THE GROWTH HORMONE / INSULIN-LIKE GROWTH FACTOR I AXIS IN INSULIN-DEPENDENT DIABETES MELLITUS DURING ADOLESCENCE

Studies of Recombinant Human Insulin-Like Growth Factor I (rhIGF-I) administration

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University of Leicester
ABSTRACT

The Growth Hormone / Insulin-Like Growth Factor I axis in Insulin-Dependent Diabetes Mellitus during Adolescence.
Studies of Recombinant Human Insulin-Like Growth Factor I administration.

Tim D. Cheetham

The fall in insulin sensitivity during adolescence is accentuated in insulin-dependent diabetes mellitus (IDDM) and has been linked to enhanced growth hormone (GH) secretion. The rise in GH release is related to low insulin-like growth factor I (IGF-I) levels and low IGF bioactivity. Abnormalities of the IGF binding proteins (IGFBP's), including low insulin-like growth factor binding protein-3 (IGFBP-3) and elevated insulin-like growth factor binding protein-1 (IGFBP-1) concentrations are also observed. The rise in GH concentrations may lead to increased insulin requirements that cannot easily be met by current treatment regimens and can result in deteriorating blood glucose control. GH release also enhances ketogenesis and has been linked to the development of microvascular complications.

The impact of a subcutaneous injection of rhIGF-I (40 μg/kg) on GH concentrations, insulin sensitivity and the IGFBP's was studied in adolescents with IDDM (n=17). A control night was compared with a night when rhIGF-I was administered at 18.00h. Blood samples were taken regularly overnight and glucose concentrations controlled by a variable-rate insulin infusion.

GH concentrations on the control night correlated with glycated haemoglobin levels. The administration of rhIGF-I led to a sustained increase in IGF-I levels, IGF bioactivity and reductions in GH secretion and the insulin infusion requirements to maintain euglycaemia. The change in GH secretion was due to reduced pulse amplitude rather than pulse frequency. The attributes assessing GH release correlated with free insulin concentrations on control and rhIGF-I nights, and the reduction in GH release was related to the fall in insulin levels. The concentrations of IGFBP-3 did not fall after rhIGF-I as they did during the control study, but IGFBP-1 levels were unchanged. In longer term studies (n=6), daily rhIGF-I administration (40 μg/kg) for one month led to a reduced isophane insulin dose and a fall in glycated haemoglobin concentrations. GH levels were reduced and IGFBP-3 concentrations rose in 5 of the 6 subjects studied.

The administration of rhIGF-I may have a therapeutic role in IDDM during adolescence by reducing GH concentrations and increasing insulin sensitivity.
DECLARATION

The original idea behind the first of the investigations described in this thesis was conceived by David Dunger. I began recruiting and then studying subjects in 1991, and after processing and analysing the data from this initial investigation I contributed to the planning and development of subsequent protocols. I performed all of the overnight profiles myself, with assistance from a research nurse when more than one individual was studied. I performed the statistical analysis with help and guidance from David Dunger and David Matthews.

The writing of the thesis was all my own work. Although the text has been reviewed by David Dunger I take responsibility for any errors contained within it.

T D Cheetham, August 1995
**SUMMARY OF ABBREVIATIONS**

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<td>ALS</td>
<td>acid-labile subunit</td>
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<tr>
<td>BG</td>
<td>blood glucose</td>
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<tr>
<td>BMI</td>
<td>body mass index (kg/m²)</td>
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<tr>
<td>CV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>GFR</td>
<td>glomerular filtration rate</td>
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<tr>
<td>GH</td>
<td>growth hormone</td>
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<tr>
<td>GHBP</td>
<td>growth hormone binding protein</td>
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<td>GHI</td>
<td>growth hormone insensitivity</td>
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<tr>
<td>GHRH</td>
<td>growth hormone-releasing hormone</td>
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<tr>
<td>HbA1</td>
<td>total glycosylated haemoglobin</td>
</tr>
<tr>
<td>HbA1c</td>
<td>the major fraction of HbA1 affected by alterations in blood glucose concentrations</td>
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<tr>
<td>IDDM</td>
<td>insulin-dependent diabetes mellitus</td>
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<td>IGF-I</td>
<td>insulin-like growth factor I</td>
</tr>
<tr>
<td>IGF-II</td>
<td>insulin-like growth factor II</td>
</tr>
<tr>
<td>rhIGF-I</td>
<td>recombinant human insulin-like growth factor I</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>the insulin-dependent small molecular weight IGF binding protein</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>the GH-dependent and principle IGF binding protein in human serum</td>
</tr>
<tr>
<td>IRMA</td>
<td>immunoradiometric assay</td>
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<tr>
<td>kDa</td>
<td>kilo-daltons</td>
</tr>
<tr>
<td>PA</td>
<td>peak amplitude</td>
</tr>
<tr>
<td>RPM</td>
<td>revolutions per minute</td>
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<tr>
<td>RIA</td>
<td>radioimmunoassay</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>SDS</td>
<td>standard deviation score</td>
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<tr>
<td>SEM</td>
<td>standard error of the mean</td>
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<tr>
<td>SMS</td>
<td>somatostatin</td>
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<tr>
<td>SPA</td>
<td>sum of peak amplitudes</td>
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ACKNOWLEDGEMENTS

I would like to thank all the people in Oxford who provided help, encouragement and support during the described studies. Special mention must go to Lynne Ahmed, Jan Gilbert, Dot Harris, Alastair Taylor and Angie Watts who have taught me a great deal about biochemical and auxological techniques. Much of what I have learnt about childhood and adolescent diabetes is down to the dedication and professionalism of Sally Strang. These studies would not have been possible without her help. David Matthews and Jeff Holly have provided considerable technical and analytical support. Both have contributed greatly to my knowledge within the field of diabetes and endocrinology.

The part played by my supervisor, David Dunger, cannot be underestimated. In recent years he has contributed much to my understanding of the problems of the young person with IDDM and the endocrinology of childhood and adolescence.

Finally, I would like to thank my parents for their help and support whilst preparing this volume.
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INTRODUCTION

The primary abnormality in Insulin Dependent Diabetes Mellitus (IDDM) in children and adolescents is insulin deficiency arising from the autoimmune destruction of pancreatic Beta cells. The impact of low portal insulin concentrations on blood glucose concentrations leads to hyperglycaemia and a characteristic clinical picture. However, insulinopaenia in IDDM has also been linked to abnormalities of the growth hormone/insulin-like growth factor I axis (GH/IGF-I axis). Reduced hepatic IGF-I generation leads to altered feedback and an increase in GH release by the pituitary gland. High circulating GH levels in IDDM have been linked to the development of insulin resistance, enhanced ketogenesis and the development of microvascular complications.

These observations have led to the hypothesis which is explored in this thesis; that IGF-I replacement might lead to improved glycaemic control, particularly during puberty when insulin resistance is most profound. IGF-I has been manufactured using recombinant techniques and the physiological and clinical studies described give an account of the effects of recombinant IGF-I (rhIGF-I) administration on the GH/IGF-I axis in IDDM and provide an exploration of its therapeutic potential.

The first chapter begins with a review of IGF physiology and is then followed by a discussion of the GH/IGF-I axis in normal subjects and individuals with IDDM. The chapter concludes with a general review of studies of rhIGF-I administration and considers what has been learnt about actions of the peptide in a number of different clinical settings.
Chapter 1.

Section 1: THE INSULIN-LIKE GROWTH FACTORS AND THEIR BINDING PROTEINS

1.1 The Insulin-like Growth Factors (IGF's)

1.1.1 The discovery of the IGF's

Insulin-like Growth Factors I and II are naturally-occurring peptides which were originally referred to as the somatomedins. The discovery of this group of peptides stemmed from experimental observations in three principal areas. In 1957 Salmon and Daughaday described how the growth promoting effects of GH were mediated by what they termed 'sulfation factor' (Salmon & Daughaday, 1957). Their studies demonstrated that sulphate uptake into cartilage could be stimulated by the serum from normal but not hypophysectomized rats. GH pre-treatment restored this activity in hypophysectomized animals whereas the addition of GH alone to the culture medium did not.

Around the same time a discrepancy between the amount of insulin-like activity detectable in serum before and after the addition of anti-insulin antibodies led to the description of 'non-suppressible insulin-like activity' (NSILA) by Froesch and co-workers (Froesch et al., 1963). In retrospect it is possible to see that these first two observations were related to the concurrent finding of specific factors in serum that promoted the replication of cell lines, referred to as 'multiplication stimulating activity' (MSA) (Pierson & Temin, 1972; Dulak & Temin, 1973). When it was realised that a single group of compounds was responsible for these three observations the term 'somatomedins' was adopted to illustrate links with somatotropin, and to indicate a role as an intermediary of somatotropic actions (Daughaday et al., 1972). The amino-acid sequences of the two principal species within this group of peptides, IGF's I and II, were described by Rinderknecht and Humbel in 1978 (Rinderknecht & Humbel, 1978a, b). IGF-II has since been found to be
homologous with MSA (Marquardt et al., 1981) and its GH dependence established as being much less than that of IGF-I. The term insulin-like growth factor is a more appropriate name for this group of peptides and is now widely used.

1.1.2 Structure of IGF-I and IGF-II

IGF-I is a single chain polypeptide of seventy amino-acid residues and a predicted molecular weight of 7649 (Rinderknecht & Humbel, 1978a). The peptide has approximately 43% sequence homology with pro-insulin and comprises ‘A’ and ‘B’ regions that are separated by a ‘C’ region, similar to pro-insulin. There are also three intrachain disulphide bridges which are identical to those of pro-insulin (Raschdorf et al., 1988). However IGF-I also has a ‘D’ domain contiguous with the A domain. Interestingly, the structure of the IGF’s are identical in some mammalian species (bovine, porcine) with only very few differences in the amino-acid sequence in others (rat, mouse) (Bell et al., 1986; Honegger & Humbel, 1986; Murphy et al., 1987; Shimatsu & Rotwein, 1987; Tavakkol et al., 1988).

IGF-II consists of sixty-seven amino-acids and has a predicted molecular weight of 7469. It shares 62% sequence homology with IGF-I and also comprises A, B, C and D regions (Rinderknecht & Humbel, 1978b). A single gene locus for IGF-I has been mapped to the long arm of chromosome 12 (Bell et al., 1985) and for IGF-II to the short arm of chromosome 11 in the vicinity of the insulin gene (Brissenden et al., 1984).

1.1.3 Circulating IGF-I levels

In contrast to insulin, where tissue stores allow for pulsatile release, IGF-I is produced at a relatively constant rate by the liver (Schwander et al., 1983). IGF-I concentrations are relatively stable in a given set of nutritional and endocrine circumstances. The production rate of circulating
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IGF-I in man has been calculated to be around 10 mg/day by one group of investigators (Guler et al., 1989b) and 3 mg/day or 40 mg/kg by others (Wilton et al., 1991).

IGF-I can be detected in the fetus beyond nine weeks gestation (D'Ercole et al., 1986). Serum levels remain relatively constant during childhood until adolescence when concentrations rise to reach a peak in late puberty (Taylor et al., 1988; Massa et al., 1992; Juul et al., 1994). Thereafter IGF-I levels decline steadily throughout adult life (Juul et al., 1994), although somewhat more rapidly in females than males according to some studies (Yamamoto et al., 1991). In normal adults IGF-I concentrations are approximately 200 ng/ml whereas towards the end of puberty concentrations are at least double this value (Taylor et al., 1988).

1.1.4 IGF-I production

IGF-I was thought initially to be produced by the hepatocyte, although it has since become clear that the peptide is also manufactured locally by many extra-hepatic tissues (Atkinson et al., 1980; D'Ercole et al., 1984; Ernst & Froesch, 1988). IGF-I mRNA can be identified in many fetal tissues (Han et al., 1988) although the expression of IGF-I genes also depends on the stage of development; lower levels are found in fetal compared to adult liver and higher amounts in fetal compared to adult brain (Sandberg et al., 1988).

GH has a central role in the control of hepatic IGF-I production in rats (Schwander et al., 1983) and GH administration to GH deficient mice results in an increase in hepatic IGF-I mRNA levels (Mathews et al., 1986). GH secretion also correlates with IGF-I concentrations in children and adolescents (Bham et al., 1993). Patients with GH deficiency have low IGF-I concentrations (usually less than 50 ng/ml) whereas in acromegaly they may exceed 1000 ng/ml (Zapf et al., 1980; Holly et al., 1991) and are closely related to prevailing GH levels (Dobrashian et al., 1993).
Insulin also has an important role in regulating hepatic IGF production and circulating IGF-I levels. GH and insulin act synergistically to stimulate IGF-I production in chicken hepatocytes (Daughaday et al., 1976; Houston & O'Neill, 1990) and insulin appears to regulate hepatic GH receptor levels in the rat (Baxter et al., 1980). IGF-I concentrations are closely linked to nutritional status; fasting and a reduced protein intake lead to a fall in IGF-I gene expression in rats (Moat-Staats et al., 1989; Straus & Takemoto, 1990) and reductions in circulating IGF-I in humans (Clemmons et al., 1981). IGF-I concentrations are low in patients with anorexia nervosa and rise with an increase in food intake (Counts et al., 1992). However the effect of insulin does not appear to be due to direct effects on IGF-I gene expression (Salamon et al., 1989).

Although hypophysectomy reduces the levels of IGF-I and IGF-I mRNA in a variety of extra-hepatic tissues (D'Ercole et al., 1984; Murphy et al., 1987), IGF-I production in these sites is not as closely linked to GH status. Other pituitary hormones (including thyroxine), extra-pituitary hormones (including glucagon), and locally produced growth factors also regulate the extra-hepatic production of IGF-I (Clemmons & Shaw, 1983; Binoux et al., 1985; Mathews et al., 1986; Kachra et al., 1991; Scheven & Hamilton, 1991). The pancreas and gonad have been identified as possible sites of GH-independent IGF-I synthesis (Mathews et al., 1986; Hernandez et al., 1992).

1.1.5 IGF receptors

The IGF type 1 receptor has a similar structure to the insulin receptor; there are two extracellular alpha-subunits of 135 kDa, and two beta-subunits of 90 kDa, linked together by disulphide bonds (Chermausek et al., 1981; Kasuga et al., 1981; Massague & Czech, 1982). The extracellular alpha-subunits are responsible for IGF-I binding and the beta subunits contain a transmembrane domain, an ATP binding site and a tyrosine kinase domain. Receptor occupancy by IGF-I increases in a linear manner until saturation is reached (Tollefsen & Thompson, 1988).
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The IGF type 2 receptor is structurally unrelated to the type 1 and insulin receptors, comprising a single polypeptide chain which is largely extracellular (Kasuga et al., 1981; Massague et al., 1981; Massague & Czech, 1982). The receptor is identical to the mannose-6-phosphate (M-6-P) receptor which mediates the transport of M-6-P containing enzymes to the lysosomes.

1.1.6 IGF-I, IGF-II, and insulin binding

The IGF type 1 (IGF-I) receptor has a high affinity for both the IGF-I and IGF-II molecules (Rechler & Nissley, 1985; Steede-Perkins et al., 1988). Insulin cross-reacts with the IGF-I receptor to a relatively minor degree. The IGF type 2 (IGF-II) receptor binds IGF-II with high affinity, IGF-I to a lesser extent and does not interact with insulin (Kasuga et al., 1981; Massague & Czech, 1982; Rechler & Nissley, 1985). The insulin receptor has greatest affinity for the insulin molecule with progressively weaker interactions with IGF-II and then IGF-I.

1.2 The Insulin-Like Growth Factor Binding Proteins (IGFBP's)

1.2.1 Introduction

IGF’s I and II circulate bound to six binding proteins (IGFBPs 1-6) (Ballard et al., 1989; Ballard et al., 1992) of which two, IGFBP-1 and IGFBP-3, will be discussed in detail in this thesis. The half-life of unbound IGF-I is around ten to twelve minutes (Guler et al., 1987), but by forming complexes with the IGFBPs the half-life is prolonged to between thirteen and twenty hours (Blethen et al., 1982; Hodgkinson et al., 1987; Guler et al., 1987).

1.2.2 IGFBP-3

IGFBP-3 has a non-reduced molecular weight of 47 - 53 kDa (Martin & Baxter, 1986; Baxter & Martin, 1989). It is the most abundant IGFBP in adolescent serum and has the highest
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affinity for IGF-I and IGF-II. The single binding site has a similar affinity for both peptides. Most circulating IGF-I occurs as a ternary complex, bound to IGFBP-3 and a protein of large molecular weight, the alpha, or acid-labile subunit (ALS) (Baxter & Martin, 1989; Baxter et al., 1989). This protein is produced by the hepatocyte, is normally present in excess and has a molecular weight of 100 to 110 kDa (Baxter, 1988; Lewitt et al., 1994; Chin et al., 1994). ALS appears to be GH rather than IGF-I dependent (Zapf et al., 1989).

The liver is one of the principle sites of IGFBP-3 production although it appears to be the portal venous and sinusoidal epithelium which manufactures the peptide rather than the hepatocyte (Chin et al., 1994). A wide variety of extra-hepatic tissues produce IGFBP-3 as well (Bachrach et al., 1989; Smith, EP et al., 1990).

IGFBP-3 concentrations are low at birth and rise to a peak at puberty, reflecting the close relationship between the binding protein and IGF-I levels. IGFBP-3 levels fall when diet is restricted (Young et al., 1992), are low in patients with hypopituitarism and increased in acromegaly (Baxter & Martin, 1986). However the factors controlling IGFBP-3 production are not yet entirely understood. GH was originally believed to be the principle regulating factor although there is evidence from in-vitro studies (Conover, 1991; Bale & Conover, 1992) and in-vivo work (Zapf et al., 1989; Jorgensen et al., 1991) to suggest that this GH dependence is mediated via IGF-I. Further studies of IGF-I administration in rats have variously reported the induction of specific GH dependent IGFBPs (Clemmons et al., 1989) or have not detected any change (Domené et al., 1993). It is possible that GH stimulation of the ALS is required to allow complex formation and maintain IGFBP-3 levels, and that IGF-I administration may protect serum IGFBP-3 from proteolysis.
1.2.3 IGFBP-1

IGFBP-1 has a non-reduced molecular weight of approximately 28 kDa and is widely distributed throughout tissues (Hill et al., 1989b). IGFBP-1 is produced principally by the liver but also locally by the endometrium and ovarian granulosa cells (Rutanen et al., 1988; Dor et al., 1992). In contrast to IGFBP-3, IGFBP-1 binding sites are largely unsaturated. Insulin has been shown to inhibit IGFBP-1 gene transcription from human hepatoma cell lines (Powell et al., 1991) and fetal liver explants (Lewitt & Baxter, 1989a). Because intracellular IGFBP-1 levels are low (Conover & Lee, 1990) insulin does not reduce circulating levels by altering release of the stored protein (Powell et al., 1991).

Circulating IGFBP-1 concentrations are inversely related to those of insulin in prepubertal and pubertal subjects (Suikkari et al., 1988; Holly et al., 1989; Batch et al., 1991), typically undergoing a diurnal variation with peak levels overnight and a nadir during the daytime. The critical insulin concentration above which IGFBP-1 concentrations start to fall appears to be between 9 and 25 mU/l (Lee et al., 1993). At this point IGFBP-1 clearance is presumed to rise beyond the rate of IGFBP-1 production. Whether insulin leads to the efflux of IGFBP-1 from the circulation is not known. A change in circulating IGFBP-1 levels can be seen ninety minutes after the alteration in insulin concentrations following an oral glucose load (Suikkari et al., 1989), and within thirty minutes of an insulin infusion (Lee et al., 1993). Steady state insulin concentrations result in stable IGFBP-1 levels after 120 - 180 minutes (Lee et al., 1993).

The concentrations of IGFBP-1 fall as puberty advances, reflecting the increase in insulin levels (Holly et al., 1989). They then remain relatively constant during early to mid adult life until sixty years of age when they start to rise again (Batch et al., 1991; Holly et al., 1990; Rutanen et al., 1993). IGFBP-1 concentrations are low in patients with insulinomas (Suikkari et al., 1988).
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It has been suggested that the relationship between insulin and IGFBP-1 concentrations weakens beyond puberty and whilst some studies have identified a relationship in adults (Conover et al., 1992), others have not (Rutanen et al., 1993). It has been estimated that in fasting young adults around 50% of the variation in IGFBP-1 concentrations can be accounted for by insulin alone (Suikkari et al., 1989).

Whilst glucagon increases IGFBP-1 production, independently of changes in insulin (Hilding et al., 1993; Denver & Nicoll, 1994), the effects of glucose are less clearly defined. There is in-vitro evidence to suggest an inverse relationship between glucose and IGFBP-1 (Lewitt & Baxter, 1989b). However Lee and colleagues were not able to demonstrate any change in IGFBP-1 production in human subjects when plasma glucose was altered in the presence of non-suppressive insulin levels, and Suikkari and colleagues were unable to demonstrate an effect of glucose concentration, glucose tolerance and rate of intracellular glucose metabolism on plasma IGFBP-1 concentrations (Suikkari et al., 1989; Lee et al., 1993). The increase in IGFBP-1 concentrations following an insulin bolus reported by Cotterill and colleagues, was believed to arise because of suppressed endogenous insulin secretion rather than because of the change in blood glucose levels (Cotterill et al., 1993b).

Although GH is not considered to be a major determinant of IGFBP-1 concentrations, an early reduction in IGFBP-1 has been identified in young women following GH administration (Tapanainen et al., 1991). Circulating (peripheral) insulin concentrations did not alter as IGFBP-1 levels fell although this may not exclude alterations in hepatic insulin delivery.
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1.3 GH and IGF function

1.3.1 Role of IGF-I in tissue growth and differentiation

GH is the principal growth-regulating factor in mammals. Excessive GH secretion leads to gigantism or acromegaly and GH deficiency to dwarfism. The connection between GH and circulating IGF-I concentrations was originally proposed by Salmon and Daughaday (Salmon & Daughaday, 1957). GH release by the pituitary gland leads to IGF-I production by the liver which, after transfer from a circulating pool, stimulates growth. The importance of IGF-I in postnatal growth is now well established (Zapf et al., 1984).

However, it has also become clear that IGF-I produced locally also has an important role in tissue growth. The local administration of GH has been shown to lead to accelerated bone growth (Isaksson et al., 1982) and, in an isolated limb preparation, the growth promoting effects of GH could be blocked by antibody to IGF-I (Schlechter et al., 1986). Although the extent to which GH mediates growth independently of local IGF-I production is not known, there is evidence that GH may promote differentiation directly by stimulating the formation of large colonies of younger cells, and that IGF-I acts at a later stage of growth and development to stimulate smaller colony formation (Isaksson et al., 1987; Lindhal et al., 1987a, b). The effects of IGF-I on cell differentiation have been demonstrated in a range of cell preparations including osteoblasts, adipocytes, oligodendrocytes, myoblasts and chondrocytes (Schmid et al., 1983, 1984; McMorris et al., 1986; Smith, PJ et al., 1988; Bhaumick & Bala, 1991). The IGF-I effects on a particular cell will therefore depend on tissue type, stage of development and relative responsiveness. Thus IGF-I may affect cell division in the proliferative phase of a given cell line, and cell differentiation at a later stage of the growth process.
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1.3.2 GH signal and IGF-I response

The nature of the GH signal is an important determinant of IGF response. Pulsatile GH administration to hypophysectomized female rats leads to increased IGF-I gene expression, a rise in circulating IGF-I concentrations and enhanced growth when compared to continuous GH infusions (Maiter et al., 1988, 1992).

Growth rate is closely linked to GH pulse amplitude in humans (Hindmarsh et al., 1987; Albertsson-Wikland & Rosberg, 1988), with changes in GH concentration of greater physiological significance than the duration of GH exposure (Hindmarsh et al., 1992). Frequent administration (of the same overall daily GH dose) is more effective at eliciting a growth response in GH deficient children (Kaasrup et al., 1983; Albertsson-Wikland et al., 1986). However, Jorgensen and colleagues (Jorgensen et al., 1990) showed that frequent injections were no more effective at generating IGF-I than a continuous infusion, which led them to conclude that the baseline GH concentration may be as important as pulsatility in determining response.

1.3.3 Growth hormone binding protein (GHBP)

A circulating high affinity growth hormone binding protein (GHBP) was reported in 1986 (Baumann et al., 1986) and is thought to be derived from the extracellular domain of the GH receptor. If GHBP concentrations are indicative of GH receptor status then they could provide information about the relationship between GH and IGF-I levels in human subjects.

GHBP concentrations are relatively stable within any given twenty-four hour period (Carlsson et al., 1993) and normally bind 40% to 60% of circulating GH (Baumann et al., 1988). The GHBP binding capacity can be saturated, and so the rise in free GH is more pronounced above certain GH concentrations. A low affinity GHBP has also been identified, although this is responsible for a minority of total GH binding (Baumann & Shaw, 1990; Tar et al., 1990). The
function of GHBP is unknown, although it may alter GH clearance and regulate hormone interaction with the GH receptor.

Interestingly, GHBP correlates with height standard deviation score (SDS) in children, adolescents and young adults (Silbergeld et al., 1989), and the response to GH in GH-deficient children is strongly related to pretreatment GHBP concentrations (Martha et al., 1992). This led the authors to suggest that the GHBP/GH receptor status may reflect the growth rate and height potential of an individual. GHBP concentrations are also known to correlate with body mass index (BMI) and are inversely related to GH release in a range of clinical settings; levels are low in anorexia nervosa when GH secretion is enhanced, and raised in obesity where GH concentrations are low (Martha et al., 1991; Hochberg et al., 1992).

GHBP concentrations rise in tandem with IGF-I concentrations during childhood but do not change significantly during puberty (Silbergeld et al., 1989; Merimee et al., 1990; Massa et al., 1992; Martha et al., 1993). The relationship with IGF-I concentrations weakens at this time (Merimee et al., 1990; Martha et al., 1991; Massa et al., 1992), which suggests that the impact of insulin and sex steroids on IGF-I concentrations may be on events distal to the GH receptor.

1.3.4 Short-term effects of IGF-I, In-vitro studies

Studies of fetal rat hepatocytes (Freemark et al., 1985) and human hepatoma cell lines (Verspohl et al., 1984) have indicated the presence of IGF-I surface receptors and effects of IGF-I on carbohydrate metabolism in these preparations. However this may not be true of adult rat or normal human hepatocytes where the absence of IGF-I receptors suggests that actions of the peptide must occur through the insulin receptor (Caro et al., 1988). It has therefore been concluded that IGF-I receptor expression does not occur in normal mature human hepatocytes (Caro et al., 1988).
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Some studies of human fat cells have indicated a specific role for the IGF-I receptor in regulating lipid metabolism (Kern et al., 1985), whilst other investigators have found that the adipocyte is devoid of IGF-I surface receptors (Bolinder et al., 1987). Bolinder and colleagues examined the actions of IGF-I in inhibiting glycerol release and glucose metabolism and concluded that effects were mediated via the insulin receptor (Bolinder et al., 1987). Their conclusions were based on the absence of any additive effect of the two peptides, a dose-dependent reduction in insulin binding in the presence of IGF-I, and because the difference in potency on a molar basis (insulin had 600 - 1000 times the potency of IGF-I) was of a similar order of magnitude to the difference in affinity for the insulin receptor. Perhaps in keeping with these observations, Sinha and colleagues suggested that specific IGF-I receptors were present on the adipocyte cell surface although they were relatively few in number when compared to those for insulin, and that a major effect on glucose metabolism through these receptors was unlikely (Sinha et al., 1989).

Evidence from in-vitro studies has suggested that IGF-I may have a physiological role in mediating glucose disposal in muscle tissue. IGF-I receptors are found in high density on muscle cells (Poggi et al., 1979; Yu & Czech, 1984) and IGF-I stimulates glucose utilisation independently of insulin (Dimitriadis et al., 1990). Dimitriadis and colleagues have also looked at the response of the soleus muscle of the rat following in-vivo IGF-I administration, and concluded that the effects of IGF-I on glucose metabolism can occur independently of insulin (Dimitriadis et al., 1992). Insulin was not found to affect glucose metabolism in the presence of maximal concentrations of IGF-I and so the authors suggest a common post-receptor mechanism for the two peptides. IGF-I also stimulates glucose disposal in human muscle preparations (Dohm et al., 1990). This effect was believed to result from interaction with the IGF-I receptor and was of a similar magnitude to that induced by insulin. In-vitro work has also provided evidence that IGF-I has a discrete role in the metabolism of specific amino-acids in muscle (Parry-Billings et al., 1993). Thus, in addition to
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studies of receptor distribution, there is other evidence to indicate that IGF-I may have an acute role in muscle metabolism which is quite distinct to that of insulin.

Rat pancreatic beta cells exhibit relatively few insulin receptors (Van Schravendijk et al., 1987) and IGF-I has been shown to reduce insulin release following interaction with the IGF-I receptor (Leahy & Vanderkerkhove, 1990; Van Schravendijk et al., 1990). The inhibition of insulin secretion in-vitro is observed at physiological IGF-I concentrations and is more pronounced as blood glucose concentrations rise (Leahy & Vanderkerkhove, 1990). As a potential regulator of insulin secretion, it is possible that IGF-I may be more closely concerned with acute fuel regulation and disposal than has previously been suspected.

1.3.5 IGF-II

The function of IGF-II in man is not known, although in some animals it is believed to have a crucial role in mediating fetal growth. In the rat, IGF-II concentrations are greatest in-utero and then decline after birth (Adams et al., 1983; Brown,AL et al., 1986), in contrast to man where serum levels rise postnatally (Enberg & Hall, 1984) and are ultimately around three to four times greater than the concentrations of IGF-I at 600 to 700 ng/ml (Zapf et al., 1981; Enberg & Hall, 1984). The levels of IGF-II are not closely related to GH status and do not change significantly at puberty. IGF-II concentrations fall to around 200 ng/ml in GH deficiency and remain relatively stable or in the low normal range in acromegaly (Zapf et al., 1981; Enberg & Hall, 1984; Bang, 1990).

The similarity between the IGF-II receptor and the M-6-P receptor has proved to be rather puzzling. The M-6-P receptor binds proteins and mediates their transport to the lysosome but a related function of IGF-II (IGF-II binds to the receptor with greatest affinity) has not been identified. Antibodies directed against the IGF-II receptor stimulate glycogen synthesis (Hari et al.,
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1987) and thymidine incorporation into cells (Kojima et al., 1988). A pathological increase in IGF-II production may cause hypoglycaemia (Daughaday et al., 1988; Zapf, 1993) and it has been suggested that by interacting with the IGF-II receptor the peptide may then have a physiological role as a mediator of glucose uptake in rat skeletal muscle (Bevan et al., 1992). However in-vivo studies, also in the rat, have concluded that the hypoglycaemic actions of IGF-II are mediated via the insulin receptor, and that a physiological role of the peptide in carbohydrate metabolism is unlikely (Stümpel & Hartmann, 1992).

There is also experimental evidence to suggest that IGF-II may normally interact with the IGF-I receptor because this is known to bind IGF-II with high affinity (Steele-Perkins et al., 1988) and because antibodies to the IGF-I receptor prevent IGF-II mediated thymidine incorporation and cell multiplication in human fibroblasts (Conover et al., 1987; Furlanetto et al., 1987). However IGF-II administration has been found to have a relatively minor impact on growth in rats (Schoenle et al., 1985).

Thus the function of IGF-II remains something of an enigma. There is little evidence to link IGF-II with the growth process in man, but involvement in acute fuel disposal cannot be excluded. The links between IGF-II, the IGF-II receptor and the lysosomal membrane may yet prove to be more physiologically relevant than potential interactions with the IGF-I and insulin receptors.

1.4 IGF action - endocrine or paracrine?

1.4.1 Introduction

In contrast to hormones such as insulin there are no readily accessible stores of the IGF’s outside the circulation. However the binding protein/IGF complex is in itself a reservoir of peptide, with the affinity of the IGFBP’s for the IGF’s preventing peptide from exerting uncontrolled insulin-like effects. But if this is the case, how do the IGF’s access tissue receptors? Is locally produced (or
paracrine) IGF-I of greater physiological importance than circulating (or endocrine) peptide? It appears that far from simply binding the IGF's and inhibiting IGF actions that the IGFBP's may provide the link between the two compartments, endocrine and paracrine. Thus the variations in the concentration of the IGFBP's and changes in their binding affinity may permit transport of the IGF's between the two compartments, and may regulate IGF actions at the tissue level. These actions will be discussed in greater detail in the following sections of this chapter.

1.4.2 The function of IGFBP-3 and circulating IGF-I

The ternary IGFBP-3 complex does not cross the capillary wall (Binoux & Hossenlopp, 1988) and inhibits the binding of the IGF's to IGF receptors (Gopinath et al., 1989). IGF-I analogues with decreased affinity for IGFBP-3 are cleared more quickly and have increased biological activity, which suggests that the IGFBP's restrict IGF-I to the vascular compartment (Ballard, FJ et al., 1991). However the effects of systemically administered IGF-I (also discussed later in this chapter) suggest that circulating IGF-I must be able to gain access to the tissues and when bound to IGFBP-3 (but not in the ternary complex with ALS), it has been shown that IGF-I can readily leave the circulation and exert acute metabolic effects (Zapf et al., 1995). Interestingly, an anti-serum to IGF-I has also been shown to increase short term protein catabolism in fasted lambs, which again suggests that circulating IGF-I has an active metabolic role in-vivo (Koca et al., 1992).

One possible link between circulating IGF-I and local IGF availability has come from the identification of serum proteases which are capable of altering the affinity of IGFBP-3 for IGF-I, and altering IGF-I distribution amongst the IGFBP's. Protease activity was first detected in pregnancy, where the effect on the IGFBP-3 complex was to increase IGF bioavailability (Giudice et al., 1990; Hossenlopp et al., 1990; Blat et al., 1994). Changes in protease activity, and subsequent
alterations in IGF tissue availability, have also been demonstrated in normal physiological states (Gargosky et al., 1992; Lalou & Binoux, 1993), at the time of severe illness (Davies et al., 1991), in childhood neoplasia (Müller et al., 1994) and during surgery, where this was thought to be a means of reducing body catabolism by enhancing tissue IGF-I uptake (Cwyfan-Hughes et al., 1992; Davenport et al., 1992).

In vitro studies have shown that the IGFBP-3/IGF-I complex may have increased activity when compared to free peptide (Blum et al., 1989; Ernst & Rodan, 1990) and IGFBP-3 could, under different circumstances, either inhibit or potentiate the actions of IGF-I in neonatal skin fibroblasts (De Mellow & Baxter, 1988). In other in vitro studies, IGFBP-3 was shown to associate closely with the surface of bovine fibroblasts and influence IGF action by altering IGF affinity and IGF receptor structure and function (Conover & Powell, 1991; Conover, 1992). The mechanisms by which circulating IGF-I reaches tissue receptors are still poorly understood. Nevertheless there is little doubt that circulating IGF-I has an active physiological role in vivo.

1.4.3 The functions of IGFBP-1

Unlike IGFBP-3 concentrations, the levels of circulating IGFBP-1 demonstrate a marked diurnal variation and the influence of insulin, and possibly glucose concentrations, on IGFBP-1 levels suggest close links with nutritional status (section 1.2.3). The daily fluctuation in IGFBP-1 concentrations has been interpreted in a number of different ways. On the one hand a fall in circulating IGFBP-1 may indicate transfer of bound IGF's to other tissues. Insulin facilitated transcapillary flux of the IGFBP-1/IGF-I complex has indeed been demonstrated in vitro (Bar et al., 1990a, b), although this mechanism might not easily be reconciled with the fact that IGFBP-1 is largely unsaturated. Alternatively, circulating IGFBP-1 may function primarily as an inhibitor of the actions of IGF-I. In the fed state insulin will reduce IGFBP-1 transcription, circulating IGFBP-1 will
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fall, and access of 'free' IGF-I to the tissues will be enhanced (Suikkari et al., 1989). In the fasting individual, the relatively high IGFBP-1 concentrations will increase IGF binding and prevent increased fuel disposal at a time when nutritional uptake from the gut is low. This proposal receives support from the experimental work of Lewitt and colleagues, who showed that administration of human IGFBP-1 led to an increase in glucose concentrations in adult rats (Lewitt et al., 1991).

IGFBP-1 has also been identified as an inhibitor of IGF actions in the porcine costal cartilage IGF bioassay (Taylor et al., 1990), and whilst IGFBP-1 is absent in endometrial tissue during the proliferative phase, it can be identified in the secretory phase when multiplication has ceased (Rutanen et al., 1988). There are other reports of the inhibitory actions of the peptide at the cellular level (Ritvos et al., 1988), although in-vitro work has also suggested that IGFBP-1 may enhance the effects of IGF-I on DNA synthesis and cell multiplication in fibroblast cultures (Elgin et al., 1987).

In summary, the function of the IGFBP's is complex and incompletely understood. Their role in the circulation seems to be that of a reservoir with the ability to enhance or prevent IGF transfer to the tissues. IGFBP's may also regulate IGF actions at the tissue level.

1.4.4 Conclusions

In addition to a fundamental role as a mediator of the relatively slow processes of growth and differentiation, IGF-I may have more acute metabolic effects on fuel disposal. Although IGF-I has a close structural relationship to insulin, pronounced insulin-like actions of the large circulating pool are usually prevented by high affinity binding proteins. Changes in the concentrations and binding affinity of the IGFBP's provide a means whereby tissues may access circulating IGF-I according to nutritional status. The presence of IGF-I receptors on muscle tissue, one of the primary target tissues of insulin action, raises the interesting question of whether there are IGF-I
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effects on fuel disposal that are relatively independent of nutritional status. The local production of IGF-I is closely linked to the growth process, but circulating peptide may have an extended role in the regulation of metabolism by mediating acute changes in IGF bioavailability.
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Section 2: THE GH/IGF-I AXIS IN NORMAL PUBERTY AND IDDM

1.5 Insulin sensitivity and the GH/IGF-I axis

1.5.1 Introduction

The endocrinological changes which occur at puberty include pronounced alterations in the concentrations of GH, IGF-I and insulin. Similar changes occur in subjects with IDDM although there are important qualitative and quantitative differences compared to normal subjects which may have important implications for metabolic control. In this part of the introduction the endocrine changes in normal adolescence are reviewed together with the ways in which GH and IGF-I concentrations differ in IDDM and how this can affect insulin requirements, blood glucose control and the well known complications of the disease which may be seen in the longer term.

1.5.2 Changes in insulin sensitivity during puberty

The onset of puberty in normal subjects is associated with a fall in insulin sensitivity, as indicated by a rise in fasting and stimulated insulin levels (Smith, CP et al., 1988; Caprio et al., 1989). The insulin sensitivity index (derived from the relationship between insulin and blood glucose concentrations) also falls steadily throughout puberty (Cook et al., 1993). Bloch and colleagues demonstrated a greater insulin response to an oral glucose tolerance test in pubertal compared with prepubertal children and then, using a euglycaemic hyperinsulinaemic clamp, calculated that the pubertal group were approximately 30% less insulin sensitive (Bloch et al., 1987).

1.5.3 The nature of insulin sensitivity at puberty—normal subjects

Amiel and colleagues studied changes in glucose turnover during puberty using the euglycaemic clamp technique, and concluded that the change in insulin sensitivity occurred primarily in peripheral tissues (Amiel et al., 1991). Hepatic glucose production was suppressed to the same
degree in both pre-pubertal and pubertal subjects, whilst peripheral glucose uptake was more pronounced in the pre-pubertal group. Insulin is an anabolic hormone (Philips et al., 1991) and skeletal muscle appears to be more sensitive to the anti-proteolytic actions of insulin than it is to the better known effects on glucose disposal (Louard et al., 1992). It has been suggested that the peripheral resistance to glucose uptake, which develops during puberty, could be a means whereby the anabolic actions of insulin, such as enhanced amino-acid uptake, are potentiated without concomitant hypoglycaemia (Amiel et al., 1991).

1.5.4 The nature of insulin sensitivity at puberty- IDDM

An decrease in insulin sensitivity during puberty can also be demonstrated in subjects with IDDM, and this is reflected in a rise in the insulin requirements necessary for the maintenance of euglycaemia. Subjects with IDDM, however, demonstrate a more profound impairment of insulin action when compared to normal subjects (Amiel et al., 1986), and the anti-lipolytic effects of insulin may also be reduced (Caprio et al., 1990).

Hepatic glucose production is enhanced in adolescents with IDDM (Arslanian et al., 1993). This may be linked to the portal to peripheral insulin ratio, which is between two and four in normals (Horwitz et al., 1975), and less than one in subjects with IDDM. However, Arslanian and colleagues found that hepatic glucose production rates were positively related to HbA1 concentrations, thereby suggesting that poor blood glucose control is associated with reduced hepatic insulin sensitivity (Arslanian et al., 1993). Adolescents with IDDM demonstrate impaired insulin-mediated glucose uptake (Arslanian et al., 1993), and hyperglycaemia also appears to decrease insulin sensitivity in subjects with IDDM by reducing the overall rate of glucose utilisation rather than glucose disposal (Fowelin et al., 1993).
1.5.5 Possible mediators of the fall in insulin sensitivity at puberty

Although the concentrations of androgens and oestrogens rise at puberty, sex steroids are not believed to be prime mediators of the fall in insulin sensitivity. Adrenarche is not associated with changes in insulin sensitivity (Smith, CP et al., 1989), and beyond puberty sex steroid concentrations remain high despite the return of insulin sensitivity to prepubertal levels. Furthermore, the menstrual cycle was not found to affect insulin-mediated glucose metabolism (Yki-Järvinen, 1984) and more direct study of the effects of androgens has shown only a minor impact on insulin sensitivity in adults (Hale et al., 1985).

GH has insulin antagonistic properties in normal subjects (Metcalf et al., 1981; Rizza et al., 1982; Sherwin et al., 1983), and the change in insulin concentrations during puberty can be related to an alteration in GH concentrations (Hindmarsh et al., 1988a). Amiel and colleagues have shown that insulin stimulated glucose metabolism is reduced in adolescents with and without diabetes and that the response to insulin is inversely related to 24-hour GH concentrations (Amiel et al., 1986). Furthermore, GH administration to subjects with IDDM leads to a rise in hepatic glucose production, a decrease in peripheral glucose utilisation and a subsequent increase in blood glucose concentrations (Press et al., 1984a, 1986). Although GH levels were not a sensitive indicator of insulin sensitivity at puberty in the studies of Cook and colleagues (Cook et al., 1993), they did find that insulin sensitivity was inversely related to the peripheral effects of GH as reflected by IGF-I concentrations. However, no account was taken of the pulsatile nature of GH release in these studies.
1.6 Circadian variations in insulin sensitivity

1.6.1 Dawn Phenomenon

In addition to the long term changes in insulin sensitivity during puberty a daily rise in insulin concentrations beyond 05.00h, which may be accompanied by changes in blood glucose levels, has been identified in some studies of normal subjects (Bolli et al., 1984; Schmidt et al., 1984). However, this has not been a consistent observation (Marin et al., 1988). A rise in overnight blood glucose concentrations has also been identified in studies of individuals with IDDM (Schmidt et al., 1981).

Hyperglycaemia at the time of waking in subjects with IDDM may have a number of different explanations. Perhaps the most obvious is a waning of insulin delivery from the injection administered earlier that night. A second possible explanation is that hyperglycaemia reflects the counter-regulatory reaction to earlier hypoglycaemia (Somogyi, 1959; Bruck & MacGillivray, 1975). It has also been proposed that sensitivity to the actions of insulin might change overnight, with an increase in blood glucose levels despite stable overnight insulin concentrations. This may represent an increase in insulin resistance above a baseline level as the night progresses, or a return to baseline following a period of increased sensitivity earlier in the night (Clore et al., 1989). Finally, it is possible that low insulin levels and rising blood glucose concentrations in IDDM could be the result of changes in insulin clearance.

Garrel and colleagues have found reduced overnight insulin sensitivity in both normal and IDDM subjects, although this was more pronounced in the IDDM group (Garrel DR et al., 1992). When insulin delivery in IDDM is kept at a constant rate overnight it has been shown that an appropriate infusion for the maintenance of initial euglycaemia is inadequate later in the night. This supports the existence of additional mechanisms besides the first two explanations, and the term 'Dawn Phenomenon' (Schmidt et al., 1981) has been used to describe the rise in the requirements
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for the maintenance of euglycaemia in the absence of declining insulin delivery and earlier
hypoglycaemia (Perrillo et al., 1991).

There is therefore good evidence to indicate that daily fluctuations in insulin sensitivity
occur in normal and IDDM subjects, and that they may be superimposed on a background of
increasing endogenous insulin production in normals during puberty, or of daily insulin requirements
in subjects with IDDM.

1.6.2 The Dawn Phenomenon: altered insulin sensitivity or clearance?

The exact mechanism responsible for the Dawn Phenomenon remains a subject of some
debate. Earlier studies, suggesting that the increased insulin delivery required for the maintenance
of euglycaemia overnight was due to a rise in insulin clearance, were probably flawed (Clarke et al.,
1980; Bright et al., 1980; Nestler et al., 1984; Bolli & Gerich, 1984). In some cases this was
because the ‘Biostator’ was used; this is a closed loop method designed to maintain steady state
blood glucose levels which at low infusion rates has been shown to lead to insulin aggregation and a
fall in immunoreactive and biologically active insulin delivery (Brennan et al., 1985). Even where
this method was not used, investigators may not have been aware of waning insulin delivery
following adherence of the peptide to the plastic of the administration apparatus (Skor et al., 1984).

More recent studies using validated infusates (Dux et al., 1985), or open delivery systems
with albumin added to the infusion to prevent adhesion (Kerner et al., 1984; Arslanian et al., 1992),
have also concluded that insulin clearance rises overnight. Some of these study findings may also be
questioned, however. For example, Arslanian and colleagues administered subcutaneous insulin as
late as 21.00h in the evening which may have led to the continued absorption of insulin from the
injection site when intravenous infusion rate calculations were undertaken. Interestingly, Dux and
colleagues provided evidence to indicate that the changes in insulin requirements were due to
reductions in insulin clearance in the early part of the night, which then rose towards the 'norm' as
the night progressed (Dux et al., 1985).

An alternative, or further explanation, is that insulin action is impaired overnight with the
rise in insulin infusion requirements in IDDM (or blood glucose concentrations when the insulin
infusion remains unchanged) reflecting altered insulin sensitivity (Campbell & Gerich, 1986; De Feo
et al., 1986). DeFeo and colleagues did not find evidence of changes in overnight insulin clearance
in their studies in normal adults or subjects with IDDM. At the same time they were able to show a
rise in the insulin requirements necessary for the maintenance of euglycaemia (De Feo et al., 1986).

1.6.3 Decreasing or increasing insulin sensitivity?

The debate as to the nature of the Dawn Phenomenon in normal and IDDM subjects has
been complicated still further by the suggestion that the rise in insulin requirements at dawn is due to
a change from the sleep state to one of greater arousal. A fall in hepatic glucose output, glucose
utilisation and in lipolysis has been described in association with sleep (Clore et al., 1989). This was
observed despite a concurrent fall in insulin secretion, and could be linked to an alteration in muscle
glycolysis in association with reduced muscle adrenergic activity. It has been proposed that the
increase in insulin requirements necessary for the maintenance of euglycaemia may therefore occur
because of a sleep related change in glucose flux (Blackard et al., 1989).

The reported information about sleep pattern in studies of the dawn phenomenon does not
usually include the details necessary to examine this hypothesis in detail, although changes in
sensitivity are commonly detected at 05.00h when it might be supposed that subjects are still asleep.
Nor does this hypothesis explain the change in insulin requirements detected when baseline nights
are compared with nights when GH secretion is suppressed (Perriello et al., 1990). This will be
considered in greater detail in subsequent sections of this chapter.
1.7 The Dawn Phenomenon and GH release

1.7.1 GH and overnight insulin sensitivity

A fall in insulin sensitivity overnight could, on a theoretical basis, be hormonal or non-hormonal in origin. The levels of some hormonal insulin antagonists are known to vary during a given 24 hour period, and could account for altered insulin sensitivity towards the dawn. Non-hormonal mechanisms include changes related to the fed as opposed to fasted state, and wake as opposed to sleep states, affecting hepatic insulin sensitivity.

Known hormonal insulin antagonists include glucagon, adrenaline, cortisol and GH. The levels of glucagon do not change overnight (Porriello et al., 1988), and the levels of adrenaline do not normally reach the threshold required to induce anti-insulin effects (Clutter et al., 1980). In addition it has been shown that alpha- and beta-adrenergic blockade does not affect the dawn phenomenon in IDDM (Campbell et al., 1985). Cortisol is not believed to play a major role in the development of insulin resistance because the dawn phenomenon can still be identified when endogenous cortisol production is suppressed (Bright et al., 1980). The rise in insulin requirements also occurs earlier than would be expected from our knowledge of the insulin antagonistic actions of cortisol.

GH has been closely linked to the changes in insulin resistance at puberty and is known to be secreted in greatest quantities overnight. A process of elimination and deduction may therefore lead one to suspect that GH is the most likely hormone responsible for the changes in insulin requirements overnight, although there is also more direct evidence leading to this conclusion.

1.7.2 The role of GH- studies in IDDM

Various groups have reported a relationship between GH concentrations and insulin sensitivity. Beaufrere and colleagues used an insulin infusion delivered by syringe pump to
demonstrate that the rise in blood glucose levels overnight in adolescents with IDDM correlated positively with nocturnal GH secretion (Beaufrere et al., 1988). Edge and colleagues used a euglycaemic insulin clamp to show a positive correlation between mean overnight GH concentrations and the change in insulin infusion requirements between 01.00h - 04.00h, when compared to 05.00h - 08.00h (Edge et al., 1990b).

Other groups have examined the effects of GH suppression on blood glucose concentrations or insulin infusion requirements. GH suppression abolishes the overnight rise in blood glucose concentrations seen on a control night in adults with IDDM (Campbell et al., 1985; Davidson et al., 1988; Perriello et al., 1990), and this rise is restored when GH is replaced (Perriello et al., 1990). Atiea and colleagues also reported a reduction in the overnight rise in blood glucose concentrations in young adults with IDDM when GH was suppressed by pirezpine, both acutely and in the longer term (Atiea et al., 1989). Similarly, the dawn phenomenon was found to be absent in subjects with IDDDM and GH deficiency, in contrast to a control group with IDDM alone (Boyle et al., 1992).

Pal and colleagues studied the effects of GH administered overnight, either continuously or in 3 discrete pulses, to young adults with IDDM following suppression of endogenous GH production by somatostatin (Pal et al., 1992). They demonstrated an increase in the intravenous insulin requirements necessary for the maintainance of euglycaemia only after pulsatile GH administration. Using cross-correlation they were able to show that the increase in insulin requirements occurred 135 minutes after the GH pulse.

Thus the investigation of overnight insulin requirements under sound methodological conditions indicates firstly, that insulin requirements change overnight, and secondly that this alteration has close links to GH release.
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1.7.3 Timing of the Dawn Phenomenon

The timing of the Dawn Phenomenon in IDDM can be assessed by establishing a stable euglycaemic period and monitoring changes in blood glucose with a constant insulin infusion rate, or preferably, varying the insulin infusion to maintain euglycaemia. The former technique was used in a small group of children and adolescents to show that mean blood glucose concentrations were stable until between 05.00h and 06.00h, beyond which they began to rise despite stable insulin levels (Beaufiere et al., 1988). De Feo and colleagues used a variable rate insulin infusion to show an increase in mean insulin requirements (and blood glucose concentrations) beyond 05.00h in a group of adults with IDDM (De Feo et al., 1986), and Edge and colleagues noted an increase in insulin requirements in adolescents with IDDM when intervals from 05.00 to 08.00h and 01.00 to 04.00h were compared (Edge et al., 1990b). The timing of the rise in insulin requirements was also assessed in a large group of adults with IDDM by Perriello and colleagues (Perriello et al., 1991); the dawn phenomenon was identified in 89% of subjects, with a rise in mean insulin requirements from approximately 05.15h (range 03.40h to 06.40h).

Thus the observations of changes in insulin sensitivity overnight in IDDM have produced surprisingly consistent results, with the increase in insulin infusion requirements, or rise in blood glucose, usually apparent at or around 05.00h.

1.7.4 Magnitude of the Dawn Phenomenon in IDDM

The elimination of GH secretion with somatostatin and subsequent GH replacement in young adults with IDDM has indicated that the fall in insulin sensitivity induced by GH is of the order of 30% (Perriello et al., 1990). When these studies were extended, Perriello and colleagues showed that the increase in insulin requirements was in the order of 20% (range 6 - 31%) in a predominantly adult population, and that this was highly reproducible within individuals (Perriello et
al., 1991). Edge and colleagues used the euglycaemic insulin clamp to show that the rise in insulin requirements overnight in adolescents with IDDM was around 25% (Edge et al., 1990b).

1.7.5 Variables affecting insulin sensitivity and the magnitude of the Dawn Phenomenon

A number of factors influencing the magnitude of the changes in overnight insulin requirements have been identified. In a study group consisting predominantly of adults with IDDM, Perriello and colleagues concluded that the Dawn Phenomenon was reduced by intensive insulin therapy and was more pronounced in those subjects with poor overall blood glucose control and shorter disease duration (Perriello et al., 1991). This latter observation was explained by the enhanced secretion of counter-regulatory hormones and reduced insulin sensitivity in subjects with disease of more recent onset. These investigators also reported that enhanced counter-regulatory responses to hypoglycaemia (induced by a standardised insulin infusion test) were linked to a greater increase in insulin infusion requirements overnight (Perriello et al., 1991).

Although the Somogyi phenomenon is not considered to be a common cause of hyperglycaemia at the time of waking, and when hypoglycaemia does occur it may not have any impact on blood glucose levels (Havlín & Cryer, 1987; Tordjman et al., 1987), counter-regulatory hormone release may nevertheless affect insulin sensitivity. Adrenaline administration reduces the glucose required to maintain euglycaemia during a hyper-insulinaemic clamp (Kollind et al., 1988) and the early (2 - 3h) changes in insulin resistance following hypoglycaemia can be suppressed by propranolol (Attvall et al., 1987a, b). Later reductions in insulin sensitivity following hypoglycaemia are due to the combined effects of GH and cortisol (Cloro et al., 1987; Fowedin et al., 1989), and these changes can in turn be abolished by the administration of somatostatin and metyrapone. Body mass index (BMI) also correlates inversely with insulin sensitivity, and may be an important variable in obese subjects (Bloch et al., 1987).
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It is clear that a wide variety of factors may affect insulin sensitivity and overnight insulin requirements in IDDM. Assessment of these factors suggests that those subjects with the greatest increase in overnight insulin requirements will include the younger, adolescent subjects with poor blood glucose control. GH is a potential link between changes in insulin sensitivity and poor blood glucose control in adolescents with IDDM (Arslanian et al., 1993), and a relationship between GH secretion and HbA1 concentrations has been identified during puberty (Nieves-Rivera et al., 1993). The actions of GH and abnormalities of GH production in IDDM will be explored in greater detail in subsequent sections of this chapter.

1.7.6 Quantifying the effects of GH - studies in normals

Although GH enhances hepatic glucose production, the reduction in peripheral glucose utilization appears to be more pronounced (Bratusch-Marrain et al., 1982; Rizza et al., 1982). Some of the GH mediated effects are on post-receptor events (Bratusch-Marrain et al., 1982; Rizza et al., 1982; Rosenfeld et al., 1982), although there is also evidence to suggest that GH reduces the affinity of insulin receptor binding (Bratusch-Marrain et al., 1982).

The temporal effects of GH on metabolism have been studied by Fowelin and colleagues using the euglycaemic clamp technique. They showed that the insulin antagonistic effects after hourly infusions of GH began after 2 hours and lasted approximately 7 hours following peak GH levels (Fowelin et al., 1991). The magnitude and duration of this effect was linked to the preceding GH concentrations, with a maximal effect at levels of approximately 50 mU/l. Bratusch-Marrain and colleagues studied the effects of GH (infusions for 2 or 12 hours with GH levels ranging from 30-90 mU/l) on glucose metabolism and insulin levels and concluded that GH reduced insulin sensitivity within between 2 and 12 hours (Bratusch-Marrain et al., 1982). The effects of a GH
pulse on forearm glucose uptake have been identified within 30 to 60 minutes (Moller, N et al., 1990; Fineberg & Merimee, 1974).

The relationship between GH and alterations in insulin sensitivity is clearly complex and dependent on a variety of factors including GH pulse amplitude. Thus, overnight changes in insulin sensitivity may reflect GH release many hours earlier.

1.7.7 Insulin-like actions of GH

Although GH has late insulin-antagonistic effects it also has insulin agonist actions (MacGorman et al., 1981). Studies demonstrating these insulin-like effects have tended to use pharmacological doses of GH (Adamson & Efendic, 1979; Fineberg & Merimee, 1974) and physiological GH levels have not always revealed such actions (Sherwin et al., 1983). Moller and colleagues recently assessed the effects of physiological doses of GH in healthy young adults and noted insulin-like effects on glucose disposal, with a fall in blood glucose of between 0.1 and 0.2 mmol/l detected within the first hour following GH administration (Moller, N et al., 1992). Although there was no control group in these studies, the authors suggest that GH administration leads to a rapid fall in glucose uptake in muscle. Because of insulin-like effects in other tissues, it was proposed that the net effect is therefore a fall in plasma glucose concentrations. The contrasting metabolic effects of GH have also led to the suggestion that changes in insulin sensitivity overnight may be linked to an insulin-like action of GH in the early part of the night (Skor et al., 1985).

It has still to be determined to what extent GH has significant short-term insulin agonist actions in-vivo, but the major effect of physiological GH concentrations in the longer term is to reduce the effects of insulin on non-hepatic tissues.
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1.7.8 Effects of GH on fat, metabolism and ketone body production

In addition to the described effects of GH on glucose metabolism, GH is also known to affect the metabolism of fat. The administration of GH to normal subjects leads to an increase in free fatty acids (FFA) by 60 minutes, and ketones and glycerol within 3 hours (Sherwin et al., 1983; Moller,N et al., 1990). Moller and colleagues extended these studies to show that the effects of GH on fat metabolism were dose dependent (Moller,N et al., 1992). Both continuous and pulsatile GH administration led to increases in acetocetate, 3-hydroxybutyrate and FFA levels in young adults with IDDM (Pal et al., 1992), and Edge and colleagues noted a profound reduction in overnight ketone concentrations in adolescents with IDDM when GH production was suppressed by the anticholinergic drug pirenzipine (Edge et al., 1993).

1.8 Abnormalities of the GH/IGF-1 axis in IDDM

1.8.1 Elevated GH concentrations in IDDM

It has been known for many years that GH concentrations are elevated in adolescents and adults with IDDM when compared to normal subjects (Hansen, 1970; Molar et al., 1972; Horner et al., 1981). The normal increase in GH concentrations during puberty is accentuated in IDDM, with elevated mean, baseline and GH peak amplitudes (Edge et al., 1990a). GH concentrations are greatest in late puberty in boys and mid to late puberty in girls (Edge et al., 1990a; Batch & Werther, 1992). Asplin and colleagues noted an increase in the parameters assessing circulating GH concentrations, including pulse frequency, in poorly controlled adults with IDDM (Asplin et al., 1989).

A rise in GH concentrations may result from changes in GH clearance or alterations in GH secretion. However alterations in pulse frequency are more likely to be the result of changes in the control of GH release at the pituitary and/or hypothalamic level. GH half-life and metabolic
clearance rate in IDDM have either been reported to be prolonged (Boucher et al., 1969; Lipman et al., 1972) or similar to normal subjects (Owens et al., 1973; Navalesi et al., 1975). Mullis and colleagues reported an increase in GH half-life and volume of distribution in young adults with IDDM when compared to methodologically similar control data (Mullis et al., 1992). In these studies, endogenous GH secretion was suppressed, and exogenous GH administered as a bolus or infusion. GH half-life increased with more prolonged GH exposure and was unaffected by glucose or insulin concentrations. Although Nieves-Rivera and colleagues noted a decrease in GH half-life in IDDM subjects in late puberty when compared to a control group, their methodology was different in that they did not suppress endogenous GH secretion, and therefore needed to make assumptions about underlying GH secretory episodes (Nieves-Rivera et al., 1993).

Even when the prolonged GH half-life reported in IDDM is used in deconvolution analysis to calculate the pattern of GH release, there is still a marked increase in both the amplitude and frequency of underlying secretory events in adolescents with IDDM (Pal et al., 1993).

1.8.2 Control of GH secretion

GH release by the pituitary gland is controlled by the interaction between the hypothalamic hormones, somatostatin (SMS) and growth hormone releasing hormone (GHRH) (Tannenbaum & Ling, 1984). Although SMS has primarily an inhibitory and GHRH a facilitatory action on GH release, SMS does not inhibit GH synthesis (Fukata et al., 1985), and may be important in optimising the ability of the pituitary gland to respond to GHRH (Hindmarsh et al., 1991). Thus it appears that GHRH pulses are required for GH release (Ho, PJ et al., 1993), but that the pulse amplitude may be regulated by the underlying SMS tone. Blood samples taken from the hypothalamic-hypophysial portal system in sheep have confirmed the importance of GHRH in mediating GH pulsatility (Frohman et al., 1990). More direct evidence for the importance of GHRH
in mediating GH secretion in humans has recently come from studies using a selective GHRH antagonist (Jaffe et al., 1993). Beta adrenergic agonists and antagonists also affect GH secretion, probably by effects on hypothalamic SMS release (Ghigo et al., 1990). There is also evidence to support the existence of a short feed-back loop, with GH increasing hypothalamic SMS release (Molitch & Hlivyalc, 1980).

IGF-I may also be an important regulator of GH release, and work has suggested that IGF-I can stimulate hypothalamic SMS release in vitro (Berelowitz et al., 1981). However the purity of the IGF preparations used in these early studies has been questioned, and particular concerns centre on whether or not they contained IGF-II as well. More recently the intracerebro-ventricular administration of IGF-I did not alter GH secretion in rats, in contrast to IGF-I in combination with IGF-II (Harel & Tannenbaum, 1992), whilst studies in ewes have suggested that the feedback effects of IGF-I on GH secretion, alone or in combination with IGF-II, do not occur at the hypothalamic level (Fletcher et al., 1995).

Studies undertaken on rat pituitary cells have shown that IGF-I inhibits GHRH elicited GH release (Ceda et al., 1987), GH gene transcription (Yamashita & Melmed, 1987) and reduces GH mRNA levels (Namba et al., 1989). IGF-II is a less potent inhibitor of GH release, and the inhibitory effects of insulin are believed to occur through one of the IGF receptors (Ceda et al., 1987). The rat is not an ideal model for the study of IDDM in relation to man, in part because GH concentrations are low when diabetes is induced by streptozotocin. Interestingly, however, IGF-I levels are elevated in the pituitary gland of such diabetic rats, despite reduced IGF-I concentrations in other tissues (Okchovsky et al., 1991).

There is an additive effect of SMS and IGF-I on the inhibition of GHRH stimulated GH secretion by rat pituitary cells (Lamberts et al., 1989; Namba et al., 1989). IGF-I also inhibits GH release from human pituitary tumours, and the additive effect with SMS in tumour cell lines further
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Supports actions of IGF-I at the level of the pituitary gland (Lamberts et al., 1989). Van Cauter and colleagues found that the magnitude of the GH response to GHRH was inversely related to IGF-I concentrations in healthy men (Van Cauter et al., 1992a), and further in-vivo evidence for the role of IGF-I in GH release comes from studies of individuals with insensitivity to the actions of GH who are short, have low levels of IGF-I, and high plasma GH concentrations (Laron et al., 1966; Zapf et al., 1980).

1.8.3 GH release in IDDM

It has been suggested that reduced hypothalamic SMS secretion is the primary abnormality of GH release in IDDM (Miller et al., 1992; Ismail et al., 1993), and an exaggerated GH response to GHRH has been demonstrated in young adults with IDDM (Krassowski et al., 1988). Ismail and colleagues showed that pre-treatment of normal individuals and subjects with IDDM with pyridostigmine (which enhances endogenous cholinergic tone) led to equivalent GH responses to GHRH stimulation, and that the response in normals was then no different to untreated subjects with IDDM (Ismail et al., 1993). As SMS release is subject to tonic cholinergic inhibition (Dieguez et al., 1988), this data supports the hypothesis that one of the principal abnormalities in IDDM is reduced hypothalamic cholinergic tone. However, other abnormalities of GH release may exist in IDDM. A resistance to physiological SMS concentrations has been demonstrated in adult males with IDDM (Cohen & Abplanalp, 1991) and although GH pre-treatment inhibits the secretory response to GHRH in normals, this effect is reduced in subjects with IDDM (Ismail et al., 1993). Further, hyperglycaemia does not suppress GH release in IDDM as it does in normals (Ismail et al., 1991; Press et al., 1984b).

The discrepancy between peripheral and portal insulin concentrations is also an important determinant of the degree of GH hypersecretion in IDDM. Peripheral hyperinsulinaemia accentuates
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the GH response to GHRH stimulation in subjects with poorly controlled IDDM (HbA1c ≥ 11.1%) when compared to those with good control (HbA1c ≤ 8.2%) (Press et al., 1992), and subjects with greater residual endogenous insulin production, as assessed by stimulated C-peptide concentrations, have lower circulating GH concentrations (Wurzburger et al., 1990). Newly diagnosed IDDM subjects, in whom there is usually significant endogenous insulin production, have comparable GH concentrations to controls (Hayford et al., 1980).

Some authors have shown a positive correlation between the GH response to GHRH and HbA1c concentrations (Giustina et al., 1990) although this has not been a consistent finding (Ismail et al., 1993).

1.8.4 Effects of intensive treatment on GH

Intensive insulin treatment has variously been reported to reduce GH concentrations (Amiel et al., 1984; Press et al., 1984b), to change the pattern of GH pulsatility with greater night-time pulse frequency but without altering mean GH levels (Miller et al., 1992), or to increase GH concentrations (Wurzburger et al., 1990). The abnormal resistance to the suppressive effects of SMS on GHRH stimulated GH release, noted in adult males with IDDM, did not change despite a period of improved control as assessed by a fall in HbA1c concentrations (Cohen & Abplanalp, 1991).

1.8.5 GH release and IDDM- conclusions

The normal interaction between hypothalamic hormone production and GH secretion by the pituitary gland is altered in IDDM. Enhanced SMS release, pituitary resistance to the actions of SMS and increased sensitivity to the actions of GHRH may all lead to an increase in GH production. However there are other circulating peptides, such as insulin and IGF-I, which may also influence GH.
release. In the following sections of this chapter the impact of the IDDM on circulating levels of the IGF's and on IGF bioactivity will be considered in greater detail, for it is the central place of these variables within the GH/IGF-I axis that may largely be responsible for mediating the rise in GH concentrations described above.

1.8.6 Abnormal IGF-I concentrations in IDDM

IGF-I concentrations are low in children and adolescents with IDDM (Amiel et al., 1984; Taylor et al., 1988; Hall et al., 1989; Massa et al., 1993; Nieves-Rivera et al., 1993), and low levels have also been reported in adults with IDDM (Asplin et al., 1989; Cohen & Abplanalp, 1991; Brismar et al., 1994). Although Blethen and colleagues noted low IGF-I concentrations only in children under 5 years of age and not between 5 and 18 years (Blethen et al., 1981), and Horner and colleagues could not detect any difference in IGF-I concentrations in a small group of teenagers with IDDM (Horner et al., 1981), the control groups in these studies were not matched according to pubertal status.

IGF-I concentrations rise during puberty in both males and females with IDDM (Blethen et al., 1981), reaching a peak at mid to late puberty in males (Massa et al., 1993). Taylor and colleagues noted peak IGF-I concentrations at mid-puberty in both males and females, although concentrations were significantly reduced at puberty stages 2, 3 and 4 when compared with puberty matched controls (Taylor et al., 1988). A reduction in urinary IGF-I excretion has also been demonstrated in prepubertal and pubertal subjects with IDDM (Quattrin et al., 1992).
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1.8.7 The relationship between IGF-I concentrations, blood glucose control and the effects of intensive insulin therapy

An inverse relationship between HbA1 or HbA1c and IGF-I concentrations in pubertal subjects with IDDM has been noted by some investigators (Blethen et al., 1981; Rogers et al., 1991), but not others (Taylor et al., 1988). Lanes and colleagues found that the IGF-I generated by GH administration was reduced in subjects with poor blood glucose control (Lanes et al., 1985).

The impact of improved glycaemic control on IGF-I levels has also been investigated. Insulin infusion following a short period of overnight insulinopenia led to a rise in circulating IGF-I concentrations in a group of adult subjects (Brismar et al., 1994), and Amiel and colleagues observed that the intensification of insulin treatment in a group of children and young adults with IDDM led to an increase in IGF-I concentrations within 7 days, but not to within the normal range (Amiel et al., 1984). A rise in IGF-I concentrations has been noted by other groups in adolescents (Miller et al., 1992) and adult males with IDDM (Cohen & Abplanalp, 1991), but this has not been a consistent observation (Hall et al., 1989).

1.8.8 Mechanisms leading to the alteration in IGF-I levels in IDDM

Low portal insulin levels may be closely linked to impaired IGF-I production in IDDM. Insulin acts synergistically with GH in mediating IGF-I production (Daughaday et al., 1976; Houston & O'Neill, 1990), and a relative resistance to the actions of GH may reflect both inappropriate insulin levels and the discordance between peripheral and portal insulin concentrations. Wurzbürger and colleagues have shown that C-peptide 'positive' subjects with IDDM (as assessed by the response to glucagon stimulation) demonstrate a more pronounced rise in IGF-I concentrations on exogenous GH injections than C-peptide 'negative' individuals with a lower endogenous insulin response (Wurzbürger et al., 1993). Further evidence in support of the
importance of the normal ratio between peripheral and portal insulin concentrations has come from a study of intraportal insulin administration (via the umbilical vein), which led to reductions in GH levels and improved glycaemic control despite a reduction in the insulin dose, when compared to the peripheral route of insulin administration (Shishko et al., 1992).

1.8.9 IGF-II concentrations in IDDM

The impact of IDDM and subsequent alterations in blood glucose control on IGF-II concentrations is not entirely clear. Normal IGF-II values have been reported in adults, adolescents and children with IDDM (Merimee et al., 1983, 1984), but increased IGF-II concentrations have also been reported during adolescence (Hall et al., 1989). Improved glycaemic control has not affected IGF-II concentrations in some studies (Merimee et al., 1984), although Amiel and colleagues identified a small sub-group of children with low IGF-II concentrations in whom a rise in levels could be identified (Amiel et al., 1984). As a whole, however, the group of children and adolescents had normal IGF-II concentrations.

1.8.10 IGF bioactivity

In addition to the described alterations in IGF-I levels in IDDM, there is evidence to suggest impaired IGF bioactivity. Winter and colleagues have shown reduced IGF bioactivity in children and adolescents with IDDM using a porcine cartilage bioassay (Winter et al., 1979), and also noted a negative relationship between IGF bioactivity and HbA1c concentrations. Similar observations on IGF bioactivity were made by Taylor and colleagues, who also noted an inverse relationship between IGF bioactivity and IGFBP-1 concentrations (Taylor et al., 1988).
1.9 GH and IGF binding in IDDM

1.9.1 IGFBP-3 and IGFBP-1

The levels of the principal IGF binding protein, IGFBP-3, are low in adults with IDDM who have poor blood glucose control (Baxter & Martin, 1986), and are reduced in adolescents with IDDM (Batch et al., 1991).

In contrast, fasting IGFBP-1 concentrations are elevated in adolescents with IDDM on standard insulin regimens when compared to normal subjects (Batch et al., 1991; Holly et al., 1990), but are usually within the normal range when overnight euglycaemia is achieved (Holly et al., 1990). Mean overnight IGFBP-1 (Batch et al., 1991), and fasting IGFBP-1 concentrations (Holly et al., 1990), are inversely related to insulin in subjects with IDDM as they are in normals. Lee and colleagues did not note any difference in the relationship between insulin and IGFBP-1 in young adults with IDDM when compared to normal subjects (Lee et al., 1993), although higher concentrations of IGFBP-1 have also been reported in the IDDM group at equivalent peripheral insulin levels (Holly et al., 1988). Although the latter authors suggest a lack of sensitivity to the effects of insulin in the diabetic subjects as a possible explanation for their findings, the discrepancy is more likely to be linked to differences in portal insulin concentrations between the two groups. It is likely that intraperitoneal insulin administration is more effective at reducing IGFBP-1 concentrations (Hopkins et al., 1993).

In normal adolescents the fall in IGFBP-1 concentrations during puberty correlates with pubertal stage (Holly et al., 1989, 1990). No such relationship was seen in subjects with IDDM when overnight IGFBP-1 levels on standard insulin therapy and puberty stage were compared (Batch et al., 1991; Holly et al., 1990). However, when euglycaemia was maintained overnight, a similar inverse relationship to that seen in normals was identified (Holly et al., 1990). A relationship between IGFBP-1 concentrations and metabolic control has also been noted by some observers.
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Fasting IGFBP-1 levels on a standard insulin regimen, relative to a night when euglycaemia was maintained, were found to correlate with HbA1c concentrations (Holly et al., 1990), and Batch and colleagues have demonstrated a correlation between overnight IGFBP-1 levels and mean 12 month HbA1c concentrations in adolescents with poorly controlled IDDM (Batch et al., 1991).

1.9.2 GHBPinIDDM

Insulin deficiency decreases hepatic GH receptors in rats with diabetes induced by streptozotocin administration (Baxter & Turtle, 1978; Baxter et al., 1980). From the noted reductions of GHBP concentrations in children, adolescents and adults with IDDM (Menon et al., 1992; Mercado et al., 1992; Holl,RH et al., 1993; Massa et al., 1993) we might infer reduced GH receptor numbers (see Section 1.3.3).

1.10 The implications of the abnormalities in the GH/IGF-I axis in adolescents with IDDM

1.10.1 Deteriorating blood glucose control

It is well recognised that blood glucose control, as assessed by HbA1c concentrations, may deteriorate during adolescence. Mann and Johnston analysed mean HbA1 levels over a 12 month period in 148 children and found that concentrations were higher in children over 12 years as opposed to those under 12 years of age (Mann & Johnston, 1982), and Rogers and colleagues demonstrated elevated HbA1 levels in pubertal (Tanner stage 3), as opposed to pre-pubertal children (Rogers et al., 1991). Elevated HbA1 concentrations have also been noted in pubertal compared to pre-pubertal subjects by other groups (Amiel et al., 1986; Quattrin et al., 1992). More recently it was reported that teenagers participating in the Diabetes Control and Complications Trial (DCCT) had higher HbA1 concentrations than young adults (Drash, 1993).
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The reasons for the deterioration in control during puberty are complex, and may include factors such as disease duration, poor compliance and psychological problems (Dunger, 1992). However the endocrinological changes of puberty, including the abnormalities of the GH/IGF-I axis discussed above, are also believed to be important. Thus the low IGF-I levels lead to GH hypersecretion and reductions in insulin sensitivity. Low portal insulin concentrations lead to the increased production of IGFBP-1 which is a known inhibitor in IGF bioassay systems. Low IGF-I bioactivity and peripheral hyperinsulinaemia may exacerbate the GH hypersecretion still further. Low circulating IGF-I concentrations, coupled with high IGFBP-1 concentrations, may impair the more acute metabolic actions of IGF-I (see Section 1.4.3). Thus there are theoretical reasons why reduced IGF-I levels may be linked to poor blood glucose control, and there is also evidence to suggest that they may be associated with impaired growth.

1.10.2 Growth impairment

Abnormal growth has long been recognised in children with IDDM. Growth failure was described in the early days of insulin therapy (Joslin et al., 1925), and a range of abnormal growth patterns have been documented since then (Salardi et al., 1987). Mauriac described a syndrome of obesity, hepatomegaly and short stature in children treated with inadequate amounts of insulin (Mauriac, 1934), and twin studies have shown a reduction in height in the affected sibling when IDDM is diagnosed before puberty (Tattersall & Pyke, 1973).

Although the more severe abnormalities of growth are uncommon with current treatment regimens, episodes of poor blood glucose control may still result in an inappropriately low growth velocity (Wise et al., 1992). Periods of improved blood glucose control and intensive insulin administration may also be associated with an increase in height velocity (Rudolf et al., 1982). A
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Reduction in the pubertal growth spurt in IDDM has been documented, particularly in girls, although this does not appear to have a profound effect on final height (Brown, M et al., 1994).

When the key role of IGF-I in mediating the growth promoting actions of GH is considered, it is perhaps surprising that abnormal growth patterns do not occur more frequently in IDDM. The explanation for this may lie in the relative roles of sex steroids and the GH/IGF-I axis in mediating the adolescent growth spurt. There is evidence to suggest that the growth spurt in males is more closely linked to androgen levels, in contrast to females where it is more closely related to changes in GH secretion (Merimee et al., 1991). Any disturbance in the GH/IGF-I axis during puberty might therefore be expected to affect growth in girls more profoundly, and indeed this appears to be the case (Brown, M et al., 1994). Local IGF-I production is also an important determinant of longitudinal bone growth (Isaksson et al., 1982; Schlechter et al., 1986), and the factors controlling IGF-I production by the chondrocyte may differ from those regulating IGF-I production by the liver (see Section 1.1.4).

1.11 GH/IGF-I and complications in IDDM

1.11.1 Retinopathy

Both GH and IGF-I have been linked to the development of the microvascular complications of diabetes. Although diabetic retinopathy may develop in the absence of GH (Rabin et al., 1984), the hormone may be important in determining the severity and rate of disease progression (Kohner et al., 1976; Merimee, 1978). Interestingly, hypophysectomy has also been shown to have a beneficial effect by delaying the progression of severe proliferative retinopathy (Lundbaek et al., 1989).

It is difficult to distinguish the effects of GH on retinopathy from those of IGF-I. Circulating IGF-I concentrations in subjects with diabetic retinopathy have variously been described as being
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elevated (Ashton et al., 1983; Merimee et al., 1983), normal (Salardi et al., 1986), or depressed (Amer et al., 1989). Circulating IGF-I concentrations were not related to the presence of proliferative retinopathy in a group of subjects with IDDM in one large study, although the same group of workers identified a positive association between circulating levels and retinopathy in a group of adults with later-onset disease (Dills et al., 1990, 1991). The patients in these two studies (comprising individuals with IDDM and NIDDM) have been followed longitudinally and more recent assessment did not identify any link between circulating IGF-I concentrations and the development or progression of retinopathy (Wang et al., 1995). Similarly, Amiel and colleagues could not identify any difference in circulating IGF levels between young subjects who did, and did not have retinopathy (Amiel et al., 1984). Grant and colleagues have reported that circulating IGF-I concentrations are directly related to those in the vitreous in subjects with diabetes, but not in normal individuals, and are increased in the vitreous of patients with retinopathy (Grant et al., 1986).

The increase in IGF-I levels in the vitreous of subjects with diabetes, despite reports of lower circulating levels, could be due to a breakdown in the blood-retinal barrier with IGF-I originating from extraocular sources. Alternatively, IGF-I could be produced locally independent of, or subsequent to, the actions of GH within the eye. IGF-I has been shown to have potent effects on vascular proliferation in vivo (Grant et al., 1993). Thus, tissue anoxia could lead to vascular neogenesis with the paracrine production of growth factors, such as IGF-I, then being a part of this regenerative process (Merimee, 1990; Meyer-Schwickerath et al., 1993). Insulin is a known mitogen (Hill & Milner, 1985), and high concentrations could also affect cell multiplication and the paracrine production of IGF-I.
1.11.2 Nephropathy

Diabetic nephropathy is a major cause of morbidity and mortality in IDDM (Borch-Johnsen & Kreiner, 1987). However, in recent years the proportion of subjects with IDDM developing this complication appears to be falling, which could be the consequence of improvements in glycaemic control (Kofoed-Enevoldsen et al., 1987; Bojestig et al., 1994).

Renal hypertrophy and glomerular hyperfiltration can be detected at an early stage in IDDM (Mogensen & Andersen, 1973; Viberti, 1979), although only a proportion of patients will subsequently develop proteinuria and renal failure, and the exact relationship with preceding hyperfiltration is not known. Those subjects who develop diabetic nephropathy appear to pass through a period of microalbuminuria (Viberti et al., 1982; Mogensen, 1987). Although microalbuminuria is clearly a risk factor for the development of nephropathy, more recent study has suggested a lower degree of specificity than had previously been indicated (Almdal et al., 1994).

Glomerular filtration rate (GFR) correlates with insulin dose and the GH secretory response to GHＲＨ in IDDM, with hyperfiltrating subjects having greater GH responses (Blanketijn et al., 1993). Exogenous GH administration leads to a rise in renal plasma flow and GFR (Hirschberg et al., 1989) and administration of SMS, which is known to reduce GH secretion, leads to a fall in GFR and renal plasma flow in normals and subjects with IDDM (Vora,DR et al., 1987; Pedersen et al., 1990). Urinary albumin excretion rate is also related to GH concentrations in subjects with acromegaly (Hoogenberg et al., 1993), although renal insufficiency is uncommon in this disease.

IGF-I receptors have been identified in human glomeruli (Chin & Bondy, 1992), and IGF-I has been shown to bind to these sites and increase mesangial cell proliferation (Conti et al., 1988; Doi et al., 1989). Although the effects of GH on the kidney are largely mediated by changes in IGF-I concentrations (Hirschberg & Kopple, 1989; Hirschberg et al., 1989), and the fetal kidney can
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manufacture IGF-I (Han et al., 1988), the ability of the adult kidney to produce the peptide is the subject of some debate (Bell et al., 1985; Aron et al., 1989; Chin & Bondy, 1992).

The overall relationship between the GH/IGF-I axis and the development of diabetic nephropathy is not known.

1.1.3 Macrovascular complications and growth factors

An association between elevated insulin levels and cardiovascular disease has been recognised for some time (Nikkilä et al., 1965). The mitogenic actions of insulin can induce proliferation and growth of arterial smooth muscle cells which are the primary cell type in atheromatous lesions (Stout et al., 1975; Pfeifle & Ditschuneit, 1981). This process, together with the accumulation of extracellular matrix, may be accelerated or indeed initiated by endothelial cell damage because of elevated blood glucose concentrations and the altered lipid profile in IDDM.

Although IGF-I binding by endothelial cells in experimental diabetes is enhanced (Kwok et al., 1993), aortic endothelium, unlike retinal endothelium, is relatively unresponsive to the growth promoting actions of IGF-I (King et al., 1985). Conversely, aortic smooth muscle cells are sensitive to IGF-I effects (King et al., 1985), and in-vitro studies have shown that smooth muscle cells can produce IGF-I which may in turn be involved in cell replication (Clemmons, 1985; Clemmons & Van Wyk, 1985). The potential impact of enhanced GH secretion on the local production of IGF-I and subsequent links with vessel plaque formation and macrovascular disease in IDDM are also uncertain.
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Section 3: STUDIES OF RECOMBINANT IGF-I ADMINISTRATION

1.12 Animal Studies

1.12.1 Introduction

The studies described in this thesis focus on the use of recombinant IGF-I (rhIGF-I) in IDDM during adolescence, and it is therefore pertinent to discuss the development, physiological study and potential uses of the recombinant peptide. In the following sections of this chapter, in-vivo studies of IGF-I (including administration of rhIGF-I) in animals and humans will be presented.

1.12.2 Recombinant peptide production

Recombinant biosynthetic techniques have been used to produce IGF-I, and the development of an analogue of the peptide (differing from human IGF-I by one amino-acid substitution) was first reported in 1985 (Peters et al., 1985). Attempts at recombinant IGF-I (rhIGF-I) production in a bacterial system (Escherichia Coli) were complicated by accumulation of denatured protein in inclusion bodies. However gene fusion with the sequence coding for an exported protein led to a protein which could be isolated relatively easily and cleaved to produce rhIGF-I (Moks et al., 1987). Yeast strains can also be used to produce recombinant peptide. A pre-propeptide is expressed and subsequently cleaved within the cell to produce peptide which is then secreted into the surrounding medium (Bayne et al., 1988). The production of rhIGF-I has provided larger quantities of the peptide than were previously available, and has enabled IGF-I actions to be studied in greater detail.

1.12.3 Growth promoting effects of rhIGF-I in animals

IGF-I (extracted from human serum) infused into hypophysectomized rats leads to an increase in epiphysial width, thymidine incorporation into costal cartilage and weight gain (Schoenle
et al., 1982), and the growth-promoting effects of IGF-I administration in the longer term have been confirmed in dwarf mice (Van Buul-Offers et al., 1986). A comparison of the effects of IGF-I and GH infusions in hypophysectomized rats has shown that GH is more effective at inducing longitudinal bone growth (Schoenle et al., 1985). The administration of rhIGF-I to GH deficient or hypophysectomized rats also results in a proportionately greater increase in the size of the kidneys, adrenals and spleen, in contrast to the uniform growth induced by GH (Guler et al., 1988; Skottner et al., 1989). Disproportionate effects on organ growth have also been documented in male sheep following an 8 week period of rhIGF-I administration (Cottam et al., 1992). In this species there were minimal effects on overall body size when compared to a control group, despite a 50% increase in circulating IGF-I concentrations. Interestingly, blood glucose concentrations were higher in the group receiving rhIGF-I.

The different effects of GH and IGF-I on bone and organ growth can be explained in a number of ways. This may indicate the importance of direct GH effects (that are IGF-independent) or of local GH-induced IGF-I production in the growth process. There is also evidence to suggest that local IGF-I production may be suppressed by exogenous IGF-I; this could be due to a reduction in GH release, or be the consequence of a more direct suppressive effect of administered peptide (Gluckman & Butler, 1994). GH also has lipolytic actions and effects on hepatic glucose production which, by increasing substrate delivery, might facilitate the growth process. The diabetogenic effect of GH may in turn stimulate insulin secretion, an anabolic hormone, in contrast to IGF-I which appears to suppress insulin secretion as will be discussed later in this chapter. Finally, GH is a regulatory factor in IGFBP production, and the maintenance of adequate circulating and local IGFBP concentrations may be essential for normal IGF function.
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1.12.4 The acute metabolic actions of rhIGF-I in animals

RhIGF-I has acute insulin-like effects in intact animals. Schmitz and colleagues studied the effects of varying intravenous boluses of insulin and rhIGF-I in rats and concluded that the effects of rhIGF-I were mediated by interaction with the insulin receptor (Schmitz et al., 1991). To achieve approximately equal rates of glucose disposal it was found that 30 times the molar dose of rhIGF-I had to be administered. Many investigators have concluded that equivalent glucose-lowering doses of rhIGF-I and insulin act in different ways, however, with a greater impact of administered rhIGF-I on glucose disposal than on glucose production (Jacob et al., 1989; Moxley et al., 1990). This was thought by some investigators to be consistent with rhIGF-I effects on glucose production that were mediated primarily by interaction with the insulin receptor, and glucose disposal by the IGF-I receptor (Moxley et al., 1990).

Observations of acute rhIGF-I effects in depancreatized dogs (Giacca et al., 1990), and lambs (Douglas et al., 1991a), have provided further support for a preferential effect of rhIGF-I on blood glucose disposal. Giacca and colleagues concluded that, in their experimental setting, rhIGF-I had around 10% of the glucose lowering actions of insulin but that there was a greater impact on muscle as opposed to liver metabolism (Giacca et al., 1990). Both sets of investigators believed that effects were mediated, in part, through the IGF-I receptor.

The effects of rhIGF-I on fat metabolism were also examined in the above studies. The peptide was found to have either no discernible effect, or a reduced impact when compared to equivalent glucose lowering doses of insulin (Jacob et al., 1989; Giacca et al., 1990; Douglas et al., 1991a). The administration of rhIGF-I has also been shown to reduce amino-acid levels which has been attributed to the inhibition of protein breakdown in the fasted rat (Jacob et al., 1989), but to a stimulatory effect on protein synthesis in fasted lambs (Douglas et al., 1991b). In-vivo rhIGF-I
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administration increases renal plasma flow and GFR and reduces renal vascular resistance in rats (Hirschberg & Kopple, 1989).

Thus the acute effects of rhIGF-I on glucose disposal appear to differ from those of insulin with respect to both relative potency and site of action. These differences reflect the in-vitro studies which have identified increased IGF-I receptor density in mammalian muscle, as opposed to hepatic and adipose tissue (Section 1.3.4).

1.12.5 Studies in experimental diabetes

IGF-I levels are low in the rat when diabetes is induced by the administration of streptozotocin (Phillips & Oravski, 1977; Franklin et al., 1979). Franklin and colleagues administered insulin to streptozotocin treated rats for 6 weeks and noted an increase in IGF-I (or non-suppressible insulin-like activity as it was then known) to control levels. Similar observations have been made in depancreatectomised dogs (Eigenmann et al., 1977).

A deficiency of insulin also appears to alter skeletal muscle IGF-I receptor expression; Bornfeldt and colleagues analysed hepatic IGF-I mRNA and skeletal muscle IGF-I receptor mRNA levels in diabetic rats and assessed the change in levels in response to a 5 day infusion of insulin or rhIGF-I (Bornfeldt et al., 1992). They found that the levels of hepatic IGF-I mRNA were reduced, and that of skeletal muscle IGF-I receptor mRNA increased. This pattern could be altered by insulin infusion, which increased hepatic IGF-I mRNA and decreased IGF-I receptor mRNA in skeletal muscle. IGF-I infusion did not affect the low levels of hepatic mRNA, but normalised skeletal muscle IGF-I receptor mRNA. This data is further confirmation of the importance of insulin in hepatic IGF-I production, and suggests that insulin may also regulate the IGF-I signalling pathway in skeletal muscle in-vivo.
Rossetti and colleagues looked at the effects of insulin and rhIGF-I on glucose metabolism in control and diabetic (partially pancreatectomized) rats (Rossetti et al., 1991). The effects of insulin and rhIGF-I on glucose uptake were similar, but not additive, in control rats. However the diabetic rats were resistant to the actions of insulin in comparison to rhIGF-I, and an additive effect of the two hormones could be demonstrated. This was thought to provide evidence of glucose disposal occurring following interaction with the IGF-I rather than the insulin receptor, and suggested a common post-receptor mechanism in control, but not diabetic rats (Rossetti et al., 1991).

The alteration in IGF-I receptor levels in skeletal muscle, and the in-vivo effects of rhIGF-I in experimental diabetes, suggest that IGF-I may have an active role in muscle metabolism, a role that may be disrupted by a reduction in circulating IGF-I or insulin concentrations.

1.13 RhIGF-I administration in normal human subjects

1.13.1 Effects of intravenous rhIGF-I administration on glucose metabolism

The first report of in-vivo IGF-I administration in normal human subjects was in 1987 (Guler et al., 1987). Intravenous bolus administration of rhIGF-I in a dose of 100 μg/kg led to hypoglycaemia, with mean peak IGF-I levels reaching 424 ng/ml of which most was free as opposed to bound peptide. In molar terms, rhIGF-I was said to have had 6% of the hypoglycaemic actions of insulin.

Since this report studies of rhIGF-I administration in normal man have been undertaken using a variety of doses, routes and lengths of administration. The studies have all been undertaken in adults rather than children, and in the following descriptions of intravenous rhIGF-I administration, euglycaemia was maintained by a glucose infusion unless stated otherwise.
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The effects of rhIGF-I infusion on glucose disposal were considered to be dose dependent in the studies of Turkalj and colleagues, where peak IGF-I levels ranged from 127 - 620 ng/ml (Turkalj et al., 1992). The principle effect of rhIGF-I was considered to be on muscle glucose uptake. Laager and colleagues showed that rhIGF-I infused over an 8h period in relatively high (30 µg/kg/h), and also low (5 µg/kg/h) doses, had 5% to 6% of the hypoglycaemic actions of insulin (Laager et al., 1993). Although insulin and glucagon secretion were suppressed by rhIGF-I administration, the peptide was thought to have had a preferential effect on glucose disposal as opposed to suppression of hepatic glucose output, and the authors concluded that effects were likely to be mediated via both the IGF-I and insulin receptors.

Other studies have suggested that rhIGF-I effects on glucose metabolism resemble those of insulin more closely (Boulware et al., 1992). Marked inhibition of hepatic glucose production was demonstrated, despite reductions in C-peptide and insulin levels, when a 20 µg/kg bolus and then 24 µg/kg/h infusion for 3h were administered (Boulware et al., 1992). Peak IGF-I levels reached 444 ng/ml of which 16% was free, unbound peptide. The reduced proportion of unbound peptide, when compared to the initial study by Guler and colleagues (Guler et al., 1987), probably reflects the extended period over which rhIGF-I was administered. Boulware and colleagues have since extended their initial studies of rhIGF-I administration in normal human subjects and again concluded that the metabolic effects of rhIGF-I were similar to those of insulin when administered in doses designed to produce a similar change in glucose uptake (Boulware et al., 1995).

Longer term studies of rhIGF-I administered in a dose of 10 µg/kg/h (without the concomitant infusion of glucose) have been shown to have a minimal effect on blood glucose concentrations (Mauras et al., 1992; Hussain et al., 1993), although there was an associated reduction in C-peptide and insulin concentrations.
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The mechanism responsible for the alteration in glucose metabolism in many of these studies is not always clear, partly because the effect of rhIGF-I on hepatic glucose output in the face of alterations in insulin and glucagon concentrations is difficult to determine. The effects of rhIGF-I on insulin and glucagon metabolism will be considered in greater detail later in this chapter.

1.13.2 Subcutaneous rhIGF-I administration

The effects of subcutaneously administered rhIGF-I on blood glucose concentrations, without concomitant glucose infusion, have also been studied. A subcutaneous infusion of rhIGF-I at a rate of 32 µg/kg/h led to hypoglycaemia at which point IGF-I levels had reached 683 ng/ml of which 18% (123 ng/ml) was free peptide (Guler et al., 1989a). Thereafter infusion at 20 µg/kg for 5 days and 6 days in 2 subjects (one of whom had received the earlier dose of 32 µg/kg) did not lead to hypoglycaemia and resulted in peak levels of between 920 and 980 ng/ml with free peptide levels in the range 50 – 80 ng/ml (5 - 8%).

Administration of a subcutaneous bolus of rhIGF-I in a dose of 100 µg/kg for 7 successive days to fed subjects resulted in a reduction in blood glucose levels but not hypoglycaemia (Takano et al., 1991). Peak IGF-I levels reached 606 ng/ml after the first injection, and 644 ng/ml after the final injection. Free IGF-I increased from 1% (2 ng/ml) to a maximum of 9% (43 ng/ml) of total IGF-I concentrations after the first injection, and were not significantly different after the final rhIGF-I dose. rhIGF-I administration by subcutaneous bolus in a dose of 80 µg/kg and 40 µg/kg, and repeated daily administration in a dose of 40 µg/kg, did not result in hypoglycaemic symptoms in fasting subjects (Wilton et al., 1991). Fasting insulin levels were unchanged but post-prandial insulin levels were lower when compared to placebo administration.
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It can be concluded that the effects of rhIGF-I administration on blood glucose concentrations depend on a number of factors. These include the route and method of administration, the dose used and whether or not subjects are fed or fasted. Thus hypoglycaemia is more likely with larger bolus doses administered intravenously, which will tend to result in increased levels of the free peptide. A proportion of free IGF-I above 10% seems to be associated with an increased incidence of hypoglycaemia.

1.13.3 Effects of rhIGF-I administration on protein metabolism

RhIGF-I infusion reduces protein catabolism as indicated by changes in leucine flux and leucine oxidation (Turkalj et al., 1992; Laager et al., 1993), and by reduced nitrogen wasting (Clemmons et al., 1992). The effects on amino-acid metabolism are dose dependent (Boulware et al., 1992; Turkalj et al., 1992), and although greater than that of insulin at equivalent glucose-lowering doses (Laager et al., 1993), the effects appear to be qualitatively similar (Boulware et al., 1992; Giordano et al., 1995). Not all investigations have identified effects of rhIGF-I administration on protein catabolism however, although in the work reported by Mauras and colleagues glucose was not infused during the period of assessment in contrast to other studies (Mauras et al., 1992). Similarly, although enhanced protein synthesis was not detected following rhIGF-I administration by Turkalj and colleagues, subjects were fasting at the time of study (Turkalj et al., 1992).

1.13.4 Effects of rhIGF-I administration on fat metabolism

Short-term intravenous rhIGF-I administration reduces plasma triglyceride (Turkalj et al., 1992), FFA concentrations (Boulware et al., 1992; Turkalj et al., 1992; Laager et al., 1993) and ketone levels (Mauras et al., 1992; Turkalj et al., 1992; Laager et al., 1993), thereby suggesting inhibition of lipolysis. The magnitude of this effect is variously described as being similar (Boulware...
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et al., 1992), or less marked (Guler et al., 1987), when compared to an equivalent glucose-lowering
dose of insulin. Short-term rhIGF-I administration does not appear to affect cholesterol, LDL and
HDL levels (Turkalj et al., 1992).

Interestingly, the longer term administration of rhIGF-I over a period of 5 days by
subcutaneous infusion led to a rise in FFA levels (Hussain et al., 1993). Current evidence suggests
that the adipocyte may be relatively devoid of IGF-I receptors (see Section 1.3.4), and a
concomitant reduction in insulin concentrations is the likely explanation for these changes. When
equivalent glucose lowering doses of insulin and rhIGF-I were compared in normal subjects, the
reduction in FFA after insulin administration was more pronounced (Guler et al., 1987).

1.13.5 Effects of rhIGF-I on insulin secretion, insulin clearance and glucagon concentrations

Administration of intravenous rhIGF-I infusions leads to a reduction in insulin
concentrations and insulin secretion as reflected by reduced C-peptide levels (Boulware et al., 1992;
Mauras et al., 1992; Turkalj et al., 1992) and to a greater extent than equivalent glucose-lowering
doses of insulin (Laager et al., 1993). This occurs in a dose-dependent manner (Turkalj et al., 1992;
Zenobi et al., 1992a) and the effects are sustained (Hussain et al., 1993). Rennert and colleagues
have shown that this suppressive effect can partially be overcome by increasing the hyperglycaemic
stimulus for insulin release (Rennert et al., 1993).

Some investigators have noted a more pronounced reduction in C-peptide as opposed to
insulin concentrations following rhIGF-I administration, and have concluded that rhIGF-I reduces
insulin clearance (Guler et al., 1990; Rennert et al., 1993). Other investigators have reported a
greater percentage reduction in C-peptide than insulin, although this was not specifically linked to
changes in insulin clearance (Boulware et al., 1992; Hussain et al., 1993). In contrast Zenobi and
colleagues did not detect any change in the C-peptide/insulin ratio in their studies, and concluded that rhIGF-I did not affect insulin clearance (Zenobi et al., 1992a).

RhIGF-I administration has been shown to reduce glucagon levels in many studies (Boulware et al., 1992; Hartman et al., 1993; Rennert et al., 1993). Laager and colleagues noted similar effects of rhIGF-I and insulin on glucagon production and concluded that the less marked suppressive effect of rhIGF-I on hepatic glucose production was not, therefore, due to a differential action on glucagon suppression (Laager et al., 1993).

1.13.6 Effects of rhIGF-I on GH levels

GH concentrations rose during the first study of rhIGF-I administration to normal human subjects, although this was not before hypoglycaemia had occurred (Guler et al., 1987). When hypoglycaemia does not occur or is prevented, GH concentrations are reduced (Zenobi et al., 1992a; Hartman et al., 1993; Bermann et al., 1994). Subcutaneous rhIGF-I administration was found to reduce circulating GH concentrations (Guler et al., 1989a; Hussain et al., 1993), to reduce the GH response to arginine stimulation (Hussain et al., 1993), and GH secretion as determined by deconvolution analysis (Hartman et al., 1993). However not all studies of rhIGF-I administration have identified a reduction in GH release (Trainer et al., 1993). There is evidence to indicate that the change in GH release following rhIGF-I administration is due to a reduction in somatotroph responsiveness to GHRH, or alterations in hypothalamic SMS release (Hartman et al., 1993; Bermann et al., 1994).

1.13.7 Effects of rhIGF-I on insulin sensitivity

The administration of rhIGF-I has been reported to increase insulin sensitivity in healthy adults (Zenobi et al., 1992a; Hussain et al., 1993). This conclusion was based on normal glucose
tolerance in the presence of reduced free insulin levels. The proposed mechanisms include an increase in insulin sensitivity in association with reduced GH concentrations because of the known insulin-antagonistic actions of this hormone (described in section 1.5.5), and receptor up-regulation following a reduction in insulin levels (Kolterman et al., 1981). However a reduction in insulin levels may also be a consequence of the more direct effects of rhIGF-I on fuel disposal, and hence not a reflection of enhanced insulin action.

1.13.8 Effects of rhIGF-I on renal function and electrolytes

The administration of rhIGF-I leads to an increase in GFR measured by creatinine and insulin clearance (Guler et al., 1989a; Hirschberg et al., 1993). Hirschberg and colleagues (administering 60 μg/kg of rhIGF-I by subcutaneous injection three times daily for three days) also demonstrated a fall in renal vascular resistance and a rise in renal plasma flow, renal phosphate reabsorption and a rise in albumin and globulin excretion, but not above the normal range. The increase in albumin excretion was not solely linked to the rise in GFR because the fractional clearance of albumin was also increased. There was no associated change in renal sodium handling, or calcium excretion, and despite the renal phosphate retention, serum phosphate levels did not change.

Repeated daily subcutaneous administration of rhIGF-I in a dose of 40 μg/kg did not alter the urinary excretion of electrolytes, urea, creatinine or C-peptide (Takano et al., 1991), although there has also been a report of a rise in serum uric acid levels following rhIGF-I administration (Hartman et al., 1993).

It has been proposed that rhIGF-I could be of some value in acute and chronic renal insufficiency because of the effects of the peptide on renal function (Hammerman & Miller, 1993), and preliminary studies to investigate the therapeutic potential of the peptide in this clinical setting have been undertaken (O'Shea et al., 1993).
1.13.9 Conclusions

Many of the described effects of rhIGF-I on glucose, fat and protein metabolism are qualitatively similar to those of insulin. However there are relative differences between the actions of insulin and rhIGF-I that may reflect the distribution of their respective receptors. Thus it appears that administered rhIGF-I does not act only through the insulin receptor. The suppression of insulin secretion following rhIGF-I administration is a consistent observation, and may explain enhanced hepatic glucose production and lipid oxidation in some studies.

1.14 RhIGF-I as a therapeutic agent

1.14.1 Growth hormone insensitivity

Many studies of rhIGF-I administration in humans have been conducted in patients with growth hormone insensitivity (GHI). These studies will not be considered in detail in this thesis, but the condition is mentioned for historical reasons, and because they have provided information of relevance when considering administration in other clinical settings.

A syndrome of insensitivity to the actions of GH was described by Laron in 1966 (Laron et al., 1966). The first reports were of an autosomal recessive disorder where affected homozygous individuals had a characteristic phenotype and high circulating GH concentrations. The postnatal course of subjects with this disorder (Laron-Type Dwarfism) was nevertheless characterised by growth failure with a markedly reduced final height. It was subsequently shown that IGF-I levels were low in affected individuals, and that GH was unable to bind to the hepatic GH receptor (Eshet et al., 1984).

As classically described subjects with this disorder have a constellation of somewhat predictable abnormalities; normal or elevated GH, low IGF-I, IGFBP-3 and low circulating IGF-II
concentrations. The levels of GHBP in subjects with GH receptor deficiency are low, and IGF-I receptor sites have been shown to be increased (Eshet et al., 1991).

The first descriptions of insensitivity to GH actions were in patients from specific ethnic groups (Laron et al., 1968; Rosenbloom et al., 1990). Further subjects from more diverse backgrounds and with other defects in GH signalling, collectively termed the syndromes of GH insensitivity (GHI), have since been reported (Savage et al., 1993). Thus the GHI syndromes are a heterogeneous range of disorders containing subgroups with defects in IGF-I generation originating from abnormalities of GH signal transduction as well as a deficiency of the GH receptor (Savage et al., 1992). For this reason, not all subjects with GHI will have low levels of GHBP (Buchanan et al., 1991).

With the advent of rhIGF-I there has been a logical approach to treating patients with GHI. The first report of intravenous bolus administration of rhIGF-I to subjects with GHI described a fall in blood glucose and a rise in GH concentrations (Laron et al., 1988). The change in GH was probably due to the fall in blood glucose concentrations and associated counter-regulatory response. RhIGF-I treatment has since been shown to decrease plasma GHRH (Laron et al., 1990) and GH levels (Laron et al., 1991; Cotterill et al., 1993a). Longer-term subcutaneous rhIGF-I administration leads to an increase in linear growth, head circumference, and an increase in body weight but with reductions in subcutaneous fat (Laron et al., 1992).

1.14.2 RhIGF-I administration in insulin resistance states

The actions of rhIGF-I on feed disposal have led to the peptide being considered as a possible therapeutic agent in conditions where the principal abnormality is one of resistance to the actions of insulin. Type A insulin resistance is characterized by glucose intolerance, hyperinsulinism, acanthosis nigricans, hyperandrogenism and virilization (Kahn et al., 1976). The disorder is due to
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genetic abnormalities of the insulin receptor (Moller, DE & Flier, 1988; Odawara et al., 1989).

RhIGF-I administration in intravenous bolus doses of 100 μg/kg to subjects with this condition led to a fall in glucose, insulin and C-peptide concentrations, which was presumed to be due to interaction between administered peptide and the IGF-I receptor (Schoenle et al., 1991). There was a concurrent reduction in GH and IGF-II concentrations. The authors comment on the reduced insulin/C-peptide ratio which was presumed to be related to impaired hepatic insulin breakdown because of the abnormal insulin receptor (C-peptide is eliminated by the kidney and not the liver). Quinn and colleagues also reported a fall in insulin, C-peptide and GH concentrations in a patient with Mendenhall's syndrome, which is characterized by short stature, acanthosis nigricans and extreme insulin resistance (Quin et al., 1994).

Administration of rhIGF-I to a 16 year old girl with an early history compatible with IDDM has also been reported (Usala et al., 1992). At the time of treatment she had experienced repeated episodes of hyperglycaemia, and was considered to have an underlying resistance to insulin action which was confirmed by in-vitro adipocyte studies. Although IGF-I concentrations were within normal limits, the weekly administration of intravenous rhIGF-I resulted in a significant therapeutic effect because of what was presumed to be an associated rise in insulin sensitivity. The authors point to a possible synergistic effect on insulin action because of the relatively low dose of insulin required to maintain euglycaemia after each weekly dose of rhIGF-I.

1.14.3 RhIGF-I administration in NIDDM

RhIGF-I administration (120 μg/kg twice daily for 5 days) to a group of adults with NIDDM led to reductions in glucose, insulin, C-peptide and triglyceride levels (Zenobi et al., 1992b). Glucagon levels were unchanged. Free IGF-I concentrations reached a maximum of 7.7% of total IGF-I levels. The C-peptide/insulin ratio was unchanged but the insulin/glucose ratio (an
index of tissue sensitivity to insulin) was decreased. Glucose and insulin concentrations were also lower in the period following rhIGF-I administration when IGF-I levels had returned to baseline. Although this indicates a sustained effect of rhIGF-I on insulin sensitivity, the mechanism is uncertain. Possible explanations include the reduction in insulin concentrations during rhIGF-I administration with subsequent insulin receptor up-regulation, enhanced insulin sensitivity in association with reduced blood glucose levels, or the result of the sustained reduction in GH concentrations. Other more direct effects of rhIGF-I at the insulin or IGF-I receptor cannot be discounted. Schalch and colleagues reported similar reductions in glucose, insulin and triglyceride following the subcutaneous administration of rhIGF-I in a dose of 90 - 160 µg/kg for 5 days (Schalch et al., 1993). Both sets of observers noted greater decrements in these parameters in subjects with higher baseline glucose concentrations. This favoured effects of rhIGF-I acting via its own receptor because insulin administration would be expected to have the opposite effect, with greater reductions in the more insulin sensitive subjects with lower blood glucose levels (Zenobi et al., 1992b; Schalch et al., 1993).

1.14.4 The effects of rhIGF-I on renal function in NIDDM and insulin-resistant states

Schalch and colleagues reported increases in creatinine clearance in their studies of patients with NIDDM (Schalch et al., 1993). Albumin excretion was unchanged. Hypophosphataemia has been reported following rhIGF-I administration in NIDDM and insulin resistant states (Usala et al., 1992; Quin et al., 1994), together with reductions in serum creatinine, magnesium and alkaline phosphatase (Quin et al., 1994). Reductions in uric acid have also been noted in contrast to studies in normal subjects (Schalch et al., 1993).
1.14.5 The side effects of administration in NIDDM

Bolus rhIGF-I administration (65 μg/kg) to a group of thirteen normal and NIDDM subjects led to dizziness, weakness, hypotension and a relative bradycardia in two individuals who were excluded from further study analysis (Laager & Keller, 1993). One of these subjects had a short, generalised tonic-clonic seizure. Details about prevailing blood glucose concentrations, and whether those concerned were in the normal or NIDDM group, were not reported. Both subjects were said to have recovered after approximately 15 minutes.

Oedema of the face and peripheries was described in studies using 90 - 160 μg/kg of rhIGF-I administered subcutaneously twice daily for 5 days (Schalch et al., 1993) and swelling and tenderness of the parotid gland and angle of the mandible has also been reported (Zenobi et al., 1992b; Young & Clemmons, 1994). An asymptomatic increase in heart rate of between 9% and 17% has been noted (Zenobi et al., 1992b; Schalch et al., 1993) together with asymptomatic postural hypotension (Schalch et al., 1993). All presumed side effects resolved when rhIGF-I was stopped.

Jabri and colleagues planned a longer term study of rhIGF-I administration in obese insulin resistant patients with NIDDM in doses of 120 - 160 μg/kg twice daily (Jabri et al., 1994). The study was terminated because of adverse effects which were observed in all subjects. Side effects included oedema of the face and peripheries, jaw tenderness, arthralgias and myalgias, tachycardia, flushing and postural hypotension. The oedema was believed to be due to fluid retention and possibly also vascular leakage.

Tachycardia, orthostatic hypotension, oedema, parotid tenderness and headaches have been observed in many studies of rhIGF-I in normal subjects and individuals with GHI. Many of these symptoms have been attributed to fluid retention, and many have been observed only when pharmacological doses of rhIGF-I have been administered. Whilst the hypoglycaemic effects of
rhIGF-I have been described earlier in this chapter (Sections 1.12.4 and 1.13.1) there is, interestingly, evidence that rhIGF-I may enhance the awareness of hypoglycaemia despite effects of the peptide on GH and glucagon secretion (Kerr et al., 1993).

1.15 Rationale for administering rhIGF-I in IDDM during adolescence

In recent years much has been learnt about the role of GH and the IGF's in health and disease. Although the fundamental abnormality in IDDM is reduced pancreatic insulin secretion it has become clear that the relationship between GH and the IGF's is also disturbed. GH secretion is abnormally high during adolescence in IDDM, and yet the concentrations of the more distal parameter in this axis, IGF-I, are reduced. Growth is not usually compromised to a major extent in IDDM, but these changes may have implications for short and longer term health. Poor metabolic control during adolescence may be related to reductions in insulin sensitivity associated with the abnormally high GH concentrations. GH hypersecretion and peripheral hyperinsulinaemia may also be adverse factors in the development of the micro- and macrovascular complications of IDDM. Altered IGF-I bioactivity may have further, perhaps more direct effects on glucose disposal in this disorder as well.

The potential benefits of reducing GH secretion in IDDM have been considered for a number of years, and anticholinergic drugs such as pirenzipine have been proposed as potential therapeutic agents (Page et al., 1987). The effect of SMS analogues on counter-regulatory hormone levels and insulin requirements has also been studied, and a reduction in postprandial hyperglycaemia and glucagon levels noted (Serrano Rios et al., 1986). The role of SMS analogues in the improvement of blood glucose control in adults may indeed require further study, although their use in growing, pubertal subjects would be expected to result in growth arrest.
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The rationale for physiological and clinical studies of rhIGF-I in adolescents with IDDM is based on the effects of rhIGF-I administration in animals and known abnormalities of the GH/IGF-I axis. It could be postulated that IGF-I replacement will lead to a rise in circulating IGF-I concentrations and IGF bioactivity. An associated fall in GH levels could lead to enhanced insulin sensitivity with the potential for an improvement in blood glucose control which may be most noticeable in the early morning when the rise in insulin requirements is not easily met by current insulin regimens. In the short term the likelihood of alternating hypo- and hyperglycaemia may be reduced, and in the longer term improved glycaemic control, together with reductions in GH levels and peripheral insulinemia, may have a beneficial impact on the development of micro- and macrovascular complications and on the development of excessive weight gain.
Chapter 2

METHODS

2.1 Introduction

The studies reported in the following chapters were undertaken in adolescents with IDDM during overnight admissions to the John Radcliffe Hospital in Oxford. In some instances the study period extended into the following day and samples were also obtained at the time of evening home visits. The basic design of the studies presented in chapters 3, 5 and 6 was a comparison of nights when rhIGF-I was administered with a control study when either no injection or a placebo was given. In these studies rhIGF-I or placebo was administered as a single bolus dose in the thigh, whilst in chapter 4 comparison is made between administration of rhIGF-I in the thigh and abdomen. All of these investigations will be referred to as the single bolus studies.

Chapter 7 describes studies where the daily administration of rhIGF-I to adolescents with IDDM was continued for 28 successive days. An extended range of data was collected during this study and the protocol is more complex. This shall be referred to as the 'longer-term' study of rhIGF-I administration to distinguish it from the single bolus investigations.

The methodology described in this chapter applies primarily to the bolus studies. This will then be extended to describe specific details of the protocol in the longer term study of rhIGF-I administration at the beginning of chapter 7. All of the described studies were approved by the Central Oxford Research Ethics Committee and subjects were recruited from the Children and Young Persons' Diabetes Clinic at the John Radcliffe Hospital in Oxford. A home visit was arranged to discuss the rationale for the studies in detail and to explain the study protocol to the family. Written and informed consent was then obtained from the subject and their parents.
2.2 Subjects and subject assessment

2.2.1 Subjects

A total of 17 subjects participated in the single bolus investigations. Details of the subject group participating in each study are given at the beginning of the relevant chapter and individual subject details are listed in Appendix 1. All subjects were in late puberty and presented with IDDM at least 3 years prior to the study to ensure minimal endogenous insulin production. All were in good general health and were non-obese (Section 2.2.2) with normal hepatic, thyroid and renal function with no proteinuria (‘Albustix’ negative; Ames, Miles Laboratories, Slough, UK). All subjects were using a combination of short and intermediate acting insulin administered either twice or four times daily. Subjects were not taking any regular medication apart from one individual who was on inhaled beclomethasone dipropionate (200 µg three times daily) for the treatment of asthma, and one subject who was on the combined oral contraceptive pill (Binovum, Ortho Pharmaceuticals Ltd; 21 day course comprising 500µg norethisterone and 35µg ethinyloestradiol for 7 days, and then 1mg norethisterone and 35µg ethinyloestradiol for 14 days). This subject was studied on two occasions, 28 days apart, at the start of respective menstrual cycles.

2.2.2 Auxological techniques

Pubertal development was assessed according to the method of Tanner (Tanner, 1962) with male subjects examined by the author and female subjects by Lynne Ahmed, the clinic auxologist. Genital stage was assessed in male subjects (G1-G5) and breast stage in females (B1-B5). Height and weight were measured by the author using techniques described by Buckler (Buckler, 1979). Body mass index (BMI) was calculated from these values (BMI = weight (kg) / height (m)^2) with obesity defined as a BMI greater than 30 kg/m^2. Height velocity was derived from measurements
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recorded at the time of the studies and from values documented by Lynne Ahmed in the children and young persons diabetes clinic.

2.3 Study design
2.3.1 Pre-study insulin arrangements

Subjects stopped all intermediate-acting insulin 36h before admission to hospital. Blood glucose concentrations were then controlled with four daily injections of soluble insulin. Subjects were advised to undertake regular pre-meal home blood glucose monitoring to help calculate the insulin dose required to maintain blood glucose concentrations close to the euglycaemic range. A possible regimen was discussed with each subject to ensure that blood glucose concentrations were controlled with minimal hypo- or hyperglycaemia; they were advised to administer the final injection of soluble insulin as late in the evening as possible, and with a large snack to prevent hypoglycaemia in the early part of the night, and hyperglycaemia at the time of waking. The final subcutaneous insulin dose was at lunchtime on the day of admission. Subjects were also advised to monitor home blood glucose concentrations regularly between the two study periods in hospital.

2.3.2 Preparation in hospital

Subjects were admitted to hospital between 16.00h and 17.00h on the day of the study. They were weighed, measured, and local anaesthetic cream was applied to prospective cannulation sites. Two 18 gauge cannulae (‘Venflon 2’, BOC Ohmeda AB, Helsingborg, Sweden) were then inserted, one into a distal forearm vein and the other into an antecubital fossa vein.

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2.3.3 rhIGF-I

The rhIGF-I used in all of these studies was produced by the yeast Saccharomyces Cerevisiae (Bayne et al., 1988). The peptide is secreted into the culture medium from where it is purified by liquid chromatography. The rhIGF-I is then sterilised by a filtration procedure and prepared, ready for administration, in glass cartridges at a concentration of 2 mg/ml.

The constituents of each cartridge, which contained 2.2 mg of rhIGF-I in a total volume of 1.1 ml, are shown in Table 2.1. The same constituents (but without rhIGF-I) were used in the preparation of the placebo injection.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount/cartridge</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhIGF-I</td>
<td>2.20 mg</td>
</tr>
<tr>
<td>Sodium phosphate, monobasic anhydrous</td>
<td>5.80 mg</td>
</tr>
<tr>
<td>(added as sodium phosphate x 1 H2O)</td>
<td></td>
</tr>
<tr>
<td>Sodium phosphate, diabasic anhydrous</td>
<td>0.98 mg</td>
</tr>
<tr>
<td>(added as sodium phosphate x 12 H2O)</td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>7.10 mg</td>
</tr>
<tr>
<td>Water for injection to a total of</td>
<td>1.1 ml</td>
</tr>
</tbody>
</table>

2.3.4 Administration of placebo or rhIGF-I

Each individual was admitted for a control night when no injection or an injection of placebo was given, and a further night or nights when rhIGF-I was administered. RhIGF-I was
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drawn up from the glass vial and administered at 18.00h in a dose of 40 μg/kg body weight by subcutaneous injection into the anterior aspect of the left thigh, or 5 cm either side of the umbilicus when administered in the abdomen.

Care was taken to ensure that the site selected did not have evidence of lipohypertrophy. The injections were administered by the author and the diabetes specialist nurse (Sally Strang) to male and female subjects respectively.

A standard, predetermined technique was used to administer the injection. A fold of subcutaneous fat was gathered between fingers and thumb of one hand, approximately 5 cm apart, and the skin entered at an angle of 45° with a 24 gauge needle.

The interval between scans was less than 3 weeks, except in the case of the subject on the combined oral contraceptive preparation who was admitted following the 21 day course of medication on each occasion. There is an interval of 7 days between the end of one monthly prescription and the beginning of the next, and so the studies were therefore 28 days apart.

2.3.5 Overnight protocol

A low fibre evening meal was consumed at 18.05h. A record was kept to ensure that this was of a similar quality on both study nights, and that the same quantity of carbohydrate was consumed on each occasion.

Subjects relaxed in the supine position throughout the evening. The lights were dimmed and the individual encouraged to sleep at the same time on both study nights. Blood samples were taken overnight between 18.00h and 08.00h.
2.3.6 Daytime protocol

Nine of the seventeen subjects remained in hospital beyond 08.00h until 16.00h in the evening (22h after rhIGF-I administration). Meals were consumed at 09.00h and 12.00h and were of similar quality and quantity during control and rhIGF-I studies. Subjects relaxed in the supine position throughout the day apart from a period of exercise on a bicycle ergometer between 11.00h and 11.15h. The exercise protocol is described in detail later in this chapter (Section 2.6).

2.4 Insulin administration

2.4.1 Introduction

An intravenous insulin infusion was commenced at the time of the evening meal in a dose that limited the associated rise in blood glucose and led to a decline towards the euglycaemic range. Beyond 20.00h the amount of insulin administered to maintain stable blood glucose concentrations was determined by one of two infusion protocols designed to maintain euglycaemia. Both recommend an infusion rate which is derived from prevailing blood glucose concentrations, but in one the infusion rate is governed by a computer programme, and in the other the rate is determined by an algorithm. The techniques are described further in the following two sections.

2.4.2 Computer-based euglycaemic 'clamp'

Using this technique blood glucose concentrations were controlled overnight by a computer programme loaded onto an Apple 2e computer. The programme was designed to maintain euglycaemia by adjusting the rate of infused insulin according to prevailing blood glucose concentrations (Matthews et al., 1990). The programme had successfully maintained overnight blood glucose concentrations within the euglycaemic range during other studies of adolescents with
IDDM that had been undertaken by members of the Paediatric Department in Oxford (Edge et al., 1990).

The programme calculates an initial insulin infusion rate based on the subject weight, sex, prevailing blood glucose concentration and the euglycaemic (or ‘clamp’) target. Glucose data are entered every 15 minutes. Thereafter the programme accumulates information on changes in blood glucose concentrations at a given insulin infusion rate, and this is then used to generate a dose response curve (an array) specific to that individual. The computer programme then selects an infusion rate according to the change in blood glucose concentration required at a particular moment in time, so that the clamp target is approached or maintained. When an infusion rate that has had an earlier dose response does not affect blood glucose concentration to the same extent at a later stage, then an infusion rate that is higher or lower, up or down the array will then be selected instead. This procedure continues as each blood glucose concentration is entered by the operator until a stable glucose in the immediate vicinity of the clamp target is reached. The clamp target in the studies where the computer programme was used to control blood glucose concentrations was 5 mmol/l. The insulin clamp programme was not introduced until beyond the evening meal to prevent the array developing during a fed, and therefore less insulin sensitive period. If the clamp programme had been used from the time of admission there would be the risk of ‘overshoot’ and subsequent hypoglycaemia as the change from fed to fasted state occurred.

The insulin infusate administered overnight consisted of 48 ml of normal saline to which 10 units of soluble insulin was added (Human Actrapid, Novo Nordisk Pharmaceuticals Ltd, Crawley, West Sussex, U.K.) together with 2 ml of the patients’ blood to prevent insulin adherence to the syringe and infusion set (Korchner et al., 1980).
2.43 Algorithm-based euglycaemic clamp

In some of the described studies insulin was administered intravenously according to the protocol described by Mokan and Gerich (Mokan & Gerich, 1992). The initial rate of insulin administration was based on the algorithm cited by these authors. Fifty units of soluble insulin (Human Actrapid, Novo Nordisk Pharmaceuticals Ltd, Crawley, West Sussex, U.K.) were added to a 500 ml bag of normal saline. 50ml of the infusate was run through the administration set before being connected to the subject in order to prevent later adherence to the plastic and loss of insulin activity (Peterson & et al., 1976).

The insulin infusion rate was intended to maintain blood glucose concentrations at 6.5 mmol/l overnight and was altered according to 30 minute blood glucose measurements. The algorithm was not always followed rigorously if it was clear that an inappropriate quantity of insulin was being administered with the risk of hypoglycaemia developing. This method of maintaining stable blood glucose concentrations was therefore not completely unbiased.

2.44 Daytime insulin administration

Early attempts at controlling blood glucose concentrations using the variable rate insulin infusion software confirmed that the reaction time of the programme was too slow to deliver appropriate quantities of insulin at meal times. The programme recommended infusion rates developed in the fasting state and did not adapt to a rise in blood glucose in time to prevent pronounced post-meal hyperglycaemia. An infusion rate was therefore selected by the author which was based on previous insulin sensitivity, prevailing blood glucose concentrations, and timing of meals. The administration of insulin during the day was not, therefore, unbiased.
2.5 Blood sampling technique

2.5.1 Introduction

The forearm used for blood sampling was maintained in a heated box to 'arterialise' the venous blood (McGuire et al., 1976; Liu et al., 1992). The constituents of venous blood will then approximate arterial samples (which have a higher blood glucose concentration) more closely. Blood also flows more easily from warm, vasodilated vessels. Blood samples were drawn at regular intervals manually, or by a mechanised, continuous sampling device.

2.5.2 Continuous sampling technique

The continuous sampling device is a means of obtaining heparinised blood samples at regular intervals. The 18 gauge cannula ('Venflon 2', BOC Ohmeda AB, Helsingborg, Sweden) inserted into the forearm was adapted by removing the rubber overhead valve using a number 7 stainless steel screw (gas sterilised with ethylene oxide prior to packaging). A double lumen was then created by inserting a luer cap on to the end of the cannula through which fine bore plastic tubing (Portex Ltd., Hythe, Kent, U.K.) had previously been fixed with epoxy-resin glue. A heparin solution (6250 units/ml) was then continuously infused through the overhead port linked to the outer lumen, and the central lumen used to withdraw samples. The central lumen of the cannula was connected to soft rubber tubing which was compressed at regular intervals by a Watson Marlow 101U peristaltic pump (Watson Marlow Ltd., Marlow, Bucks, U.K.). The negative pressure generated from aspirated heparinised blood in quantities that could be adjusted by speeding up or slowing down the rate at which the compressing arms rotate. Aliquots were collected every 15 minutes into a fraction collector (LKB fraction collector, LKB Instruments Ltd., Surrey, U.K.). The rate of the peristaltic pump was increased at 45 minutes past each hour to ensure that sufficient sample was collected for the assays undertaken every 60 minutes.
2.5.3 Manual intermittent sampling

Heparinised normal saline (1 u/ml) was infused at a low rate (approximately 5 ml per hour) through an extension set attached to a cannula via a 3-way tap (Vygon, Ecoven, France). Samples were obtained every 15 minutes from the forearm cannula by intermittent aspiration. Each sample was obtained by attaching a syringe to the vacant port of the 3-way tap, aspirating the dead space filled with heparinised saline (1 - 2 ml), and then attaching a fresh syringe to take the required sample volume. The dead space (filled with a mixture of heparinised saline and blood) was then replaced to reduce blood loss.

2.6 Exercise protocol

Between 11.00h and 11.15h on the morning after rhIGF-I administration subjects underwent a period of exercise to assess the GH secretory response to a standardised stimulus. Calculating a subject's maximum oxygen consumption (VO$_2$ max) and deriving a workload from this figure is the most accurate way of ensuring a standardised exercise test. This is a relatively complex procedure, however, and would have necessitated an extra visit to hospital before the first study night. It was felt that a more straightforward and yet reproducible exercise test was required. The test had to result in marked tachycardia (greater than 150 beats per minute), sweating but not exhaustion (despite varying degrees of fitness in the participating subjects), and be practicable with 2 cannulae in situ.

These criteria were satisfied by a protocol used in an earlier study of subjects with diabetes (Jefferson et al., 1985). This consisted of a 15 minute period of exercise on a bicycle ergometer at a setting of 2 Watts/kg body weight. The load was generated at a rate of 50 revolutions per minute (rpm). As no subject was obese (BMI greater than 27 kg/m$^2$) a calculation based on body weight was considered acceptable. The bicycle was calibrated according to the manufacturer's instructions.
Chapter 2.

(Tunturi, Finland), and adjusted so that with the pedal at the lowest point the subject's leg was fully extended with the ankle at an angle of 90°. Each subject was carefully observed during the protocol to ensure that the degree of exertion was at this pre-determined level.

2.7 Laboratory techniques

2.7.1 Introduction

The principal laboratory methods used in these studies are described in the following sections. The remaining analytical methods are described in Appendix 2.

Assays were undertaken in the laboratories at the John Radcliffe Hospital in Oxford apart from the IGF-I and IGF bioassay which were undertaken by Jennifer Jones and Alastair Taylor at the Institute of Child Health in London, U.K., the IGFBP-3 assay and chromatography studies which were undertaken by Jeff Holly and Sian Cwylan-Hughes at the Department of Medicine, Bristol Royal Infirmary, Bristol, U.K., the IGF-II assay which was undertaken by Professor Werner Blum at the University Children's Hospital, Tubingen, Germany and the ALS assay undertaken by Robert Baxter at the Kolling Institute of Medical Research, St Leonards, New South Wales, Australia.

Table 2.2 indicates the frequency with which the principle biochemical variables were measured during the overnight studies.

2.7.2 Glucose

Whole blood glucose was determined at the bedside by the glucose oxidase method using a Yellow Springs analyser (YSI analyser, Clandon Scientific Ltd, Aldershot, Hants, U.K.). The inter-assay coefficients of variation (CV's) were 3.4% at 4.9 mmol/l and 2.4% at 18.5 mmol/l.

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Table 2.2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sampling interval (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>15 or 30</td>
</tr>
<tr>
<td>GH</td>
<td>15</td>
</tr>
<tr>
<td>Insulin</td>
<td>60</td>
</tr>
<tr>
<td>Ketones and lactate</td>
<td>60</td>
</tr>
<tr>
<td>IGF-I</td>
<td>30 or 60</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>120</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>60</td>
</tr>
<tr>
<td>IGF-II</td>
<td>120</td>
</tr>
<tr>
<td>IGF bioactivity</td>
<td>180</td>
</tr>
<tr>
<td>ALS</td>
<td>12 (hourly)</td>
</tr>
</tbody>
</table>

Blood samples for the measurement of IGF-I concentrations were also taken at home from some subjects at +24, +48 and +72h following the rhIGF-I injection.

2.7.3 IGF-I

Plasma IGF-I levels were determined by radioimmunoassay (RIA) following acid ethanol extraction (Taylor et al., 1988). The IGF-I antiserum used was raised in a rabbit by immunisation with 125 μg of approximately 20% pure human IGF-1 from Cohn fraction IV and from a post-chromatofocusing stage (Morrell et al., 1986). 125I-labelled IGF-I was prepared by the iodogen...
method. All samples were assayed against a serum standard obtained from a pool of healthy normal adult males, and defined as one unit of IGF-I/ml (1 unit of IGF-I = 182 ng of IGF-I/ml). The maximum sensitivity of the assay was 18.2 ng/ml and 50% inhibition of binding of $^{125}$I-labelled IGF-I was observed at a concentration of 109.2 ng/ml. The intra-assay CV's were 5.2% and 4.8% at analyte levels of 27.5 ng/ml and 220 ng/ml respectively. The interassay CV's were 12.7% and 10.6% at analyte levels of 77 ng/ml and 242 ng/ml respectively.

2.7.4 IGF bioassay

Overall IGF bioactivity was determined by the method described by Spencer & Taylor and Taylor and colleagues (Spencer and Taylor, 1978; Taylor et al., 1990).

Cartilage sulphation activity of a pooled serum standard was measured by the uptake of $^{35}$S sulphate by small uniform discs of pre-adolescent porcine costal cartilage. The serum standard from a serum pool of healthy adult males (S4) was diluted to varying degrees with Hams F12 nutrient medium. By definition 1 ml of S4 contains 1 U of IGF. Samples from the subjects studied were then added to a series of S4/F12 medium dilutions and compared to S4 serum alone. The intrinsic stimulatory or inhibitory activity of the study serum was therefore indicated by the change in the potency of $^{35}$S sulphate uptake. Statistical parameters for the assay were: index of precision (Fieller’s g) 0.06 and the index of variation (lambda) 0.18.

2.7.5 Plasma free insulin

Plasma free insulin was measured by RIA as follows; 1.0ml of whole blood was added immediately after sampling to 0.6ml of ice-cold 25% polyethylene glycol (PEG; molecular weight 6000, Sigma Ltd, Poole U.K.) (Collins & Pickup, 1985) and then spun at 3000 rpm for 20 minutes before separation. The plasma was stored at -20° C and assayed by double-antibody RIA (Guildhay...
Chapter 2.

Antisera Ltd., Guildford, UK) modified from Morgan and Lazarow (Morgan & Lazarow, 1963). Interassay CV's at 12.2 mU/L and 47.2 mU/L were 5.5% and 8.6% respectively.

2.7.6 Intermediate metabolites

For ketone (3-hydroxybutyrate, acetoacetate) and lactate measurements, 1.0 ml of whole blood was added immediately after sampling to 3.0 ml of ice-cold 6% perchloric acid (PCA). After storage at 0-4°C overnight the samples were spun and the precipitate discarded prior to neutralisation by 2% and 20% potassium hydroxide and 6% PCA. The samples were assayed within one month by standard enzymatic techniques (Guttman & Wahlefeld, 1974; Mellanby & Williamson, 1974; Williamson & Mellanby, 1974). Inter- and intra-assay CV's in the 3-hydroxybutyrate (3-OHB) assay were 8.3% and 2.2% and in the acetoacetate (AcAc) assay were 5.3% and 2.2% respectively. Lactate was measured by standard enzymatic techniques. Lactate inter and intra-assay CV's were 5.1% and 1.0% respectively.

2.7.7 Growth hormone

Samples for GH assay were kept at room temperature until the profile was complete and then were spun, separated, and the plasma frozen at -20°C until assay. Plasma GH concentrations were measured by immunoradiometric assay (NETRIA) using an international reference standard 80/505. All the samples from each individual profile were analysed in the same batch. The inter-assay CV's at GH concentrations of 3.5, 15.2, and 77.4 mU/L were 9.4%, 7.7%, and 10.5% respectively and the intra-assay CV's at GH concentrations of 2.9, 14.3, and 69.4 mU/L were 8.0, 2.0 and 3.4% respectively.
2.7.8 IGFBP-3

Serum IGFBP-3 levels were determined using a double antibody RIA. IGFBP-3 antiserum (SCH-2/5, Celtrix Pharmaceuticals, Santa Clara, California, U.S.A.) was used at a final concentration of 1:8000. Recombinant glycosylated IGFBP-3 (Celtrix Pharmaceuticals, Santa Clara, California, U.S.A.) was used for standards, giving a range of 5 ng/ml to 500 ng/ml. Bound and free $^{125}$I-labelled glycosylated IGFBP-3 were separated using a donkey anti-rabbit SAC-CEL second antibody (I.D.S. Ltd. Bolden Business Park, Bolden, Tyne and Wear, U.K.). The inter-assay CV was 5.1% at 5 μg/ml and the intra-assay CV was 4.3% at 5 μg/ml.

2.7.9 IGFBP-1

Serum IGFBP-1 levels were determined by RIA as previously described by Holly and colleagues (Holly et al., 1988) using antisera and purified antigen provided by Dr H. Bohn (Behringwerke, Marburg, Germany). Tracer was prepared by iodination of IGFBP-1 using the chloramine-T method, followed by separation on a short Sephadex G75 column. Antiserum was used at a final dilution of 1:4000 which bound approximately 50% of iodinated tracer. Bound and free antigen were separated using a solid-phase second antibody (donkey anti-rabbit coated cellulose). The limit of detection of the assay was 6 μg/l. The intra-assay CV’s were 10.3% and 9.1% at 9 μg/l and 353 μg/l respectively. Interassay CV’s were 10.6% and 7.0% at 106 μg/l and 253 μg/l respectively.

2.7.10 IGF-II

IGF-II concentrations were also determined after acid ethanol extraction in the laboratories of Prof. W. Blum (University Children’s Hospital, Tubingen, Germany) using a specific RIA which
Chapter 2.

utilises excess IGF-I to block interference from IGF binding proteins (Blum et al., 1988). The inter-assay CV at 50% of maximum binding capacity was 12.2%, and the minimum detection limit was 0.018 ng.

2.7.11 ALS

The ALS was measured by a specific RIA using an antisera raised in New Zealand White rabbits following the injection of purified human ALS. Serum samples were incubated with antiserum, $^{125}$I-labelled ALS and RIA buffer as described by Baxter (Baxter, 1990). The standard curve was derived from incubations containing pure ALS. Bound and free $^{125}$I-labelled alpha-subunit were separated and counted after the addition of goat anti-rabbit immunoglobulin and normal rabbit serum. The intra-assay CV's were 3.4% at 5.1 mg/ml, 3.3% at 20.8 mg/ml and 3.4% at 42.7 mg/ml. The inter-assay CV's were 10.5% at 5.3 mg/ml, 5.4% at 24.0 mg/ml and 6.5% at 57.5 mg/ml.

2.7.12 IGF Chromatography

The distribution of IGF-I in the plasma of one subject were analysed at baseline and 3, 6, 12 and 18 h following the rhIGF-I injection and on the control night. Plasma samples were separated on a Superose-12 HR 10/30 column (Pharmacia LKB Biotechnology, Uppsala, Sweden) under neutral conditions. The flow rate of the column was 0.5 ml/min with 0.25 ml fractions collected. These fractions were then extracted and assayed for IGF-I immunoreactivity as described above.
2.7.13 Haemoglobin A1 (HbA1)

HbA1 was measured by agar electrophoresis using Ciba-Comin standards. The interassay CV at an HbA1 concentration of 7% was 7%, and at a concentration of 13% was 5.4%. The laboratory normal range was 4.5% to 8.0%.

2.7.14 Haemoglobin Alc (HbAlc)

HbAlc was measured by High Pressure Liquid Chromatography (HPLC) (Diamat, BioRad Laboratories Ltd, Hemel Hempstead, U.K.). The intra-assay CV’s were 1.9% and 2.2% at HbAlc levels of 6.9% and 11.5% respectively. The interassay CV’s were 2.7% and 2.3% at HbAlc levels of 7.0% and 11.6% respectively.

2.8 Analytical methods; Standard deviation scores

The impact of rhIGF-I administration on IGF-I concentrations was assessed by calculating a standard deviation score (SD score). SD scores can be used to compare variables that change during puberty by relating them to normal data at the appropriate puberty stage. A valid comparison between subjects at different stages of pubertal development can then be made. The standard formula for calculating the IGF-I concentration SD score (or IGF-I SD score) is therefore:

\[
\text{IGF-I SD score} = \frac{\text{IGF-I concentration (diabetes)} - \text{IGF-I concentration (normal)}}{\text{SD (normal)}}
\]

Normal reference data used by the Paediatric Endocrine Group in Oxford and collected from a longitudinal study of normal puberty were used in this calculation (Preece et al., 1884). This normal data set was accumulated from a large number of subjects and, importantly, distinctions
Chapter 2.

made between the early and later stages of Tanner puberty stage 5. IGF-I concentrations tend to
decline once late puberty has been reached and the normal data is divided according to the number
of years within a given puberty stage. The IGF-I assay used in the set of normal data was also
undertaken in the same laboratory, and using the same method, as the studies reported in this thesis.

2.9 Analytical methods; GH profile analysis

2.9.1 Introduction

GH profiles from the two studies were first assessed by comparing mean levels. This
approach can be easily applied to any set of hormone profiles, but does not provide information
about pulsatility and the hormone baseline characteristics. More sophisticated methods, described in
the following sections, were therefore applied as well.

2.9.2 Pulsar

The Pulsar program is a means of quantifying pulsatile hormone release and is a more
objective assessment of pulsatility than that gained by visual inspection (Merriam & Wachter, 1982).
The output provides information about pulse frequency and the hormone profile baseline. These
indices are closely linked to the hormone assay characteristics and can also be linked to probability
theory. Further details of the Pulsar programme and of the coefficients that were used in the
associated profile assessment are given in Appendix 3.

2.9.3 Distribution analysis

Distribution analysis (Matthews et al., 1991) is an observer unbiased method of analysing a
hormone profile. The profile is characterised according to the proportion of data points within a set
of concentration bands. This can then be expressed as a frequency, or cumulative frequency
Chapter 2.

distribution, the appearance of which will vary according to the profile characteristics. Further
details of this method of hormone profile analysis are given in Appendix 3.

2.9.4 Fourier Transformation

Fourier transformation describes a profile as the sum of a series of sinusoids. This
description has amplitude and frequency components and is a useful and unbiased means of
assessing a hormone profile providing more detailed information about pulsatility than pulse
counting methods (Matthews, 1988). The technique of Fourier analysis is described in more detail
in Appendix 3.

2.9.5 Deconvolution analysis

Deconvolution analysis determines the secretion rate underlying a hormone profile
(Hindmarsh et al., 1990), although information about GH half-life is required before this can be
undertaken. A variable GH half-life which is dependent on length of exposure and volume of
distribution data, determined by previous studies of adolescents with IDDM, were used in this
analysis (Mullis et al., 1992; Pal et al., 1993).

Deconvolution analysis was undertaken on 22h data but Pulsar, distribution analysis and
Fourier transformation were undertaken on 12h overnight data because of the known effects of
feeding and exercise on GH pulsatility.

2.10 Statistical methods

The principle means of determining whether parametric or non-parametric statistical
techniques were employed was by inspection of the respective data set. However with very small
samples (generally less than 7 or 8 single or paired observations) non-parametric tests were usually employed.

Where data were normally distributed then parametric statistical tests were used in the analysis; paired and unpaired Student’s t-test, one- and two-way analysis of variance and standard least squares linear regression. In some instances non-normally distributed data could be normalised by the process of log-transformation in which case parametric statistical techniques were also applied.

Data that was not normally distributed were analysed by non-parametric statistical techniques. The Wilcoxon matched pairs signed rank test was used for the analysis of paired samples, the Mann-Whitney U-test for non-paired samples and the Kruskal-Wallis one-way analysis of variance for changes with time.

A probability of less than 5% has been taken as evidence against the null hypothesis although attention may also be brought to values approaching this figure.

Where data is normally distributed then results are listed in the text as mean ± standard error of the mean (SEM). A range is also included with smaller samples where parametric techniques have been used. Data that is not normally distributed is expressed as a median and range.

Further statistical techniques are described in relevant chapters and in Appendix 4.
Chapter 3

**IGF-I LEVELS, GH CONCENTRATIONS AND INSULIN SENSITIVITY FOLLOWING rhIGF-I ADMINISTRATION**

### 3.1 Introduction

The primary aims of the studies described in this chapter were to assess the impact of subcutaneous rhIGF-I administration on IGF-I and GH concentrations in adolescents with IDDM. By maintaining blood glucose concentrations within the euglycaemic range overnight the insulin requirements during control and rhIGF-I studies could also be compared.

### 3.2 Subjects and Methods

#### 3.2.1 Design

The study was double-blind and placebo controlled. Both injections (placebo or rhIGF-I in a dose of 40 μg/kg) were administered subcutaneously in the thigh at 18.00h. The computer-based euglycaemic insulin clamp was used to control blood glucose concentrations (Section 2.4.2). The continuous blood sampling technique was used to collect samples overnight and the intermittent technique employed during the day (Sections 2.5.2 and 2.5.3). Subjects exercised for a 15 minute period on a bicycle ergometer between 11.00h and 11.15h (Section 2.6) but were otherwise in the supine position during the study. Individuals were carefully supervised by the author throughout the period in hospital. The study protocol is illustrated in Figure 1.

#### 3.2.2 Study size

The following data were used when calculating the study size; pirenzipine (a cholinergic antagonist) administration leads to a median reduction in GH concentrations of approximately 50% (Edge et al., 1990b). Overnight mean GH concentrations in late pubertal adolescent males and
Figure 1.

Protocol assessing the impact of placebo or rhIGF-I administration on the GH/IGF-I axis in adolescents with IDDM.
females with IDDM are around 30mU/l (Edge et al., 1990a). The CV of GH concentrations is 35% with an SD of 9mU/l (Saini et al., 1991). Assuming that rhIGF-I reduces GH concentrations by an overall mean of 33%, then to detect this at the 5% level the number of subjects required to demonstrate a difference in GH secretion between placebo and rhIGF-I administration, with a power of 80%, is then greater than or equal to ten.

3.2.3 Subject characteristics

A total of ten late pubertal adolescents with IDDM aged between 14 and 18 years volunteered to take part in the study. Nine subjects completed the two 22h periods in hospital, with one subject withdrawing on the first night because of difficulties obtaining samples from the cannula. This subject had not, as it later transpired, receive rhIGF-I. Data refer to the nine subjects who completed the study. The full subject characteristics (subjects 1 to 9) are listed in Appendix 1 and are summarised in Table 3.1.

3.2.4 Exclusion criteria

The threshold at which counter-regulatory hormone release occurs will vary in a heterogeneous group of adolescents with IDDM such as those recruited in this study. Subjects with blood glucose concentrations of less than 3.5 mmol/l, and all subjects who experienced symptoms of hypoglycaemia during the study, were excluded from the insulin infusion, insulin, GH and ketone analysis. The rationale for selecting 3.5 mmol/l as the hypoglycaemic ‘threshold’ is discussed later in this chapter.
Table 3.1

Subject characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>14.0-18.9</td>
<td>5.1</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>3.0-13.8</td>
<td>6.6</td>
</tr>
<tr>
<td>C-peptide (nmol/l) (BG&gt;7.0mmol/l)</td>
<td>0.02-0.27</td>
<td>0.04</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18.8-25.9</td>
<td>22.3</td>
</tr>
<tr>
<td>Insulin dose (u/kg)</td>
<td>0.8-1.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (number of subjects)</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Puberty stage (genital/breast):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G4 B4</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>G5 B5</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

3.3 - 3.6 Results

3.3 Side effects of injection

The injection of placebo and rhIGF-I was associated with mild to moderate discomfort at the site of administration. This was said to be of an ‘aching’ or ‘stinging’ nature, and usually subsided within 5 minutes. Although the extent of the discomfort varied between individuals there was no obvious difference in intensity between the two injections in any one subject. This therefore suggests that symptoms were the result of the vehicle and not because of recombinant peptide.
Local discomfort at the site of rhIGF-I administration has since been reported in patients with GH insensitivity syndrome receiving a similar rhIGF-I preparation (Ranke & Wilton, 1994). There were no other overt problems following administration of placebo or rhIGF-I, and no subject experienced severe hypoglycaemic symptoms or an increase in the frequency of mild hypoglycaemia between or following the two study periods in hospital.

Of the nine subjects participating in these studies, five received rhIGF-I during the first study period.

3.4 IGF-I levels and IGF bioactivity

3.4.1 IGF-I concentrations

Baseline IGF-I concentrations were similar at the start of the two nights and did not alter significantly following the injection of placebo. IGF-I concentrations were elevated by 30 minutes after administration of rhIGF-I and reached a peak after 5.5h (Figure 2a). Maximum concentrations in individual subjects were reached between 3.5h and 12h post-injection. There was no relationship between baseline IGF-I concentrations and the time to peak levels (r= 0.23; p= 0.5). IGF-I concentrations were greater after rhIGF-I administration than placebo in all subjects at the end of the 22h period in hospital and were still elevated at the time of the final sample at +48h (n= 8).

IGF-I SD scores were used to assess the change in mean IGF-I concentrations following rhIGF-I administration. IGF-I concentrations were measured every 30 minutes initially and then at 60 minute intervals beyond 08.00h (14h after rhIGF-I administration). In the 22h period following administration of placebo the IGF-I SD scores (based on hourly IGF-I values) ranged from -4.48 to -0.21 (median -2.04) and were therefore all below the fiftieth percentile when compared to normal subjects. After rhIGF-I administration, IGF-I SD scores ranged from -1.92 to +1.46 (median -0.16)
Figure 2a.

Mean (± SEM) IGF-I concentrations for the 22h study period following administration of placebo (•) or rhIGF-I (O).

Figure 2b.

IGF-I standard deviation scores (SDS's) derived from 22h data following placebo and rhIGF-I administration.
Chapter 3.

and were therefore within normal limits (± 2SD). This data is illustrated in Figure 2b and the IGF-I data is summarised in Table 3.2.

3.4.2 IGF-I half-life

IGF-I half-life was calculated by estimation of the elimination rate constant by linear regression. The steepest gradient beyond the peak concentration (for a time interval of not less than 6h and not more than 9h) was selected from each individual data set by visual inspection of the calculated regression slope. Further details of the method used to calculate IGF-I half-life are included in Appendix 4.

The mean half-life of administered rhIGF-I was 21.4 ± 1.0h (range 17.4h - 26.1h). If the IGF-I concentration at 48h was included with the first and final data points from this calculation, then the derived half-life was considerably greater (73.2 ± 13.1h with a range of 31 - 150h). This suggests that second order pharmacokinetics, where log transformation of values describes a straight line, is not applicable over the longer time interval.

There was no relationship between rhIGF-I half-life and initial IGF-I concentrations.

3.4.3 IGF bioactivity

IGF bioactivity, measured at 2 or 3 hourly intervals during the 22h study period in 6 subjects, is illustrated in Figure 3.

Baseline IGF bioactivity tended to be higher on the night when rhIGF-I was administered; median 0.47 U/ml (range 0.38 - 0.96 U/ml) on the placebo night versus 0.86 U/ml (range 0.59 - 0.98 U/ml) on the rhIGF-I night (0.1< p >0.05). This discrepancy may have been due to the pooling of some of the blood samples taken at 18.00h and 18.15h because of insufficient baseline blood.
Figure 3.

Mean (± SEM) 22h IGF bioactivity following placebo (●) and rhIGF-I (○) administration
Table 3.2

IGF-I data during placebo and IGF-I studies (ng/ml). Mean ± SEM (range).

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>rhIGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline IGF-I</strong></td>
<td>195 ± 17</td>
<td>223 ± 19</td>
</tr>
<tr>
<td>(140 - 296)</td>
<td>(140-316)</td>
<td>p=0.72</td>
</tr>
<tr>
<td><strong>IGF-I +30'</strong></td>
<td>202 ± 19</td>
<td>252 ± 18</td>
</tr>
<tr>
<td>(147 - 298)</td>
<td>(165 - 340)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td><strong>Maximum IGF-I (Mean</strong></td>
<td>222 ± 26.0</td>
<td>413 ± 28</td>
</tr>
<tr>
<td><strong>of maximum values)</strong></td>
<td>(at 2.5h)</td>
<td>(at 5.5h)</td>
</tr>
<tr>
<td><strong>IGF-I+22h</strong></td>
<td>187 ± 18</td>
<td>292 ± 30</td>
</tr>
<tr>
<td>(123 - 271)</td>
<td>(205 - 495)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td><strong>IGF-I+48h</strong></td>
<td>186 ± 18</td>
<td>292 ± 30</td>
</tr>
<tr>
<td>(131 - 271)</td>
<td>(205 - 495)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td><strong>Mean IGF-I (18.00-08.00)</strong></td>
<td>206 ± 22</td>
<td>359 ± 26</td>
</tr>
<tr>
<td></td>
<td>(135 - 347)</td>
<td>(277 - 518)</td>
</tr>
<tr>
<td><strong>Mean IGF-I (18.00-16.00)</strong></td>
<td>204 ± 21</td>
<td>341 ± 26</td>
</tr>
<tr>
<td></td>
<td>(137 - 327)</td>
<td>(263 - 509)</td>
</tr>
</tbody>
</table>

Overall IGF bioactivity during the 22h study (beyond the baseline sample) was a median of 45% of the serum standard after administration of placebo (0.45 U/ml; range 0.23 - 0.76 U/ml p< 0.05) compared to 91% following rhIGF-I administration (0.91 U/ml; range 0.62 - 1.03 U/ml). Peak bioactivity was seen 5h after rhIGF-I administration (1.01 U/ml). A clear reduction in IGF bioactivity was apparent by 14.00h, approximately 12h after peak IGF-I levels. Nevertheless IGF bioactivity was still elevated at the end of the 22h study period following rhIGF-I administration.
when compared to placebo (median 0.36 U/ml; range 0.18 - 0.65 U/ml after placebo, and a median of 0.68 U/ml; range 0.40 - 0.95 U/ml after rhIGF-I administration, p = 0.05).

Overall IGF bioactivity data in individual subjects beyond the baseline value is detailed in Table 3.3.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Placebo (median and range)</th>
<th>IGF-I (median and range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.25 (0.15 - 0.66)</td>
<td>0.97 (0.58 - 1.17)</td>
</tr>
<tr>
<td>5</td>
<td>0.68 (0.39 - 0.89)</td>
<td>0.97 (0.75 - 1.29)</td>
</tr>
<tr>
<td>6</td>
<td>0.73 (0.57 - 0.96)</td>
<td>0.97 (0.65 - 1.23)</td>
</tr>
<tr>
<td>7</td>
<td>0.50 (0.28 - 0.84)</td>
<td>0.94 (0.89 - 1.47)</td>
</tr>
<tr>
<td>9</td>
<td>0.25 (0.16 - 0.29)</td>
<td>0.74 (0.68 - 0.88)</td>
</tr>
<tr>
<td>10</td>
<td>0.31 (0.22 - 0.68)</td>
<td>0.64 (0.40 - 0.77)</td>
</tr>
</tbody>
</table>

The IGF-I bioassay compared study samples with a standard derived from adults. Although rhIGF-I administration led to an increase in IGF bioactivity the level remained low for adolescents in late puberty where mean values of approximately 1.7 U/ml and 2.2 U/ml have been reported (Taylor et al., 1988).

3.5 Effects of rhIGF-I on glucose, insulin infusion, insulin and ketone levels

3.5.1 Introduction

Overall data for the 22h study and for the separate periods of day and night will be presented in subsequent sections. The data has been divided in this way because it was hoped to achieve strict euglycaemia overnight whilst subjects slept. Blood glucose concentrations were not
expected to be as closely controlled during the daytime because subjects were fed, and because of
the period of exercise. Insulin infusion rates, free insulin and ketone levels are more easily
interpreted when the confounding effects of differing blood glucose concentrations are eliminated.
These variables will therefore be discussed in greatest detail for periods of stable euglycaemia.

Subjects spent some of the period between 08.00h and 09.00h in the bathroom, and were
detached from the insulin infusion for some of this time. This interval has not been included in the
insulin infusion calculation.

3.5.2 Blood glucose

Most subjects found that home blood glucose measurements tended to be higher than usual
prior to their arrival in hospital. Hyperglycaemia at the time of waking was a particular problem
following the change to a regimen comprising soluble insulin alone.

One individual (Subject 3) developed asymptomatic hypoglycaemia on both study nights
(whole blood glucose concentration below 3.5 mmol/l; 3.3 mmol/l after placebo and 2.5 mmol/l
after rhIGF-I administration). There were no other episodes of biochemical or symptomatic
hypoglycaemia throughout the 22h study period in any of the other subjects. Mean blood glucose
concentrations in the nine subjects taking part in the study are shown in Figure 4.

Mean blood glucose concentrations were reduced between 23.30h and 00.30h following
rhIGF-I administration when compared to the placebo night (5.4 ± 0.4 mmol/l after placebo and 4.5
± 0.2 after rhIGF-I administration; p= 0.04). The lowest mean blood glucose occurred slightly
earlier after rhIGF-I administration (5.4 ± 0.4 mmol/l at 00.15h after placebo compared to 4.4 ± 0.3
mmol/l at 23.30h after rhIGF-I administration). There was no difference in the lowest blood glucose
concentration reached in individual subjects when the two study periods were compared (3.9 ± 0.1
mmol/l after placebo and 4.0 ± 0.2 mmol/l after rhIGF-I administration; p= 0.9).
Figure 4.

Mean (± SEM) blood glucose concentrations for the 22h study period (n=9) after placebo (●) or rhIGF-I (○) administration.
Stable euglycaemia was reached by 02.00h on both nights. One-way analysis of variance and two-way analysis of variance, within and between placebo and rhIGF-I groups, did not reveal any difference in blood glucose values between 02.00h and 08.00h. Hypoglycaemia excluded subjects from the analysis of insulin infusion requirements, free insulin levels and ketone data, and so the same analysis was undertaken in the eight subjects who did not become hypoglycaemic. Again, there were no differences in blood glucose concentrations between 02.00h and 08.00h. Mean blood glucose concentrations in the eight subjects who did not become hypoglycaemic are shown in Figure 5.

The daytime blood glucose concentrations were, as anticipated, greater in all subjects after placebo and rhIGF-I administration when compared to the euglycaemic period overnight. There was no difference in blood glucose concentrations between the two groups during the day or for the overall study period. Blood glucose data during the study is summarised in Table 3.4.

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Placebo</th>
<th>rhIGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.00 - 16.00h (22h)</td>
<td>7.9 ± 0.3 (6.3 - 9.5)</td>
<td>7.8 ± 0.4 (6.4 - 10.4)</td>
</tr>
<tr>
<td>n=9</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>20.00 - 08.00h (12h)</td>
<td>6.3 ± 0.3 (5.3 - 8.1)</td>
<td>6.1 ± 0.2 (5.2 - 6.8)</td>
</tr>
<tr>
<td>n=8</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>02.00 - 08.00h (6h)</td>
<td>5.6 ± 0.1 (5.1 - 6.2)</td>
<td>5.5 ± 0.1 (5.2 - 5.8)</td>
</tr>
<tr>
<td>n=8</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>09.00 - 16.00h (7h)</td>
<td>10.2 ± 0.7 (7.0 - 15.7)</td>
<td>10.0 ± 0.9 (7.1 - 11.3)</td>
</tr>
<tr>
<td>n=8</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
Figure 5.

Mean blood glucose concentrations during the period of stable euglycaemia (02.00h - 08.00h) following placebo (●) or rhIGF-I (○) administration.

Subject 9 has been excluded because of overnight hypoglycaemia.
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3.5.3 Insulin infusion

There was no difference in the insulin infused between the time of placebo or rhIGF-I administration and the establishment of a stable insulin clamp at 02.00h (0.57 ± 0.08 mU/kg/min versus 0.51 ± 0.06 mU/Kg/min respectively; p= 0.4)

Analysis of the insulin infusion during the stable clamp between 02.00h and 08.00h (n= 8; Figure 6a) revealed a significant reduction in the insulin requirements to maintain euglycaemia after rhIGF-I when compared to the placebo night (0.25 ± 0.12 mU/kg/min versus 0.31 ± 0.07 mU/Kg/min; p= 0.03). The mean insulin infusion requirement was more stable following rhIGF-I administration, and there was absence of the rise beyond 05.00h seen on the placebo night (Figure 6b).

There was no difference in the insulin infused during the day (09.00h - 16.00h; 1.03 ± 0.12 mU/kg/min after placebo and 0.91 ± 0.08 mU/kg/min after rhIGF-I; p= 0.1).

3.5.4 Free insulin levels

Mean plasma free insulin levels were reduced after rhIGF-I administration in the period between 02.00h and 08.00h (61.3 ± 15.0 mU/l after placebo administration versus 30.7 ± 2.6 mU/l after rhIGF-I; p= 0.001, Figure 7) and during the 12h period overnight between 20.00h and 08.00h (41.3 ± 5.4 mU/l versus 82.6 ± 20.9 mU/l; p< 0.001). There was no relationship between change in free insulin and C-peptide levels (p> 0.1). Plasma free insulin levels demonstrated a similar pattern to the insulin infusion data during the period of stable euglycaemia, with relatively stable concentrations on the night of rhIGF-I administration, but a sharp rise beyond 06.00h following injection of placebo.
Figure 6a.
Mean insulin infusion data (02.00h - 08.00h) during the period of stable euglycaemia in individual subjects on placebo and rhIGF-I nights.

Figure 6b.
Mean (± SEM) insulin infusion rate during the period of stable euglycaemia (02.00h - 08.00h) after placebo (●) or rhIGF-I (○) administration in the 8 subjects who did not become hypoglycaemic.
Figure 7.

Plasma free insulin concentrations for the period of stable euglycaemia (02.00h - 08.00h) following placebo (•) or rhIGF-I (○) administration in the 8 subjects who did not become hypoglycaemic.
Daytime free insulin concentrations were elevated following injection of placebo when compared to rhIGF-I administration (09.00h - 16.00h; 99.4 ± 14.6 mU/l after placebo and 69.2 ± 8.5 mU/l after rhIGF-I; p = 0.02).

3.5.5 The relationship between insulin infusion and free insulin levels

A similar relationship between the amount of insulin infused and free insulin concentrations was expected after placebo and rhIGF-I administration in the absence of changes in the rate of insulin clearance. Throughout the study the insulin infusion rate was assessed every 15 minutes and the free insulin concentration every hour. Although circulating insulin has a relatively short half-life, the hourly insulin concentration will be influenced by the insulin infusion rate prior to the preceding 15 minute value. A comparison was therefore made between hourly insulin concentrations and the insulin infusion rate over both the preceding 15, and also the preceding 60 minutes.

The hourly free insulin concentration and the preceding 15 minute insulin infusion rate were related during the period of stable euglycaemia (02.00h - 08.00h) on both placebo and rhIGF-I nights (r = 0.25, p = 0.05 after placebo and r = 0.68, p < 0.001 after rhIGF-I administration). Hourly free insulin levels and the mean hourly insulin infusion rate were also more closely related after rhIGF-I administration than on the placebo night (r = 0.26, p = 0.05 after placebo and r = 0.73, p < 0.001 after rhIGF-I administration; Figure 8). One explanation for this discrepancy might be a more stable insulin infusion requirement after rhIGF-I administration, with a weaker relationship when the insulin infusion rate is altered more regularly following administration of placebo. The variance of the insulin infusion rate was, in fact, greater following placebo administration in 4 of the 8 subjects, and so there may be other explanations well.
The relationship between mean hourly insulin infusion rate and log-transformed plasma free insulin concentrations for the period of euglycaemia (02.00h - 08.00h) in the 8 subjects who did not become hypoglycaemic. The relationship following placebo administration is represented in the upper section of the figure (●) and following rhIGF-I in the lower section of the figure (○).
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Examination of the relationship between insulin infusion rate and free insulin levels during placebo and rhIGF-I studies shows similar regression slopes (illustrated in Figure 8), and does not support major changes in insulin clearance following rhIGF-I administration.

3.5.6 Ketones

There was no clear evidence of an effect of rhIGF-I administration on ketone (3-beta hydroxybutyrate, acetoacetate) and lactate concentrations. 3-beta hydroxybutyrate, acetoacetate and lactate were not significantly different during the euglycaemic period between 02.00h and 08.00h in the 8 subjects who did not become hypoglycaemic (3-hydroxybutyrate: 184 ± 58.8 mmol/l versus 151 ± 34.4 mmol/l, p = 0.4; acetoacetate: 132 ± 33.3 mmol/l versus 98.4 ± 16.7 mmol/l, p = 0.4; lactate: 745 ± 71.1 mmol/l versus 701 ± 50.8 mmol/l after placebo and rhIGF-I administration respectively, p = 0.7). Ketone and lactate concentrations are illustrated in Figure 9.

3.6 Effects of rhIGF-I on GH levels and GH secretion

3.6.1 Introduction

GH data will be presented for the complete 22h study period, but a more detailed analysis of GH pulsatility will be undertaken for the 12h period overnight (20.00h - 08.00h) when subjects were fasting and either relaxed in the supine position or asleep. GH is usually released in greatest quantities in association with sleep, and an examination of concentrations overnight may therefore be more likely to detect alterations in GH production. There is also evidence that intermittent sampling with an interval of 15 minutes may overlook GH pulses (Evans et al., 1987; Holl,RW et al., 1991) and the continuous sampling technique that was used overnight (in comparison to the intermittent technique during the day) is therefore a more robust means of collecting samples for
Figure 9.

Mean (+ SEM) 3-hydroxybutyrate, acetoacetate and lactate concentrations during the period of stable euglycaemia (02.00h - 08.00h) following placebo (♀) or rhIGF-I (O) administration (n=8).
detailed GH hormone profile analysis. Although GH pulse amplitude may be attenuated, a peak will always be 'embraced' by the continuous sampling technique (Matthews, 1988).

3.6.2 Quality of sleep

Sleep has a major impact on GH secretion (Clore et al., 1989; Holl, RW et al., 1991), and a first night in hospital may not be associated with sleep of the same quality or duration as subsequent nights when the circumstances and surroundings are more familiar (and usually more relaxed). The lights were therefore dimmed and conversation discouraged at similar times on both nights in hospital. Sleep pattern was documented, and the duration was similar on both the first and second nights in hospital. There was no difference in sleep duration when placebo and rhIGF-I nights were compared either. Data on sleep duration are shown in Table 3.5

<table>
<thead>
<tr>
<th>Night</th>
<th>Mean ± SEM</th>
<th>(median, range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.8 ± 0.9</td>
<td>(6.7, 0 - 9.0)</td>
</tr>
<tr>
<td>2</td>
<td>5.5 ± 0.8</td>
<td>(6.2, 0 - 9.7)</td>
</tr>
<tr>
<td>Placebo</td>
<td>5.8 ± 0.8</td>
<td>(6.2, 0 - 9.7)</td>
</tr>
<tr>
<td>rhIGF-I</td>
<td>5.4 ± 0.9</td>
<td>(6.7, 0 - 9.0)</td>
</tr>
</tbody>
</table>
3.6.3 Mean GH concentrations

Mean 22h GH concentrations during control and rhIGF-I studies in individual subjects are shown in Figure 10. GH concentrations were significantly reduced following rhIGF-I administration (27.8 ± 4.9 mU/l after placebo versus 16.9 ± 3.2 mU/l after rhIGF-I administration; p = 0.01).

The GH profiles showed considerable inter-individual variation in terms of mean concentrations and profile characteristics, but the impact of the 15 minute period of exercise on overall mean levels was clear, with a pronounced GH peak evident beyond 11.00h (Figure 11). 12h overnight mean GH levels between 20.00h and 08.00h were also reduced after rhIGF-I when compared to placebo administration (33.6 ± 5.7 versus 19.3 ± 4.0 mU/l; p = 0.01). GH concentrations were high on the baseline night when compared to levels normally seen in late pubertal adolescents, but were reduced to more appropriate levels following rhIGF-I administration (Edge et al., 1990a). There was also evidence of a relatively early impact on GH concentrations with reduced levels within the first six hours following rhIGF-I administration (mean GH levels between 18.00h and 24.00h: 28.0 ± 5.7 mU/l after placebo versus 10.7 ± 1.4 mU/l after rhIGF-I administration; p = 0.02).

3.6.4 Pulsar

Analysis of the GH profiles by the Pulsar programme revealed a significant reduction in the maximum GH concentration, the calculated baseline, mean GH peak amplitude (above the baseline calculated by Pulsar and above the assay threshold), following rhIGF-I administration. There was no difference in the other Pulsar parameters, including pulse frequency and pulse length. Thus it was the attributes assessing GH pulse amplitude and baseline concentration that were reduced by rhIGF-I administration. The results of Pulsar analysis of the 12h overnight GH data (20.00h - 08.00h) are shown in Table 3.6.
Figure 10.

A comparison of mean 22h GH concentrations in individual subjects following placebo or rhIGF-I administration.

Figure 11.

Overall mean 22h GH concentrations in the 8 subjects who did not become hypoglycaemic following placebo (●) or rhIGF-I (○) administration.
Table 3.6

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Mean ± SEM (Range)</th>
<th>rhIGF-I Mean ± SEM (Range)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean GH</td>
<td>33.6 ± 5.7 (14.2 - 62.7)</td>
<td>19.3 ± 4.0 (5.1 - 37.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>Maximum GH</td>
<td>150.1 ± 28.2 (50.2 - 293.1)</td>
<td>103.9 ± 27.0 (28.8 - 229.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>Minimum GH</td>
<td>1.2 ± 0.3 (0.5 - 3.6)</td>
<td>1.4 ± 0.4 (0.5 - 3.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline</td>
<td>8.1 ± 2.5 (1.2 - 22.8)</td>
<td>4.0 ± 1.2 (0.8 - 9.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Maximum baseline</td>
<td>17.0 ± 7.2 (1.5 - 64.1)</td>
<td>6.3 ± 1.9 (0.8 - 13.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Minimum baseline</td>
<td>1.8 ± 0.7 (0.5 - 6.1)</td>
<td>1.4 ± 0.4 (0.5 - 4.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Peaks</td>
<td>5.7 ± 0.5 (4.0 - 8.0)</td>
<td>6.3 ± 0.5 (4.0 - 8.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Amplitude</td>
<td>62.0 ± 10.9 (24.7 - 118.2)</td>
<td>37.2 ± 6.6 (17.4 - 62.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>Peak Length</td>
<td>92 ± 5 (65 - 138)</td>
<td>73 ± 4 (56 - 96)</td>
<td>NS</td>
</tr>
<tr>
<td>Interpeak interval</td>
<td>126 ± 11 (83 - 180)</td>
<td>131 ± 16 (102 - 240)</td>
<td>NS</td>
</tr>
<tr>
<td>SOPA (above assay threshold)</td>
<td>405 ± 70 (37 - 651)</td>
<td>270 ± 51 (104 - 536)</td>
<td>NS</td>
</tr>
<tr>
<td>Derived mean PA (above assay threshold)</td>
<td>51 ± 7 (34 - 120)</td>
<td>35 ± 3 (22 - 67)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

(SOPA = sum of peak amplitude, PA = peak amplitude)
3.6.5 Distribution analysis

Distribution analysis of the GH profiles overnight (20.00h - 08.00h) showed that the reduction in GH levels occurred over a wide range of concentrations, and was not solely due to alterations in peak or baseline values (Figure 12). The assessment of GH data by this technique was therefore consistent with the Pulsar analysis. Statistical analysis at three percentage points demonstrated significant reductions in the GH concentration below which 95% and 50% of the GH data points lay (OC_{50} and OC_{50}), but not the 5% value (OC_{5}). The distribution data is summarised in Table 3.7.

Table 3.7

| Distribution of GH concentrations (mU/l) overnight (20.00 - 08.00; n= 8). |
|----------------|-----------------|--------|
|                | Placebo         | IGF-I  |
| Mean           | 33.6 ± 5.8      | 19.4 ± 4.0 | 0.01 |
| OC_{50}        | 318 ± 93        | 118 ± 22 | 0.02 |
| OC_{50}        | 18.7 ± 4.8      | 9.0 ± 2.5 | 0.01 |
| OC_{5}         | 1.3 ± 0.3       | 1.2 ± 0.5 | >0.1 |

3.6.6 Deconvolution analysis

Deconvolution analysis determines the pattern of hormone release underlying a concentration profile and is therefore a means of establishing changes in GH secretion. Deconvolution analysis of the 22h profiles showed that the reduction in circulating GH levels following rhIGF-I administration was a reflection of a fall in GH secretion, and not of changes in GH clearance. Mean 22h secretory rates were 2.98 ± 0.47 mU/min after placebo versus 1.81 ± 0.30 mU/min after rhIGF-I administration; p = 0.01. Dividing the 22h study period into four blocks of
Figure 12.

Distribution analysis of 12h overnight GH concentration data (02.00h - 08.00h) expressed as a cumulative frequency curve following placebo (●) or rhIGF-I (O) administration (n=8).
equal duration indicated that changes in GH secretion were sustained, with significantly reduced secretory rates for the initial and also final time intervals (Table 3.8).

Table 3.8
GH secretion (mU/min) during the 22h study.

<table>
<thead>
<tr>
<th>Time interval</th>
<th>placebo</th>
<th>rhIGF-I</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.00 - 23.30h:</td>
<td>2.91 ± 0.63</td>
<td>1.07 ± 0.11</td>
<td>0.02</td>
</tr>
<tr>
<td>23.30 - 05.00h:</td>
<td>3.97 ± 1.24</td>
<td>2.38 ± 0.75</td>
<td>0.05</td>
</tr>
<tr>
<td>05.00 - 10.30h:</td>
<td>2.28 ± 0.54</td>
<td>2.28 ± 0.54</td>
<td>0.1</td>
</tr>
<tr>
<td>10.30 - 16.00h:</td>
<td>2.76 ± 0.61</td>
<td>1.51 ± 0.30</td>
<td>0.02</td>
</tr>
</tbody>
</table>

When the percentage reduction in GH secretion after rhIGF-I administration over the 22h study period was compared with initial IGF-I levels at the start of the rhIGF-I study night, a trend suggesting a greater reduction in secretion with lower initial IGF-I levels was observed ($r=-0.63$; $p=0.09$).

3.6.7 Characteristics of pulsatility

Of further interest was the similarity in the pattern of GH secretion when the two profiles from one individual were compared. Although the pulse amplitude was reduced after IGF-I administration, an underlying pattern which was peculiar to that individual was found to remain in most subjects. This was particularly so in subject numbers 1, 2, 4, 6 and 7, as illustrated in Figure 13 (a-b).
Figure 13a.
Individual GH secretory profiles determined by deconvolution analysis for the period 20.00h - 11.00h (up to the period of exercise) following administration of placebo (●) and rhIGF-I (○). Subjects 1, 2, 4, 5.
Figure 13b.
3.6.8 Fourier transformation

Fourier transformation analysis of the GH concentration profiles (20.00h - 08.00h) revealed that the change in levels following rhIGF-I administration was primarily amplitude related. The pulse periodicity (the time between pulses) was between 150 and 200 minutes on both study nights (Figure 14).

Fourier transformation of the deconvoluted GH data (which should correlate more closely with GH secretory events) confirmed a reduction in GH pulse amplitude following rhIGF-I administration. The dominant pulse periodicity appeared to be somewhat shorter than that indicated by analysis of the raw concentration profiles, with calculated values of between 120 and 180 minutes (Figure 15). There was no difference in pulsatility between the two nights, placebo and rhIGF-I.

3.6.9 GH relationships with other variables

Mean 22h GH levels during the placebo study correlated with HbA1 concentrations (r= 0.8; p= 0.005) but there was no relationship with IGF-I concentrations, C-peptide, insulin dose or overnight free insulin levels before or after rhIGF-I administration (p> 0.1).

Overnight GH secretion determined by deconvolution analysis correlated with HbA1 concentrations (r= 0.74; p= 0.03) but there was no relationship with BMI, IGF-I concentrations, IGF-I SD score, C-peptide, or overnight free insulin levels.

3.6.10 GH response to exercise

Mean GH concentrations were greater at the start of the period of exercise at 11.00h during the placebo as opposed to the rhIGF-I study. The change in GH secretion in response to exercise was therefore examined rather than the alteration in absolute GH levels.
Figure 14.

Absolute Fourier Transform of overnight GH concentration profiles (20.00h - 08.00h) in the 8 subjects following administration of placebo (●) and rhIGF-I (○).

Figure 15.

Absolute Fourier Transform of overnight GH secretory data derived from the GH profiles by deconvolution analysis (20.00h - 08.00h) following administration of placebo (●) and rhIGF-I (○).
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Eight of the nine subjects exercised between 11.00h and 11.15h. A comparison of GH secretory response between 11.00h - 12.00h (determined by deconvolution analysis), compared with the period between 10.00h - 11.00h, demonstrated a rise in GH release in 6 out of 8 subjects following placebo, and 7 out of 8 following rhIGF-I administration (27.9 ± 6.7 mU/min versus 11.0 ± 5.6 mU/min; p= 0.05 during the placebo study and 17.7 ± 4.0 mU/min versus 3.0 ± 1.4 mU/min; p= 0.005 after rhIGF-I administration). There was no significant difference in GH secretion between 11.00h and 12.00h when the two groups, placebo and IGF-I, were compared (p= 0.2; Figure 16). The GH secretory response to exercise was greater in 5 out of the 8 subjects following placebo administration, and 3 of the 8 after rhIGF-I.

3.7 Discussion

3.7.1 IGF-I levels

The administration of rhIGF-I in the subcutaneous dose of 40 µg/kg increased mean IGF-I levels in 9 subjects to within the normal physiological range during the 22h study. The mean half-life of 21.4 ± 1.0h in subjects with IDDM is slightly longer than the figure of 18.4h calculated in normal adults given the same dose (Wilton et al., 1991). It is difficult to comment on the significance of this difference, but the baseline adjusted increase in mean IGF-I concentrations of 190 ng/ml in the group with IDDM was also 40 ng/ml greater than that reported in normals, and maximum values occurred 1.5h earlier (Wilton et al., 1991). This may reflect normal variation between two separate study groups, but could also indicate differences in peptide absorption, or altered IGF binding in the IDDM subjects (Section 1.9.1). Interestingly endogenous IGF-I was shown to have a half-life of 20.7 ± 2.3h in GH deficient children treated with GH (Blethen et al., 1982).

The calculated half-life of 73.2h when the +48h sample was included in the calculation deserves some mention. A decline in plasma IGF-I concentrations indicates that elimination and
Figure 16.

GH response to exercise. GH secretion between 11.00h and 12.00h in individual subjects following placebo or rhIGF-I administration. Subjects exercised on a bicycle ergometer between 11.00h and 11.15h.
redistribution of peptide from the bloodstream exceeds the combined rates of absorption and endogenous peptide production. Half-life was calculated using a single compartment, mono-exponential model which will be influenced by continued peptide absorption and alterations in the rate of endogenous IGF-I production. The impact of these variables in the calculation was reduced by selecting the interval during which the decline in peptide concentrations was most pronounced. Before this interval and the calculation is affected to a greater extent by continuing peptide absorption, and endogenous production will become increasingly significant as circulating concentrations fall towards baseline levels. The single-compartment model was not an appropriate method for calculating half-life when the 48h sample was included because this was clearly beyond the interval of mono-exponential decay.

Despite IGF-I concentrations increasing to within the normal range for late pubertal adolescents, the rise in IGF bioactivity was relatively modest. This suggests that there are other factors affecting IGF bioactivity besides the absolute concentration of circulating IGF-I. There is evidence to implicate abnormal IGF binding in the reduced IGF bioactivity in IDDM (Taylor et al., 1990) and this will be discussed in greater detail in a subsequent chapter.

3.7.2 Hypoglycaemic threshold and blood glucose nadir

Many factors influence the glycaemic threshold at which a biochemical and symptomatic hypoglycaemic response occurs in subjects with IDDM. Hypoglycaemia may be a stimulus for the release of GH, glucagon, adrenaline and cortisol. The presence of these counter-regulatory hormones may, in turn, affect insulin sensitivity. Thus the analysis of insulin requirements and GH concentrations in a study such as this requires the exclusion of subjects who become hypoglycaemic.
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Adrenaline release was noted at blood glucose concentrations of less than 2.5 mmol/l in a group of young adult IDDM subjects with respectable glycaemic control compared to a threshold of greater than 3.1 mmol/l in subjects with poor control (Amiel et al., 1987b). In further studies of young subjects with IDDM the threshold for adrenaline release fell from 3.7 mmol/l to 2.5 mmol/l, with intensified insulin therapy accompanied by a fall in mean HbA1c concentrations from 9.6 to 7.1% (Amiel et al., 1988). Although other counter-regulatory hormones including GH, cortisol and glucagon may be released at slightly lower blood glucose levels there is considerable variability between individuals (Amiel et al., 1987a). In the case of GH, the threshold for release has been shown to range from 2.1 to 3.5 mmol/l (Amiel et al., 1987a, 1988). Tests of cerebral function have also been used to determine the threshold at which 'hypoglycaemia' occurs. Electroencephalography (Pramming et al., 1988), visual reaction time (Herold et al., 1985) and neuropsychological tests (Holmes et al., 1984) have indicated that neuroglycopenia occurs at blood glucose concentrations of around 3.0 mmol/l. A more recent study using auditory event-related potentials indicated a somewhat higher threshold of 4.0 mmol/l (De Feo et al., 1988). This particular investigation also revealed that the hormonal response could be influenced by the duration of hypoglycaemia. The threshold for counter-regulatory hormone release was approximately 3.3 mmol/l with a short stimulus (lasting minutes), and 4.0 mmol/l when the stimulus was sustained for at least 120 minutes. Gender and prevailing insulin concentrations are further factors that may influence the counter-regulatory response to hypoglycaemia (Mellman et al., 1992; Amiel et al., 1993; Davis et al., 1993; Diamond et al., 1993).

The selection of a threshold of 3.5 mmol/l was therefore a compromise between the need to exclude subjects developing hypoglycaemia, whilst at the same time recognising the difficulty in establishing euglycaemia without values drifting below the euglycaemic target of 5.0 mmol/l at some
Chapter 3.

point overnight. The detailed assessment of counter-regulatory hormone production was not undertaken in these studies.

Despite the slightly lower blood glucose nadir after rhIGF-I administration there was no difference in the lowest blood glucose concentrations reached in individual subjects to suggest a stimulus for the production of counter-regulatory hormones on one night and not the other. Although counter-regulatory hormone production may have accompanied cannula insertion and the injection of placebo or rhIGF-I, there was no indication that the administration of one vial was more uncomfortable than the other.

Other explanations for the reduced mean blood glucose nadir include early insulin-like actions of rhIGF-I and a fall in insulin sensitivity because of the reduction in GH concentrations apparent within the first hours of the study. These mechanisms will be considered in greater detail later in this discussion.

One further feature of the insulin infusion data leading up to the stable euglycaemic period deserves mention. There was a tendency for the insulin infusion programme to recommend an infusion rate which would lead to 'overshoot' beyond the euglycaemic target. This was particularly apparent when initial blood glucose concentrations were high. Blood glucose concentrations were greater at the time of admission on the evening when rhIGF-I was administered, and may have been a further factor leading to the more pronounced mean blood glucose nadir following rhIGF-I administration.

If the lower mean blood glucose nadir after rhIGF-I administration had led to enhanced counter-regulatory hormone production, then this would be expected to lead to increased GH secretion and reduced insulin sensitivity on the placebo night. Such changes were not observed in these studies.
3.7.3 Insulin infusion requirement

The insulin infusion requirement for the maintenance of euglycaemia was reduced in all subjects following rhIGF-I administration. Endogenous insulin production is suppressed by rhIGF-I (Section 1.13.5) but it was hoped that the exclusion of subjects with more recent disease onset would reduce the potential impact of such an effect. If rhIGF-I administration were to reduce insulin production to a significant extent then a rise, and not the observed fall in insulin infusion requirements, would have been observed.

Studies in normal subjects have reported reductions in insulin clearance following rhIGF-I administration (Guler et al., 1989a; Rennert et al., 1993), but other investigators have not detected these changes (Zenobi et al., 1992a). Both insulin infusion rate and free insulin concentrations were reduced after rhIGF-I administration, and the two variables were therefore analysed to establish whether the relationship between them had altered. Although the slope of the regression line was increased following rhIGF-I administration (suggesting reduced insulin clearance) the correlation coefficient after administration of placebo was low (0.23 after placebo versus 0.73 after rhIGF-I administration). Conversely, the reduced insulin concentrations during the day with a similar infusion rate following rhIGF-I administration might at first sight indicate an increase in insulin clearance. However strict glycaemic control was not maintained at this time. The evidence does not, therefore, suggest that an alteration in insulin clearance is the explanation for the change in insulin infusion requirements observed in these studies.

Glucagon production and the glucagon response to hypoglycaemia in normals and subjects with NIDDM is inhibited by rhIGF-I administration (Takano et al., 1991; Kerr et al., 1993; Laager & Keller, 1993). In IDDM subjects with longer disease duration the glucagon response to a hypoglycaemic stimulus is reduced (Bolli et al., 1983), although baseline glucagon concentrations are similar to those seen in normals (Bolli et al., 1983; Amiel et al., 1987a). A reduction in baseline
Chapter 3.

Glucagon levels could reduce hepatic glucose output with subsequent effects on glucose homeostasis, and although glucagon concentrations were not measured in these studies this may have contributed to the observed change in free insulin levels.

Finally, the reduction in insulin infusion requirements and free insulin concentrations following rhIGF-I administration may be linked to the insulin-like actions of the peptide (Section 1.13.1 and 1.13.2) or to changes in GH secretion (Section 1.13.6). These factors will be considered in greater detail in the next two sections of this discussion.

3.7.4 The insulin-like effects of rhIGF-I

An estimation of the insulin-like actions of the dose of rhIGF-I administered in these studies can be gained from the work of other investigators. Guler and colleagues calculated that administered rhIGF-I had approximately 6% of the hypoglycaemic actions of insulin (Guler et al., 1987). Using the mean subject weight of 62.5 kg, the average dose of rhIGF-I administered in the described studies of adolescents with IDDM was 2500 μg (62.5 x 40 μg/kg). 2500 μg of rhIGF-I is equivalent to 357 nmol and by assuming that rhIGF-I has 6% of the hypoglycaemic actions of insulin this could be expected to have similar hypoglycaemic activity to 21.6 nmol of insulin, or approximately 3.1 units. The calculated half-life of administered rhIGF-I was 21h and IGF-I concentrations were still elevated at 48h. Assuming that one eighth of the rhIGF-I dose is available for interaction with the insulin receptor during the 6 hour period between 02.00h and 08.00h then this will then have the direct hypoglycaemic effect of 0.38 units of insulin. The discrepancy in mean insulin infusion requirements during the euglycaemic period was 0.06 mU/kg/min (approximately 1.3 units of insulin). A ‘raw’ calculation of the insulin-like actions of rhIGF-I can therefore be estimated to account for between one third and one quarter of the difference in insulin requirements.
detected following rhIGF-I administration. A more appropriate estimation would need to assess free and bioavailable peptide but such data is not yet available.

In addition to possible interactions at the insulin receptor, IGF-I may also have a direct effect on glucose disposal through interaction with its own receptor or by indirect effects on post-receptor mechanisms (Giacca et al., 1990; Rossetti et al., 1991). The effects of a reduction in insulin levels following rhIGF-I administration would need to be offset by increased glucose disposal by this and other means to explain the difference in insulin infusion requirements observed in these studies. Although it is difficult to assess these effects collectively, the minor impact of subcutaneously administered rhIGF-I on glucose tolerance when administered in similar doses to normal subjects points towards further, additional explanations for the fall in insulin infusion requirements seen in adolescents with IDDM (Wilton et al., 1991).

3.7.5 GH analysis

GH has insulin antagonistic effects which have close links with the changes in insulin sensitivity seen overnight in adolescents with IDDM (Beaufriere et al., 1988; Edge et al., 1990b). All the methods of GH profile analysis demonstrated reductions in GH pulse amplitude and in the baseline GH concentrations. Pulse amplitude has been identified as a principal mediator of GH induced alterations in insulin sensitivity, with an interval between pulse and subsequent effect of between 2h and 5h (Fowelin et al., 1991; Pal et al., 1992). The change in insulin infusion requirements could be linked to the alterations in GH secretion, although there was no direct correlation between change in mean GH concentrations and change in insulin infusion requirements in these preliminary studies (see Chapter 5).

An estimate of the extent to which reducing GH levels will affect insulin requirements during puberty in adolescents with IDDM can also be made. The magnitude of the dawn
Chapter 3.

phenomenon of a rise in insulin requirements has been estimated to be approximately 20 to 25% in
subjects with IDDM (Edge et al., 1990b; Perriello et al., 1991). The 20% reduction in insulin
infusion requirements in these current studies is of a similar order of magnitude. If GH had
significant insulin-like action in these studies (Moller,N et al., 1992) then, in the absence of other
factors affecting insulin sensitivity, evidence of a reduction in insulin infusion requirements would be
expected on the control night. This was clearly not the case.

The change in GH release in these studies provides some insight into the effects of IGF-I on
the hypothalamic-pituitary axis. The determination of alterations in GH pulsatility is most
appropriately undertaken on deconvoluted data which, by taking into account alterations in half-life
and volume of distribution, determines the pattern of underlying GH secretory events. A reduction
in GH pulse amplitude and GH baseline with similar periodicity could be explained in a number of
different ways. The administration of rhIGF-I could reduce the magnitude of hypothalamic GHRH
pulses or attenuate the pituitary response to the released peptide. Both mechanisms receive some
experimental support (Shibasaki et al., 1986; Ceda et al., 1987). However recent evidence has also
suggested that the principal effect of rhIGF-I in humans is to alter SMS release and increase overall
SMS tone (Bermann et al., 1994). Without the means to measure GHRH and SMS directly this is
difficult to prove in man.

A striking feature of the GH profiles was the similarity in the timing and the pattern of GH
secretory events between the two study nights. Although the magnitude of GH release was reduced
after rhIGF-I administration, the pattern of secretion on the two nights was remarkably similar in
the majority of subjects when the deconvoluted profiles were studied. Although earlier studies in
prepubertal children and adults have shown poor intraindividual reproducibility of overnight GH
profiles in terms of mean levels and amplitude of GH peaks (Salani et al., 1991; Albertsson-Wikland
& Rosberg, 1992), similarities in the timing of secretory events have been noted (Salani et al.,
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1991). There is evidence that the primary abnormality in IDDM is altered SMS tone or a resistance to SMS actions (Cohen & Abplanalp, 1991; Ismail et al., 1993), and if low IGF bioactivity is the principle abnormality underlying the abnormal GH release in IDDM, then the similar pattern of pulsatile events in the short and longer term is compatible with alterations in the magnitude of SMS release, or of reduced pituitary sensitivity to SMS actions.

It has been suggested that pulse frequency is increased in IDDM during adolescence with a peak periodicity in the order of 90 minutes (Pal et al., 1993), compared to normal subjects where values ranging from 100 to 200 minutes have been reported (Hindmarsh et al., 1988b; Goji, 1993). Despite the difficulty in establishing specific alterations in periodicity from the Fourier transforms this appeared to be somewhat greater than 90 minutes. There was no evidence of an increase in the interval between pulses following rhIGF-I administration.

Despite the evidence to suggest that a change in IGF-I levels led to the alteration in GH secretion, a direct relationship between the two variables was not identified. Hepatic resistance may result in discordance between GH and IGF-I levels and thus explain the absence of any such relationship on the placebo night. However IGF-I concentrations do not parallel GH secretion closely in normals. Peak GH levels occur in females in mid-puberty whilst maximum IGF-I concentrations occur in late puberty (Taylor et al., 1988; Edge et al., 1990a), and other factors such as gonadal steroid concentrations clearly influence GH secretion (Merimee et al., 1991).

Glucose has a suppressive effect on GH secretion in normal subjects but not to the same degree in IDDM (Ismail et al., 1991; Pal et al., 1993). There was no overall difference in mean blood glucose concentrations between the two 12h periods overnight, and no correlation between glucose and GH concentration in individual subjects to suggest that this was a factor affecting GH secretion.
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Insulin does not have consistent effects on GH release (Section 1.8.3) but may enhance secretion in subjects with elevated HbA1 concentrations (Press et al., 1992). Glycaemic control varied considerably in the subjects studied, and higher circulating free insulin concentrations on the placebo night may have enhanced GH secretion in some individuals. There was no identifiable relationship between mean overnight GH and insulin concentrations, and although C-peptide concentrations were low in this group of subjects there was no relationship between C-peptide and GH release. However C-peptide status was not assessed by glucagon stimulation, in contrast to the studies reported by Wurzburger and colleagues who showed a direct relationship between these variables (Wurzburger et al., 1990).

The GH secretory responses to exercise and other provocative stimuli are enhanced in IDDM (Hansen, 1970; Speroni et al., 1983; Krassowski et al., 1988). However, despite the reduction in overnight GH concentrations there was no significant difference in the response to exercise following rhIGF-I administration when compared to placebo. GH concentrations at the start of the exercise stimulus were higher during the placebo study, but although the response to GHRH following GH pretreatment appears to be attenuated in normals (Ross et al., 1987), this is not the case in subjects with IDDM (Ismail et al., 1993). If rhIGF-I does indeed alter SMS tone then one may have anticipated a reduced GH response to exercise. Unfortunately exercise is not a reliable stimulus for the release of GH and, further, the exercise test was not performed in ideal circumstances because it was not linked directly to maximum oxygen consumption.

3.7.6 Ketones

Acetoacetate and 3-hydroxybutyrate concentrations tended to be lower during the period of euglycaemia following rhIGF-I administration, although the differences did not reach statistical significance. There are theoretical reasons why ketone concentrations might increase or decrease
following rhIGF-I administration. GH has pronounced ketogenic effects in IDDM (Press et al., 1984a), and a reduction in GH concentrations following rhIGF-I administration might therefore lead to a fall in ketone levels. Insulin also suppresses ketone formation (Gerich et al., 1976), and the reduction in free insulin levels following rhIGF-I administration could, conversely, increase ketone formation. The adipocyte appears to have few IGF-I receptors, and a direct suppressive effect of administered rhIGF-I on FFA output (with a consequent reduction in ketone formation) is unlikely to have contributed greatly to these observations.

3.7.7 Summary

These initial studies have shown that the subcutaneous administration of rhIGF-I in a dose of 40 µg/kg to adolescents with IDDM leads to a sustained reduction in GH secretion and in the insulin requirements for the maintenance of euglycaemia in adolescents with IDDM. Although a direct relationship between changes in mean GH levels and insulin sensitivity was not demonstrated, the magnitude and nature of the reduction in insulin requirements suggests that changes in GH release could explain some of the study findings. The possible direct effects of administered rhIGF-I on glucose disposal and effects on glucagon release also require careful consideration, although the impact of these variables was not addressed in detail by the study protocol.
Chapter 4

A COMPARISON OF rhIGF-I ADMINISTRATION IN THE ABDOMEN AND THIGH

4.1 Introduction

The site, tissue, tissue thickness, temperature and peptide concentration may all affect the absorption of peptide hormones (Binder et al., 1984; Laursen et al., 1994; Sindelka et al., 1994). Insulin is absorbed more quickly from the abdomen than the thigh (Koivisto & Felig, 1980) and so subjects with IDDM may be advised to administer soluble pre-meal insulin into the abdomen to enhance insulin concentrations at the time of food intake. Conversely, insulin preparations with a longer duration of action may best be administered into the thigh from where absorption will be more prolonged.

The longer-term investigation of rhIGF-I administration in IDDM requires information about how the site of injection affects peptide pharmacodynamics, and it was intended that this question be addressed by the study described in this chapter. Administration in the thigh and abdomen were compared because these sites are frequently used by adolescents when administering insulin injections. It was also hoped that information about effects on IGF-I levels and rhIGF-I half-life in IDDM (described in Sections 3.4.1 and 3.4.2) could be extended by measuring IGF-I concentrations up to 72h after rhIGF-I administration.

4.2 Subjects and methods

4.2.1 Subjects

Eight late-pubertal female subjects with IDDM took part in the study. All were accustomed to administering insulin injections in the abdomen and thigh. The study group characteristics are
Chapter 4.

shown in table 4.1. Further individual patient details are given in Appendix 1 where they are listed as subjects 10 to 17.

<table>
<thead>
<tr>
<th>Table 4.1</th>
<th>Subject characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Age (years)</td>
<td>13.5 - 18.6</td>
</tr>
<tr>
<td>Duration of Diabetes (years)</td>
<td>3.6 - 12.5</td>
</tr>
<tr>
<td>C-peptide (nmol/l) (BG&gt;7.0mmol/l)</td>
<td>0.01 - 0.10</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.0 - 29.4</td>
</tr>
<tr>
<td>Insulin dose (u/kg)</td>
<td>0.7 - 1.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (number of subjects)</td>
<td>0</td>
</tr>
</tbody>
</table>

Puberty stage (genital/breast):

<table>
<thead>
<tr>
<th>G4 B4</th>
<th>G5 B5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

4.2.2 Design

Each individual was admitted for two study periods when rhIGF-1 in a dose of 40 µg/kg was administered at 18.00h into the anterior aspect of the left thigh or, alternatively, 5 cm to the left or right side of the umbilicus. A baseline night when no injection was given preceded the two nights of rhIGF-1 administration. The results from the baseline study will not be discussed in this chapter but form part of the studies presented in subsequent chapters (Chapters 5 and 6). The rhIGF-1
preparation was identical to that described in Section 2.3.3, and the injection technique is described in greater detail in section 2.3.4. The sequence of the two injections of rhIGF-I was randomised, with the order revealed to the investigator at the time of the first study night. The syringe was weighed before and after the injection to accurately assess the amount of rhIGF-I administered.

The algorithm-based insulin infusion method was used to control blood glucose concentrations overnight (section 2.4.3).

IGF-I levels were measured at half-hourly intervals for the first 6h following rhIGF-I administration, and then hourly until 08.00h (14h following rhIGF-I administration). Samples of blood were then taken from subjects at home at +24h, +48h and +72h to assess IGF-I concentrations beyond the study period in hospital.

4.4 Results

4.4.1 Side effects

The injection of rhIGF-I was associated with discomfort in the vicinity of the injection site which resolved within 10 minutes. There were no obvious side effects at the injection site during or after the study period in hospital.

4.4.2 Administered rhIGF-I dose

Calculation of the syringe weight before and after the injection of rhIGF-I did not reveal any difference in the amount of rhIGF-I administered on one night when compared to the other and there was no significant alteration in subject weight from one night to the next; 61.4 ± 3.7 kg on the night of rhIGF-I administration in the abdomen and 61.1 ± 3.6 kg following rhIGF-I administration in the thigh. A mean of 40.5 ± 3.4 μg/kg of rhIGF-I was injected in the abdomen and 40.0 ± 5.6 μg/kg on the night of administration in the thigh.
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4.4.3 Pharmacokinetics

Plasma IGF-I concentrations following rhIGF-I administration in the thigh and abdomen are shown in Figure 17. There was no difference in baseline IGF-I concentrations when the two study nights were compared. The overall mean peak IGF-I concentration was reached 90 minutes earlier after injection in the abdomen when compared to the thigh (518 ± 47 ng/ml at 21.30h following injection in the abdomen, and 516 ± 51 ng/ml at 23.00h following injection in the thigh), but there was no statistically significant difference in the baseline adjusted IGF-I area under the curve (AUC) in the first 3.5h following rhIGF-I administration (156 ± 23 ng/ml/min following administration in the abdomen, and 130 ± 22 ng/ml/min after administration in the thigh).

The time to peak IGF-I concentrations in individual subjects was no different either, although the mean of these individual values revealed that peak concentrations were achieved at 3.9 ± 0.5h (approximately 22.00h) after injection in the abdomen (range; 2.0h - 5.5h post rhIGF-I injection), compared to 5.6 ± 0.6h (approximately 23.30h) after injection in the thigh (range; 3.0h - 8.0h post rhIGF-I injection). When the maximum IGF-I concentration in individual subjects was adjusted according to baseline levels, the maximum increase in concentrations was 285 ± 34 ng/ml after administration in the abdomen and 274 ± 32 ng/ml after injection in the thigh. There was no overall difference in values for the IGF-I AUC during the 14h stay in hospital (137 ± 15 mg/ml/min after administration in the abdomen and 169 ± 28 ng/ml/min after administration in the thigh). Data for the first 3.5h of the study period and for the complete 14h period in hospital are shown in Table 4.2 overleaf.
Figure 17.

Mean (± SEM) IGF-I concentrations following administration of rhIGF-I in the abdomen (■) or thigh (○).
Table 4.2

IGF-I area under the curve (ng/ml/min)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SEM</th>
<th>Range</th>
<th>N.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial 3.5h (18.00 - 21.30)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdomen</td>
<td>156 ± 23</td>
<td>(82 - 287)</td>
<td></td>
</tr>
<tr>
<td>Thigh</td>
<td>130 ± 22</td>
<td>(41 - 247)</td>
<td></td>
</tr>
<tr>
<td>14h data (18.00 - 08.00)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdomen</td>
<td>137 ± 15</td>
<td>(78 - 187)</td>
<td></td>
</tr>
<tr>
<td>Thigh</td>
<td>169 ± 28</td>
<td>(70 - 344)</td>
<td></td>
</tr>
</tbody>
</table>

4.4.4 IGF-I levels up to 72h post-rhIGF-I administration

Samples were obtained from all participating subjects at +48h and from 7 of the 8 subjects at +24h and +72h. IGF-I concentrations were significantly elevated compared to baseline at +24h after rhIGF-I administration in the thigh but not after injection in the abdomen. There were no differences in IGF-I concentrations at 48h and 72h following rhIGF-I administration at either site when compared to baseline. IGF-I concentrations at the 3 sampling points (+24h, +48h, +72h) were no different when the two sites of administration were compared (Table 4.3).

It was hoped that continued sampling beyond the 14h period in hospital would provide further information about rhIGF-I half-life beyond that obtained in the 9 original subjects (described in Section 3.4.2). However, blood samples were not obtained during the interval between +14h and +24h and could not be collected from all participating subjects after leaving hospital. IGF-I
concentrations were also found to be below baseline values at +24h in 3 subjects following rhIGF-I administration in the abdomen, and increased beyond the +24h sample in 2 subjects after injection in the thigh. Although further information about the impact of rhIGF-I administration on IGF-I levels was provided by this study, these factors meant that the detailed analysis of IGF-I half-life (using the methods employed in Section 3.4.2.) was not possible. The individual IGF-I profiles following rhIGF-I administration in the abdomen and thigh are shown in Figure 18 (a, b).

### Table 4.3

IGF-I concentrations (ng/ml) - extended sampling data.

<table>
<thead>
<tr>
<th>Time(h)</th>
<th>Abdomen</th>
<th>Thigh</th>
<th>Site of administration (median and range):</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>272</td>
<td>230</td>
<td>n=8 (172 - 453) (174 - 478) NS</td>
</tr>
<tr>
<td>24</td>
<td>316</td>
<td>340*</td>
<td>n=7 (242 - 540) (220 - 549) NS</td>
</tr>
<tr>
<td>48</td>
<td>284</td>
<td>315</td>
<td>n=8 (156 - 582) (149 - 480) NS</td>
</tr>
<tr>
<td>72</td>
<td>229</td>
<td>318</td>
<td>n=7 (172 - 516) (200 - 431) NS</td>
</tr>
</tbody>
</table>

* p< 0.05 compared to time 0

### 4.4.5 rhIGF-I clearance

By combining the data points beyond 08.00h with the AUC for the first 14h a total IGF-I AUC following rhIGF-I administration was calculated for individual subjects. This was then used to
Figure 1. IGF-I concentrations following rhIGF-I administration in the abdomen (■) or thigh (O). Subjects 10 - 13.
Figure 18b.
IGF-I concentrations following rhIGF-I administration in the abdomen (■) or thigh (○).
Subjects 14 - 17.
Chapter 4.

derive the clearance rate of IGF-I after the two injections (total serum clearance equals the amount of rhIGF-I administered divided by the IGF-I AUC). Although the clearance of IGF-I was greater after administration in the abdomen compared to the thigh, the differences were not statistically significant (17 ± 2 ml/min versus 11 ± 1 ml/min respectively; p > 0.1). The overall clearance of rhIGF-I, obtained by combining data from the two separate injection sites, was 14 ± 1 ml/min (range 7 - 30 ml/min).

4.5 Discussion

The absorption of subcutaneously injected insulin and GH occurs more rapidly from the abdomen than from the thigh (Koivisto & Felig, 1980; Binder et al., 1984; Laursen et al., 1994). Increasing skin temperature (Thow et al., 1989; Sindelka et al., 1994), and injecting into a thinner layer of subcutaneous tissue (Vora, JP et al., 1992; Sindelka et al., 1994) have been shown to increase insulin absorption as well. Decreasing the concentration of injected insulin has a similar effect on peptide absorption (Sindelka et al., 1994).

Investigations undertaken in normal human subjects have shown that the bioavailability of the rhIGF-I preparation used in these studies is 100% (Wilton et al., 1991). There is no reason to suspect significant subcutaneous degradation of a first injection of rhIGF-I in subjects with IDDM, and so one would anticipate similar bioavailability in the studies described in this chapter. There were no easily identifiable factors, other than the injection site itself, that could promote more rapid absorption following administration in one site when compared to another. Exercise was prohibited and subjects were dissuaded from massaging the skin after the injection, in spite of the local discomfort. Although the range of BMI was considerable (over 10 kg/m²) patients' weight did not differ significantly between study nights.
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The absorption of rhIGF-I from the thigh was relatively slow, with peak levels achieved after approximately 5h. This is similar to the original study where peak IGF-I concentrations were seen after 5.5h (Section 3.4.1). Peak IGF-I concentrations were reached 90 minutes later following injection in the thigh when compared to injection in the abdomen, and concentrations were still above baseline 24h later (but not after administration in the abdomen). Thus one may suspect that, like other peptides, rhIGF-I is absorbed more rapidly from more ‘central’ body sites (Binder et al., 1984). It is possible with a larger study group that this would be demonstrated more clearly.

The half-life data generated by the more intensive period of profiling in the earlier study of administration in the thigh (Section 3.4.2) demonstrated a relatively stable and sustained increase in IGF-I levels over a 22h period. As the subcutaneous administration of rhIGF-I in the abdomen appears to lead to more rapid peptide absorption (with IGF-I concentrations returning to baseline 24h after injection) it can be concluded that the thigh is a more appropriate site for the daily administration of rhIGF-I in longer term studies in adolescents with IDDM. It is of interest that the time to peak levels and the peptide half-life are not unlike that seen following administration of an insulin such as isophane, which has an intermediate duration of action (Binder et al., 1984).

The pharmacokinetics of rhIGF-I will depend on peptide distribution (including IGF binding) and metabolism (Binder et al., 1984) as well as absorption. Effects of exogenous IGF-I on endogenous peptide production may also affect the pharmacokinetic profile of administered peptide (Gluckman & Butler, 1994), although repeated daily rhIGF-I administration in normals does not suggest that major changes in production rate occur. The binding of administered rhIGF-I is a complex process (Guler et al., 1989b), and will be considered in greater detail later in this thesis. However there is no reason to suspect that this will be affected by the site of administration.

The calculation of IGF-I clearance was based on single IGF-I measurements beyond the period in hospital. Although the extrapolation of the rate of decay over many hours will not
Chapter 4.

generate precise values in individual subjects, the mean value of 14 ml/min is similar to that derived from a study of bolus rhIGF-I administration in normal subjects (Wilton et al., 1991). It is worth noting the similarity in IGF-I profile, half-life (section 3.4.2) and clearance between normals and adolescents with IDDM in contrast to subjects with GHI where profound abnormalities of IGF binding are described (Vaccarello et al., 1993). The binding of IGF-I by the IGFBP's in IDDM will be discussed in greater detail in Chapter 6.
Chapter 5

THE EFFECTS OF rhIGF-I ON GH CONCENTRATIONS, GLYCAEMIC CONTROL, AND INSULIN SENSITIVITY

5.1 Introduction

A relationship between GH concentrations and insulin sensitivity (Sections 1.5.5 and 1.7.1), and blood glucose control as assessed by HbA1 levels (Section 1.7.5), has been identified in IDDM. Although GH concentrations were reduced following rhIGF-I administration in the initial placebo controlled studies (Section 3.6), a direct relationship between change in GH levels and insulin infusion requirements was not identified.

To examine the potential link between GH concentrations and insulin sensitivity further, all of the overnight profiles where blood glucose concentrations were closely controlled were included in a further analysis to identify factors leading to altered GH secretion. It was hoped that this would, in turn, further elucidate the mechanism behind altered insulin sensitivity following rhIGF-I administration.

5.2 Subjects and methods

5.2.1 Subjects

Sixteen subjects were admitted for both control and rhIGF-I study nights. Only nights where rhIGF-I was administered in the thigh were included in the analysis. Eight of the sixteen individuals formed the study group described in Chapter 3 (excluding subject 3 who became hypoglycaemic) and received placebo and rhIGF-I injections (Section 3.2.3; subjects 1, 2 and 4 - 9 in Appendix 1). The remaining eight subjects participated in the studies described in Chapter 4. These individuals were admitted for a control night and a night when rhIGF-I was administered in
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the thigh (Section 4.2.1; subjects 10 - 17 in Appendix 1). Thus, a total of 16 paired studies were assessed.

Table 5.1

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>Range</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.5-18.9</td>
<td>15.3</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>3.0-13.8</td>
<td>5.9</td>
</tr>
<tr>
<td>C-peptide (nmol/L) (HG&lt;7.8mmol/L)</td>
<td>0.02-0.27</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>18.8-29.42</td>
<td>2.3</td>
</tr>
<tr>
<td>Insulin dose (u/kg)</td>
<td>0.73-1.50</td>
<td>1.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex (number of subjects)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Puberty stage (genital/breast)</th>
<th>G4 B4</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G5 B5</td>
<td>3</td>
</tr>
</tbody>
</table>

5.2.2 Design

All subjects were admitted for a control night (a baseline night or a night when placebo was administered) and a night when rhIGF-I was injected subcutaneously in the thigh at 18.00h in a dose of 40 µg/kg. Overnight euglycaemia was maintained by an intravenous insulin infusion governed by the euglycaemic insulin clamp programme or the infusion algorithm (Sections 2.4.2 and 2.4.3) and the insulin infusates were therefore prepared by one of two different methods. When analysing the
data the methodological differences were overcome by using free insulin concentrations as an index of insulin sensitivity during periods of euglycaemia, rather than the insulin infusion requirement.

5.2.3 GH Analysis

Mean overnight GH levels (20.00h - 08.00h) were used to assess GH release together with a more detailed assessment of GH pulsatility by the Pulsar peak detection programme (Section 2.8.2 and Appendix 3). These indices were compared with free insulin concentrations during the period of stable euglycaemia on the respective study nights.

5.3 Results

5.3.1 IGF-I concentrations

IGF-I concentrations (18.00h - 08.00h) were 242.0 ± 20.0 ng/ml on the control night and 400.4 ± 25.4 ng/ml on the night of rhIGF-I administration (p< 0.001). The pattern following rhIGF-I administration was similar to that described in the placebo controlled and injection site studies (Sections 3.4.1 and 4.4.3), with peak IGF-I levels achieved after 5h and a maximum increase in IGF-I concentrations of 210 ng/ml.

5.3.2 Glucose

Stable euglycaemia was achieved between 03.30h and 07.30h. During this interval blood glucose concentrations were 6.1 ± 0.2 mmol/l on the control night and 5.9 ± 0.2 mmol/l after rhIGF-I administration. Overall blood glucose concentrations (18.00h - 08.00h) were 8.8 ± 0.6 mmol/l on the control night and 8.1 ± 0.4 mmol/l after rhIGF-I administration (Figure 19).
Figure 19.
Mean (± SEM) blood glucose concentrations in the 16 subjects who did not become hypoglycaemic on control (●) and rhIGF-I (O) nights. Stable euglycaemia was achieved between 03.30h and 07.30h.

Figure 20.
Mean overnight free insulin concentrations on control (●) or rhIGF-I (O) nights. Comparable stable euglycaemia was achieved in the two study groups between 03.30h and 07.30h and is indicated by the parallel arrow.
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5.3.3 Plasma free insulin

During the euglycaemic period (03.30h - 07.30h) insulin concentrations were elevated on the control night when compared to the night when rhIGF-I was administered. Insulin concentrations were 34.2 ± 6.5 mU/l (range 7.4 - 99.2 mU/l) on the control night versus 23.5 ± 3.0 mU/l (range 10.3 - 46.4 mU/l) following rhIGF-I administration (p= 0.04). There was a pronounced rise in mean insulin concentrations on the control night beyond 06.00h whilst concentrations did not change during the euglycaemic period after rhIGF-I administration (Figure 20).

5.3.4 GH

Mean GH concentrations on the control night (20.00h - 08.00h) were 24.9 ± 3.6 mU/l and correlated positively with HbA1 levels (r= 0.63; p< 0.01. Figure 21), but not IGF-I concentrations (r= -0.1; p= 0.6). Following rhIGF-I administration, there was an overall reduction in GH levels to 17.4 ± 2.2 mU/l (p= 0.02).

Pulsar analysis of the GH profiles revealed that rhIGF-I administration led to more frequent GH pulses (reflected by an increase in peak number and reduced peak interval) which were of reduced amplitude and duration. Mean GH concentrations and the Pulsar analysis of the 12h overnight GH profiles are shown in Table 5.2.

The change in pulse frequency following rhIGF-I administration was small, but statistically significant, and is in contrast to the Fourier and Pulsar analysis of the original data set (Section 3.6), which did not demonstrate any change in peak number. In other respects the Pulsar assessment of the GH data demonstrated similar changes to the earlier analyses.
Figure 21.

The relationship between 12h overnight mean GH and HbA1 concentrations on the control night.
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<table>
<thead>
<tr>
<th>Pulsar parameter</th>
<th>Control</th>
<th>rhlGF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SEM (Range)</td>
<td>Mean±SEM (Range)</td>
</tr>
<tr>
<td>Mean GH</td>
<td>24.9 ± 3.6 (11.9 - 62.7)</td>
<td>17.4 ± 2.2 (5.1 - 62.7)</td>
</tr>
<tr>
<td>Maximum GH</td>
<td>103.9 ± 18.9 (32.5 - 293.1)</td>
<td>84.2 ± 14.4 (28.8 - 229.4)</td>
</tr>
<tr>
<td>Minimum GH</td>
<td>1.6 ± 0.3 (0.5 - 4.9)</td>
<td>1.2 ± 0.2 (0.5 - 3.7)</td>
</tr>
<tr>
<td>GH Baseline</td>
<td>6.1 ± 1.3 (1.2 - 22.8)</td>
<td>4.0 ± 0.8 (0.8 - 12.0)</td>
</tr>
<tr>
<td>Maximum baseline</td>
<td>11.4 ± 3.8 (1.5 - 64.1)</td>
<td>6.4 ± 1.3 (0.8 - 18.2)</td>
</tr>
<tr>
<td>Minimum baseline</td>
<td>1.9 ± 0.4 (0.5 - 6.1)</td>
<td>1.3 ± 0.2 (0.5 - 4.4)</td>
</tr>
<tr>
<td>Peak Number</td>
<td>5.3 ± 0.2 (4.0 - 8.0)</td>
<td>6.5 ± 0.4 (4.0 - 11.0)</td>
</tr>
<tr>
<td>Peak Amplitude</td>
<td>45.0 ± 7.1 (17.3 - 118.2)</td>
<td>31.0 ± 3.9 (11.7 - 62.0)</td>
</tr>
<tr>
<td>Peak Length</td>
<td>100± 5 (65 - 138)</td>
<td>77 ± 4 (45 - 105)</td>
</tr>
<tr>
<td>Interpeak interval</td>
<td>39 ± 7 (83 - 180)</td>
<td>60 ± 10 (45 - 240)</td>
</tr>
<tr>
<td>Sum of PA</td>
<td>286 ± 47 (90 - 651)</td>
<td>228 ± 28 (93 - 536)</td>
</tr>
<tr>
<td>Derived mean PA</td>
<td>51 ± 7 (20 - 120)</td>
<td>35 ± 3 (15 - 67)</td>
</tr>
</tbody>
</table>
5.3.5 Determinants of GH suppression

An assessment of the GH response in individual subjects revealed that GH concentrations were reduced in 12, and increased in 4 of the 16 subjects following rhIGF-I administration (Figure 22). The two groups of subjects (those who did and those who did not suppress following rhIGF-I administration) were therefore analysed in an attempt to determine factors predicting the GH response to rhIGF-I. This included an assessment of variables known to influence GH secretion such as insulin levels and C-peptide status. The comparison between the two groups is shown in Table 5.3.

### Table 5.3
Overnight GH response to rhIGF-I administration (mean ± SEM and range).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Suppressed</th>
<th>Not suppressed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(20.00h-08.00h)</td>
<td></td>
</tr>
<tr>
<td>n=12</td>
<td>n=4</td>
<td></td>
</tr>
<tr>
<td>GH (mU/l)</td>
<td>28.4 ± 4.4</td>
<td>14.6 ± 1.2</td>
</tr>
<tr>
<td>(control night)</td>
<td>(12.0 - 62.7)</td>
<td>(13.1 - 18.5)</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>243.7 ± 22.2</td>
<td>237.9 ± 50.3</td>
</tr>
<tr>
<td>(136.5 - 374.9)</td>
<td>(161.9 - 385.8)</td>
<td></td>
</tr>
<tr>
<td>C-peptide (nmol/l)</td>
<td>0.08 ± 0.02</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>(0.01 - 0.28)</td>
<td>(0.01 - 0.08)</td>
<td></td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>73.8 ± 15.4</td>
<td>36.8 ± 9.0</td>
</tr>
<tr>
<td>(26.0 - 217.0)</td>
<td>(23.4 - 63.3)</td>
<td></td>
</tr>
<tr>
<td>HbA1 (%)</td>
<td>12.8 ± 0.7</td>
<td>13.4 ± 1.0</td>
</tr>
<tr>
<td>(8.4 - 17.0)</td>
<td>(11.3 - 15.8)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 22.

The GH response to rhIGF-I administration in individual subjects. Mean overnight GH concentrations (20.00h - 08.00h) are shown on control and rhIGF-I nights.
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Those subjects who did not suppress following rhIGF-I administration therefore had reduced GH concentrations on the control night. Endogenous insulin production and prevailing insulin concentrations, both of which have previously been identified as factors affecting GH secretion in IDDM, were not significantly different when the two groups were compared. There was no relationship between GH levels and prevailing blood glucose concentrations.

5.3.6 The relationship between GH and insulin sensitivity

A relationship between overnight GH concentrations and insulin requirements between 05.00h and 08.00h has been described in adolescents with IDDM (Edge et al., 1990b), and other groups studying the dawn phenomenon have detected changes in insulin sensitivity at a similar time (Beaufere et al., 1988; Perriello et al., 1991). The Pulsar parameters, derived from overnight GH levels (20.00h - 08.00h), were therefore compared with free insulin concentrations during the euglycaemic period between 05.00h and 07.00h. A relationship between the parameters assessing GH pulsatility and insulin sensitivity (as assessed by free insulin concentrations) was identified on both control and rhIGF-I nights. The data are shown in Table 5.4, and the relationship between the mean peak amplitude above the assay threshold, maximum GH and free insulin concentrations are also illustrated graphically in Figures 23a and 23b.

The percentage reduction in mean GH peak amplitude (above the assay threshold) following rhIGF-I administration was found to correlate with the reduction in free insulin concentrations between 05.00 and 07.00h (r= 0.53; p= 0.03. Figure 24). There were no associations on control or rhIGF-I nights with Pulsar parameters assessing the GH baseline.
Figure 23a.
The relationship between mean GH peak amplitude (above the assay threshold) determined by the Pulsar programme and log-transformed mean plasma free insulin concentrations (05.00h - 07.00h) on the control night (●; r=0.65, p<0.01) and following rhIGF-I administration (○; r=0.64, p<0.01).

Figure 23b.
The relationship between the maximum GH concentration between 20.00h and 08.00h and log-transformed plasma free insulin data between 05.00h and 07.00h on the control night (●; r=0.48, p=0.05) and following rhIGF-I administration (○; r=0.53, p=0.03).
Figure 24.

The relationship between change in mean overnight GH peak amplitude (above the assay threshold) and the change in plasma free insulin concentrations (05.00h - 07.00h) following rhIGF-I administration when compared to the control night.
Table 5.4
The relationship between GH pulsatility and free insulin concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>rhIGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r = )</td>
<td>( p = )</td>
</tr>
<tr>
<td>Max GH</td>
<td>0.48</td>
<td>0.05</td>
</tr>
<tr>
<td>Peak Amplitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(above calculated baseline)</td>
<td>0.64</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Peak amplitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(above assay threshold)</td>
<td>0.65</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SOPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(above assay threshold)</td>
<td>0.49</td>
<td>0.05</td>
</tr>
</tbody>
</table>

5.4 Discussion

A relationship between the fall in insulin sensitivity during puberty and elevated mean GH concentrations has been demonstrated in normals and subjects with IDDM by Amiel and colleagues (Amiel et al., 1986). Poor blood glucose control in IDDM is also associated with enhanced GH release (Giustina et al., 1990) and reduced hepatic insulin sensitivity (Arslanian et al., 1993). A positive relationship between mean overnight GH levels and HbA1 concentrations was identified in this study, although the precise sequence of events leading to this association are not clear. One explanation might be that GH hypersecretion is the result of inappropriate insulin delivery. Hyperglycaemia does not suppress GH secretion in IDDM as it does in normal subjects (Press et al., 1984b; Ismail et al., 1991) and, furthermore, a reduction in insulin concentrations at adolescence could lead to a rise in GH secretion because of reduced endogenous IGF-I production (Section 1.1.4) and altered feedback control. Thus the rise in GH and HbA1 levels may occur independently, but with the shared underlying link of portal hypoinsulinaemia. Alternatively, GH hypersecretion at
puberty could have a more fundamental role in initiating insulin resistance with the insulin antagonistic actions of the peptide affecting glycaemic control as a primary event. GH release might then be enhanced still further by peripheral hyperinsulinaemia (Press et al., 1992). These mechanisms will be considered further in Chapter 8.

There were methodological differences within the study group which require further consideration when interpreting the GH data. Only eight of the paired studies were of a randomised, double-blind design, and in the remainder the control night always preceded the night of rhIGF-I administration. Subjects received rhIGF-I on the second night in 12 of the 16 paired studies and, interestingly, the baseline night preceded the night when rhIGF-I was administered in all instances when GH was not suppressed. There is evidence that GH secretion may be affected by hospitalisation (Donaldson et al., 1989), and reduced concentrations during a first night in hospital may be followed by a rise in GH levels when sleep is of enhanced quality and duration at the time of a second night when surroundings are more familiar. There may be other explanations for the lack of a consistent effect of rhIGF-I administration on mean GH levels. Overnight GH profiles show significant intraindividual variability (Albertsson-Wikland & Rosberg, 1992) which may lead to a rise in GH concentrations despite a concomitant increase in IGF-I levels and IGF bioactivity.

Furthermore, all subjects with increased GH concentrations on the night of rhIGF-I administration were female. Oestrogen can influence GH levels with a rise in concentrations enhancing GH release (Ho,KY et al., 1987). Two of the four subjects who did not suppress following rhIGF-I administration were in the follicular phase of the menstrual cycle on the control night and in the luteal phase (when endogenous oestrogen production is enhanced) when rhIGF-I was administered. Of the remaining two subjects, one individual was at the same point in the menstrual cycle on both study nights and one subject had a five month history of secondary amenorrhoea. Fasting is a

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further variable influencing GH secretion (Ho, KY et al., 1988) but the timing of meals and calorie intake was similar on the two study nights.

Subjects who did not suppress on the night of rhIGF-I administration also had lower baseline GH concentrations. Low subcutaneous doses of rhIGF-I do not always suppress GH secretion in normal subjects (Trainer et al., 1993) and the impact of an increase in IGF-I levels may be identified more easily when the GH/IGF-I axis is most disturbed. C-peptide status is an important determinant of GH secretion (Wurzburger et al., 1990), but no relationship between C-peptide status and either GH secretion or IGF-I levels was identified. However it is of note that, firstly, C-peptide levels were not assessed by glucagon stimulation as they were in the study by Wurzburger and colleagues (glucagon stimulation may be a more effective means of accurately quantifying the endogenous insulin reserve) and secondly, the number of subjects in the group who did not suppress following rhIGF-I administration was small.

An increase in GH pulse frequency following rhIGF-I administration was identified by the peak detection programme in this study but not by deconvolution and time-series analysis earlier in this thesis (Section 3.6). This discrepancy could be a consequence of improved sleep quality and GH pulsatility when rhIGF-I was administered in 12 of the paired studies on the second study night (Van Cauter et al., 1992b). Alternatively, this may reflect an increase in the resolution of GH profiles. According to this hypothesis, the breakdown of complex GH pulses into more discrete peaks following rhIGF-I administration may lead to an increase in peak identification by Pulsar.

In three out of the four subjects where mean GH levels were not reduced following rhIGF-I administration the free insulin concentrations were not reduced either. Although the insulin antagonistic properties of GH are well-established it is the nature of GH pulsatility, rather than mean GH levels, that appear to determine insulin resistance (Fowelin et al., 1991; Pal et al., 1992), as well as the effects of GH on somatic growth (Hindmarsh et al., 1987, 1992). A relationship between the
attributes assessing GH pulse amplitude and insulin sensitivity was identified when control and rhIGF-I nights were studied, and between the change in GH pulse amplitude and change in insulin sensitivity following rhIGF-I administration when compared to the control night. These observations provide strong evidence of the links between reduced IGF bioactivity, GH hypersecretion and the changes in insulin sensitivity detected in IDDM. However, free insulin levels were reduced following rhIGF-I when compared to the control night in the first six hours of the study. Although comparable euglycaemia had not been established, this suggests that more direct effects of administered rhIGF-I on glucose disposal may have occurred as well.

In one subject a reduction in free insulin concentrations was observed despite an increase in mean GH levels, and a reduction in GH was also seen in one individual without a concomitant fall in free insulin concentrations. This discordance was also seen when the attributes assessing GH pulsatility were examined. Factors such as the GH baseline concentration may need to be included in the description of GH effects on insulin sensitivity, although the discrepancy may also reflect effects of rhIGF-I on glucose disposal by GH-independent means (Section 3.7.4).

A direct correlation between GH concentrations and IGF-I levels was not identified in these studies. Potential inhibitors of IGF-I action, including the IGF binding protein IGFBP-1, have been identified in IDDM (Taylor et al., 1990) and it is possible that IGF bioactivity is a more important determinant of feedback drive than absolute IGF-I levels. The impact of rhIGF-I administration on the IGFBP’s will be considered in Chapter 6.

In summary, this analysis has shown that one of the principle determinants of the GH response to rhIGF-I administration is baseline GH levels, and has demonstrated the importance of GH pulse amplitude in determining insulin sensitivity in adolescents with IDDM. The reduction in insulin requirements following rhIGF-I is linked to the change in GH pulse amplitude, although this does not exclude other more direct actions of administered peptide on glucose disposal.
Chapter 6
THE IMPACT OF rhIGF-I ADMINISTRATION ON THE IGF BINDING PROTEINS

6.1 Introduction

Studies of the GH/IGF-I axis in health and disease have shown that the IGFBP's are important regulators of IGF action (Section 1.4). Reduced IGF-I concentrations in adolescents with IDDM are accompanied by alterations in the circulating levels of the IGFBP's (Section 1.9). However, altered IGFBP's in IDDM might also affect IGF availability and bioactivity independently of changes in the absolute levels of the IGF's. In this chapter the impact of rhIGF-I administration on the IGFBP's and levels of the other components of the IGF/IGFBP complex will be presented and discussed.

6.2 Subjects and methods

The data in this chapter is derived from the seventeen subjects who participated in the studies of bolus rhIGF-I administration. Nine individuals (Subjects 1 - 9 in Appendix 1) participated in the studies described in Chapter 3, and the remaining eight individuals participated in the studies described in Chapter 4 (Subject numbers 10 - 17 in Appendix 1).

IGF-II and IGFBP data following rhIGF-I administration in the thigh will be compared with control studies when either an injection of placebo was administered (Subjects 1 - 9) or no injection at all (Subjects 10 - 17).

The assays described in this chapter were not undertaken in all subjects. Sometimes there was insufficient sample, and in other instances an assay was undertaken before the study group was extended to include 17 individuals. In most sections the effects of rhIGF-I administration are therefore considered in a subgroup of the 'core' 17 subjects.
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Short term alterations in glucose and insulin concentrations do not have a significant impact on IGF-II, IGFBP-3 and ALS concentrations and so these variables have been assessed irrespective of short term glycaemic control. IGFBP-1 concentrations are closely related to those of insulin and so IGFBP-1 data will be analysed in greatest detail during periods of strict euglycaemia. The development of hypoglycaemia was not a criterion for exclusion from the IGF-II or IGFBP analysis.

6.3 Results

6.3.1 IGFBP-1

The IGFBP-1 concentrations in subjects 1-9 were measured during the 22h period in hospital and underwent a diurnal change with lower levels during the day than overnight. This was observed during control and rhIGF-I studies, and it was apparent that low IGFBP-1 levels were seen around three to four hours after each meal at 18.00h, 09.00h and 12.00h (Figure 25). An inverse relationship between levels of free insulin and IGFBP-1 concentrations has been described (Holly et al., 1988), and increased insulin delivery at mealtimes would explain the elevated IGFBP-1 concentrations overnight with reduced levels during the day. The mean early morning IGFBP-1 levels at 08.00h of approximately 50 μg/l on both control and rhIGF-I nights are similar to those described in normal puberty (Holly et al., 1990). These values are lower than those seen in a group of predominantly pre-pubertal individuals ( Cotterill et al., 1988), and may reflect higher insulin concentrations in the pubertal subjects described in this chapter.

Because serum IGFBP-1 levels are strongly related to insulin in normal and diabetic subjects, IGFBP-1 concentrations were analysed in greatest detail during the period of strict euglycaemia achieved between 02.00h and 08.00h in subjects 1 to 9 (Figure 25). Mean IGFBP-1 levels during this interval were 49.3 ± 7.6 μg/l on the control night, and 59.3 ± 8.2 μg/l after rhIGF-I administration. Repeated measures analysis of variance did not identify significant differences
Figure 25.
Mean (+ SEM) 22h IGFBP-1 concentrations after placebo (○) or rhIGF-I (O) administration (n=9). The timing of meals is indicated by the vertical arrows.

Figure 26.
Mean 12h overnight IGFBP-1 concentrations (n=17) on the control night (●) and following administration of rhIGF-I at 18.00h (O).
between the two sets of data, although visual inspection and paired statistical testing of mean values for the interval between 02.00h - 08.00h indicated a trend suggestive of increased IGFBP-1 concentrations following rhIGF-I administration (p= 0.08).

A further analysis of IGFBP-1 concentrations was therefore undertaken in all 17 subjects during the 12h period from 20.00h to 08.00h. Blood glucose concentrations were similar during this interval (7.6 ± 0.5 mmol/l on the control night and 7.0 ± 0.4 mmol/l after rhIGF-I) and yet free insulin concentrations were reduced (62.1 ± 11.8 mU/l on the control night and 35.5 ± 3.8 mU/l after rhIGF-I administration; p< 0.01). Despite this marked reduction in insulin concentrations no difference in IGFBP-1 levels was identified; 53.4 ± 7.3 μg/l on the control night and 61.7 ± 12.9 μg/l after rhIGF-I administration (Figure 26).

IGFBP-1 has inhibitory activity in most bioassay systems, and the presence of elevated concentrations during control and rhIGF-I studies may be implicated in the relatively low IGF bioactivity (Section 3.4.3). However a direct relationship between IGF bioactivity and IGFBP-1 concentrations was not identified in these studies.

6.3.2 The relationship between IGFBP-1 and insulin

The relationship between insulin and IGFBP-1 was assessed in the 9 subjects in whom strict euglycaemia was achieved between 02.00h and 08.00h. An inverse relationship between mean free insulin and IGFBP-1 levels was identified in individual subjects on the control night (r= -0.79, p= 0.01), and following rhIGF-I administration (r= -0.88, p= 0.001; figure 27).

The temporal relationship between insulin and IGFBP-1 was assessed in more detail by examining the relationship between hourly insulin and IGFBP-1 concentrations in addition to mean values overnight. Each study night then generated seven points for comparison rather than a single mean value. A significant relationship between the two variables was identified on the control night
Figure 27.
The relationship between log transformed mean overnight insulin and IGFBP-1 concentrations in individual subjects (02.00h - 08.00h) following placebo (•) or rhIGF-I (○) administration.

Figure 28.
The relationship between insulin concentrations (24.00h - 06.00h) and IGFBP-1 concentrations 2h later (02.00h - 08.00h) following placebo (•) or rhIGF-I administration (○). Both sets of data have been log-transformed.
(r = -0.39, p = 0.001), but not following rhIGF-I administration (r = -0.22, p = 0.08). However, when insulin concentrations 2h earlier were compared with the same set of IGFBP-1 data, a more robust relationship was identified (r = -0.56, p < 0.001 on the control night and r = -0.74, p < 0.001 after rhIGF-I; Figure 28). The relationship weakened when insulin concentrations 1h and also 3h prior to IGFBP-1 levels (02.00 - 08.00h) were used in the analysis. Insulin alters hepatic IGFBP-1 production, and there is an inherent delay before a change in insulin concentration alters IGFBP-1 transcription and then circulating IGFBP-1 levels. The observed delay of 2h is similar to the interval described in other experimental settings (Lee et al., 1993).

The levels of IGFBP-1 were the same on the two study nights despite a significant reduction in free insulin concentrations following rhIGF-I administration. Although this may lead one to suspect that administered rhIGF-I alters IGFBP-1 levels directly, visual inspection of the data in Figure 28 does not suggest that rhIGF-I administration led to major changes in the relationship between the two variables. Although the slope of the regression lines on control and rhIGF-I nights were different (-1.49 on the control night versus -0.88 after rhIGF-I administration), the correlation coefficient on the control night was relatively low (-0.56), and one would expect direct effects of administered rhIGF-I at the insulin receptor to increase and not decrease this slope.

6.3.3 The inter-relationship between IGF-I, insulin and IGFBP-1 concentrations

The inter-relationship between IGF-I, IGFBP-1 and insulin was assessed in the 9 subjects in whom strict euglycaemia was maintained overnight. Mean values (between 02.00h and 08.00h) were used in this comparison and the results are shown in Table 6.1. There were strong negative relationships between IGFBP-1 and insulin as described, an inverse relationship between IGFBP-1 and IGF-I, and a positive relationship between insulin and IGF-I concentrations. This data is also represented graphically in Figure 29.
Figure 29.

The relationship between: a) mean insulin and IGFBP-1 concentrations, b) mean insulin and IGF-I concentrations and c) IGFBP-1 and IGF-I concentrations after placebo (●) or rHIGF-I (○) administration. Each data point is derived from mean values during the period of euglycaemia (02.00h - 06.00h). Insulin and IGFBP-1 data have been log transformed. Table 6.1 lists further details of the relationship between variables.
Table 6.1

The relationship between IGF-I (ng/ml) and log-transformed insulin (mU/l) and IGFBP-1 (μg/l) concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>rhIGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insulin versus IGF-I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r= 0.74</td>
<td>r= 0.82</td>
<td></td>
</tr>
<tr>
<td>p= 0.02</td>
<td>p= 0.006</td>
<td></td>
</tr>
<tr>
<td>slope= 0.66</td>
<td>slope= 3.41</td>
<td></td>
</tr>
<tr>
<td><strong>Insulin versus IGFBP-1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r= -0.79</td>
<td>r= -0.88</td>
<td></td>
</tr>
<tr>
<td>p= 0.01</td>
<td>p= 0.001</td>
<td></td>
</tr>
<tr>
<td>slope= -0.71</td>
<td>slope= -1.61</td>
<td></td>
</tr>
<tr>
<td><strong>IGFBP-1 versus IGF-I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r= -0.65</td>
<td>r= -0.72</td>
<td></td>
</tr>
<tr>
<td>p= 0.05</td>
<td>p= 0.027</td>
<td></td>
</tr>
<tr>
<td>slope= -1.01</td>
<td>slope= -2.11</td>
<td></td>
</tr>
</tbody>
</table>

('r' = correlation coefficient, 'p' = probability value, 'slope' = incline of the regression line)

Thus the relationship between insulin and IGF-I concentrations changes following rhIGF-I administration, with a given IGF-I value then corresponding to a reduced insulin concentration (Figure 29b). The reduction in insulin concentrations following rhIGF-I administration is reflected by the rise in IGFBP-1 which can be seen in Figure 29c.
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6.3.4 IGF-II

IGF-II levels were measured over a period of 22h in subjects 1-9, and are shown in Figure 30. There were no differences in baseline IGF-II levels, but there was a decline in concentrations in both control and rhIGF-I groups as the evening progressed to a nadir at 04.00h (p< 0.01). The decline was more pronounced following rhIGF-I administration (p< 0.01). There was no difference in IGF-II concentrations between the two nights 14h after rhIGF-I administration, and no overall difference in mean IGF-II levels (18.00h - 16.00h) between the study two groups (Table 6.2).

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>rhIGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong> (18.00h)</td>
<td>626 ± 48 (432 - 843)</td>
<td>609 ± 40 (484 - 857)</td>
</tr>
<tr>
<td><strong>Nadir</strong> (04.00h)</td>
<td>559 ± 40 (402 - 791)</td>
<td>473 ± 32 (325 - 650)</td>
</tr>
<tr>
<td><strong>Final sample</strong> (16.00h)</td>
<td>650 ± 54 (392 - 862)</td>
<td>628 ± 73 (381 - 1010)</td>
</tr>
<tr>
<td><strong>Overall mean</strong> (18.00h-16.00h)</td>
<td>598 ± 44 (410 - 875)</td>
<td>554 ± 29 (448 - 697)</td>
</tr>
</tbody>
</table>

6.3.5 IGFBP-3

Overnight IGFBP-3 data (assessed in 17 subjects) is represented graphically in Figure 31. Although there was no difference in baseline IGFBP-3 concentrations, they fell between 18.00h and 03.00h on the placebo night whilst remaining stable after rhIGF-I administration (p< 0.01). Levels
Figure 30.

Mean (± SEM) 22h IGF-II concentrations (n=9) after administration of placebo (●) or rhIGF-I (○).
Figure 31.

Overnight (± SEM) IGFBP-3 concentrations (18.00h - 08.00h) in the 17 subjects involved in the single bolus studies on the control night (●) and following administration of rhIGF-1 (○).
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were comparable again by 06.00h the following morning, 12h after rhIGF-1 administration. The overall mean IGFBP-3 concentrations were increased on the night of rhIGF-1 administration when compared to the placebo night (p= 0.04; Table 6.3).

Table 6.3

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>rhIGF-1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (18.00h)</td>
<td>5.6 ± 0.2</td>
<td>5.3 ± 0.3</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>(4.3 - 7.0)</td>
<td>(4.0 - 9.4)</td>
<td></td>
</tr>
<tr>
<td>Final sample (08.00h)</td>
<td>4.9 ± 0.2</td>
<td>5.1 ± 0.3</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>(3.2 - 6.7)</td>
<td>(3.7 - 8.1)</td>
<td></td>
</tr>
<tr>
<td>Overall mean (18.00 - 08.00h)</td>
<td>4.9 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>p=0.04</td>
</tr>
<tr>
<td></td>
<td>(3.3 - 6.8)</td>
<td>(3.9 - 7.5)</td>
<td></td>
</tr>
</tbody>
</table>

6.3.7 ALS

ALS concentrations were measured in 16 of the 17 subjects on both control and rhIGF-1 nights at 18.00h and 08.00h. There was no difference in baseline ALS concentrations when control and rhIGF-1 nights were compared, and no apparent change in ALS concentrations during the control study. However a small but statistically significant decrease in the ALS occurred overnight following rhIGF-1 administration (Table 6.4). The ALS was clearly present in excess of the levels of IGFBP-3 because although the molecular weight is nearly twice that of IGFBP-3, the concentrations were approximately eight-fold greater.
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Table 6.4

<table>
<thead>
<tr>
<th></th>
<th>18.00h</th>
<th>08.00h</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>47.8±3.2</td>
<td>45.7±3.1</td>
<td>0.2</td>
</tr>
<tr>
<td>rhIGF-I</td>
<td>48.5±2.8</td>
<td>44.5±3.2</td>
<td>0.04</td>
</tr>
</tbody>
</table>

6.3.7 The relationship between the IGF’s, IGFBP-3 and ALS

IGFBP-3 is believed to be largely saturated by IGF-I and IGF-II, and so the sum of IGF-I and IGF-II, when expressed in molar terms, is approximately equal to the circulating concentration of IGFBP-3 in normal subjects. The free IGF’s have a short half-life of around 10 minutes and the amount of IGF-I and II bound to other IGFBPs is relatively small. When the concentrations of the IGF’s and IGFBP-3 were measured at the same time during the first 12h of the study, relationships were identified between the combined amount of IGF’s I and II and IGFBP-3 levels during the placebo, but not the rhIGF-I studies (Table 6.5). The molecular weights of IGF-I and IGF-II were assumed to be 7.6 kDa and 7.4 kDa respectively (Section 1.1.2) and the molecular weight of IGFBP-3 was assumed to be 53kDa (Section 1.2.2). This analysis could only be undertaken in the nine subjects in whom IGF-II concentrations had been measured.
Table 6.5

The relationship between the sum of the molar amounts of IGF-I and IGF-II, and those of IGFBP-3.

<table>
<thead>
<tr>
<th>Placebo</th>
<th>rhIGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>18.00h</strong></td>
<td></td>
</tr>
<tr>
<td>r = 0.78</td>
<td>r = 0.55</td>
</tr>
<tr>
<td>p = 0.01</td>
<td>p = 0.12</td>
</tr>
<tr>
<td>slope= 0.93</td>
<td>slope= 0.77</td>
</tr>
</tbody>
</table>

| **24.00h** |         |         |
| r = 0.6  | r = 0.16 |
| p = 0.07 | p = 0.67 |
| slope= 1.05 | slope= 0.18 |

| **06.00h** |         |         |
| r = 0.70 | r = 0.57 |
| p = 0.03 | p = 0.10 |
| slope= 1.13 | slope= 0.98 |

('r' = correlation coefficient, 'p' = probability value, 'slope' = incline of the regression line)

On the placebo night the slope of the regression line at each of the three points (18.00h, 24.00h and 06.00h) was approximately 1.0. This was not unexpected when considering the relationship between the IGF's and IGFBP-3 in normal subjects. Following rhIGF-I administration this relationship was no longer observed however, indicating that significant amounts of the IGF's
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were bound to other BP's besides IGFBP-3, or alternatively were circulating in the unbound 'free' form. The absence of an identifiable relationship at 18.00h, prior to the administration of rhIGF-I, was probably due to sample pooling because of insufficient baseline blood. Mean overnight IGF-I concentrations were related to IGFBP-3 concentrations on the placebo night ($r= 0.48$, $p= 0.05$), but not on the night of rhIGF-I administration ($r= 0.4$, $p= 0.12$).

ALS concentrations correlated with IGFBP-3 levels in individual subjects on both control and rhIGF-I nights at 18.00 and 08.00h (Table 6.6).

| Table 6.6 |
| The relationship between IGFBP-3 and ALS concentrations. |
| Control | rhIGF-I |
| 18.00h | $r= 0.63$, $p= 0.008$ | $r= 0.52$, $p= 0.036$ |
| 08.00h | $r= 0.70$, $p= 0.002$ | $r= 0.68$, $p= 0.003$ |

There was no relationship between change in overnight ALS concentrations and change in the levels of IGFBP-3 on either night ($r= 0.18$, $p= 0.49$ on control night and $r= -0.31$, $p= 0.24$ following rhIGF-I administration). However, the sum of IGF-I and IGF-II concentrations were related to ALS concentrations at baseline ($r= 0.8$, $p= 0.01$ at 18.00h on both nights) but not the following morning ($r= 0.22$, $p= 0.5$ on the baseline night and $r= 0.24$, $p= 0.2$ after rhIGF-I).
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6.3.8 IGF-I chromatography

Detailed analysis by gel filtration chromatography in one individual (subject number 1 who received placebo or rhIGF-I injections), demonstrated increased in IGF-I in the fractions corresponding to the 150 kDa IGFBP-3 ternary complex 3h after rhIGF-I administration. The peak corresponding to this complex had approximately doubled by 6h, and remained elevated after 18h. IGF-I in fractions corresponding to the 50 kDa binary complexes fell on the control night by around 50% but remained relatively stable following rhIGF-I administration throughout the 22h study.

Fractions corresponding to unbound IGF-I had increased 3h after rhIGF-I administration, with levels twice that seen on the control night at 6h (20 ng/ml versus 10 ng/ml), but with similar amounts by 12h and 18h. Free IGF-I levels, expressed as a percentage of total IGF-I, ranged from approximately 3% to 7%. The change in IGF-I distribution according to molecular weight band following placebo and rhIGF-I administration is shown in Figure 32, whilst Table 6.7 indicates the increase in IGF-I levels above baseline values after the rhIGF-I injection.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>150kDa</th>
<th>50kDa</th>
<th>7.5kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>28</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>48</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>18</td>
<td>24</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>
Figure 32.
IGF-I chromatography studies in one subject followed sequentially for 18h. The figure illustrates IGF-I distribution in the molecular weight bands corresponding to that primarily bound to IGFBP-3 (fraction numbers 42 - 46 = 150 kDa), that bound to IGFBP-1 (fraction numbers 50 - 54 = 50kDa) and free IGF-I (fraction numbers 59 - 62 = 7.5kDa).
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The chromatography technique is a useful means of determining temporal trends, although the absolute IGF-I concentrations have to be interpreted cautiously. However, rhIGF-I administration appeared to lead to a relatively brisk rise and then fall in levels of the free peptide in the one subject studied, with a more pronounced and sustained rise in IGF-I levels within the 150kDa complex.

6.4 Discussion

6.4.1. Baseline IGFBP-3 concentrations

Approximately 80% of circulating IGF-I can be identified as a ternary, 150K complex with IGFBP-3 and the ALS (Baxter & Martin, 1989; Sara & Hall, 1990). IGF-I binds first to IGFBP-3 which in turn can then bind the ALS (Baxter et al., 1992). Complex formation does not occur between IGFBP-3 and ALS alone. IGFBP-3 is saturated with IGF-I and IGF-II (Baxter & Martin, 1986), and the half-lives of the peptides are reduced when in the free form (Guler et al., 1989b; Lewitt et al., 1993). GH appears to regulate IGFBP-3 production directly, although some animal studies have indicated that this effect may be mediated via IGF-I (Zapf et al., 1989; Blum et al., 1993).

Low IGFBP-3 levels in IDDM were first reported in adults (Baxter & Martin, 1986), and later confirmed in the adolescent age group (Batch et al., 1990). The studies described in this chapter have also demonstrated low baseline IGFBP-3 concentrations in adolescents with IDDM (Baxter & Martin, 1986; Cwyfan-Hughes et al., 1992). The close relationship between the IGFs and IGFBP-3 may explain this observation, with reduced IGF-I production leading to a fall in IGFBP-3 concentrations because of altered kinetics of the unbound protein. According to this hypothesis, low IGFBP-3 concentrations arise as a secondary, rather than as a primary event.
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There are other potential explanations for low IGFBP-3 concentrations in IDDM as well. Thus they may, like reduced levels of IGF-I, reflect portal insulin deficiency. A reduction in hepatic GH receptor numbers, such as that arising in IDDM, could then result in impaired generation of GH dependent factors (Baxter et al., 1981; Batch et al., 1991). A more direct link between low portal insulin concentrations and impaired IGFBP-3 production has not been identified, however (Brismar et al, 1994). It has also become clear that the affinity of IGFBP-3 for IGF-I and the distribution of IGF-I amongst the IGFBP's may change in both normal and abnormal physiological states (Section 1.4.2). Alterations in affinity have been linked to the activity of serum proteases which appear to change IGF availability (Blat et al., 1994). Structural alterations in the IGFBP-3 molecule induced by protease activity (with subsequent changes in ligand affinity) may also account for some of the methodological problems described when determining IGFBP-3 levels (Cwyfan-Hughes et al., 1992). It is possible that alterations in protease activity also account for the low IGFBP-3 concentrations observed at the start of the studies described in this thesis.

An overnight fall and then rise in IGFBP-3 concentrations was seen in the studies described in this chapter. IGFBP-3 concentrations levels are normally relatively stable in the short term and do not undergo diurnal change. Calorie restriction leads to reductions in IGFBP-3 band intensity as assessed by ligand blotting techniques, but only over a period of days (Young et al., 1992). The observed decline in IGFBP-3 concentrations overnight may therefore reflect changes in tissue availability of the IGF's and IGFBP's in subjects with IDDM. Although the ternary complex is confined within the circulation, the binary complexes are not, and the fall in IGFBP-3 may thus represent redistribution rather than clearance (Binoux & Hossenlopp, 1988). The changes observed overnight may also reflect alterations in serum protease activity. Circulating or locally produced IGFBP-3 might then participate in the regulation of IGF action at the tissue level (Conover & Powell, 1991; Conover, 1992). Thus it is possible that regulatory mechanisms, similar to that seen in
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catabolic states, operate in subjects with IDDM as well. It could also be argued that a decline in overnight IGFBP-3 levels reflects reduced IGF or IGFBP-3 production and is, again, a pathological consequence of reduced portal insulin levels. The assessment of protease activity in these studies may help to resolve these issues.

6.4.2 IGFBP-3 levels following rhIGF-I administration

IGFBP-3 concentrations did not fall after rhIGF-I administration as they did on the control night. A change in IGFBP-3 concentrations could indicate an increase in formation of the relatively stable IGF-I/IGFBP-3 complex or a change in the rate of complex breakdown. There is evidence to suggest that IGF-I concentrations may be the limiting factor in the formation of the more stable ternary complex (Lewitt et al., 1994) and rhIGF-I administration to diabetic rats has been shown to lead to enhanced ternary complex formation (Lewitt et al., 1993b). The difference in IGFBP-3 levels in these studies was brief, with similar IGFBP-3 levels on control and rhIGF-I nights by 06.00h. This rapid but short-lived change points to altered kinetics rather than altered rates of protein production. This could be explained by increased formation of the stable complex in the presence of increased IGF-I delivery, or reflect changes in protease activity preventing complex breakdown and IGF redistribution. Interestingly, the slightly longer rhIGF-I half-life in IDDM subjects when compared to normals (Sections 3.4.2 and 3.7.1) could be linked to enhanced ternary complex formation and altered IGF-I kinetics. A direct relationship between IGFBP-3 concentrations and IGF-I half-life was not identified, however.

An alteration in production rate of one of the components of the binding complex may also explain the alteration in IGFBP-3 levels on the rhIGF-I night. Administration of rhIGF-I to normal subjects led to a decrease in IGFBP-3 concentrations as determined by radioimmunoassay (Baxter et al., 1993; Kupfer et al., 1993), and yet when measured by immunoblotting and autoradiography,
or ligand blotting, increases in IGFBP-3 levels were identified (Lieberman et al., 1992; Young et al., 1992). Although the explanation for at least some of the discrepancies may lie in the technique used to assess binding protein levels (Gargosky et al., 1992), the combination of rhIGF-I and GH together has been shown to increase IGFBP-3 concentrations (determined by RIA), in contrast to rhIGF-I alone (Kupfer et al., 1993).

Determining the impact of rhIGF-I administration on IGFBP-3 concentrations in patients with GHI has not been straightforward. An increase in IGFBP-3 (measured by Western ligand blotting) with longer term rhIGF-I administration led to the conclusion that IGFBP-3 could be induced by IGF-I alone (Kanety et al., 1993), and yet when measured by RIA no alteration in mean levels was identified (Fielder et al., 1993). These reports have served to emphasise the apparent complexities of IGFBP-3 production, regulation and measurement.

6.4.3 ALS

There is little data in the literature about the impact of the abnormalities of the GH/IGF-I axis in IDDM on the third component of the ternary complex, the ALS. ALS is reportedly under GH regulation and appears to circulate in excess in diabetic rats and in normal human subjects (Baxter, 1988; Lewitt et al., 1993a). Reductions in ALS have been noted in calorie restricted adults following intravenous rhIGF-I administration on successive days. However concentrations tended to rise when GH and IGF-I were administered together (p= 0.07) (Kupfer et al., 1993). ALS concentrations are low in GHI subjects, and appear to be unaffected by rhIGF-I administration (Gargosky et al., 1993). The low ALS concentrations in GHI may prevent ternary complex formation and explain why rhIGF-I appears to have little impact on IGFBP-3 levels in this condition.
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Baseline ALS concentrations were high in this group of adolescents with IDDM. Levels fell overnight following rhIGF-I administration although the magnitude of the change was small. It is possible that the reduction in GH secretion and GH levels following rhIGF-I administration may account for these observations. If the rise in IGFBP-3 levels following rhIGF-I administration was associated with an increase in ternary complex formation, then this suggests that ALS is indeed present in excess in IDDM.

6.4.4 IGF-II

Baseline IGF-II concentrations were not increased in these studies, and so there was no clear evidence to indicate a rise in IGF-II production as a means of compensating for the low levels of IGF-I (Hall et al., 1989).

Although IGF-I concentrations were stable on the control night there was a significant decline in the levels of IGF-II which occurred at a similar time to the fall in IGFBP-3. The alteration in IGF-II concentrations could be associated with redistribution to other IGFBP's, and there is also the theoretical potential for acute metabolic effects on glucose disposal (Shapiro et al., 1990). The more pronounced decline in IGF-II concentrations following rhIGF-I administration, when compared to the control night, is consistent with displacement of the peptide from IGFBP-3 (Guler et al., 1989a; Zenobi et al., 1992a).

The nadir in IGF-II levels occurred somewhat later than peak IGF-I concentrations, suggesting that in the interim period IGF-I was either in the free form, or was bound to other IGFBP's besides IGFBP-3. Interestingly, an increase in the concentrations of other binding proteins besides IGFBP-1 and IGFBP-3 has been demonstrated in studies of rhIGF-I administration in adults with NIDDM (Young & Clemmons, 1994). A close relationship between IGFBP-3 and the IGF's was not identified following rhIGF-I administration in contrast to the control night. This is further
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Evidence of changes in the levels of free peptide and that bound to other IGFBPs following rhIGF-I administration. The chromatography data demonstrated a rise in the low molecular weight bands corresponding to IGFBPs other than IGFBP-3 after rhIGF-I, and a modest increase in bands corresponding to free peptide. IGFBP-1 is largely unsaturated under normal circumstances and, together with the other IGFBPs, represents a potential source of binding sites for the IGF's.

6.4.5 IGFBP-1

The inverse relationship between insulin and IGFBP-1 is well described (Cotterill et al., 1988; Holly et al., 1988), and was observed during control and rhIGF-I studies. IGFBP-1 transcription and production in the liver is primarily regulated by portal insulin delivery, with an increase in circulating insulin levels resulting in a reduction in the concentrations of IGFBP-1 between one and two hours later (Suikkanen et al., 1989; Brismar et al., 1994). The relationship between insulin and IGFBP-1 was maximal at 2h, and the nature of the relationship was also similar during control and rhIGF-I studies. This latter observation is surprising in view of the reduction in insulin levels after rhIGF-I administration but with similar IGFBP-1 concentrations during the two studies. High doses of rhIGF-I administered to subjects with NIDDM have been shown to lead a reduction in IGFBP-1 levels, and it was hypothesised that this could be due to interactions between rhIGF-I and the insulin receptor (Young & Clemmons, 1994). A similar effect of administered rhIGF-I may have occurred in these studies, although not to the extent of being clearly identified by calculating the slope of the respective regression lines. In-vitro studies of the effects of rhIGF-I on IGFBP-1 levels have revealed conflicting results (Hill et al., 1989a; Lewitt & Baxter, 1989a). There is experimental evidence to suggest that IGFBP-1 may modulate IGF-I activity in response to changes in blood glucose rather than indirectly via insulin (Yeoh & Baxter, 1988), and it is
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noteworthy that the relationship between insulin and IGFBP-1 was studied during nights of comparable glycaemic control.

It has been suggested that IGFBP-1 is a regulator of the acute metabolic actions of IGF-I, inhibiting IGF bioactivity by increased circulatory binding when insulin levels are low, and possibly transporting the peptide to the tissues after meals when insulin levels are high (Holly, 1991). IGFBP-1 levels are elevated in IDDM (Batch et al., 1991), particularly when metabolic control is poor. These observations could be linked to the altered peripheral/portal insulin ratio in IDDM. Baxter and colleagues have reported a pronounced increase in IGFBP-1 levels following rhIGF-I administration to fasting normal subjects, but this could be linked to the higher dose of rhIGF-I used in that study (100 µg/kg) and, more significantly, the suppression of endogenous insulin production (Baxter et al., 1993).

An interesting observation derived from the period of euglycaemia was that higher IGF-I concentrations were associated with greater free insulin and lower IGFBP-1 concentrations. IGF-I concentrations rise during puberty in subjects with IDDM and are maximal in late puberty. At this time IGF-I concentrations are not 'static', and in some of the older and more mature subjects may be undergoing a slow but steady decline. The subjects with the greatest IGF-I concentrations may be the most insulin resistant because insulin resistance and IGF-I concentrations tend to rise in tandem, and then fall to lower values in young adults (Caprio et al., 1989; Cook et al., 1993). To identify the effect of other variables on insulin concentrations may require a more homogenous group at a more discrete stage of development. Because subjects with the greatest concentrations of IGF-I also have the highest insulin levels, they might also be expected to have reduced levels of IGFBP-1. This was, indeed, found to be the case.
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6.4.6 Summary

Reduced portal insulin delivery is the principal reason for the rise in IGFBP-1 concentrations in IDDM, and low IGF-I levels are closely linked to the reduction in levels of IGFBP-3. The most plausible explanation for the transient increase in IGFBP-3 concentrations that was observed following rhIGF-I administration in this group of adolescents with IDDM is a change in complex stability, rather than a change in the rate of IGFBP-3 production. The high concentrations of the ALS do not suggest that this is a rate limiting factor in ternary complex formation (with IGF-I and IGFBP-3) in IDDM, and the small reduction in the ALS following rhIGF-I administration provides some support for the role of GH in ALS regulation. There was a linear relationship between the sum of the IGF's and IGFBP-3 in IDDM. This relationship persisted overnight despite a reduction in IGF-II concentrations, probably because of a reduction in IGFBP-3 levels. A direct relationship between the fall in IGF-II and fall in IGFBP-3 concentrations was not identified, although the different sampling intervals prevented a more detailed comparison between these variables.

Following rhIGF-I administration IGF-I levels rose, IGF-II concentrations fell, and IGFBP-3 concentrations remained stable. A relationship between the sum of the IGF's and IGFBP-3 was not seen, probably because the pronounced rise in IGF-I was not matched by an equivalent fall in IGF-II levels, and because, as shown by the chromatography data, not all of the administered rhIGF-I was carried in the large 150kDa complex with IGFBP-3. Although ALS concentrations were related to IGFBP-3 levels at the beginning and end of control and rhIGF-I studies, an overnight change in the concentration of the IGF's appears to have contributed to the weakening of a relationship identified in the early evening between the ALS and the sum of IGF-I and IGF-II. A change in the normal inverse relationship between insulin and IGFBP-1 was not observed following administration of rhIGF-I.
Chapter 7

THE EFFECTS OF REPEATED rhIGF-I ADMINISTRATION IN IDDM DURING ADOLESCENCE

7.1 Introduction

The studies described in earlier chapters provide evidence to support the original hypothesis that rhIGF-I administration may have a therapeutic role in IDDM during adolescence. A single subcutaneous injection of rhIGF-I in a dose of 40 µg/kg led to an increase in IGF-I concentrations to within the normal physiological range, a reduction in GH secretion and in the insulin requirements for the maintenance of euglycaemia without any overt side effects.

The next stage in the investigation of rhIGF-I 'replacement' in adolescents with IDDM was to examine the effects of longer-term administration in a small group of subjects. The change in IGF-I concentrations in the single bolus studies suggested that 40 µg/kg of rhIGF-I administered by subcutaneous injection in the thigh on a daily basis would be an appropriate dose to use in the longer term.

7.2 Subjects and methods

7.2.1 Subjects

Male subjects were recruited because animal toxicology data on the effects of rhIGF-I during pregnancy was limited at that time. Only subjects with less than ideal blood glucose control were considered eligible, and individuals with HbA1c concentrations of less than 8.0% were not approached. The six adolescent males recruited were all in mid to late puberty (stages 3 - 5) and presented with IDDM at least 4 years before the studies began. All were in good general health, without proteinuria on strip testing and had normal fundoscopy. None had a recent history of severe
hypoglycaemia. Subjects were not taking any regular medication apart from one individual with acquired hypothyroidism who was biochemically euthyroid on 200 μg of thyroxine daily.

The overall characteristics of the six subjects are shown in Table 7.1. Further details of individual subjects are listed in Appendix 1 (Subjects 18 - 23).

<table>
<thead>
<tr>
<th>Subject characteristics.</th>
<th>Range</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.6-19.4</td>
<td>15.6</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>4.5-16.0</td>
<td>10.0</td>
</tr>
<tr>
<td>C-peptide (nmol/l) (BG&lt;7.0mmol/l)</td>
<td>0.02-0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.8-26.9</td>
<td>23.6</td>
</tr>
<tr>
<td>Insulin dose (u/kg)</td>
<td>0.9-1.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (number of subjects)</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Puberty stage</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>G3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>G4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G5</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>
Chapter 7.

7.2.2 Objectives

The study was designed to provide preliminary data about the safety and efficacy of longer-term administration of rhIGF-I in adolescents with IDDM. The impact of rhIGF-I administration on renal function and biochemical indices would be addressed, whilst close contact with participating subjects during the study would rapidly identify potential side-effects such as hypoglycaemia. An assessment of the effects of longer term rhIGF-I administration on circulating IGF-I levels, subcutaneous insulin requirements and GH concentrations was also part of the study protocol.

7.2.3 Study Design

The study was of an open and non-randomised design, and subjects were made aware of the possible effect of rhIGF-I administration on insulin requirements. The study lasted 12 weeks and consisted of a run-in period followed by a period of rhIGF-I administration and then a run-out, all of 4 weeks duration.

7.2.4 Protocol

All recruited subjects were on a ‘basal/bolus’ multiple injection regimen comprising three pre-meal injections of soluble insulin and intermediate acting insulin (human isophane) at bed-time. During the 4 week run-in period (Wks -4 to day 0) blood glucose control was optimised by regular home blood glucose testing and regular discussion of the results with the author. After the run-in period, rhIGF-I in a dose of 40 µg/kg was administered by subcutaneous injection in the thigh at approximately 18.00h for 28 successive days (day 0 to Wk +4). Reductions in the basal insulin dose were made at the start of the period of rhIGF-I administration in anticipation of a change in subcutaneous insulin requirement. The studies of bolus rhIGF-I administration led to an overnight reduction in insulin infusion requirements of approximately 20%. Each subject was therefore
advised to reduce the evening (pre-bedtime) dose of isophane insulin by this amount, and
subsequently to adjust the insulin dose according to early morning blood glucose test results. The
period of rhIGF-I administration (day 0 to Wk +4) was followed by a run-out period of a further
four weeks duration (Wks +4 to +8). The study protocol is illustrated in Figure 33.

Subjects were instructed to undertake blood glucose testing on at least two occasions each
day throughout the 12 week study, and to pay particular attention to values before breakfast.
Subjects kept a record of home blood glucose test results, the dose of insulin administered and of
hypoglycaemic symptoms throughout the study in a specially prepared diary, and were advised to
adjust the evening isophane dose if blood glucose concentrations were outside a range from 4 to 8
mmol/l on successive mornings. A significant change in the three, pre-meal soluble insulin bolus
injections was not anticipated, although subjects were advised to adjust the dose administered if this
was indicated by home blood glucose test results.

Close contact with the author was maintained throughout the study. This comprised regular
phone calls and intermittent home visits.

7.2.5 rhIGF-I injection

The rhIGF-I preparation was the same as that used in the single bolus studies. Subjects were
shown how to draw up the peptide from the vial at the time of the first injection in hospital, and the
second injection of rhIGF-I was supervised at home the following day. Subjects were warned of
possible discomfort at the time of rhIGF-I administration because the vehicle for the recombinant
peptide had not been altered following the single-bolus studies. They were advised to administer the
rhIGF-I injection at approximately 18.00h each day.
Figure 33.

Illustration of the study protocol devised to assess the safety and effects of rhIGF-I administered to adolescents with IDDM for 28 successive days.
7.2.6 Overnight profiles

Three overnight sampling profiles were conducted in hospital. These were at the beginning of the final week of the run-in period (Wk -1), and at the time of the first (day 0), and final injections of rhIGF-I (Wk +4). Blood sampling began at 20.00h on the baseline night (Wk -1), and from 18.00h (the time of rhIGF-I administration) on the subsequent two nights.

Subjects remained on their current insulin regimen on the day of admission to hospital, and were encouraged to eat meals and snacks as they would under normal circumstances before and during the profile. The profiles were otherwise conducted as for the single bolus studies using the intermittent blood sampling technique.

The following parameters were assessed during the three overnight profiles;

At 15 minute intervals: Glucose, GH
60 minute intervals: insulin, ketones, lactate
120 minute intervals: IGF-I, IGFBP-3, IGFBP-1

7.2.7 Assays

The indices assessed at day 0 and Wks +4 and +8 are shown in Table 7.2 (assay details are included in Appendix 2). HbA1c levels were measured at the beginning and end of each of the three 4 week study periods (Wks -4, -1, day 0, and Wks +1, +4, +8). GFR was measured byulin clearance and real-time renal and splenic ultrasound undertaken for the measurement of renal volume and splenic length at the end of the first and final profiles in hospital (Wk -1 and Wk +4).

Further ultrasonography details are included in Appendix 5.

Three overnight urine collections were collected at the end of weeks 1, +4 and +8 to assess the urinary albumin/creatinine ratio.
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Table 7.2

Haematological and biochemical indices measured at day 0 and Wks +4 and +8

<table>
<thead>
<tr>
<th>Haematological indices</th>
<th>Haemoglobin (Hb)</th>
<th>Red cell count (RCC)</th>
<th>Haematocrit (Hct)</th>
<th>White cell count (WCC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal function:</td>
<td>Sodium (Na⁺)</td>
<td>Potassium (K⁺)</td>
<td>Urea</td>
<td>Creatinine</td>
</tr>
<tr>
<td>Hepatic function:</td>
<td>Albumin</td>
<td>Aspartate aminotransferase (AST)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid function:</td>
<td>Thyroxine (T₄)</td>
<td>Thyroid stimulating hormone (TSH)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

7.3-7.7 Results

7.3 Hypoglycaemia and side-effects

Subject compliance during the study appeared to be high. Two individuals each forgot to administer the rhIGF-I injection on one occasion part way through the 28 day period of administration (volunteering this information themselves). The first two subjects participating in the study reported local discomfort at the time of rhIGF-I injection but found that this could be reduced if the vial of peptide was removed from the refrigerator 30 minutes or more prior to administration.
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It was concluded that a low preparation temperature was responsible for some of the discomfort at the time of injection, and subjects were subsequently advised accordingly.

Nobody experienced any overt side effects whilst receiving rhIGF-I injections or commented on alterations in symptomatic awareness of hypoglycaemia. There was no alteration in the frequency of hypoglycaemic episodes, and there were no severe hypoglycaemic symptoms at any stage during the study. Standard fundoscopic examination (undertaken by the author) was normal in all subjects at the beginning and end of the study.

7.4 Anthropometry

Two individuals were growing rapidly as part of the pubertal growth spurt at the time of the study. This was evident from measurements taken as part of the routine clinic assessment before the study began. Subjects' height was measured at weeks -1 and +8 (9 weeks apart) and this increased in 5 out of the 6 participants. It is clearly not possible to determine the impact of administered rhIGF-I on growth rate in a study of this size and design.

Weight (measured using the same set of scales on each occasion) increased in 4, and fell in 2 individuals during the 4 week period of rhIGF-I administration (BMI also rose and fell in the same subjects). The change in body weight did not reach statistical significance at any point during the study (Table 7.3).

7.5 Blood glucose control and insulin dose

7.5.1 Home blood glucose tests

Subjects recorded a mean of 49 home blood glucose values before breakfast during the 12 week study period (range; 24 - 84 tests/12wks). Mean home blood glucose test results before breakfast were all within the euglycaemic range when the study periods were compared (Table 7.4).
Table 7.3

Subject weight (kg).

<table>
<thead>
<tr>
<th>Week (day)</th>
<th>Subject 18</th>
<th>Subject 19</th>
<th>Subject 20</th>
<th>Subject 21</th>
<th>Subject 22</th>
<th>Subject 23</th>
</tr>
</thead>
<tbody>
<tr>
<td>(day) 0</td>
<td>78.0</td>
<td>38.5</td>
<td>83.8</td>
<td>68.7</td>
<td>52.9</td>
<td>69.1</td>
</tr>
<tr>
<td>+4</td>
<td>79.3</td>
<td>41.5</td>
<td>81.5</td>
<td>70.0</td>
<td>55.7</td>
<td>68.1</td>
</tr>
<tr>
<td>+8</td>
<td>79.8</td>
<td>40.5</td>
<td>85.4</td>
<td>69.9</td>
<td>57.2</td>
<td>67.8</td>
</tr>
</tbody>
</table>

Table 7.4

Pre-breakfast blood glucose concentrations (mmol/l) for the three study periods.

<table>
<thead>
<tr>
<th>Interval</th>
<th>mean ± SEM</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wk -4 to day 0</td>
<td>6.7 ± 0.5</td>
<td>5.1 - 8.4</td>
</tr>
<tr>
<td>Wk 0 to +4</td>
<td>7.3 ± 1.1</td>
<td>5.6 - 10.6</td>
</tr>
<tr>
<td>Wk +4 to +8</td>
<td>7.4 ± 0.8</td>
<td>5.0 - 10.1</td>
</tr>
</tbody>
</table>
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7.5.2 Insulin Dose

There was no change in the isophane insulin dose during the run-in period but a significant reduction occurred during the period of rhIGF-I administration. The isophane dose then increased again during the run-out period. The isophane and total insulin dose during the study is shown in Table 7.5.

Table 7.5

<table>
<thead>
<tr>
<th>Week</th>
<th>Isophane dose (U/kg)</th>
<th>Total dose (U/kg)</th>
<th>'p' value</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>0.51±0.03</td>
<td>1.08±0.05</td>
<td>p=0.5</td>
</tr>
<tr>
<td>0</td>
<td>0.51±0.02</td>
<td>1.07±0.04</td>
<td>p=0.03</td>
</tr>
<tr>
<td>+4</td>
<td>0.41±0.02</td>
<td>0.96±0.05</td>
<td>p=0.03</td>
</tr>
<tr>
<td>+8</td>
<td>0.47±0.02</td>
<td>1.03±0.04</td>
<td></td>
</tr>
</tbody>
</table>

Two subjects felt that they had made consistent changes to the soluble insulin component during the study, and in both cases this was confirmed by their home blood glucose test records. One subject had reduced each of the 3 pre-meal injections by approximately 2 units during the period of rhIGF-I administration. He then increased the dose by the same amount during the run-out because of elevated blood glucose concentrations. The other subject who increased the soluble insulin dose began the school vacation at the same time as starting rhIGF-I and changed the insulin dose at this time because of the associated change in routine. He normally ate more and exercised...
less than on a typical school day, and was accustomed to altering the insulin dose on such occasions. The overall reduction in the isophane insulin dose in the six subjects (calculated in U/kg) ranged from 9 to 26%.

The total insulin dose (the sum of the isophane dose and the quantity of soluble insulin administered most frequently in the final week of each 4 week period) did not change during the run-in period, fell in five subjects during the period of rHIGF-I administration and then increased in all six subjects during the run-out.

### 7.5.3 HbA1c

HbA1c data is shown in Table 7.6.

<table>
<thead>
<tr>
<th>HbA1c data (%) during the 12 Week study</th>
<th>Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-4</td>
</tr>
<tr>
<td>18</td>
<td>8.8</td>
</tr>
<tr>
<td>19</td>
<td>12.5</td>
</tr>
<tr>
<td>20</td>
<td>9.4</td>
</tr>
<tr>
<td>21</td>
<td>9.0</td>
</tr>
<tr>
<td>22</td>
<td>15.6</td>
</tr>
<tr>
<td>23</td>
<td>12.2</td>
</tr>
<tr>
<td>mean</td>
<td>11.2</td>
</tr>
<tr>
<td>'p' value</td>
<td>p=0.24</td>
</tr>
</tbody>
</table>
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There was a reduction in HbA1c levels during the run-in period in 5 of the 6 subjects. The overall mean HbA1c changed from 11.2 ± 1.0% at week -4 to 10.4 ± 1.9% at day 0 (p=0.24). HbA1c levels then fell in all 6 subjects during the period of rhIGF-I administration to a mean of 9.4 ± 1.9% (p=0.03). At the end of the run-out period HbA1c levels had risen in 4 of the 6 subjects, with an overall mean value of 9.7 ± 2.0% at week +8 (p=0.24). The isophane insulin dose and HbA1c data during the study are also represented graphically in Figure 34.

7.6 Overnight profiles

7.6.1 IGF-I

Mean 12h overnight IGF-I levels were 198 ± 16 ng/ml (range; 147 - 249 ng/ml) on the baseline night and 331 ± 16 ng/ml (range; 274 - 376 ng/ml) after the first dose of rhIGF-I (p= 0.03). After the final dose of rhIGF-I, IGF-I concentrations were still significantly higher in all 6 subjects when compared to both the baseline and first night of administration at 422 ± 18 ng/ml (range; 367 - 485 ng/ml) (p= 0.03).

Although mean IGF-I values following rhIGF-I administration were normal for late pubertal subjects, the two individuals at puberty stage III had mean overnight levels of 367 ng/ml and 485 ng/ml after the final night of rhIGF-I. The latter of these two values is above the 90th centile for normal males at this puberty stage (Taylor et al., 1988). The increase in overnight IGF-I concentrations after the final injection of rhIGF-I ranged from 150% to 260% when compared to the baseline night.

7.6.2 IGF bioassay

The overall mean IGF bioassay (20.00 - 08.00h) was 0.77 U/ml (0.53 - 1.12 U/ml) on the baseline night and 0.88 U/ml (0.63 - 1.46 U/ml) on the final night after the last injection of rhIGF-I.
Figure 34.

Intermediate-acting insulin (isophane) dose (upper figure) and HbA1c concentrations (lower figure) during the 12 week study period in individual subjects.
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There was no difference in IGF bioactivity between the two nights, although IGF bioactivity tended to be higher after the final dose of rhIGF-I in the early part of the night (Figure 35).

7.6.3 Blood glucose

Hypoglycaemia (defined as a blood glucose < 3.5 mmol/l, Section 3.7.2) occurred in four subjects during the baseline profiles and in two subjects during both the first and second nights of rhIGF-I administration.

Mean blood glucose concentrations were 9.6 ± 1.0 mmol/l (range; 4.9 - 12.9 mmol/l) at week -1, 8.6 ± 1.2 mmol/l (range; 4.8 - 12.8 mmol/l) at week 0 and 9.7 ± 1.4 mmol/l (range; 4.0 - 14.2 mmol/l) at week +4. These values were not significantly different.

7.6.4 Insulin, IGFBP-1 and intermediate metabolites

Although mean overnight plasma free insulin concentrations were higher at the time of the first dose of rhIGF-I when compared to baseline (Wk -1), the difference was not statistically significant. After the final injection of rhIGF-I insulin concentrations were reduced when compared to day 0 (p=0.04), but not the baseline profile (p=0.07). There were no clear trend in overnight ketone, lactate or IGFBP-1 levels when the three nights were compared. Values for the three nights are shown in Table 7.7.

7.6.5 GH

Mean GH levels at Wk -1, day 0 and Wk +4 were 14.0 ± 3.1 mU/L (range; 3.8 - 25.5 mU/L), 9.2 ± 2.0 mU/L (range; 4.0 - 15.2 mU/L) and 7.6 ± 1.7 mU/L (range; 2.5 - 14.3 mU/L) respectively. These differences were not significantly different (p=0.07 when comparing either night of rhIGF-I administration to baseline) although it is of note that overnight GH concentrations were
Figure 35.
Mean overnight IGF bioactivity (U/ml) on the baseline night (Week -1; •) and following rhIGF-I administration (Week +4; O).

Figure 36.
Mean (± SEM) overnight IGF-II concentrations on the baseline night (Week -1; •) and after the final night of rhIGF-I (Week +4; O).
reduced in 5 of the 6 subjects on the first night of rhIGF-I administration, and 4 of the 6 subjects after the final injection of rhIGF-I (remaining the same in 1 of the 2 remaining individuals) when compared to baseline values at Wk -1. Interestingly, the one subject who demonstrated increased GH concentrations when compared to baseline was growing most rapidly during the study, with a height velocity of 6.7 cm/year. The 4 subjects with the highest baseline GH levels all suppressed following repeated daily IGF-I administration. Mean overnight GH concentrations of 7 mU/l are similar to levels reported in normal subjects (Edge et al., 1990a).

Table 7.7

Alterations in insulin-dependent variables during the three study nights.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week-1 (baseline)</th>
<th>p</th>
<th>Week 0 (first dose)</th>
<th>p</th>
<th>Week+4 (final dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>9.6 ± 1.08</td>
<td>0.24</td>
<td>8.6 ± 1.2</td>
<td>0.24</td>
<td>9.7 ± 1.4</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>15.7 ± 1.3</td>
<td>0.17</td>
<td>19.3 ± 4.8</td>
<td>0.04</td>
<td>14.3 ± 1.8</td>
</tr>
<tr>
<td>IGFBP-1 (mcg/l)</td>
<td>66.7 ± 28.7</td>
<td>0.46</td>
<td>41.9 ± 10.5</td>
<td>0.60</td>
<td>54.8 ± 11.9</td>
</tr>
<tr>
<td>Acetoacetate (mmol/l)</td>
<td>57 ± 8</td>
<td>0.75</td>
<td>44 ± 4</td>
<td>0.17</td>
<td>49 ± 4</td>
</tr>
<tr>
<td>3-hydroxybutyrate (mmol/l)</td>
<td>47 ± 7</td>
<td>0.46</td>
<td>39 ± 10</td>
<td>0.46</td>
<td>48 ± 8</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>653 ± 54</td>
<td>0.60</td>
<td>656 ± 43</td>
<td>0.75</td>
<td>662 ± 48</td>
</tr>
</tbody>
</table>

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7.6.6 IGF-II

Overall mean IGF-II concentrations (20.00 - 08.00h) were reduced in 5 subjects after the final injection of rhIGF-I when compared to the baseline night. Mean overnight IGF-II concentrations were 630 ± 50 ng/ml (range; 452 - 739 ng/ml) on the baseline night and 494 ± 29 ng/ml (range; 413 - 623 ng/ml) on the final night of rhIGF-I administration (N.S.). Further analysis of the overnight data showed that the final dose of rhIGF-I (Wk +4) led to a decline in IGF-II levels until a nadir was reached at 02.00h (p= 0.05 when compared to levels on the baseline night). IGF-II data are illustrated in Figure 36.

7.6.7 IGFBP-3

Mean overnight IGFBP-3 levels were 4.5 ± 0.3 mg/ml (3.5 - 6.0 mg/ml) on the baseline night and increased in 5 of the 6 subjects to 4.9 ± 0.3 mg/ml (4.1 - 6.0 mg/ml) following the first injection of rhIGF-I (p=0.04). After the final dose of rhIGF-I the concentrations of IGFBP-3 were still above baseline values in 5 (different) subjects with an overnight mean of 5.1 ± 0.4 mg/ml (3.9 - 7.1 mg/ml; p= 0.1 when compared to baseline at Wk -1). The one subject in whom overnight IGFBP-3 concentrations did not increase was also the individual with IGF-I concentrations above the 90th centile (Section 7.6.1), and in whom GH levels were increased at week +4 (Section 7.6.5). IGF-I, GH and IGFBP-3 data are shown in Figure 37.

When IGFBP-3 concentrations were analysed with respect to the combined levels of IGF-I and IGF-II a similar pattern was identified to that seen in the single bolus studies. On the baseline night the relationship between the variables was much closer than after the final injection of rhIGF-I (r= 0.76, p= 0.07 at Wk -1 and r= -0.36, p= 0.4 at Wk +4).
Figure 37.

Mean overnight IGF-I, GH and IGFBP-3 concentrations (20.00h - 08.00h) at the time of the baseline profile (Wk -1), and after the first (day 0) and final (Wk +4) dose of rhIGF-I.
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7.6.8 ALS

There was no alteration in concentrations of the ALS when the baseline night and final night of rhIGF-I administration were compared. At the start of the baseline (Wk -1) and final (Wk +4) overnight profiles ALS concentrations were 32.7 ± 3.2 mg/ml and 32.2 ± 2.6 mg/ml respectively, and in the morning (08:00h) they were 32.2 ± 2.6 mg/ml after the baseline profile, and 30.5 ± 1.8 mg/ml following the final injection of rhIGF-I.

7.7 Renal assessment and toxicology

7.7.1 Toxicology

Biochemical and haematological indices were measured at Wks -1, +4 and +8. The mean and range of values are shown in Appendix 6. The only parameter to change in all subjects from one assessment to the next (an increase or decrease in all 6 subjects) was a rise in thyroxine levels from Wk +4 to Wk +8.

7.7.2 Ultrasound

Renal volume and splenic length did not change significantly during the period of rhIGF-I administration (Table 7.8).

7.7.3 GFR

GFR was above, or towards the upper end of the normal range, in all subjects at the time of the baseline profile at week -1. Small reductions in GFR were seen in 5 individuals after the final injection of rhIGF-I but there was no overall change during the period of rhIGF-I administration. Mean GFR was 154 ± 4 ml/min/1.73m² (range 145 - 168 ml/min/1.73m²) at the time of the baseline
profile (Wk -1), and 150 ± 6 ml/min/1.73m² (range 138 - 178 ml/min/1.73m²) after the final dose of rhIGF-I (p=0.24). The data are illustrated in Figure 38.

Interestingly, the one subject whose GFR rose following rhIGF-I administration was the individual who did not demonstrate a reduction in overnight GH levels (subject 22).

<table>
<thead>
<tr>
<th>Renal and splenic ultrasound assessment (mean ± SEM and range).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week-1</strong></td>
</tr>
<tr>
<td>Renal volume (cm³):-</td>
</tr>
<tr>
<td>left kidney</td>
</tr>
<tr>
<td>(76 - 186)</td>
</tr>
<tr>
<td>right kidney</td>
</tr>
<tr>
<td>(77 - 160)</td>
</tr>
<tr>
<td>Splenic length (mm)</td>
</tr>
<tr>
<td>(72 - 129)</td>
</tr>
</tbody>
</table>

7.7.4 Albumin excretion

The urinary albumin/creatinine ratio fell following rhIGF-I administration in 5 of the 6 subjects although the overall change was not statistically significant (p=0.07). In one individual the urinary albumin/creatinine ratio was above the normal upper limit of 3 mg/mmol throughout the study (Rowe et al., 1990). Values in this subject had fallen, but were still abnormal, at the end of the period of rhIGF-I administration (Table 7.9) and then at Wk +8 were more suggestive of frank albuminuria (usually detected by 'Albustix' testing).
Figure 38.

Glomerular filtration rate (GFR) in individual subjects before (Week -1) and after the 28 day period of rhIGF-I administration (Week +4).
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Table 7.9

<table>
<thead>
<tr>
<th>Subject</th>
<th>Wk-1</th>
<th>Wk+4</th>
<th>Wk+8</th>
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<td>0.7</td>
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<td>1.3</td>
<td>0.3</td>
<td>0.5</td>
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<tr>
<td>23</td>
<td>1.0</td>
<td>0.8</td>
<td>0.9</td>
</tr>
</tbody>
</table>

7.7.5 Urinary Calcium excretion

Consistent alterations in urinary calcium excretion were not seen. The calcium/creatinine ratio rose in 5 of the 6 subjects following rhIGF-I administration but the differences were not statistically significant (Table 7.10).

Table 7.10

<table>
<thead>
<tr>
<th></th>
<th>Week -1</th>
<th>Week +4</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SEM</td>
<td>0.34 ± 0.09</td>
<td>0.51 ± 0.17</td>
<td>0.53 ± 0.22</td>
</tr>
<tr>
<td>Range</td>
<td>(0.10 - 0.72)</td>
<td>(0.06 - 1.28)</td>
<td>(0.20 - 1.6)</td>
</tr>
<tr>
<td>'p' value</td>
<td>0.17</td>
<td>0.89</td>
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</tbody>
</table>
Chapter 7.

7.8 Discussion

7.8.1 Introduction

This preliminary study was undertaken in a small number of subjects because of uncertainty as to the effects of repeated daily rhIGF-I administration in adolescents with IDDM, and to ensure that individuals could be carefully supervised. Although the subject number was small, some interesting and potentially important observations were made.

7.8.2 Overnight profiles

The rise in IGF-I concentrations with repeated daily rhIGF-I administration was sustained during this study, as they were with repeated daily rhIGF-I administration to normal volunteers and subjects with NIDDM (Wilton et al., 1991; Young & Clemmons, 1994). Following rhIGF-I administration overnight IGF-I concentrations were appropriate for late pubertal adolescents, but were rather high for subjects at earlier pubertal stages. There was no evidence of side-effects such as those reported in subjects with NIDDM where larger doses of rhIGF-I were administered, and supraphysiological IGF-I concentrations attained (Jahri et al., 1994).

It was surprising that, despite the increase in IGF-I levels, IGF bioactivity remained rather low on the final night of rhIGF-I administration. Overnight IGFBP-1 levels were not related to IGF bioactivity, nor were they high when compared to normal values (Holly et al., 1989). This suggests that there are other inhibitors of IGF bioactivity in IDDM subjects besides IGFBP-1, a conclusion which was also reached in the single bolus studies (Section 3.7.1).

There was a significant rise in IGFBP-3 concentrations following a single dose of rhIGF-I in keeping with earlier observations (Section 6.3.5), and overnight IGFBP-3 levels rose in 5 of the 6 subjects with longer-term rhIGF-I administration. Although the difference was not statistically significant when compared to baseline values, the overall figure was greater than after the first dose
Chapter 7.

of rhIGF-I. This apparent rise in IGFBP-3 with longer-term administration is in contrast to the suppressive effects of rhIGF-I administration on IGFBP-3 levels observed in normal subjects (Baxter et al., 1993; Kupfer et al., 1993) and subjects with NIDDM (Young & Clemmons, 1994). The combined administration of rhIGF-I and GH was found to increase IGFBP-3 levels in healthy volunteers (Kupfer et al., 1993), and in the studies of subjects with IDDM the GH levels were not suppressed below the appropriate physiological range as they might be in normals, and subjects with NIDDM, when rhIGF-I is administered alone. However the increase in overnight IGFBP-3 concentrations was relatively modest, and levels did not rise to within the normal adolescent range.

The rise in IGF-I levels after the final injection of rhIGF-I was less pronounced than in the single bolus studies. There was still evidence of a fall in IGF-II concentrations and discordance between the sum of the IGFs and IGFBP-3 levels following repeated daily rhIGF-I administration as there was after a single rhIGF-I dose (Section 6.3.7). There was also discordance between these variables at the time of admission prior to the final injection of rhIGF-I, which raises the possibility of IGF binding to other IGFBP's besides IGFBP-3 at this stage. The administration of rhIGF-I increases IGFBP-2 concentrations in NIDDM, and a rise in IGFBP-2 may also be linked to GH suppression (Young & Clemmons, 1994). There was evidence of IGF-I binding in lower molecular weight bands appropriate to the size of IGFBP-2 in the single bolus studies (Section 6.3.8), and this is a plausible explanation for some of the observations following longer term rhIGF-I administration.

The GH data is difficult to interpret because of the study design. The known intraindividual variation in GH secretion (Albertsson-Wikland & Rosberg, 1992) and the effects of hypoglycaemia (particularly during the baseline profiles) could be confounding variables. Nevertheless, the overall reduction in mean GH concentrations on the final and first night of rhIGF-I administration in this
Chapter 7.

small number of subjects is consistent with a continued suppressive effect of administered rhIGF-I on GH secretion.

There were no differences in overnight insulin concentrations on the first night of rhIGF-I administration compared to baseline, despite the reduced isophane dose. Altered insulin clearance is an unlikely explanation for these observations (Section 3.5.5), and it is possible that this first night did not accurately represent insulinosstatin at other times. Free insulin levels were reduced after the final injection, however. The development of hypoglycaemia during many of the profiles, the reduction in isophane dose on the first night of rhIGF-I administration, and the wide variation in blood glucose concentrations has to be considered when interpreting the insulin, IGFBP-1 and ketone data. Overnight hypoglycaemia is not uncommon in IDDM (Bolli et al., 1993), although the episodes in the studies described in this chapter may also reflect a change of routine and dietary intake.

The administration of rhIGF-I has been shown to affect the counter-regulatory response to hypoglycaemia in normals and subjects with NIDDM. GH and glucagon responses may be attenuated, and sympathetic responses enhanced following rhIGF-I administration (Kerr et al., 1993; Laager & Kellor, 1993). There was no objective or subjective evidence to suggest altered responses to hypoglycaemia in these studies. However this was not addressed in detail by the study protocol, and subjects might be unable to identify subtle differences in these parameters.

7.8.3 Metabolic control

The improvement in metabolic control in 5 subjects during the 4 week run-in period was not unexpected because of the known effects of study participation (Worth et al., 1982). This improvement occurred without alterations in their isophane dose, and was presumably the result of improved compliance. After the initial reduction in isophane insulin at the time of the first injection
Chapter 7.

of rhIGF-I, subjects adjusted the insulin dose according to the results of home blood glucose tests.
Four individuals subsequently altered the isophane insulin dose during the 28 day period of rhIGF-I
administration, but this remained below baseline values (Wk -4 to Wk 0) in all subjects. There was
no alteration in blood glucose control as assessed by morning home blood glucose test results
during the period of rhIGF-I administration, and despite the reduction in the isophane insulin dose
(and total daily insulin dose in 5 of the 6 subjects), HbA1c concentrations continued to fall in all
subjects until the end of the period of rhIGF-I administration (Wk 4). A continuation of the ‘run-in
effect’ induced by study participation may have contributed to the change in HbA1c concentrations,
but in view of the reduced insulin dose, the impact of rhIGF-I administration must be considered as
well. The isophane and total insulin dose rose in all subjects during the run-out period, and the mean
HbA1c concentrations increased (in 5 of the 6 subjects). These changes may indicate a relative
‘relaxation’ by participating subjects at this time, but are also compatible with effects of rhIGF-I on
insulin sensitivity and blood glucose concentrations.

The expected insulin-like actions of administered rhIGF-I in this study can be calculated
using the same estimation of insulin-like potency used in the single bolus studies (Section 3.7.4).
The average reduction in insulin dose was 0.11 units/kg or the equivalent of 7.3 units of insulin
each day. The expected insulin-like potency of rhIGF-I in the administered dose is 3.2 U/day. Thus
the insulin-like actions of rhIGF-I alone cannot, according to this calculation, account for the
change in insulin dose and the reduction in HbA1c concentrations. Altered subject compliance with
changes in factors such as diet and the timing of the insulin injection may have contributed to
improved metabolic control, but reductions in GH hypersecretion and alterations in insulin
sensitivity induced by rhIGF-I administration are also plausible explanations.
7.8.4 Toxicology and renal function

There are hypothetical reasons why alterations in IGF-I, GH and insulin levels, following rhIGF-I administration, may affect the GFR and renal and splenic size. The increase in renal volume observed in IDDM subjects has been linked to GH hypersecretion, and a disproportionate increase in splenic size has also been noted in studies of rhIGF-I administration in animals. GH hypersecretion (Blankestijn et al., 1993), peripheral hyperinsulinism and rhIGF-I administration (Hirschberg et al., 1993) have also been linked to renal hyperfiltration.

Subjects demonstrated significant renal hyperfiltration at the time of the base-line profile and there was little overall change in GFR or renal size following rhIGF-I administration. Unfortunately, the assessment of renal volume and splenic length was undertaken by 2 different radiologists, but it is interesting to note that the one subject who did not demonstrate reduced GH levels at the end of the period of rhIGF-I administration was the only person in whom the GFR rose.

Urinary albumin excretion (as assessed by albumin/creatinine ratio) fell in 5 of the 6 subjects during the period of rhIGF-I administration. Although glomerular hyperfiltration is well recognised in IDDM, the relationship with the later development of diabetic nephropathy is uncertain. The evidence suggesting that elevated albumin excretion precedes nephropathy is somewhat stronger (Almdal et al., 1994).

There was an increase in thyroxine levels in all subjects beyond the period of rhIGF-I administration, and a decrease in thyroxine levels was also observed in 5 individuals during the course of rhIGF-I injections. This is consistent with previous studies suggesting an increase in peripheral T4 conversion to T3 during rhIGF-I administration (Trainer et al., 1993). There was no clear alteration in any other biochemical or haematological parameters during the period of rhIGF-I administration (no other parameter increased or decreased in all subjects from one assessment to the next), in contrast to earlier studies in normal subjects (Lieberman et al., 1992). RhIGF-I
administration has been shown to have pronounced effects on phosphate excretion, although this was not assessed in these studies (Hirschberg et al., 1993). There were no consistent changes in urinary calcium excretion or circulating calcium levels.

In summary, there was evidence of an improvement in glycaemic control on a reduced isophane insulin dose during a 28 day period of rhIGF-I administration, although the mechanisms responsible for these observations are not clear. Data from overnight profiles suggest that a reduction in GH release is a possible contributing factor. There was no evidence of a deleterious effect of administered rhIGF-I on GFR and albumin excretion, and no apparent side effects with the daily dose used in this study.
Chapter 8

CONCLUDING DISCUSSION

8.1 RhIGF-I, insulin and glycaemic control in adolescence

8.1.1 Introduction

The importance of glycaemic control in determining the progression of the microangiopathic complications in patients with IDDM has recently been demonstrated (The Diabetes Control and Complications Trial Research Group, 1993). The results of this study have served to emphasise the importance of developing new strategies and treatments to safely improve blood glucose control in people with this disease.

The studies described in this thesis were the first to explore the therapeutic potential of rhIGF-I administration in adolescent subjects with IDDM. They were designed to assess the hypothesis that restoring IGF-I concentrations in adolescents with IDDM would reduce GH hypersecretion. A subsequent alteration in insulin sensitivity and reductions in the magnitude of the ‘dawn phenomenon’ would then permit more appropriate insulin delivery and improve glycaemic control. The studies have confirmed that all the central tenants of this hypothesis are correct, but in this final chapter I hope to place the abnormalities of the GH/IGF-I axis into the context of the overall problems of IDDM during adolescence, and to explore the potential therapeutic impact of regular rhIGF-I administration.

8.1.2 Insulin and the GH/IGF-I axis

When considering the GH/IGF-I axis in IDDM it should be emphasised that the principle underlying abnormality is the inadequate delivery of insulin into the portal vein, and not a reduction in IGF-I concentrations. In normal subjects the portal/peripheral vein insulin ratio will be greater than one, and in subjects with IDDM using the conventional, peripheral, route of insulin
administration this ratio will be less than one. Subjects with residual beta cell function have lower GH concentrations (Madsbad et al., 1982; Wurzburger et al., 1990), show an elevated IGF-I response to exogenous GH administration (Wurzburger et al., 1993), and are less likely to decompensate (Madsbad et al., 1979, 1982). Insulin is an important determinant of GH receptor function (Baxter & Turtle, 1978), and because the amount of circulating GHBP appears to reflect GH receptor numbers, it is therefore not surprising that levels are reduced in IDDM (Menon et al., 1992). The alteration in receptor expression leads to a reduction in the amount of IGF-I that may be generated in response to a GH pulse. This is not overcome by an increase in GH concentrations and the normal inverse relationship between GH and GHBP is lost (Clayton et al., 1994). Although changes at the levels of the GH receptor may explain some of the abnormalities of the GH/IGF-I axis in IDDM, a direct relationship between GHBP and IGF-I does not exist in adolescents with, or indeed without, IDDM (Massa et al., 1993; Clayton et al., 1994). It is likely that hepatic post-GH receptor defects are present in IDDM as well (Maes et al., 1986), although they may also be closely linked to reduced endogenous insulin production.

Under normal circumstances the alteration in sex steroids, GH and insulin concentrations interact and mediate the change in IGF-I levels during puberty (Link et al., 1986; Smith et al., 1989; Merimee et al., 1991). The impact of a reduction in portal insulin delivery, at a time when IGF-I levels normally undergo rapid change, is likely to be particularly pronounced.

8.1.3 The GH/IGF-I axis and deteriorating glycaemic control

There are theoretical reasons why changes in the GH/IGF-I axis may affect growth, blood glucose control and the complications of diabetes. The described studies have focused on blood glucose control during adolescence, and this aspect will be considered in greatest detail.
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The deterioration in blood glucose control during adolescence was also recently highlighted by the DCCT (The Diabetes Control and Complications Trial Research Group, 1993; Drash, 1993). Two questions stem from this observation. Firstly, to what extent is this an inevitable consequence of the changes in insulin requirements during puberty? The evidence for this is largely circumstantial (Amiel et al., 1986) but it is likely that a rising insulin requirement will disrupt an established routine. Alterations to the insulin regimen will be made once changes in glycaemic control are identified, but there will be an interval before these changes are detected. Psychological considerations may be particularly relevant in the adolescent age group, but the appropriate delivery of an increasing insulin dose is likely to be more demanding.

The second question hinges on whether or not low IGF-I concentrations and excessive GH secretion aggravate the problems of appropriate insulin delivery. Circulating IGF-I levels are inversely related to HbA1 concentrations during puberty, and a correlation between GH and HbA1 concentrations in adolescents with IDDM has been identified in the studies described in this thesis. The increase in GH levels may be a manifestation of reduced IGF-I concentrations, which in turn may reflect poor insulin delivery and an associated deterioration in blood glucose control. As such the rise in GH may be a secondary phenomenon, following on from low insulin concentrations, and not the primary event leading to poor glycaemic control. However there is a clear potential for the rising insulin requirement and further portal hypoinsulinaemia to enhance GH secretion and reduce insulin sensitivity still further.

8.1.4 Intensive insulin therapy

The standard approach to deteriorating glycaemic control during puberty has been to intensify insulin treatment. Intensive insulin regimens usually entail an increase in insulin dose and attempts at a more physiological insulin delivery. This often involves the introduction of a regimen
comprising a pre-meal soluble insulin bolus superimposed on a background of steady insulin release. However, many consider the impact of intensive insulin treatment disappointing (Davies & Baum, 1988; Edge et al., 1990), and there is little evidence to suggest that an alteration in insulin delivery without a change in insulin dose will improve glycaemic control in the longer term (Tubiana-Rufi et al., 1989).

Intensified insulin treatment may increase IGF-I concentrations and growth velocity (Rudolf et al., 1982; Amiel et al., 1984), although an improvement in blood glucose control appears to be a prerequisite for these changes. The relationship between GH production and glycaemic control is less clear. Intensified treatment has a variable impact on GH levels and might even increase GH secretion (Molnar et al., 1972; Arias et al., 1984; Hermansen et al., 1987; Press et al., 1992). The variable response to such therapy may reflect the relationship between endogenous insulin production, peripheral insulin levels and baseline glycaemic control. The factors determining whether GH levels ultimately fall with intensive insulin treatment, and the extent to which improved glycaemic control can occur in the face of elevated GH levels, remain largely unresolved (Arslanian et al., 1993). Central to this uncertainty may be the fundamental problem of generating adequate portal insulin delivery, and normalising the relationship between GH and hepatic IGF-I production without associated peripheral hyperinsulinaemia. Current insulin regimens may aim for, and indeed achieve near-normal blood glucose levels, but the discrepancy in circulating insulin levels will remain. The disadvantages of intensified insulin treatment, which include the potential for an increase in hypoglycaemic episodes and weight gain (The Diabetes Control and Complications Trial Research Group, 1993), may partly reflect this problem. Increased insulin delivery also leads to a temporary acceleration in the development of microvascular complications (Dahl-Jorgensen et al., 1985; The Diabetes Control and Complications Trial Research Group, 1993). This may also be linked to peripheral hyperinsulinaemia, or enhanced GH secretion with or without a local increase in
IGF-I production. Whatever the exact mechanism, the benefits of an overall improvement in blood glucose control appear to predominate beyond this initial period.

8.1.5 RhIGF-I administration and the impact on GH concentrations and glycaemic control

Although the impact of excessive GH secretion in IDDM has been studied most extensively in relation to overnight changes in blood glucose concentrations or insulin requirements (calculated to be in the order of 20% to 25%; Edge et al., 1990b; Perriello et al., 1991), the normal rise in insulin resistance at puberty is also closely linked to GH concentrations (Amiel et al., 1986; Hindmarsh et al., 1988a). The rise in GH levels leads to a fall in insulin sensitivity with further, daily fluctuations resulting in a circadian pattern which will be superimposed on this general trend. The consequences of reducing GH secretion to more appropriate levels at a given puberty stage will not, therefore, only affect insulin requirements overnight. This is an important point, because it could be argued that a reduction in early morning insulin requirement by 20% beyond 05.00h will not have a major impact on glycaemic control; a rising blood glucose concentration might be effectively interrupted by the first insulin injection of the day 2 to 3 hours later. Will a reduction in blood glucose in the order of 20% over a three hour period justify an extra, daily injection, it could be argued? If an overall rise in insulin sensitivity throughout the day is coupled with a further reduction in overnight insulin requirements, at a time when increased insulin delivery cannot easily be achieved, then intervention to reduce GH concentrations may have a more profound impact on blood glucose control.

The studies in this thesis provide some support for the beneficial effect of reducing GH levels during adolescence. A relationship between GH levels and insulin sensitivity was established in the single bolus studies, and there were reductions in the insulin requirements to achieve equivalent (short-term) or improved (longer-term) glycaemic control. The effect on insulin
requirements overnight was of an order of magnitude similar to that predicted from previous studies of the Dawn Phenomenon. The magnitude of the longer term effects of rhIGF-I on blood glucose control are harder to quantify because both insulin dose and HbA1c altered during the 4 Wk period of rhIGF-I administration. The impact on HbA1c may only be judged when treatment is continued for at least 3 months, and assessed in the setting of a double-blind controlled study.

8.1.6 The direct effects of rhIGF-I on metabolism

Although rhIGF-I has 'insulin-like' effects, the objective of studies in adolescents with IDDM was not to mimic these actions, but to enhance IGF-I effects that are qualitatively different to insulin. The acute metabolic actions of the peptide may, nevertheless, have contributed to observations made in earlier chapters. There were instances when reduced free insulin levels were seen following rhIGF-I administration in the presence of an increase in the parameters assessing GH pulsatility (Section 5.4), and it has been shown that rhIGF-I can enhance glucose disposal in normal human subjects despite reduced insulin levels (Rennert et al., 1993). The simple extrapolation of insulin-like potency may not accurately represent peptide effects in-vivo, and a preferential effect of rhIGF-I on glucose disposal, as opposed to hepatic glucose production (when compared to insulin), has been reported (Laager et al., 1993). This may reflect IGF-I receptor distribution and the relatively high numbers in muscle tissue (Dohn et al., 1990). Hepatic glucose production is more sensitive to the actions of insulin than glucose disposal (Amiel et al., 1991) and, after rhIGF-I administration, glucose production may remain relatively suppressed when compared to glucose uptake, in spite of reduced insulin concentrations (Hassain et al., 1993). A reduction in insulin requirements after rhIGF-I administration, independent of changes in GH secretion, may then be observed. A reduction in circulating insulin levels may also affect the tissue sensitivity to insulin action in the longer term. Interestingly, the trend towards a reduction in ketone formation following
rhIGF-I administration in the single bolus studies, does not suggest an adverse effect of reduced insulin concentrations on ketogenesis in the presence of increased IGF-I and reduced GH levels.

The impact of rhIGF-I on glucose disposal is complex. An increase in our understanding of the functions of the IGFBP’s has confirmed that the simple assessment of peptide concentration, and presence or absence of the appropriate receptor, is unlikely to be an accurate reflection of the impact of administered rhIGF-I on cellular function. The administration of rhIGF-I to subjects with IDDM alters the distribution of circulating peptide amongst the IGFBP’s, and the implications of this are, as yet, unknown.

8.1.7 RhIGF-I administration and the adolescent

Many clinicians believe that the major problem in adolescence is poor adherence to the rigours of the diabetic regimen, with missed insulin injections the rule rather than the exception. According to this school of thought, elevated HbA1 concentrations indicate poor compliance in all but the most exceptional of circumstances. What, it might be asked, can be gained by rhIGF-I administration that cannot be achieved by intensified insulin regimens, or measures taken to improve compliance? Can those adolescents who are unable to undertake more intensive insulin treatment be expected to administer an extra injection of rhIGF-I?

A significant therapeutic effect of rhIGF-I in IDDM may depend on the extent to which administered peptide interrupts a cycle where elevated GH concentrations lead to a rise in blood glucose concentrations, hepatic insulin resistance and peripheral insulin levels which will then further hinder adequate insulin delivery. In some patients it is conceivable that the increase in IGF-I concentrations may be of value, particularly if a spiral of insulin resistance leading to further deterioration in glycaemic control has been entered. A reduction in circulating insulin concentrations may also attenuate weight gain at a time when this can be particularly troublesome for the
adolescent. However rhIGF-I administration must always be viewed as an additional and supplementary measure, and not as a replacement for an otherwise appropriate insulin dose.

8.2 Further investigation of rhIGF-I in IDDM

8.2.1 Selecting subjects for IGF-I

The subjects taking part in these studies were a heterogeneous group. Blood glucose control, as assessed by HbA1 concentrations, varied from the respectable (7.1%) to poor (17.0%). Those subjects with the greatest GH concentrations tended to have worse glycaemic control and showed the greatest suppression following rhIGF-I administration, and it may be possible to identify other factors which will determine who might benefit from rhIGF-I administration.

Potential recipients might be individuals with low serum IGF-I concentrations and poor glycaemic control (both of which are associated with an increase in GH levels), despite appropriate increases in insulin dose during puberty (Hindmarsh et al., 1988). Subjects with a relatively short disease duration, or questionable compliance, would require special consideration.

8.2.2 Future study design

The pharmaco-kinetics of rhIGF-I are compatible with once daily administration. The timing of the injection may not be critical because the impact on GH secretion is sustained. The rhIGF-I dose requires further refinement so that a balance between an increase in circulating IGF-I levels, and reductions in GH concentrations, might be achieved. However the relationship between IGF-I and GH levels will be influenced by a number of variables, and is not likely to be of a simple linear nature. The design of the short and longer-term studies prevents conclusions about sex differences in IGF-I response, although this is an issue that may need to be addressed.
Further studies of rhIGF-I administration will need to be of a double-blind placebo controlled design and of sufficient power to establish whether there is a therapeutic effect as judged by a change in HbA1c concentrations. The possible effects of rhIGF-I administration on microvascular complications and renal function will require careful consideration, and must be a major focus of any future study. The sequence of events leading to the development and progression of complications is not sufficiently clear at the present time to be certain about the impact of increasing circulating IGF-I levels in this way.

8.3 Conclusion

These preliminary physiological studies have provided information about the effects of low dose subcutaneous rhIGF-I administration in adolescents with IDDM. A reduction in pulsatile GH release and a fall in insulin requirements was observed following short term rhIGF-I administration, and similar changes were identified with daily injections over a period of 1 month. The change in insulin requirements was linked to alterations in insulin sensitivity in association with reduced GH secretion. No complications due to rhIGF-I administration and the associated increase in IGF-I levels were identified.

These preliminary investigations should prompt further exploration of the use of rhIGF-I as an adjunct to insulin in adolescents with IDDM. Long-term placebo controlled studies will determine whether rhIGF-I has a role in the management of IDDM.
APPENDICES

Appendix 1: Subject details

<table>
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</table>
Appendix 2: Assay techniques

2.1 C-peptide

C-peptide was measured by RIA from Diagnostic Products (DPL Division, EUROWDPC Ltd, Glyn Rhonwy, Llanberis, Caernarfon, Gwynedd, Wales). Measurements were made at the Institute of Child Health, London, UK. The assay sensitivity was 0.072 nmol/l. The intrasay CV's were 3.4% and 3.0% at 0.29 nmol/l and 2.92 nmol/l respectively with interassay CV's of 10.0% and 1.9% at 0.29 nmol/l and 2.92 nmol/l respectively.

2.2 Creatinine

Creatinine was measured in blood and urine by an automated kinetic Jaffé reaction in the Biochemistry Department, the John Radcliffe Hospital, Oxford.

2.3 Fructosamine

Fructosamine was measured by a colorimetric assay using a reaction with nitroblue tetrazolium. The kit assay was obtained from Roche Diagnostics, Herts, UK. Non-diabetic subjects will usually have values below 285 μmol/l (mean + 2 SD’s). The inter assay CV’s were 3.2% and 2.4% at 294.2 μmol and 561.1 μmol/l respectively.

2.4 Thyroid Stimulating Hormone and Thyroxine

Measurement of TSH and T4 was undertaken at the Endocrine Laboratories, the Radcliffe Infirmary, Oxford. TSH was measured by a monoclonal antibody-coated IRMA (Immunodiagnostic Systems Limited, Bolden Business Park, Tyne and Wear, UK). The assay sensitivity was 0.03 mU/l with inter-assay CV’s of 6.0, 3.9 and 3.9% at concentrations of 1.2, 4.8 and 24.6 mU/l respectively. The laboratory normal range was 0.25 - 4.3 mU/l. Thyroxine (T4) was measured
by RIA (Lifescreen Limited, Watford, UK). The assay sensitivity was 4 nmol/l with inter-assay CV's of 3.3, 4.5 and 4.0% at concentrations of 40, 85 and 136 nmol/l respectively. The laboratory normal range was 70 - 140 nmol/l.

2.5 Lipids

Total cholesterol was measured by an enzymatic method. Following the addition of precipitating agent (dextran sulphate and magnesium chloride) high-density lipoprotein cholesterol (HDL) was measured by the same technique. After triglyceride (TG) had been measured (also by an enzymatic method) low-density lipoprotein (LDL) could be calculated from the formula:

\[
LDL = Total \text{ cholesterol} - HDL \text{ cholesterol} - 0.46 \times TG
\]

2.6 Urinary albumin concentrations

Urinary albumin concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using rabbit anti-human albumin coating antibody and horseradish peroxidase conjugated antibody (DAKO). The intra-assay CV was 4.2% at 10 mg/ml and the inter-assay CV was 11.9% at 10 mg/ml.

2.7 Other biochemical variables

Urea and electrolytes and liver function tests were assayed by standard techniques at the Department of Biochemistry, John Radcliffe Hospital, Oxford, UK.
2.8 Glomerular filtration rate (GFR)

GFR was measured by inulin clearance. After administration of an intravenous bolus of inulin (Inustest, IDIS Pharmaceuticals) in a dose of 75mg/kg, blood samples were then taken at intervals over the subsequent 3 hours. Inulin cannot be metabolised, and is excreted only by renal clearance. The decline in circulating inulin concentrations will reflect renal function and with a knowledge of subject weight and the inulin dose administered the GFR can be calculated. Normal GFR ranges from $125 \pm 25$ ml/min/1.73m$^2$ (mean ± 2SD).
Appendix 3: GH analysis

3.1 The peak-counting programme Pulsar

The peak counting programme Pulsar (Merriam & Wachter, 1982) was used to analyze GH profiles overnight between 20.00h and 08.00h. The programme is a more objective means of interpreting hormone profiles than visual inspection, and uses the assay standard deviation to determine what is a 'pulse' and what is variation due to assay 'noise'.

The programme detects hormone pulses as deviations from a smoothed detrended baseline using the assay standard deviation (SD) as a scale factor. The intrassay SD coefficients were calculated from large single pool assays. When setting the cut-off parameters, the objective was to strike a balance between sensitivity and specificity which would then be supported by GH plasma pool analysis. The cut-off parameters to detect peaks with a width of one to five points were 3.4, 2.6, 1.9, 1.5, 1.2 times the assay SD. The cut-off for splitting peaks was set at 2.5 times the assay SD, and the weight assigned to peaks in the smoothing iterations was 0.05. With these settings the program did not detect any peaks when four plasma GH pools were analysed.

3.2 Distribution analysis

Distribution analysis (Matthews et al., 1991) is an observer unbiased method of describing hormone profiles in terms of their time concentration attribute. The number of observations in each of a set of intervals is expressed as a percentage of the total. This is a particularly useful approach to the analysis of the baseline hormone concentration. The observed concentrations below which 95%, 50%, and 5% of the values lie (‘OC95’, ‘OC50’ and ‘OC5’ ) were selected as previously described by Matthews and colleagues (Matthews et al., 1991). As an example; when a profile has a prominent baseline which is close to the assay threshold, with few pulses above this baseline, then so the OC95 value will also be near to the assay threshold.
3.3 Fourier transformation

Fourier transformation, one of the methods of time series analysis (Chatfield, 1984), is an observer unbiased means of assessing pulsatile hormone release. Absolute Fourier transforms describe an oscillatory profile in terms of amplitude and periodicity by separating the data series into different frequencies. It therefore provides different information from that provided by pulse counting techniques such as Pulsar. GH secretion is described in terms of period, rather than frequency, as this provides an assessment independent of total sampling interval. Linear stationarisation was used to detrend the data before analysis (Matthews, 1988).

Fourier transformation was performed on 12h overnight GH profiles both before and after deconvolution analysis. The daytime profiles were not analysed in this way because of the increase in GH concentrations which was expected at the time of the exercise protocol. The release in GH, so generated, could influence the Fourier analysis and lead to erroneous conclusions regarding hormone pulsatility.
Appendix 4: Statistical techniques

4.1 Hardware and software

Statistical analyses were undertaken using OXSTAT II (Microsoft Corporation, 1985), SAS (SAS Institute, Cary, NC, USA) and SPSS (SPSS Inc. Chicago, IL 60611, U.S.A.) on a personal computer.

4.2 IGF-I

Overall mean IGF-I concentrations on the two nights were compared using paired students ‘t’ test and the relationship between variables by standard least squares linear regression. The IGF bioassay data during the two studies (analysed in 6 subjects only) was compared using Wilcoxon’s matched pairs signed rank test.

4.3 IGF-I half-life

The half-life of administered rhIGF-I was calculated using a one-compartment, mono-exponential model with first-order absorption and then with elimination from the circulating pool (Wilton et al., 1991). Linear regression analysis of log₁₀-transformed data is then used to calculate half-life using the following formula;

\[
\text{Half-life} = \frac{\log_{10}(0.5)}{\text{regression slope}}
\]

The steepest gradient beyond the peak concentration, over a time period of not less than 6h and not more than 9h, was selected from each individual data set by visual inspection of log-transformed values. This was considered to be an appropriate compromise between the need to select a substantial number of data points beyond the time when most of administered peptide had
been absorbed, but without the number being so small as to be influenced by the limitations of the IGF-I assay.

4.4 rhIGF-I: comparison of thigh versus abdominal administration

The area under the IGF-I curve (AUC) was calculated using Tai's model (Tai, 1994; Wolever, 1994) which, although based upon the trapezoid rule, is also designed to accommodate changes in sampling interval as occurred in this particular study. The accuracy of this method compares well with other techniques (Tai, 1994). The comparison of IGF-I AUC and of concentrations at individual time points was made by Wilcoxon's matched pairs signed rank test.

IGF-I clearance was calculated from the dose of rhIGF-I administered in individual subjects divided by the total AUC. In the calculation of AUC, IGF-I concentrations beyond the period in hospital were extrapolated to the point where the original baseline level was reached. The rhIGF-I dose was that actually administered (based on the alteration in syringe weight) rather than the dose calculated prior to the injection.

4.5 Blood glucose concentrations

Changes in blood glucose data with time were analysed by one and two-way repeated measures analysis of variance. The stable euglycaemic period was not selected until concentrations had settled according to visual inspection, and then analysis of variance undertaken to ensure that no differences between groups or within groups were present.
4.6 Insulin and ketone data

Insulin and ketone data were normally distributed following log-transformation and so parametric statistical techniques (paired ‘t’ tests) were used to compare overall mean values and repeated measures analysis of variance the changes with time.

4.7 GH data

Comparison of mean GH and pulsar data between the two nights was undertaken by paired ‘t’ tests.

The links between pulsar and log-transformed insulin parameters were analysed by standard least squares linear regression.

4.8 IGFBP and IGF-II data

Log transformation normalised the IGFBP-1 data. IGF-II and IGFBP-3 data was normally distributed. Overall mean values were compared using paired ‘t’ tests with changes with time assessed by repeated measures two-way analysis of variance and one-way analysis of variance. Standard least squares linear regression was used to compare variables.

4.9 Statistical Analysis: chapter 7

Because of the small numbers in this study, non-parametric statistical techniques (Wilcoxon’s matched pairs signed rank test) have been used to compare paired observations. This applies to the HbA1c and insulin dose data and when mean values from two overnight profiles are compared. The raw data is frequently presented in the tables and figures to provide a better appreciation of the changes observed in individual subjects and in the study group as a whole.
Appendix 5; Renal and splenic ultrasound

Renal length, width and depth together with splenic length were measured using an Acuson 128 ultrasound machine (Acuson, Mountain View, California). Subjects were examined in the supine and prone positions to obtain maximum dimensions when the image was frozen on screen.

Renal volume was calculated from the formula for the volume of an ellipsoid:

\[
\text{length} \times \text{width} \times \text{depth} \times 0.523 = \text{volume (ml)}
\]
Appendix 6: Biochemical and Haematological indices.

6.1 Biochemical indices

The following table lists the biochemical indices that were measured before and after the 28 day period of rhIGF-I administration described in chapter 7. rhIGF-I was administered between weeks 0 and +4 and the values are mean ± SEM and the range.

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<th>Week -1</th>
<th>Week +4</th>
<th>Week +8</th>
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<tr>
<td>Sodium (mmol/l)</td>
<td>140 ± 1 (136 - 143)</td>
<td>140 ± 1 (137 - 144)</td>
<td>139 ± 1 (137 - 143)</td>
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<tr>
<td>Potassium (mmol/l)</td>
<td>3.8 ± 0.1 (3.1 - 4.5)</td>
<td>4.1 ± 0.1 (3.7 - 4.6)</td>
<td>4.0 ± 0.1 (3.7 - 4.7)</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>4.7 ± 0.5 (3.4 - 7.1)</td>
<td>4.9 ± 0.4 (3.7 - 6.6)</td>
<td>5.3 ± 0.4 (3.5 - 6.6)</td>
</tr>
<tr>
<td>Creatinine (mmol/l)</td>
<td>67 ± 5 (54 - 91)</td>
<td>70 ± 6 (56 - 89)</td>
<td>74 ± 4 (63 - 93)</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>41 ± 1 (38 - 47)</td>
<td>42 ± 1 (34 - 46)</td>
<td>47 ± 1 (44 - 52)</td>
</tr>
<tr>
<td>Alkaline Phosphatase (iu/ml)</td>
<td>442 ± 105 (152-822)</td>
<td>467 ± 119 (176-982)</td>
<td>539 ± 129 (205-975)</td>
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<tr>
<td>Aspartate Aminotransferase (iu/ml)</td>
<td>31 ± 5 (20 - 58)</td>
<td>27 ± 4 (16 - 47)</td>
<td>24 ± 2 (18 - 33)</td>
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<td>Cholesterol (mmol/l)</td>
<td>3.7 ± 0.2 (3.2 - 4.8)</td>
<td>3.9 ± 0.3 (2.8 - 5.0)</td>
<td>4.4 ± 0.3 (3.6 - 5.5)</td>
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<td>Triglyceride (mmol/l)</td>
<td>0.7 ± 0.1 (0.4 - 0.9)</td>
<td>0.7 ± 0.1 (0.4 - 1.2)</td>
<td>1.3 ± 0.2 (0.7 - 2.2)</td>
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<td>High-Density Lipoprotein (mmol/l)</td>
<td>1.1 ± 0.1 (1.0 - 1.7)</td>
<td>1.0 ± 0.1 (0.5 - 1.5)</td>
<td>1.3 ± 0.2 (0.6 - 1.9)</td>
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<tr>
<td>Low-Density Lipoprotein (mmol/l)</td>
<td>1.8 ± 0.1 (1.6 - 2.1)</td>
<td>1.8 ± 0.2 (1.5 - 2.4)</td>
<td>1.9 ± 0.3 (1.5 - 2.6)</td>
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<tr>
<td>Thyroxine (nmol/l)</td>
<td>80 ± 8 (51 - 99)</td>
<td>71 ± 4 (55 - 83)</td>
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<td>TSH (mU/l)</td>
<td>1.9 ± 0.4 (0.5 - 3.7)</td>
<td>1.5 ± 0.4 (0.3 - 2.9)</td>
<td>2.2 ± 0.5 (0.7 - 4.4)</td>
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6.2 Haematological indices

The following table lists the haematological indices measured in the same study of rhIGF-I administration referred to in chapter 7. Values are mean ± SEM and a range.

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<th>Parameter</th>
<th>Week -1</th>
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<td>Haemoglobin (g/dl)</td>
<td>14.5 ± 0.3</td>
<td>13.4 ± 0.6</td>
<td>13.8 ± 0.6</td>
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<td>(13.3 - 15.2)</td>
<td>(11.8 - 15.1)</td>
<td>(12.2 - 15.4)</td>
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<td>White cell count (x 10^9/l)</td>
<td>5.1 ± 0.5 (3.6 - 6.7)</td>
<td>4.8 ± 0.4 (3.5 - 6.0)</td>
<td>7.0 ± 1.5 (2.9 - 0.4)</td>
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<td>Platelets (x 10^9/l)</td>
<td>232 ± 17 (181-285)</td>
<td>248 ± 10 (229-282)</td>
<td>245 ± 26 (170-286)</td>
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<tr>
<td>Red cell count (x 10^{12} /l)</td>
<td>5.0 ± 0.1 (4.6 - 5.4)</td>
<td>4.7 ± 0.1 (4.1 - 5.0)</td>
<td>4.7 ± 0.2 (4.2 - 5.2)</td>
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<tr>
<td>Haematocrit l/l</td>
<td>0.43 ± 0.1</td>
<td>0.41 ± 0.02</td>
<td>0.40 ± 0.02</td>
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<td>(0.38 - 0.46)</td>
<td>(0.35-0.44)</td>
<td>(0.36 - 0.43)</td>
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