, placebo.

MEASUREMENTS Circulating levels were measured, over 24 hours, of GH, LH, FSH, PRL, TSH, cortisol, ACTH, glucose, IGF-I, IGF-II, insulin, C-peptide; IGF binding proteins by Western ligand blotting; total IGF bioactivity using FRTL-5 thyroid cells; and glucose by the glucose oxidase method.

RESULTS Recombinant IGF-I increased AUC for plasma IGF-I, measured by radioimmunoassay (rhIGF-I mean 7065 $\pm$ SEM 33 *vs* 3895 $\pm$ 204 μg/l, $P < 0{\cdot}0001$) and IGF bioactivity (22·5 $\pm$ 3·4 *vs* 14·2 $\pm$ 1·8 U/ml, $P < 0{\cdot}001$) but plasma IGF-II fell (9308 $\pm$ 403 *vs* 11052 $\pm$ 451 μg/l, $P < 0{\cdot}0001$). There was no biochemical or clinical evidence of hypoglycaemia and no difference in mean glucose levels. No difference existed in AUC for GH, LH, FSH, ACTH and cortisol between rhIGF-I and placebo; additionally, pulse number and amplitude for GH and LH were unaffected. TSH fell following rhIGF-I (33·0 $\pm$ 3·36 *vs* 42·5 $\pm$ 5·98 mU h/l, $P = 0{\cdot}01$). Both mean plasma C-peptide (0·73 $\pm$ 0·06 *vs* 0·91 $\pm$ 0·05 nmol/l, $P = 0{\cdot}03$), and insulin (10·81 $\pm$ 1·02 *vs* 15·36 $\pm$ 1·18 mU/l, $P = 0{\cdot}03$) were lower following rhIGF-I. There was no change in IGFBPs.

CONCLUSION A single injection of 40 μg/kg of subcutaneous rhIGF-I does not cause hypoglycaemia. IGF bioactivity was increased without inhibition of GH secretion. The only change observed in anterior pituitary function was a fall in plasma TSH.

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Insulin-like growth factor-I (IGF-I) has been shown to be the mediator of many of the actions of growth hormone while having a high degree of sequence homology with human proinsulin (Daughaday *et al.*, 1972; Rinderknecht & Humbel, 1978). Growth hormone is the principal determinant of circulating IGF-I with levels being high in acromegaly and low in growth hormone deficiency and Laron's syndrome (Hall & Sara, 1984). The metabolic actions of IGF-I can be subdivided into insulin-like effects and growth promotion (Froesch & Zapf, 1985). Although insulin and IGF-I have their own receptors, some cross-affinity exists which may account for the mechanism by which IGF-I, in high doses, can induce hypoglycaemia (Humbel, 1990). Circulating IGF-I in serum binds to specific binding proteins (IGFBPs), of which six have been identified, such that under normal circumstances less than 1% is unbound and physiologically active (Ballard *et al.*, 1989).

Recombinant gene technology has made human sequence IGF-I (rhIGF-I) available for clinical studies. Possible therapeutic uses include the treatment of conditions such as Laron's syndrome, stress induced catabolic states, diabetes mellitus, renal impairment and hyperlipidaemia.

Studies of the effect of IGF-I on pituitary function have been almost exclusively limited to its effects on GH secretion.

The highest concentrations in the rat brain of both type I and type II IGF receptors are to be found in the median eminence of the hypothalamus (Lesniak *et al.*, 1988), the site of secretion of both growth hormone-releasing hormone and somatostatin. The anterior pituitary has equally high concentrations of both receptors, suggesting a possible second site of regulation of pituitary function by IGF-I (Lesniak *et al.*, 1988).

It has been suggested that growth hormone therapy in children with short stature causes earlier onset of puberty and accelerated bone maturation with consequent loss of growth potential (Darendeliler *et al.*, 1990; Stanhope *et al.*, 1991; Russell *et al.*, 1989). The underlying mechanism is ill understood but may relate to the elevation of circulating

IGF-I levels acting at the hypothalamus or pituitary. It is therefore necessary to clarify the impact of rhIGF-I on anterior pituitary function before embarking on long-term therapeutic trials, particularly in peri-pubertal patients.

This study was designed to assess any acute effects on anterior pituitary function of rhIGF-I administered by the therapeutic route, namely subcutaneously, in a dose that does not cause hypoglycaemia.

Materials and methods

Twelve healthy non-obese male volunteers (mean age 23·4 years, range 21·3–27·6, mean body mass index 22·9 kg/m^2, range 19·6–25·4) were studied on two occasions in a randomized double blind cross-over design. The interval between studies was 2 weeks.

Subjects fasted from midnight prior to attending the investigation ward at 0800 h. They lay on a bed and an antecubital vein was cannulated. At 0900 h a s.c. injection of either rhIGF-I (40 μg/kg) or placebo (diluent only) was administered. Blood was obtained every 20 minutes for 24 hours for measurement of GH, LH, FSH and at hourly intervals for PRL, TSH, cortisol, ACTH and glucose assay. Plasma IGF-I, IGF-II, insulin, C-peptide and IGF binding proteins were measured at 6-hourly intervals throughout the day. Twenty-four hours after rhIGF-I administration, an i.v. bolus of 100 μg of GHRH was administered and blood obtained for GH measurement at 15-minute intervals for a further 2 hours. Bedside glucose monitoring (BM Stix, Reflolux S, Beohringer Mannheim, Brighton, UK) was also performed at hourly intervals throughout the study. All blood samples were collected into lithium heparin tubes, cold spun and rapidly frozen.

One hour after the s.c. injection a standard breakfast was provided and the subjects were thereafter allowed to move freely within the ward. Meals were served at standardized times and lights were extinguished at 2230 h and the subjects went to sleep.

The study was approved by the Research Ethics Committee of the City and Hackney Health Authority and Medical College of St Bartholomew's Hospital.

Assays

All assays were performed on plasma. Cortisol was measured by radioimmunoassay, intra-assay coefficient of variation (CV) 6% at both 100 and 1000 nmol/l. GH (CV 8·9% at 1·2 mU/l, 4·3% at 24 mU/l), TSH (CV 4·1% at 1·1 mU/l, 2·3% at 10 mU/l, LH (CV 3·5% at 2·2 mU/l, 2·3% at 9·6 mU/l), FSH (CV 7·0% at 2·6 mU/l, 4·4% at 9·6 mU/l), ACTH (CV 9·5% at 58 ng/l), and PRL (CV 5·3% at 151 mU/l, 2·9% at 460 mU/l) were measured by immunoradiometric assays (NETRIA, London, UK). Blood glucose was measured by the glucose oxidase method. Radioimmunoassays were used to meausure IGF-I, IGF-II (CV 4·7% at 510 μg/l), insulin (CV 3·9% at 38 mU/l) and C-peptide (CV 7·1% at 0·42 pmol/l). IGF bioactivity was measured by the eluted stain assay system using the stable, non-transformed thyroid cell line FRTL-5 (Claffey *et al.*, 1994). In common with other bioassays it does not discriminate between IGF-I and IGF-II bioactivity. IGF binding proteins were estimated by Western ligand blotting (Davies *et al.*, 1991), and quantified by densitometry (KLB Ultrascan XL, model 2400 Laser Densitometer).

Statistical analysis

Analysis was performed according to the standard cross-over model described by Jones and Kenward (1989).

The area under the curve (AUC) was calculated by the trapezoidal method for GH, LH, FSH, TSH, ACTH, PRL, IGF-I and IGF-II between time zero and 24 hours. AUC was also calculated for GH for the 2 hours following the bolus of GHRH. Samples below the level of assay sensitivities were assigned a value of half the limit of assay sensitivity.

The pulsatilities of GH, LH and FSH were analysed by the Pulsar program (Merriam & Wachter, 1982).

Comparisons of AUC and means were performed as appropriate by either Student's paired *t*-test and/or Wilcoxon's signed rank test, statistical significance being defined by a P value of $\leqslant 0{\cdot}05$.

Results

Analysis for a treatment–period interaction indicated there was neither an order nor carry-over effect between the two limbs.

There was a significant increase of 80% in AUC for plasma IGF-I measured by radioimmunoassay following rhIGF-I administration compared with placebo (Table 1). Mean plasma IGF-I immunoactivity peaked at 6 hours and thereafter gradually declined but was still above baseline at 24 hours, although within the normal range (Fig. 1). A significant reduction of approximately 15% in AUC for plasma IGF-II was seen following rhIGF-I. AUC for IGF bioactivity was significantly increased by 30% following rhIGF-I administration. As with IGF-I immunoreactivity the peak occurred at 6 hours and thereafter decreased (Fig. 1).

There was no biochemical or clinical evidence of hypoglycaemia. The lowest laboratory blood glucose

Table 1 The 24-hour AUC for IGF-I and II measured by radioimmunoassay and IGF bioactivity (mean ± SEM).

Drug	IGF-I(RIA) (μg/l)	IGF-II(RIA) (μg/l)	IGF(bio) (U/l)
rhIGF-I	7065 ± 33*	9308 ± 403	22·5 ± 3·4†
Placebo	3895 ± 204	11052 ± 451*	14·2 ± 1·8

*$P < 0{\cdot}0001$, †$P < 0{\cdot}001$.

recorded was 3·9 mmol/l, and there was no difference in mean blood glucose between the two limbs of the study (rhIGF-I mean 5·43 mmol/l SEM ± 0·06 *vs* placebo 5·55 ± 0·06) (Fig. 2).

No difference existed in AUC, pulse number or pulse amplitude for plasma GH, LH or FSH between the two limbs of the study (Table 2). The AUC for GH following intravenous GHRH (100 μg) was not significantly different but the peak GH response was greater following rhIGF-I (Fig. 3). Administration of rhIGF-I resulted in a significant fall in AUC for plasma TSH (Table 2) and at each individual time-point mean plasma TSH was lower following rhIGF-I than placebo (Fig. 4). The circadian rhythm of TSH remained intact. There was no change in free thyroxine or free triiodothyronine 24 hours after rhIGF-I administration. No change was seen in AUC for plasma ACTH, cortisol or prolactin (Table 2).

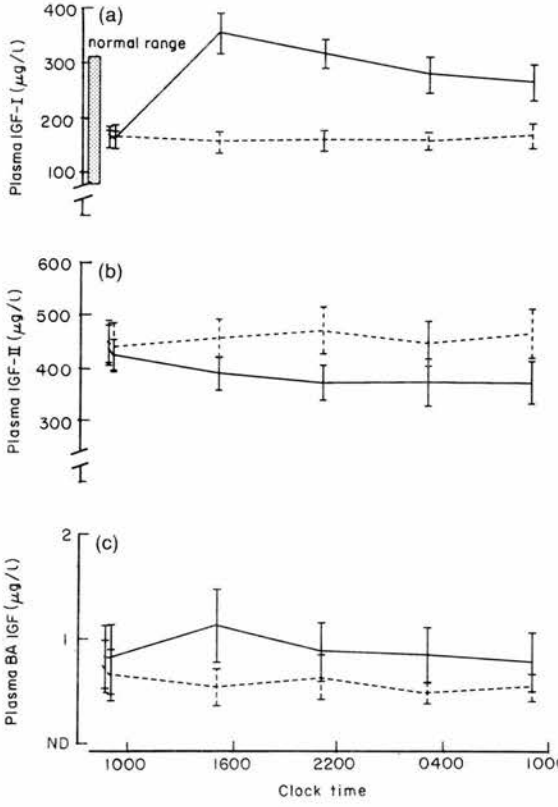

Fig. 1 Twenty-four-hour profiles mean for mean plasma a, IGF-I ($P < 0{\cdot}0001$) and b, IGF-II ($P < 0{\cdot}0001$) measured by radioimmunoassay and c, mean plasma IGF bioactivity ($P < 0{\cdot}001$) (mean ± SEM shown, —— rhIGF-I (40 μg/kg at 0900 h), – – – – placebo).

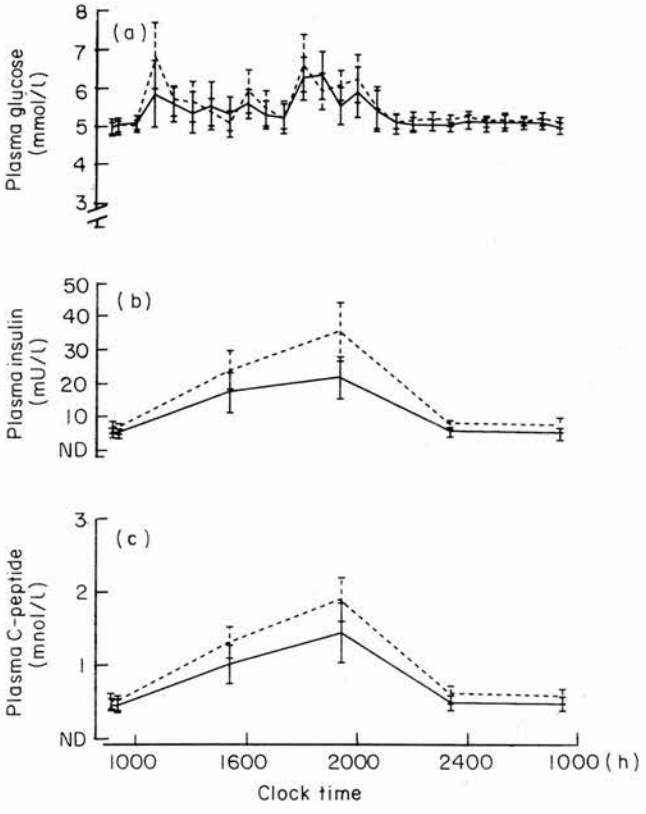

Fig. 2 Twenty-four-hour profiles mean for mean plasma a, glucose (P NS); b, insulin ($P = 0{\cdot}03$) and c, C-peptide ($P = 0{\cdot}03$) (mean ± SEM shown, —— rhIGF-I (40 μg/kg at 0900 h), – – – – placebo).

Both mean plasma C-peptide (0·91 ± 0·05 *vs* 0·73 ± 0·06 nmol/l, $P = 0{\cdot}03$, Wilcoxon signed rank test) and insulin (15·36 mU/*l* ± 1·18 *vs* 10·81 ± 1·02, $P = 0{\cdot}03$, Wilcoxon signed rank test) were lower following rhIGF-I (Fig. 2). As C-peptide and insulin were measured only at 6-hourly intervals, it is not possible to be precise about the time scale of the changes. However, plasma levels had returned to baseline 18 hours after rhIGF-I.

IGFBP-3 was the most abundant binding protein identified on the Western ligand blots and did not alter

Table 2 The 24-hour AUC for each of the anterior pituitary hormones (mean ± SEM)

	Area under curve		Pulse number		Pulse amplitude	
	placebo	IGF-I	placebo	IGF-I	placebo	IGF-I
GH (mU h/l)	86·6 ± 14·7	79·2 ± 14·6	4·1 ± 0·9	2·7 ± 0·4	22·3 ± 4·5	28·5 ± 6·7
LH (mU h/l)	123 ± 13·2	122 ± 10·5	10·8 ± 0·6	11·2 ± 0·8	2·8 ± 0·2	2·8 ± 0·2
FSH (mU h/l)	85·0 ± 26·5	83·9 ± 23·4				
TSH (mU h/l)	42·5 ± 5·98	33·0 ± 3·36*				
ACTH (ng h/l)	279·8 ± 18·0	272·9 ± 22·8				
Cortisol (nmol h/l)	4986 ± 290	4849 ± 314				
Prolactin (mU h/l)	7201 ± 2336	7152 ± 2250				

*$P = 0{\cdot}01$.

with administration of rhIGF-I. The evaluation of IGFBP-1 levels showed no discernible change with rhIGF-I (data not shown).

All subjects experienced transient discomfort at the injection site, due to the pH of the diluent. No side-effects were encountered with IGF-I; specifically, no symptoms of hypoglycaemia were reported. Short-lived facial flushing following GHRH was uniform.

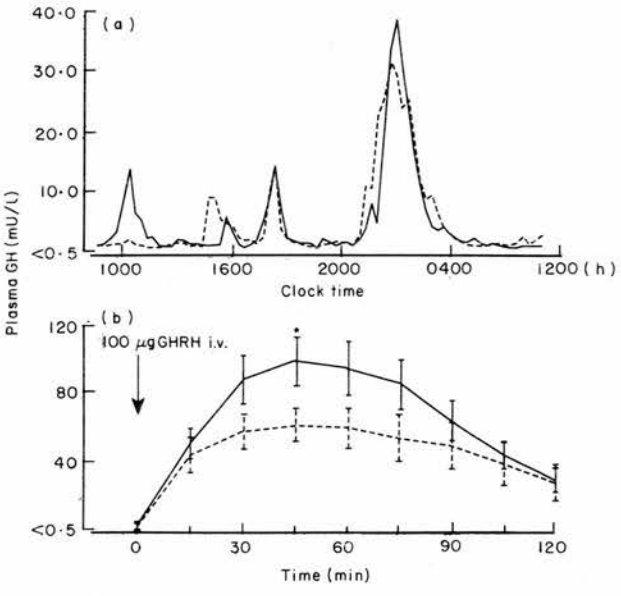

Fig. 3a, Twenty-four-hour profiles mean for mean plasma GH (P NS) (error bars not shown, —— rhIGF-I (40 μg/kg at 0900 h), – – – – placebo); b, the GH response to i.v. GHRH (100 μg) 24 hours after IGF-I or placebo (*$P = 0{\cdot}05$) (mean ± SEM shown, —— rhIGF-I, – – – – placebo).

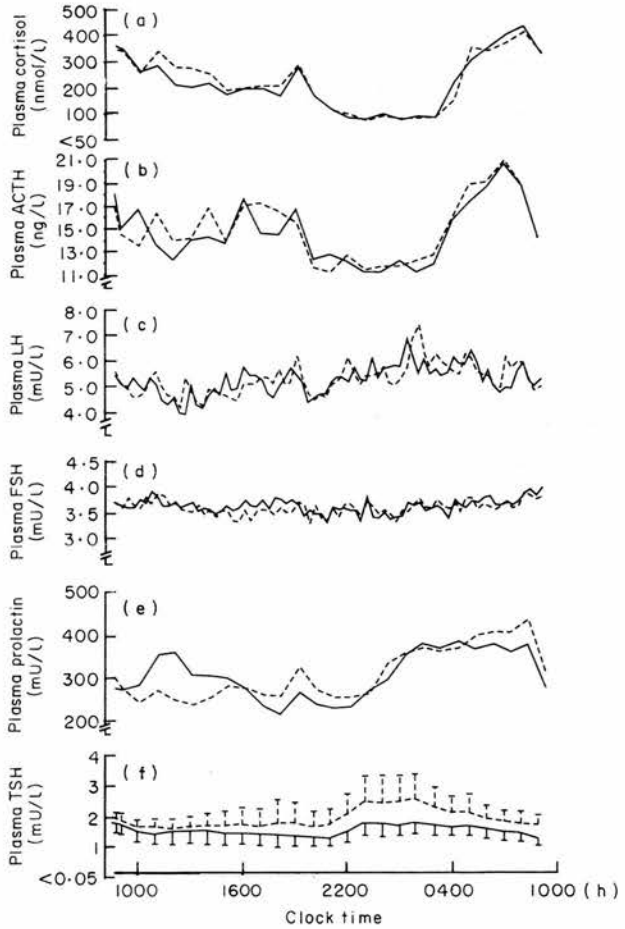

Fig. 4 Twenty-four-hour profiles mean for mean plasma a, cortisol; b, ACTH; c, LH; d, FSH; e, prolactin and f, TSH (P a–e, NS; $Pf = 0{\cdot}01$) (error bars shown only for TSH, —— rhIGF-I (40 μg/kg at 0900 h), – – – – placebo).

Discussion

This study was designed to investigate the effect of rhIGF-I on basal anterior pituitary function using a dose (40 μg/kg) which was unlikely to cause hypoglycaemia. This dose was sufficient to raise the circulating levels of bioactive IGF and resulted in changes in plasma insulin, C-peptide, TSH.

The fall seen in plasma IGF-II following rhIGF-I administration has been reported previously (Walker *et al.*, 1991; Guler *et al.*, 1989). IGF-I and II have similar affinities for IGFBP-3 and the fall in IGF-II is probably a result of the displacement of IGF-II by IGF-I from the IGFBPs, principally IGFBP-3 (Martin & Baxter, 1986), with the dislodged IGF-II being rapidly degraded.

Although it seems likely that GH can autoregulate its secretion independently of IGF-I (Ross *et al.*, 1987), at the same time IGF-I can negatively modulate GH secretion. *In vitro*, IGF-I has been shown to be involved in the feedback regulation of GH secretion with reported effects on both the hypothalamus and pituitary. Berelowitz *et al.* (1981) demonstrated that isolated rat hypothalami incubated with highly purified somatomedin-C released somatostatin in a dose-dependent manner. Studies on the effect of IGF-I on GHRH secretion have produced contradictory results (Aquila, 1991; Shibasaki *et al.*, 1986). Both GH mRNA and secretion are reduced by incubation with highly purified somatomedin-C (Berelowitz *et al.*, 1981; Yamashita & Melmed, 1986). Further, when given intracerebroventricularly (i.c.v.), highly purified somatomedin-C has been shown to inhibit GH secretion in conscious rats (Tannenbaum *et al.*, 1983; Abe *et al.*, 1983). However, it is now believed that the preparations of highly purified somatomedin-C used in many of these early experiments contained both IGF-I and II. In contrast, i.c.v. recombinant IGF-I, in doses up to 10 μg, failed to alter GH secretion in rats. Likewise, i.c.v. recombinant IGF-II did not inhibit GH secretion. However, 1 μg IGF-I administered i.c.v. with 1 μg IGF-II resulted in a significant inhibition of GH secretion (Harel & Tannenbaum, 1992a,b). The synergy of centrally administered IGF-I and II in inhibiting GH secretion in rats underlines the importance of the hypothalamus in the regulation of GH secretion, possibly via modulating somatostatin secretion.

In a study of two euglycaemic men, a s.c. infusion of rhIGF-I at 20 μg/kg/h inhibited nocturnal and GHRH induced GH secretion in one (Guler *et al.*, 1989). Likewise, plasma GH levels were inhibited in a single subject with Laron's syndrome by an infusion of up to 24 μg/kg/h of rhIGF-I (Walker *et al.*, 1991). Cotterill *et al.* (1993a) demonstrated inhibition of GH secretion in the hours immediately after 120 μg/kg rhIGF-I in two children with Laron's syndrome. The inhibition was short-lived as the low IGFBP-3 levels associated with Laron's syndrome resulted in rapid clearance of rhIGF-I from the plasma. An i.v. infusion of 10 μg/kg/h to fasted normal volunteers, during euglycaemic clamps, resulted in suppression of GH secretion rates within 60 minutes when total plasma IGF-I levels were increased 1·6-fold (Hartman *et al.*, 1993). However, the interpretation is complicated by the increase in GH secretion and IGFBP-1 associated with fasting (Cotterill *et al.*, 1993b). The lack of effect of IGF-I on GH secretion in this study in normal volunteers, is in accord with the results of Miell *et al.* (1992). Using the same dose of rhIGF-I, they failed to demonstrate a change in GH secretion in patients catabolic after surgery.

It seems likely that rhIGF-I in larger doses than we have used may inhibit GH secretion but the route of administration may also be important. Our data indicate that it is possible to increase circulating IGF bioactivity without inhibiting GH secretion. The potentiation by rhIGF-I of the GH response following GHRH has not previously been reported and needs to be further explored. It is unlikely to be a result of the falling IGF levels even though this was from an elevated level towards normal.

While GH, PRL, LH and FSH levels or patterns of pulsatility did not change, there was a significant reduction in plasma TSH of the order of 30%. This has previously been reported in adults with Laron's syndrome as well as in healthy volunteers (Laron *et al.*, 1990; Lieberman *et al.*, 1992).

Reduced TSH secretion might have been a consequence of IGF-I acting on the hypothalamus to stimulate secretion of somatostatin, an important regulator of TSH secretion (Hall *et al.*, 1973), but this seems unlikely because no associated change in GH was seen. A more plausible possibility is a peripheral action of IGF-I to increase conversion of T4 to T3, with T3 being the more active at the hypothalamus and pituitary to inhibit TSH secretion. In support of the latter hypothesis, Salomon and colleagues (1989) observed that in GH-deficient adults treated with GH, several subjects on fixed doses of thyroxine replacement therapy developed symptoms of thyrotoxicosis associated with a rise in plasma T3. In a group of normal subjects given 0·125 mg GH daily for 4 days, a fall in plasma TSH and T4 and a rise in T3 were seen (Grunfeld *et al.*, 1988). Similar changes in thyroid function plus an increase in metabolic rate have been reported following one week of rhIGF-I therapy in normal volunteers (Zenobi, 1993); however, no change was seen in our study in plasma free T4 or free T3. This may indicate that any change in the values of plasma thyroid hormones, with the limitation of free thyroid assays, may be too small to detect by

24 hours. Tachycardia on commencing rhIGF-I therapy has been reported (Vasconez *et al.*, 1994). It seems probable that IGF-I causes increased peripheral conversion of T4 to T3 and possible mild transient thyrotoxicosis. The effects of IGF-I on thyroid function need to be studied further. For example, changes in thyroid function may account for alterations in lipid profiles with IGF-I therapy and thyroid dysfunction itself alters IGFBPs (Miell *et al.*, 1993).

Plasma insulin and C-peptide levels fell in parallel following rhIGF-I and in the absence of any change in blood glucose. Suppression of insulin secretion in the absence of hypoglycaemia has been reported previously (Guler *et al.*, 1989; Walker *et al.*, 1991; Lieberman *et al.*, 1992) and Boulware *et al.* (1992) demonstrated insulin suppression during a 5 mmol/l euglycaemic clamp in subjects receiving an i.v. infusion of 20 μg/kg/h rhIGF-I. It is difficult to differentiate between the relative influences of increased glucose clearance caused by IGF-I (Moxley *et al.*, 1990; Jacob *et al.*, 1989) and a secondary fall in plasma insulin resulting from a direct effect of IGF-I on pancreatic islet cells to inhibit insulin secretion, as has been shown by Leahy and Vanderkerkhove (1990) *in vitro*. Fifty μg/kg rhIGF-I 8-hourly in lambs resulted in a small but significant rise in blood sugar, presumably secondary to the documented suppression of insulin secretion (Cottam *et al.*, 1992), and similarly Walker *et al.* (1991) noted post-prandial hyperglycaemia in a single patient with Laron's syndrome treated by a continuous i.v. infusion of IGF-I. Hyperglycaemia in combination with low plasma insulin levels suggests IGF-I inhibits insulin secretion *in vivo*. In our study, there was no evidence of hyperglycaemia.

Plasma IGFBP-1 levels are inversely related to circulating insulin levels and therefore an increase would be expected secondary to the inhibition of insulin secretion by rhIGF-I seen in this study (Holly *et al.*, 1988). That no change was observed in IGFBP-1 may reflect that insulin levels fell by only 30% and a corresponding increase in IGFBP-1 is unlikely to be detected on Western ligand blotting.

In conclusion, our data confirm 40 μg/kg of s.c. rhIGF-I does not cause hypoglycaemia but does increase IGF-I bioactivity. The data support the belief that IGF-I acts directly to inhibit insulin secretion. No change other than a fall in plasma TSH was seen in anterior pituitary function. The influence of rhIGF-I on thyroid function requires further study. The GH response to GHRH was enhanced 24 hours after the IGF-I administration. There was no evidence of GH suppression at any time during the study.

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References

Abe, H., Molitch, M.E., Van Wyk, J.J. & Underwood, L.E. (1983) Human growth hormone and somatomedin C suppress the spontaneous release of growth hormone in unanesthetized rats. *Endocrinology*, **113,** 1319–1324.

Aquila, M.C. (1991) Insulin-like growth factor I regulates somatostatin (SRIF) release and messanger ribonucleic acid levels in rat hypothalamus. *74th American Endocrine Society (San Antonio)*, 1283. (Abstract)

Ballard, J., Baxter, R., Binoux, M., Clemmons, D., Drop, S., Hall, K., Hintz, R., Rechler, M., Rutanen, E. & Schwander, J. (1989) On the nomenclature of the IGF binding proteins. *Acta Endocrinologica Copenhagen*, **121,** 751–752.

Berelowitz, M., Szabo, M., Frohman, L.A., Firestone, S., Chu, L. & Hintz, R.L. (1981) Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. *Science*, **212,** 1279–1281.

Boulware, S.D., Tamborlane, W.V., Matthews, L.S. & Sherwin, R.S. (1992) Diverse effects of insulin-like growth factor I on glucose, lipid, and amino acid metabolism. *American Journal of Physiology*, **262,** 130–133.

Claffey, D., Yateman, M., Lane, P., Ealey, P., Wass, J., Marshall, N. & Holly, J. (1994) The development and characterization of an eluted stain assay (ESTA) for insulin-like growth factors. *Growth Regulation*, **3,** 215–225.

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

Cotterill, A.M., Camachohubner, C., Holly, J.M.P. & Savage, M.O. (1993a) Rapid communication—the effect of recombinant human insulin-like growth factor-I treatment on growth hormone secretion in two subjects with growth hormone insensitivity (Laron syndrome). *Clinical Endocrinology*, **39,** 119–122.

Cotterill, A.M., Holly, J.M.P., Amiel, S. & Wass, J.A.H. (1993b) Suppression of endogenous insulin secretion regulates the rapid rise of insulin-like growth binding protein (IGFBP)-1 levels following acute hypoglycaemia. *Clinical Endocrinology*, **38,** 633–639.

Darendeliler, F., Hindmarsh, P.C., Preece, M.A., Cox, L. & Brook, C.G. (1990) Growth hormone increases rate of pubertal maturation. *Acta Endocrinologica*, **122,** 414–416.

Daughaday, W.H., Hall, K., Raben, M.S., Salmon, W.D.J., Van den Brande, J.L. & Van Wyke, J.J. (1972) Somatomedin: Proposed designation for sulphation factor. *Nature*, **235,** 107.

Davies, S.C., Wass, J.A., Ross, R.J., Cotterill, A.M., Buchanan, C.R., Coulson, V.J. & Holly, J.M. (1991) The induction of a specific protease for insulin-like growth factor binding protein-3 in the circulation during severe illness. *Journal of Endocrinology*, **130,** 469–473.

Froesch, E.R. & Zapf, J. (1985) Insulin-like growth factors and insulin: comparative aspects. *Diabetologia*, **28,** 485–493.

Grunfeld, C., Sherman, B.M. & Cavalieri, R.R. (1988) The acute effects of human growth hormone administration on thyroid function in normal men. *Journal of Clinical Endocrinology & Metabolism*, **67,** 1111–1114.

Guler, H.P., Schmid, C., Zapf, J. & Froesch, E.R. (1989) Effects of recombinant insulin-like growth factor I on insulin secretion and renal function in normal human subjects. *Proceedings of the National Academy of Sciences, USA*, **86,** 2868–2872.

Hall, K. & Sara, V.R. (1984) Somatomedin levels in childhood, adolescence and adult life. *Journal of Clinical Endocrinology and Metabolism*, **13,** 91–112.

Hall, R., Schally, A.V., Evered, D., Kastin, A.J., Mortimer, C.H., Tunbridge, W.M.G., Weightman, D., Phenekos, C., McNeilly, A.S., Goldie, D.J., Coy, D.H. & Besser, G.M. (1973) Action of growth-hormone-release inhibitory hormone in healthy men and in acromegaly. *Lancet*, **11,** 581–584.

Harel, Z. & Tannenbaum, G.S. (1992a) Synergistic interaction between insulin-like growth factors-I and -II in central regulation of pulsatile growth hormone secretion. *Endocrinology*, **131,** 758–764.

Harel, Z. & Tannenbaum, G.S. (1992b) Centrally administered insulin-like growth II fails to alter pulsatile growth hormone secretion or food intake. *Neuroendocrinology*, **56,** 161–168.

Hartman, M.L., Clayton, P.E., Johnson, M.L., Celniker, A., Perlman, A.J., Alberti, K.G.M.M. & Thorner, M.O. (1993) A low dose euglycemic infusion of recombinant human insulin-like growth factor-I rapidly suppresses fasting-enhanced pulsatile growth hormone secretion in humans. *Journal of Clinical Investigation*, **91**, 2453–2462.

Holly, J.M., Biddlecombe, R.A., Dunger, D.B., Edge, J.A., Amiel, S.A., Howell, R., Chard, T., Rees, L.H. & Wass, J.A. (1988) Circadian variation of GH-independent IGF-binding protein in diabetes mellitus and its relationship to insulin. A new role for insulin? *Clinical Endocrinology*, **29,** 667–675.

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

Jacob, R., Barrett, E., Plewe, G., Fagin, K.D. & Sherwin, R.S. (1989) Acute effects of insulin-like growth factor I on glucose and amino acid metabolism in the awake fasted rat. Comparison with insulin. *Journal of Clinical Investigation*, **83,** 1717–1723.

Jones, B. & Kenward, M.G. (1989) Design and Analysis of Cross-over Trials. *Chapman & Hall.*

Laron, Z., Klinger, B., Silbergeld, A., Lewin, R., Erster, B. & Gil-ad, I. (1990) Intravenous administration of recombinant IGF-I lowers serum GHRH and TSH. *Acta Endocrinologica*, **123,** 378–382.

Leahy, J.L. & Vandekerkhove, K.M. (1990) Insulin-like growth factor-I at physiological concentrations is a potent inhibitor of insulin secretion. *Endocrinology*, **126,** 1593–1598.

Lesniak, M.A., Hill, J.M., Kiess, W., Rojeski, M., Pert, C.B. & Roth, J. (1988) Receptors for insulin-like growth factors I and II: autoradiographic localization in rat brain and comparison to receptors for insulin. *Endocrinology*, **123,** 2089–2099.

Lieberman, S.A., Bukar, J., Chen, S.A., Celniker, A.C., Compton, P.G., Cook, J., Albu, J., Perlman, A.J. & Hoffman, A.R. (1992) Effects of Recombinant Human Insulin-like growth factor-I (rhIGF-I) on total and free IGF-I concentrations, IGF-binding proteins, and glycaemic response in humans. *Journal of Clinical Endocrinology and Metabolism*, **75,** 30–36.

Martin, J.L. & Baxter, R.C. (1986) Insulin-like growth factor-binding protein from human plasma. Purification and characterization. *Journal of Biological Chemistry*, **261,** 8754–8760.

Merriam, G.R. & Wachter, K.W. (1982) Algorithms for the study of episodic hormone secretion. *American Journal of Physiology*, **243,** E310–E318.

Miell, J.P., Taylor, A.M., Jones, J., Buchanan, C.R., Rennie, J., Sherwood, R., Leicester, R. & Ross, R.J.M. (1992) Administration of human recombinant insulin-like growth factor-I to patients following major surgery. *Clinical Endocrinology*, **37,** 542–551.

Miell, J.P., Taylor, A.M., Zini, M., Maheshwari, H.G., Ross, R.J.M. & Valcavi, R. (1993) Effects of hypothyroidism and hyperthyroidism on insulin-like growth factors (IGFs) and growth hormone-binding and IGF-binding proteins. *Journal of Clinical Endocrinology & Metabolism*, **76,** 950–955.

Moxley, R.T., Arner, P., Moss, A., Skottner, A., Fox, M., James, D. & Livingston, J.N. (1990) Acute effects of insulin-like growth factor I and insulin on glucose metabolism *in vivo*. *American Journal of Physiology*, **259,** E561–E567.

Rinderknecht, E. & Humbel, R.E. (1978) The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *Journal of Biological Chemistry*, **253,** 2769–2776.

Ross, R.J., Tsagarakis, S., Grossman, A., Nhagafoong, L., Touzel, R.J., Rees, L.H. & Besser, G.M. (1987) GH feedback occurs through modulation of hypothalamic somatostatin under cholinergic control: studies with pyridostigmine and GHRH. *Clinical Endocrinology*, **27,** 727–733.

Russell, R.E., Parks, J.S., McKean, M.C., Bell, G.I., Keret, R., Kelijman, M. & Laron, Z. (1989) Laron-type dwarfism is associated with normal growth hormone and insulin-like growth factor-I gene restriction patterns. *Israel Journal of Medical Sciences*, **25,** 342–344.

Salomon, F., Cuneo, R.C., Hesp, R. & Sonksen, P.H. (1989) The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *New England Journal of Medicine*, **321,** 1797–1803.

Shibasaki, T., Yamachi, N., Hotta, M., Masuda, A., Imaki, T., Demura, H., Ling, N. & Shizume, K. (1986) *In vitro* release of growth hormone-releasing factor from rat hypothalamus: effects of insulin-like growth factor-I. *Regulatory Peptides*, **15,** 47–53.

Stanhope, R., Uruena, M., Hindmarsh, P., Leiper, A.D. & Brook, C.G.D. (1991) Management of growth hormone deficiency through puberty. *Acta Paediatrica Scandinavica*, **372,** 47–52.

Tannenbaum, G.S., Guyda, H.J. & Posner, B.I. (1983) Insulin-like growth factors: a role in growth hormone negative feedback and body weight regulation via brain. *Science*, **220,** 77–79.

Vasconez, O., Martinez, V., Martinez, A., Hidago, F., Diamond, F.V., Rosenbloom, A.L., Rosenfeld, R.G. & Guevara-Aguiere, J. (1994) Heart rate increases in patients with growth hormone receptor deficiency treated with insulin-like growth factor I. *Acta Paediatrica Scandinavica* **(Suppl. 399)**, 137–139.

Walker, J.L., Ginalska-Malinowska, M., Romer, T.E., Pucilowska, J.B. & Underwood, L.E. (1991) Effects of the infusion of insulin-like growth factor I in a child with growth hormone insensitivity syndrome (Laron dwarfism). *New England Journal of Medicine*, **324,** 1483–1488.

Yamashita, S. & Melmed, S. (1986) Insulin-like growth factor I action on rat anterior pituitary cells: suppression of growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology*, **118,** 176–182.

Zenobi, P.D. (1993) Insulin-like growth factor (IGF)-I alters the profile of thyroid hormones *in vivo*. *75th American Endocrine Society (Las Vegas)*, **Abstract 1317**.

Pyridostigmine partially reverses dexamethasone-induced inhibition of the growth hormone response to growth hormone-releasing hormone

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ABSTRACT

The GH response to insulin-induced hypoglycaemia and growth hormone-releasing hormone (GHRH) has been shown to be impaired in subjects with Cushing's syndrome and in healthy volunteers given oral glucocorticoids. Pyridostigmine is an anticholinesterase that stimulates GH secretion, probably by inhibition of hypothalamic somatostatin secretion. This work was designed to study the site of action of glucocorticoids in inhibiting the secretion of GH.

Eight healthy male volunteers were studied on three occasions in random order. They took 2 mg oral dexamethasone or placebo at precisely 6-hourly intervals for 48 h before receiving 120 mg oral pyridostigmine or placebo, followed 60 min later by GHRH (100 μg) i.v. Samples for measuring GH were obtained at 15 min intervals for 2 h.

The 'area under the curve' (AUC) for each of the treatments was significantly different: dexamethasone–pyridostigmine–GHRH (mean ± S.E.M., 1938 ± 631 mU/min per l), dexamethasone–placebo–GHRH (634 ± 211) and placebo–placebo–GHRH (4267 ± 1183) ($P < 0{\cdot}02$, Wilcoxon test).

In conclusion, dexamethasone given for 48 h significantly inhibited the AUC for GH following treatment with GHRH. However, pretreatment with pyridostigmine significantly reversed the inhibition although this was still partial. Our data suggested that this short-term suppressive effect of dexamethasone was independent of GHRH, and most probably relates to stimulation of the release of somatostatin.
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INTRODUCTION

The inhibitory effects of supraphysiological levels of glucocorticoids on growth have long been known, both when they have been used for the treatment of inflammation as well as in spontaneous Cushing's syndrome (Friedman & Strang, 1966; McArthur, Cloutier, Hayles & Sprague, 1972; Preece, 1976). Basal levels of growth hormone (GH) and the response to insulin-induced hypoglycaemia and GH-releasing hormone (GHRH) are impaired in Cushing's syndrome (Demura, Demura, Nunokawa *et al.* 1972; Smals, Pieters, Benraad & Kloppenborg, 1986; Burguera, Muruais, Penalva *et al.* 1990) and in healthy volunteers given oral glucocorticoids to simulate Cushing's syndrome (Hartog, Gaafar & Fraser, 1964; Nakagawa, Horiuchi & Mashimo, 1969; von Werder, Hane & Forsham, 1971; Burguera *et al.* 1990). However, the site of action of corticosteroids in this situation remains uncertain.

Pyridostigmine, an anticholinesterase, stimulates GH secretion both *in vivo* and *in vitro* (Locatelli, Torsello, Redaelli *et al.* 1986; Penalva, Muruais, Casanueva & Dieguez, 1990*b*). The mode of action of pyridostigmine is believed to be the inhibition of somatostatin secretion secondary to the increased hypothalamic cholinergic tone. Previous studies have indicated that the failure of GHRH to stimulate GH secretion in the presence of exogenous GH, glucose, free fatty acids and obesity can be reversed, at least partially, with pyridostigmine (Ross, Tsagarakis, Grossman *et al.* 1987; Penalva, Burguera, Casabiell *et al.* 1989; Penalva, Gaztambide, Vazquez *et al.* 1990*a*; Castro, Vieira, Chacra *et al.* 1990), implying that the reduction in GH under these conditions,

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without pyridostigmine, is due to increased somatostatinergic tone.

The study was designed to investigate the mechanism of the inhibitory effect of short-term sustained increased corticosteroid levels on stimulated GH secretion.

MATERIALS AND METHODS

Eight healthy non-obese male volunteers were studied on three occasions in random order. They received 2 mg oral dexamethasone (Organon Laboratories Ltd, Cambridge, U.K.) or placebo at precisely 6-hourly intervals (09.00, 15.00, 21.00, 03.00, 09.00, 15.00, 21.00, 03.00 h) for 48 h before reporting to our investigation ward at 08.00 h, having eaten and drunk nothing since midnight. A forearm cannula was inserted and the subjects remained supine for the duration of the study. Thirty (−90 min) and 60 min after cannulation, and again immediately before taking pyridostigmine or placebo (−60 min), basal blood samples were obtained and the subjects then took orally either 120 mg pyridostigmine (Roche, Welwyn Garden City, Herts, U.K.) or placebo. A further 60 min later 100 μg GHRH(1–29)NH_2 (Serono Laboratories Ltd, Welwyn Garden City, Herts, U.K.) was administered as a single bolus, and thereafter blood was sampled for estimation of serum GH every 15 min for 2 h. The three parts of the study were placebo–placebo–GHRH (experiment 1), dexamethasone–pyridostigmine–GHRH (experiment 2) and dexamethasone–placebo–GHRH (experiment 3). All studies were carried out at a minimum of weekly intervals in a randomized double-blind manner.

The study was approved by the Research Ethics Committee of St Bartholomew's Medical College and the City and Hackney Health Authority. The subjects all gave informed consent in writing.

Assay

GH was measured by an immunoradiometric assay (NETRIA, St Bartholomew's Hospital, London, U.K.). The intra-assay coefficient of variation at 1·2 mU/l was 8·9% and at 24 mU/l 4·3%, and the interassay coefficient of variation was 10·3% at 2·7 mU/l and 6·1% at 30 mU/l. The lower limit of detection of the assay was 0·5 mU/l (Knott, Hourd & Edwards, 1985). All samples were assayed in duplicate, the samples for each subject being included in the same assay.

Statistics

The 'area under the curve' (AUC) for GH was calculated by the trapezoid method, and the values were compared by the non-parametric Wilcoxon test (Siegel, 1956). Significance was taken at 5% confidence limits. All data are given as means ± S.E.M. In the analysis, GH samples with a value of <0·5 mU/l were assigned the value zero.

RESULTS

Figure 1 shows the changes in AUC for GH following GHRH for each of the experiments. Values are means ± S.E.M. Pretreatment with 2 mg dexamethasone at 6-hourly intervals for 48 h (experiment 3) produced a clear and significant attenuation in the AUC for GH response to GHRH compared with placebo treatment (634 ± 211 vs 4267 ± 1183 mU/min per l, $P<0·02$). The AUC for the GH response to GHRH after dexamethasone was significantly greater when preceded by 120 mg pyridostigmine rather than placebo (1938 ± 631 vs 634 ± 211 mU/min per l, $P<0·02$). However, the response to GHRH was still significantly less after dexamethasone and pyridostigmine than in the placebo control study (1938 ± 631 mU/min per l to 4267 ± 1183 mU/min per l, $P<0·02$).

Figure 2 shows the effect of 48 h of dexamethasone or placebo and pyridostigmine or placebo on the GH response to GHRH for each of the experiments. Means ± S.E.M. are shown.

All subjects experienced transient facial flushing with GHRH, two subjects complained of nausea with pyridostigmine but no side effects were encountered with dexamethasone.

DISCUSSION

The inhibitory effects of sustained elevated plasma glucocorticoid levels on linear growth and GH secretion are well known. However, confusion exists as to the acute effects of glucocorticoids on GH secretion. Casanueva, Burguera, Muruais & Dieguez (1990) have shown that dexamethasone given at 4 mg intravenously or 8 mg orally stimulates secretion of GH. The same workers have, in addition, shown that 4 mg dexamethasone i.v. potentiates the GH response to GHRH administered 3 h later (Burguera *et al.* 1990). In contrast, 25 mg oral cortisone acetate administered 60 min before GHRH has been reported to ablate the GH response (Giustina, Doga, Bodini *et al.* 1990*a*). Dexamethasone (8 mg) given orally 12 h before

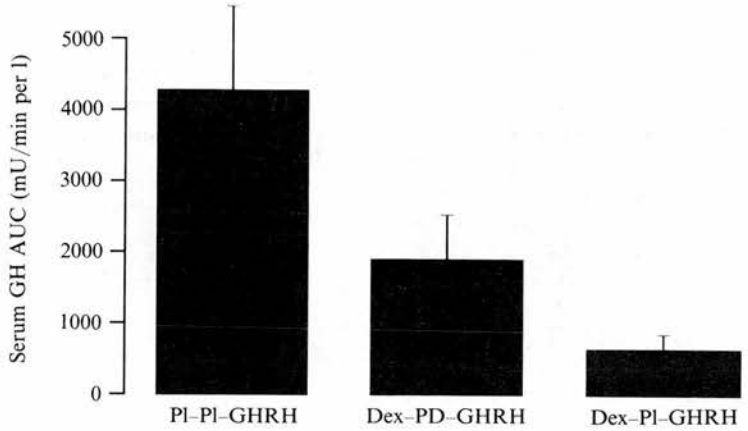

FIGURE 1. The 'area under the curve' (AUC) for GH following GH-releasing hormone (GHRH) administration in eight healthy subjects given dexamethasone or placebo for 48 h and 120 mg pyridostigmine or placebo 60 min before GHRH. There were three experiments: placebo–placebo–GHRH (Pl–Pl–GHRH), dexamethasone–pyridostigmine–GHRH (Dex–PD–GHRH) and dexamethasone–placebo–GHRH (Dex–Pl–GHRH). Values are means ± S.E.M.

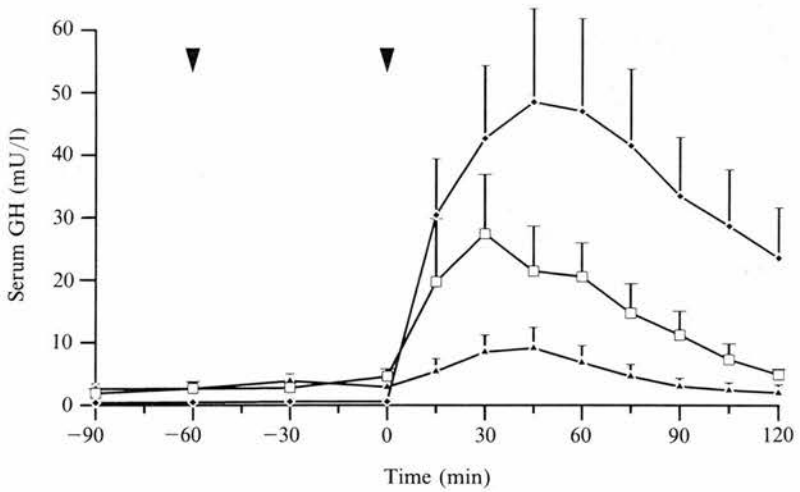

FIGURE 2. The GH response to GH-releasing hormone (GHRH) (100 μg) in eight subjects given dexamethasone or placebo for 48 h and 120 mg pyridostigmine or placebo 60 min (first arrow) before the GHRH (second arrow). Means ± S.E.M. are shown. There were three experiments: placebo–placebo–GHRH (◆), dexamethasone–pyridostigmine–GHRH (□) and dexamethasone–placebo–GHRH (▲).

GHRH or 22 mg given over 48 h have both been shown to inhibit the GH response to GHRH, although 1 mg 9 h earlier had no effect (Burguera *et al.* 1990; Del Balzo, Salvatori, Cappa & Gertner, 1990; Rupprecht, Niehaus & Lesch, 1990).

The results of this study demonstrate a loss of the GH response to GHRH in healthy volunteers after 48 h of elevated plasma glucocorticoid levels, a result in accordance with the findings of Burguera *et al.* (1990). The failure of a supraphysiological dose of GHRH(1–29)NH_2 to restore GH secretion makes it unlikely that the glucocorticoid-induced inhibition of GH secretion is due to suppression of hypothalamic GHRH.

Pyridostigmine increases hypothalamic cholinergic tone and hence reduces somatostatin secretion (Locatelli *et al.* 1986). In this study the response to GHRH is significantly, although only partially, restored by 120 mg oral pyridostigmine administered 60 min before the GHRH, a result in accordance with others (Del Balzo *et al.* 1990; Giustina, Girelli, Alberti *et al.* 1991). Giustina, Girelli, Doga *et al.* (1990*b*) demonstrated that 50 mg oral cortisone acetate administered 60 min before GHRH could inhibit the subsequent GH response, and that the addition of pyridostigmine fully restored the GH response. It therefore seems probable that dexamethasone acts to inhibit GH secretion by increasing hypothalamic somatostatin secretion.

The failure of pyridostigmine to reverse the dexamethasone-induced inhibition of GH secretion fully is in accord with the finding that 120 mg pyridostigmine also failed to restore the GH response to GHRH in subjects pretreated with glucose and GH (Penalva *et al.* 1989; Ross *et al.* 1987). Castro *et al.* (1990) showed a greater peak GH response after administration of 180 mg pyridostigmine than after administration of 120 mg in obese subjects, although this did not reach statistical significance. Evidence exists that 150 and 180 mg pyridostigmine stimulate greater secretion of GH than 120 mg (G. Delitala, personal communication). However, more detailed investigation of this, and adoption of a larger dose in clinical studies, has been limited by the side effects. The inability of pyridostigmine to reverse the dexamethasone-induced inhibition of GH release completely may be dose-related, 120 mg being insufficient to inhibit hypothalamic somatostatin secretion entirely.

The partial reversibility of the GH response to GHRH seen in this study after 48 h of dexamethasone administration contrasts with the full reversibility reported by Giustina *et al.* (1990*b*) 60 min after cortisone acetate administration. It seems possible that after 2 days of elevated plasma glucocorticoid levels, the exposure to increased somatostatin may have substantially depleted the readily releasable pool of GH. In patients with Cushing's syndrome, pyridostigmine was unable, even partially, to restore the GH response to GHRH (Leal-Cerro, Pereira, Garcia-Luna *et al.* 1990), indicating that somatostatin secretion is less important in inducing inhibition of GH secretion in subjects with chronically elevated circulating glucocorticoids. However, a direct effect of dexamethasone on somatostatin cannot be excluded.

In conclusion, this study has demonstrated that 2 mg dexamethasone administered every 6 h for 48 h inhibits the GH response to GHRH. The effect of GHRH can, in part, be restored by 120 mg pyridostigmine, indicating that the mechanism of the inhibition may be increased hypothalamic somatostatin secretion. The failure of pyridostigmine to restore the GH response fully may be due to a submaximal dose of pyridostigmine being used, or alternatively to the fact that other mechanisms are also involved, such as reduced pituitary stores of readily releasable GH.

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REFERENCES

Burguera, B., Muruais, C., Penalva, A., Dieguez, C. & Casanueva, F. F. (1990). Dual and selective action of glucocorticoids upon basal and stimulated growth hormone release in man. *Neuroendocrinology* **51,** 51–58.

Casanueva, F. F., Burguera, B., Muruais, C. & Dieguez, C. (1990). Acute administration of corticoids: A new and peculiar stimulus of growth hormone secretion in man. *Journal of Clinical Endocrinology and Metabolism* **70,** 234–237.

Castro, R. C., Vieira, J. G. H., Chacra, A. R., Besser, G. M., Grossman, A. B. & Lengyel, A. M. J. (1990). Pyridostigmine enhances, but does not normalise, the GH response to GH-releasing hormone in obese subjects. *Acta Endocrinologica* **122,** 385–390.

Del Balzo, P., Salvatori, R., Cappa, M. & Gertner, J. M. (1990). Pyridostigmine does not reverse dexamethasone-induced growth hormone inhibition. *Clinical Endocrinology* **33,** 605–612.

Demura, R., Demura, H., Nunokawa, T., Baba, H. & Miura, K. (1972). Responses of plasma ACTH, GH, LH and 11-hydroxycorticosteroids to various stimuli in patients with Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* **34,** 852–859.

Friedman, M. & Strang, L. B. (1966). Effect of long-term corticosteroids and corticotrophin on growth in children. *Lancet* **ii,** 569–572.

Giustina, A., Doga, M., Bodini, C., Girelli, A., Legati, F., Bossoni, S. & Romanelli, G. (1990*a*). Acute effects of cortisone on growth hormone response to growth hormone-releasing hormone in normal adult subjects. *Acta Endocrinologica* **122,** 206–210.

Giustina, A., Girelli, A., Alberti, D., Bossoni, S., Buzi, F., Doga, M., Schettino, M. & Wehrenberg, W. B. (1991). Effects of pyridostigmine on spontaneous and growth hormone-releasing hormone stimulated growth hormone secretion in children on daily glucocorticoid therapy after liver transplantation. *Clinical Endocrinology* **35,** 491–498.

Giustina, A., Girelli, A., Doga, M., Bodini, C., Bossoni, S., Romanelli, G. & Wehrenberg, W. B. (1990*b*). Pyridostigmine blocks the inhibitory effect of glucocorticoids on growth hormone-releasing hormone stimulated growth hormone secretion in normal man. *Journal of Clinical Endocrinology and Metabolism* **70,** 580–584.

Hartog, M., Gaafar, M. A. & Fraser, R. (1964). Effects of corticosteroids on serum growth hormone. *Lancet* **ii,** 376–378.

Knott, J. A., Hourd, P. & Edwards, R. (1985). The development of an immunoradiometric assay for the measurement of human growth hormone. *Journal of Endocrinology* **104** (Suppl.), Abstract No. 209.

Leal-Cerro, A., Pereira, J. L., Garcia-Luna, P. P., Astorga, R., Cordido, F., Casanueva, F. F. & Dieguez, C. (1990). Effect of enhancement of endogenous cholinergic tone with pyridostigmine on growth hormone (GH) response to GH-releasing hormone in patients with Cushing's syndrome. *Clinical Endocrinology* **33,** 291–295.

Locatelli, V., Torsello, A., Redaelli, M., Ghigo, E., Massara, F. & Müller, E. E. (1986). Cholinergic agonist and antagonist drugs modulate the growth hormone response to growth hormone-releasing hormone in the rat: evidence for mediation by somatostatin. *Journal of Endocrinology* **111,** 271–278.

McArthur, R. G., Cloutier, M. D., Hayles, A. B. & Sprague, R. G. (1972). Cushing's disease in children. *Mayo Clinic Proceedings* **47,** 318–326.

Nakagawa, K., Horiuchi, Y. & Mashimo, K. (1969). Responses of plasma growth hormone and corticosteroids to insulin and arginine with or without prior administration of dexamethasone. *Journal of Clinical Endocrinology and Metabolism* **29,** 35–40.

Penalva, A., Burguera, B., Casabiell, X., Tresguerres, J. A. F., Dieguez, F. F. & Casanueva, F. F. (1989). Activation of cholinergic neurotransmission by pyridostigmine reverses the inhibitory effect of hyperglycaemia on growth hormone (GH) releasing hormone-induced GH secretion in man: does acute hyperglycaemia act through release of somatostatin? *Neuroendocrinology* **49,** 551–554.

Penalva, A., Gaztambide, S., Vazquez, J. A., Dieguez, C. & Casanueva, F. F. (1990*a*). Role of cholinergic muscarinic pathways on the free fatty acid inhibition of GH response to GHRH in normal men. *Clinical Endocrinology* **33,** 171–176.

Penalva, A., Muruais, C., Casanueva, F. F. & Dieguez, C. (1990*b*). Effect of enhancement of endogenous cholinergic tone with pyridostigmine on the dose–response relationships of growth hormone (GH)-releasing hormone-induced GH secretion in normal subjects. *Journal of Clinical Endocrinology and Metabolism* **70,** 324–327.

Preece, M. A. (1976). The effect of administered corticosteroids on the growth of children. *Postgraduate Medical Journal* **52,** 625–630.

Ross, R. J. M., Tsagarakis, S., Grossman, A., Nhagafoong, L., Touzel, R. J., Rees, L. H. & Besser, G. M. (1987). GH feedback occurs through modulation of hypothalamic somatostatin under cholinergic control: studies with pyridostigmine and GHRH. *Clinical Endocrinology* **27,** 727–733.

Rupprecht, R., Niehaus, A. & Lesch, K.-P. (1990). The influence of dexamethasone on growth hormone (GH). Response to GH-releasing hormone in normal man. *Journal of Neural Transmission* **79,** 51–57.

Siegel, S. (1956). *Non-parametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill Book Company.

Smals, A. E. M., Pieters, G. F. F. M., Benraad, T. J. & Kloppenborg, P. W. C. (1986). Human pancreatic growth hormone releasing hormone fails to stimulate growth hormone both in Cushing's disease and in Cushing's syndrome adrenocortical adenoma. *Clinical Endocrinology* **24,** 401–407.

von Werder, K., Hane, S. & Forsham, P. H. (1971). Suppression of the hypothalamic-pituitary-adrenal axis and growth hormone release with dexamethasone. *Hormone and Metabolic Research* **3,** 171–173.