~~Growth, Growth Hormone-Insulin-Like Growth Factor Axis and Protein Turnover in children with Chronic Renal Failure and Renal Transplants~~

GROWTH, GROWTH AXES AND BODY COMPOSITION BEFORE AND AFTER USE OF GROWTH HORMONE IN CHILDREN WITH CHRONIC RENAL FAILURE.

Thesis submitted for degree of
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To my dear family and friends
without whose support I would not have
had the courage, patience or dedication
to complete this work.

Abstract:

The studies in this thesis were designed to elucidate the role of clinical and endocrinological factors in the growth suppression that is a recognised feature of chronic renal failure (CRF) and renal transplantation, and to determine whether the mechanism of action of recombinant growth hormone (rhGH) is by improving endocrine disturbances or via a direct anabolic effect.

Endocrine parameters (IGF-I, IGF-II, IGF bioactivity, IGFBP-1, IGFBP-2, IGFBP-3, ALS, insulin and c-peptide) were studied in 3 groups of children - those with renal transplants and those with reduced glomerular filtration rate (GFR), moderate and severe. Clinical variables (age, gender, body mass index - BMI), aetiology of renal failure, steroid dose and renal modality – (CRF/ transplant/ dialysis) were also included in the analysis. All 3 groups had elevated mean IGF-II, IGFBP-2 and -3 levels. IGF-I levels, although in the normal range, would be considered low in this population as GH levels are elevated. IGF-II and ALS had a significant positive influence on growth, whilst renal modality, age, steroid treatment and duration of dialysis all had a significantly negative effect. Collectively these parameters explained 38.4% of the variability in height (Ht) standard deviation score (SDS) in the population studied. Of all the parameters studied, only transplantation had a significant influence on Ht velocity (Vel) SDS. This was positive and accounted for 26% of the variability in Ht Vel SDS.

Protein turnover was measured in 8 fasting children (4 CRF, 4 transplant) using stable isotopes (^{13}C) incorporated into leucine. Turnover was lower at baseline, and remained so despite any increases with rhGH treatment, in transplanted children compared with those with CRF. The body mass index of transplanted children who were on steroid treatment was higher, but their resting energy expenditure (REE) was lower than CRF patients. RhGH improved growth rate, arm muscle area and REE significantly whilst fat area decreased. C-peptide and IGFBP-3 showed a more consistent increase than IGF-I whilst IGFBP-1 decreased with rhGH.

The studied parameters can only partially explain the observed variability in growth rate suggesting the involvement of other factors. RhGH has anabolic effects which are apparent despite variation in endocrine responses between individuals. This variation could be attributed to differing levels of resistance to the actions of hormones.

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List of abbreviations

Acq	acquired renal disease
ALP	alkaline phosphatase
ALS	acid labile subunit
ARPKD	autosomal recessive polycystic kidney disease
BMD	bone mineral density
BMI	body mass index
C	congenital renal dysplasia
^{13}C	stable isotope of carbon
CNS	congenital nephrotic syndrome
Creat	creatinine
CRF	chronic renal failure
DBP	diastolic blood pressure
ESRD	end stage renal disease (failure)
FFM	fat free mass
FRR	fractional recovery rate
FSGS	focal segmental glomerulosclerosis
GCMS	gas chromatography mass spectrometry
GFR	glomerular filtration rate
GH	growth hormone
H	hybritech assay
HCO_3^-	bicarbonate

Ht	height
Ht Vel	height velocity
IGF	insulin-like growth factor (-I/ -II)
IGFBP	insulin like growth factor bind protein (-1, -2, -3)
IRMA	immunoradiometric assay
IRMS	isotope ratio mass spectrometry
LBM	lean body mass
MAC	mid-arm muscle circumference
MRNA	mitochondrial ribonucleic acid
NS	nephrotic syndrome
PUV	posterior urethral valves
PTH	parathyroid hormone
RhGH	recombinant human growth hormone
RhIGF-I	recombinant human insulin-like growth factor-I
REE	resting energy expenditure
RIA	radioimmunoassay
SBP	systolic blood pressure
SDS	standard deviation scores
TCO_2	total body carbon dioxide
VUR	vesicoureteric reflux

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Chapter 1

Introduction

Introduction

1.1 Normal growth and influencing factors

The general inference of the term " growth" is of linear increase in length. As the visual result of a complex process, it provides an easy method of documenting change and progress that reflects general health (1, 2). Growth however also encompasses an increase in body weight and size of the organs (1). It is a dynamic process that varies on a daily basis, and has a seasonal preponderance as well (3, 4). The velocity of growth differs at various ages, and has a predominantly biphasic pattern in life as it is most rapid in the first two years of life and during puberty, although a gradual deceleration in the former phase that continues until puberty can be noted (4). The linear accruement of height ceases with closure of the epiphysis at the end of puberty. Growth is determined by genetic and environmental influences. Genetic influences may lead directly to hormonal deficiencies, imbalances or insensitivity as in Growth Hormone (GH) Insensitivity Syndrome (Laron Dwarfism) (5), or affect the development of organs such as the kidneys leading to chronic illnesses. Environmental factors encompass nutrition, acquired illnesses, particularly those with long term consequences, and psychological

deprivation. The association between parental and off spring height is well documented.

Ethnic differences in growth and development are recognised (1, 6). Seasonal variations are also detectable in the growth pattern (7).

With increasing knowledge of growth disorders, it is becoming apparent that the common pathway for the above factors to influence growth is via the endocrine system, and therefore it is important to understand the normal growth pattern in order to manage abnormalities.

1.1.1 Normal growth

Growth is most rapid in utero, when the fertilised zygote increases tremendously in size, and then starts to decline rapidly after birth, over the next three years. Apart from a slight acceleration in mid-childhood, growth velocity shows a steady decline until the pubertal growth spurt takes place. Most rapid growth, therefore, takes place during early childhood and at puberty, although the former stage is a gradual deceleration of the growth rate continuing throughout childhood until puberty.

In 1759 Count Phillip DeMontbeillard started to plot his son's height, and produced the first chart that recorded growth velocity (figure 1.1) (7). In 1965 Tanner and Whitehouse carried out a survey of the heights and weights of healthy Caucasian school children in the SE of England and especially the London area, and plotted the normative data obtained to produce reference curves from the distribution of these measures (8,9). Due to secular increases in size and earlier maturity, new height and weight reference curves were produced based on national data obtained between 1978-1990 (10). Data from non-white children was excluded due to ethnic differences in size.

Using reference charts, it can be determined whether a child's growth is of concern. Height and weight have a normal distribution, and most children's auxology can be plotted on these curves. Using parental height (cm), a target height range can be derived using the following equation:

Target height range (cm) = mean of parental height (cm) ± 8.5 cm

where the mean of parental height has been adjusted for the sex of the child by adding 13cm to the maternal height for male children, or subtracting 13 cm from the paternal height for female children to obtain the mid-parental centile. Using the chronological age of the child, height

and bone age, assuming the child maintains the existing rate of growth, an adult height can be predicted (11,12) and compared to the target height derived from the parental heights. Concern arises if the predicted height lies outside the parental target range, or below 3rd centile reference curve or, more unusually, above the 97th centile reference curve (7). Further information can be obtained from monitoring rate of growth or height velocity. This may fluctuate during the year, and from year to year. For accuracy it is advisable to leave a minimum gap of 6 months between measurements in order to determine velocity over a year, although velocity over 12 months provides the greatest accuracy. Records of velocity show that there is no correlation in successive years, with oscillations around the 50th centile in normal children. Data must be interpreted in the context of the child's maturity. A persistently high or low rate of growth will alter the height of the child, and velocities constantly below the 25th centile will result in short stature and are regarded as abnormal (7).

Variations of normal growth due to genetic influences can be observed. A striking example of this is the difference in height observed between, and indeed within, various ethnic groups (13). Environmental factors such as nutrition and exercise contribute significantly to growth as well (1). The level of contribution of genetic and environmental influences cannot be apportioned at present, with secular changes observed in growth trends in subsequent generations and with migration (14).

Abnormalities in growth can also arise from genetic and environmental influences. Ill health can occur due to genetic and/or environmental factors, and will interfere with growth, which may show "catch up" with an increased velocity in the recovery period, especially when the illness occurs during the phase of slower growth (15). Chronic illness leads to a more complex picture as the disruption maybe complete (cessation of growth) or incomplete (slowing of growth rate), the overall impact of which is dependent on the phase of growth during which the worst effects of the illness are experienced and the duration of illness. Chronic illness can lead to alterations in the normal physiological and biochemical processes of the body, particularly puberty (16,17). Poor growth may be the only manifestation of underlying illness (18). The growth suppression present may appear disproportionate to the degree of illness, and one thought on this is that this maybe a survival mechanism for the body to diminish "non-essential metabolic" functions (19). Delayed and poor pubertal progression may also be a manifestation of this adaptation.

Growth in chronic renal failure (CRF) has evoked interest as survival rates and life expectancy have improved. The number of children under 2 starting renal replacement therapy in Europe has doubled over 15 years (20), whilst in N. America 21% of children who receive renal transplants are under 6 years of age (21), highlighting the need to focus on the quality of life of these patients. The aetiology of CRF can be attributed to

either genetic and/or environmental influences. The complications arising secondary to the CRF such as anorexia, uraemia (22), acidosis (23), hormonal imbalances (19) and anaemia (24) all contribute to the poor growth observed.

1.2 Chronic renal failure and growth

1.2.1 Chronic Renal Failure

CRF is signified by reduced glomerular filtration rate (GFR)- ranging from mild failure (40-60 mls min^{-1}1.73 m^2); moderate (20 - 40 mls min^{-1} 1.73 m^2); severe (<20 mls min^{-1}1.73 m^2) and end-stage (<10 mls min^{-1}1.73 m^2). End stage renal failure (ESRD/ ESRF) necessitates the introduction of supportive measures such as dialysis or, if possible, renal transplantation. The latter increases quality of life and life expectancy (25).

1.2.2 Growth in children with chronic renal failure

Congenital abnormalities are the commonest cause of CRF in childhood (26), and those with congenital renal dysplasia may have reduced birth weight (27, 28).

Reduction in GFR leads to rise in creatinine. A rise of 1mg/dl (90 μmol/ml) is associated with a decrease of -0.15 SDS in height (29). Vast improvements in the conservative management of complications arising secondary to CRF such as poor nutritional status, acidosis, bone disease, and anaemia have taken place in the last 10 -15 years, and are associated with improvements in growth rate (30, 28). More invasive measures, in the form of dialysis and renal transplantation, are required as end-stage renal failure approaches.

Transplantation has had a radical impact on the quality of patients' lives, and patients can go on to lead normal lives, including having children (31). A retrospective study looked at the outcome of 150 children who had received renal transplants between 1970 to 1993. Adult height was available for 100 of these. Eighty-four adults had non cystinotic disease and 37 of these, and all 16 with cystinosis, had growth stunting with a final height less than -2 SDS below the mean (31). Children who receive a transplant between the ages of 2- 5 years show catch-up growth, but this trend decreases as age at transplantation increases (29). Other than age at which transplantation takes place, use of immunosuppression (particularly steroids), pre-transplantation dialysis treatment, graft function, mildness of growth retardation pre-transplantation and number of transplants are factors adversely associated with growth post transplantation (32, 29, 33).

CRF can occur at any age from birth onwards. Foetal manifestations of CRF can be limited to oligohydramnios and its complications, due to placental replacement for the kidney in utero. Many infants are now diagnosed antenatally using ultrasound. However there is evidence that congenital renal disease results in lower birth weight (30). Children with congenital renal disease are particularly vulnerable to growth stunting as a direct effect of the illness at a time of rapid growth, and lack of appropriate management in non-specialist centres especially with regard to nutrition (27).

The aetiology of CRF can be variable, from infection (haemolytic uremic syndrome, sepsis), glomerulonephritis, secondary to drug usage (chemotherapy, antibiotics), genetic (nephronophthisis, polycystic kidneys, glomerulopathies), or malignancy. However the commonest cause of CRF in childhood is congenital renal dysplasia with or without reflux or obstruction (26).

The increase in the numbers of children with early onset CRF surviving to adulthood has highlighted the effects of the illness on growth and nutrition. Retrospective studies carried out in 1995 showed that the mean final adult height of males was -1.98 SDS and -1.41 SDS for females (34). In 1974 Chantler demonstrated, using animal models, that rats rendered uremic by 5/6 nephrectomy were smaller, and weighed less than controls (35). Kleinknecht et al in 1983 noted from their prospective

study that significant deceleration of growth velocity occurred within the first two years of life in children developing CRF in infancy, resulting in short stature, despite normalisation of the growth velocity in subsequent years (28). This has been confirmed by other studies (30). Catch up growth, reflected by growth velocity rate above that of age related peers, to compensate for the loss during the maximal growth period is rarely observed.

Since the early eighties, significant improvement in the therapeutic management of CRF has taken place. Knowledge of the importance of adequate nutritional status, not only in CRF (36, 37, 38), but any chronic disease, has increased (39). This is true not only in children (38), but also for adults (40). Multinational, cross sectional studies have shown that adult patients with ESRD manifest protein calorie malnutrition typified by reduced body weight, skin fold thickness and mid arm circumference (41). Severe malnutrition as characterised by decreased serum albumin levels is associated with higher mortality rates (42). In children, poor growth can be a manifestation of malnutrition (1). Supplementation of calories to 120% of daily requirements has been shown to slow the rate of decrease of height velocity and improve weight gain in infants with chronic renal failure (30). This improvement in Ht SDS, although greater in supplemented children with mild rather than severe CRF, and better in those supplemented compared to non-supplemented with the same degree of CRF, still remains below that of normal controls (30).

Despite supportive measures such as dialysis, other complications such as acidosis, bone disease (43), and anaemia can occur. These can be present, albeit in a milder form, when CRF is established, increasing in severity with progressive deterioration in GFR (43). Improvements in the management of CRF aim to identify and prevent or minimise the onset and progression of complications. It is also important to bear in mind that both dialysis and transplantation are associated with their own complications.

1.2.2.1 Acidosis

Acidosis is a complication of reduced GFR, and many patients with CRF require bicarbonate supplementation. Studies have shown that reduced serum bicarbonate is associated with decreased GH secretion and weight gain in rats (44), neonates (45, 46) and in healthy volunteers rendered acidotic (47). Protein catabolism is also higher (48, 49) and exogenous recombinant growth hormone (rhGH) less effective in improving the rate of growth (50) in acidotic states.

1.2.2.2 Anaemia

Anaemia occurs a complication of CRF, secondary to decreased erythropoietin (51), leading to reduced exercise tolerance, impaired cardiac function and insufficient tissue oxygenation. Replacement therapy is administered to correct the anaemia with improvements in growth (24).

1.2.2.3 Renal osteodystrophy

Phosphate retention, hypocalcaemia and hyperparathyroidism lead to renal osteodystrophy (52). Bone disease arises partly from the electrolyte abnormalities present in CRF secondary to the reduced GFR, but also due to the loss or diminished action of the kidney 1α-hydroxylase in the conversion of 25 (OH) D_3 to its active metabolite 1,25 $(OH)_2D_3$, which enhances calcium resorption from the renal tubules, absorption from the gastrointestinal tract and increases the action of osteoblasts and osteoclasts (53). Hypocalcaemia leads to hyperparathyroidism (54). Treatment is by dietary phosphate restriction, phosphate binders and the prescription of activated vitamin D. However the effect of renal osteodystrophy on growth in CRF is controversial (55).

1.2.2.4 Dialysis and renal transplantation

ESRD necessitates the commencement of dialysis or renal transplantation. Malnutrition and growth suppression are maximal in ESRD (56). Some improvement may be demonstrated with the commencement of peritoneal dialysis compared to the immediate predialysis phase in height standard deviation score (Ht SDS) as observed over a six month period (57). The growth of children on continuous ambulatory peritoneal dialysis is better than those on haemodialysis (57, 56). However, dialysis is an invasive process and increases the risk of infection in a patient group with an already compromised immune function (41), thus compounding the problems with malnutrition and growth. The other complications described above are maximal in this group, leading to increased morbidity and mortality (42).

Transplantation results in an improvement in nutrition, growth and general well-being. The height at the time of transplant, GFR after transplant (if < or > 50 mls/min/1.72m^2) (59) and age at time of transplant (32) are important determinants of progress in growth after transplantation, and a positive association is observed with higher values of the former two, whilst the optimal age of the latter for catch-up growth

is between 2-5 years. Immunosuppressive medication such as glucocorticoids used following the transplant to prevent organ rejection also has a detrimental effect on growth (60).

1.2.3 Endocrine factors in CRF

Despite preventing or minimising the above factors, children with CRF still demonstrate problems with growth (30). This has lead to more detailed attention to endocrine factors in CRF. Initial findings showed normal or elevated growth hormone (GH) levels, but closer scrutiny has revealed insensitivity to the actions of GH (61, 62), and abnormalities of the GH-insulin-like growth factor (IGF) axis (63, 64). (See Chapter 3).

1.2.4 Relevance of bone age in CRF

Bone age in chronic illnesses, particularly in CRF can be unreliable as the bone architecture can be altered by renal osteodystrophy (65). This can also occur in a population with use of steroids following renal transplantation (65). Therefore bone age in CRF is not a helpful measurement (65).

1.3 Consequences of short stature

Studies have demonstrated that adults with short stature have low self-esteem (66), but children with CRF do not appear to have a major preoccupation with growth although this becomes more of a problem with increasing age (67). This may be due to the limited awareness children have of life experiences. Children appear to be more concerned about the impact of their illness on other areas of their life, particularly their family (67). Parents of these children also place more emphasis on future health rather than growth (67).

1.4 Recombinant human growth hormone

Use of rhGH and trials of recombinant IGF-I (rhIGF-I) in GH deficiency, GH resistance and chronic illnesses have been closely monitored for efficacy and safety. This has increased knowledge of the benefits of rhGH in areas other than linear growth alone. RhGH has been effective in improving lean body mass, affect and in some reports cardiovascular function in adult GH deficient patients (68). It also appears to be beneficial in osteoporotic bone disease (69). Some of these benefits have been clearly demonstrated in GH deficient children (70). Attention has therefore been focused on how rhGH can be of benefit to children with CRF and those subjected to prolonged steroid therapy. Data on final

height of short children with CRF following use of rhGH therapy is now emerging and shows that response of individuals can vary (71, 72).

1.4.1 Indications for GH therapy

It has been accepted since the fifties that replacement therapy for GH deficiency is required. About 25 years ago, GH therapy was restricted to replacement in GH deficiency due to the limited availability and expense of GH as it was obtained from the pituitaries of cadavers. With DNA technology, it became possible to mass produce recombinant GH (rhGH) which was more cost effective and safer. RhGH is now licensed for use in CRF and in girls' with Turner's Syndrome, in addition to GH deficiency.

Some centres have also used rhGH in the treatment of children with idiopathic short stature with some improvement in growth rate (73), and although preliminary data on final height appears promising (74) there is still controversy regarding use of rhGH in these children. It is now accepted that adults with GH deficiency benefit from replacement therapy (68).

Adolescents are capable of giving informed consent, and rarely withhold this for rhGH although compliance may then become an issue. Younger children commence rhGH on the basis of consent provided by parents.

1.4.2 Recombinant growth hormone in children with CRF and renal transplant

RhGH is effective in children with CRF and those post renal transplantation and preliminary data of its influence on final height appears promising (72,75), but is limited. RhGH is used in supraphysiological doses in CRF (4 iu/m^2/day or 0.07 mg/kg/day). It is not clear whether protein kinetics in children with CRF are altered with increase of catabolism, decrease of synthesis or a combination of the two. RhGH has an anabolic effect on lean body mass which maybe direct or by stimulation of its intermediaries, particularly IGF-I.

1.5 Nature of work for MD thesis

The work for this MD has involved children with CRF, ESRD, post renal transplant, and for comparison, children on steroid treatment for nephrotic syndrome or vasculitis without current renal involvement.

Multiregression analysis has been applied to determine which parameters of clinical and endocrinological origin have a significant influence on the Ht SDS of all the children and Ht Vel SDS of prepubertal children. Protein turnover in short children, an area that has been studied little in children, particularly in those with renal impairment, was

determined before and after the commencement of rhGH and interpreted in the context of hormonal involvement.

1.5.1 Hypotheses

I. Levels of endocrine factors comprising the GH-IGF-IGFBP axis and their importance to growth are altered by deteriorating GFR and steroid usage and this will influence the Ht SDS and Ht Vel SDS of patients with CRF, ESRD and renal transplants.

II. Use of rhGH in children with CRF and renal transplants will improve growth, lean body mass, reduce adipose tissue and increase protein turnover that maybe reduced compared to healthy individuals.

1.5.2 Aims of the work for this thesis

I. To study children with CRF, ESRD treated with dialysis and post renal transplant with or without growth retardation, to determine which clinical and endocrinological (GH-IGF-IGFBP axis) parameters are important to growth.

II. To study protein turnover in children with CRF and those with a renal transplant, and study the effects of rhGH therapy on protein turnover, growth and endocrine factors.

1.6 Ethical approval

Ethical approval for all the studies in the thesis was granted by the Ethics Committee at Great Ormond Street Hospital.

Written informed consent was obtained from the parents, and when appropriate from the children.

Clearance for use of stable isotopes was obtained from the dangerous drugs and therapeutics committee.

Figure 1.1 Growth velocity chart

Chapter 2

Patients

Patients

2.1 Introduction

This chapter provides details of the principal epidemiological and clinical features of the children recruited for the studies described in this thesis in subsequent chapters. Studies undertaken on the children had both clinical and laboratory aspects.

2.2 Growth and the Growth-Hormone-Insulin-like Growth Factor axis

2.2.1 Patients

Patients for this study were recruited on the basis of their renal status of which there were four categories: chronic renal failure (CRF), end-stage renal disease (ESRD) necessitating dialysis support, renal transplant and those with a normal glomerular function (GFR) – but on steroid treatment.

Criteria for recruitment were

i) None of the children were acutely unwell (pyrexial, septic or being treated for acute transplant rejection).

ii) None of the children were on recombinant growth hormone therapy.

iii) Children on steroid therapy had not received steroids at a dosage > 1mg/kg/day in the fortnight prior to sample collection.

iv) Children with a history of proteinuria did not have more than 1+ on dipstick testing of their urine on the morning of sample collection, nor had they had more than 2+ intermittently in the fortnight prior to sample collection.

v) All patients with CRF had a GFR less than 40ml/min/1.73m^2.

vi) Children were able to fast for the study as required, and if on overnight nasogastric tube feeds, parents were amenable to stopping the feed at least 6 hours prior to the sample collection time which was usually at or soon after 9.00 am.

2.2.1.1 Chronic renal failure group (appendix 2.1)

Thirty one children (7 females) with CRF aged (mean ± SD) 11.7 ± 3.6 years were recruited. Patient demographics are as detailed in appendix 2.1, and table 2.1 .

2.2.1.1.1 Primary diagnosis

Fourteen children had congenital renal dysplasia (8 also had vesicoureteric reflux - VUR). Four had posterior urethral valves, 4 had autosomal recessive polycystic kidney disease (ARPKD), 2 had focal segmental glomerulosclerosis (FSGS), 2 had acquired CRF (birth asphyxia, post cardiac surgery), 1 had CRF of unknown cause, 1 had a remnant kidney after treatment for Wilm's tumour, 1 had Alport's syndrome, 1 had VATERs syndrome and 1 had cystinosis.

2.2.1.1.2 Pubertal status and auxology

The pubertal status (mean ± SD) of the children was 2 ± 1. There were 15 children who had commenced pubertal development. Of these 4 had pubertal delay (pubertal stage below 3rd centile for genital/ breast development for age determined using age and gender appropriate

Tanner and Whitehouse centile charts for pubertal development). Two females were pre-pubertal (stage 1) at 12.5 and 13 years and thus would also be considered as delayed.

Mean ± SD auxological parameters of all the children were as follows-: body mass index (BMI) standard deviation score (SDS) 0 ± 1.2, Height (Ht) SDS -1.4 ± 1.5, Ht velocity (Vel) SDS -0.4 ± 3.4, and weight (Wt) SDS -0.9 ± 1.7. Of these children, ten had a Ht SDS < - 2.0 SDS.

2.2.1.1.3 Renal function and acid-base status.

The mean (SD) glomerular filtration rate (GFR) of the patients was 22.5 ± 7.4 mls/min/1.73 m^2. The total body carbon dioxide (TCO_2), a measure of the acid-base status of the children, was 22.9 ± 3.3 mmol/l (normal range 20 to 26 mmol/l).

2.2.1.1.4 Serum albumin

The serum albumin of the children was (mean ± SD) 41.0 ± 3.8 g/l. Serum albumin is a indication of nutritional status in patients with chronic illnesses, but can also reflect disease activity in children with nephrotic

syndrome. Levels increase with age and for children aged 3 – 15 years can range from 35 – 56 g/l.

2.2.1.2 Children with renal transplants (appendix 2.2)

Twenty seven children (6 females) with a renal transplant were recruited to the study. Patient details are in appendix 2.2, table 2.1. The (mean ± SD) age of the children was 12.3 ± 2.8 yrs.

Samples had been obtained previously from two children in this group prior to transplantation, when one had CRF and the other ESRD.

2.2.1.2.1 Primary diagnosis

Underlying diagnoses in this group were renal dysplasia -16 (including 4 with VUR), posterior urethral valves - 3, focal segmental glomerulosclerosis (FSGS) – 5, mesangiocapillary glomerulonephritis - 1, oto-branchio-renal syndrome – 1, and 1 with unknown cause of CRF.

2.2.1.2.2 Pubertal status and auxology

The pubertal status (mean ± SD) of the children was 2 ± 1. Nine children were established in puberty and of these four (one female) had pubertal delay. There were three children (one female) who were inappropriately prepubertal at 14 years, 16.3 years and 13.5 years (female).

Auxological parameters of all the children were as follows: (mean ± SD) BMI SDS 0.9 ± 1.2, Ht SDS -1.6 ± 1.2, Ht Vel SDS 0.6 ± 3.7 and Wt SDS -0.2 ± 1.3. Ten children had a Ht SDS < -2 SDS.

2.2.1.2.3 Renal function and acid- base status

The GFR of the children was 43.8 ± 22.4 mls/min/1.73 m^2. Three children had had two or more renal transplants. Mean (± SD) time since transplantation was 42.6 (± 26.7) months.

The mean (±SD) TCO_2 level of the children was 21.6 (± 1.9) mmol/l.

2.2.1.2.4 Serum albumin

The mean ± SD level of serum albumin of the children was 41.0 ± 2.3 g/l.

2.2.1.2.5 Steroid dosage

Children were on prednisolone at a dose of 0.3 ± 0.2 mg/kg/alt day.

2.2.1.3 End-stage renal disease group (appendix 2.3)

Ten children (5 females) were enrolled into this sub-group (demographic details in table 2.1). The mean (± SD) age of the children was 10.7 (± 4.5) yrs.

2.2.1.3.1 Primary diagnosis

Diagnoses ranged from renal dysplasia – 5 (including 2 with VUR), posterior urethral valves - 1, congenital nephrotic syndrome - 1, focal segmental glomerulosclerosis - 2 and acquired disease (haemolytic uremic syndrome) in 1 case.

2.2.1.3.2 Pubertal status and auxology

The pubertal status (mean ± SD) of the children was 1 ± 1. Three children were established in puberty, and of these two children had delayed development. One female at 14.3 years was prepubertal and thus also delayed.

The mean ± SD Ht SDS of all the children was -1.8 ± 1.2, whilst their Ht Vel SDS was -0.3 ± 2.4, BMI SDS was 0.1 ± 1.4 and Wt SDS was -1.0 ± 1.3.

2.2.1.3.3 Renal function and acid- base status

All patients were on dialysis treatment (2 on haemodialysis, 8 on peritoneal dialysis). Mean ± SD time since commencement of dialysis was 29.3 ± 22.1 months.

The mean ± SD TCO_2 level was 26.4 ± 2.7 mmol/l.

2.2.1.3.4 Serum albumin

The mean (SD) albumin of the children in this group was 34.5 (± 2.6) g/l. This was at the lower end of the normal range (35 – 56 g/l for children aged 3 – 15 years). In dialysis patients this can be difficult to interpret as fluid overload can produce a dilutional effect.

2.2.1.4 Normal GFR group on steroid treatment (appendix 2.4)

Ten children (3 females), age (mean ± SD) 11.8 ± 1.7 years (median 11.4) were enrolled into the study (details as per table 2.1). This group with normal GFR was initially intended to be a control group to determine the effect of steroids without renal failure but was then used in the multiregression analysis as a control group with normal GFR.

2.2.1.4.1 Primary diagnosis

Eight patients had steroid dependent nephriotic syndrome (SSNS), whilst two had vasculitis without renal involvement. None of these children were acutely unwell and those with SSNS were in remission.

2.2.1.4.2 Pubertal status and auxology

The (mean ± SD) pubertal status of the children was 2 ± 1. Three children were established in puberty with appropriate development. One female at 12.5 years was prepubertal and the only one who thus had pubertal delay.

The auxological (mean ± SD) measurements of all the children were as follows: Ht SDS was 0.2 ± 1.3, Ht Vel SDS -0.9 ± 2.2, Wt SDS 0.3 ± 1.2 and BMI SDS was 0.4 ± 1.8.

2.2.1.4.3 Renal function and acid-base status

None of the children had diseases that affect GFR and creatinine levels ranged from 35 - 76 μmol/l (mean ± SD: 54.4 ± 13.8 μmol/l) and were normal when compared to age matched reference ranges supplied by the laboratory. Levels increase with age (35 – 80 μmol/l at 13 years of age)

The acid-base status was normal for all children with a mean of 24.3 and SD of ± 2.9 mmol/l.

2.2.1.4.4 Serum albumin

This was normal for children (mean ± SD) at 37.4 ± 3.9 g/l.

2.2.1.4.5 Steroid dose

All children were receiving steroid treatment, and the mean (SD) dose was 0.4 (±0.2) mg/kg per day. Eight patients received alternate day steroids and 2 (vasculitis) had steroids (0.4 and 0.6 mg/kg) on a daily basis.

2.3 Effects of rhGH on growth and protein turnover (appendix 2.5)

Twelve patients (1 female) (appendix 2.5). were recruited to this study, and underwent preliminary studies on fractional recovery of 1-^{13}C during

an infusion of 1-^{13}C sodium bicarbonate prior to proceeding on to the protein turnover studies (see introduction to Chapter 4 for further details). These patients were also included in the study of the GH-IGF-IGFBP axis (chapter3) where they were 8-12c, 4d, 4t, 6-9t and 2t, where c=CRF; t=transplant and d=ESRD (Patient 2t had CRF at the time of the fractional recovery study) (appendix 2.5). Four patients (j,k,l,m) did not proceed to the protein turnover studies due to needle phobia (1), change of renal status with renal transplantation and cessation of rhGH (2) and change of renal status with development of ESRD and cessation of rhGH (1).

2.3.1 Patient selection

Patients were selected using the following criteria:

i) Clinical indication for rhGH treatment due to short stature. The criteria were height standard deviation score (Ht SDS) more than 2SD below the mean (or below the 2nd percentile), despite adequate nutrition, correction of metabolic abnormalities, adequate dialysis and, if post transplant, reduction of prednisolone to a minimum.

ii) Amenable to fasting in accordance with protocol requirements.

iii) Old enough to cooperate with protocol requirements.

iv) Not acutely unwell.

2.3.2 Age and pubertal status

The median (range) age of the children was 13.2 years (7.5 – 17.1), and their pubertal status ranged from 1 - 3 (median 1). Five children were established in puberty. Three of these (all male) were delayed with development below the 3rd centile appropriate for age as determined using appropriate age and gender pubertal centile charts. One male was prepubertal at 15. 2 years of age and thus also delayed in puberty.

2.3.3 Auxological and anthropometrics parameters

The Ht SDS of the children ranged from -5.7 to -2.0 (median -2.7) SDS. Their Ht Vel SDS range was -7.1 to 8.8 (median 0.6) SDS.

The median value for the BMI for the group was 18.7 (14.9 – 28.7) kg/m^2.

Anthropometrics were determined only in the children who proceeded on to the protein turnover work. The median (range) value of triceps skinfold thickness of the children was 16.6 (-7.0 - 32.0) mm, and of mid-arm circumference was 246 (180 - 334) mm.

2.3.4 Renal function

Six patients (a,c,f,h,j,l) had CRF and 5 (b,d,e,g,m) had a renal transplant. One patient (k) was on peritoneal dialysis. The median (range) GFR of the children with CRF was 21 (12 – 34) ml/min/1.73m^2, and of those with renal transplants was 27 (7 – 85) ml/min/1.73m^2. Overall the median (range) was 25 (7 – 85) ml/min/1.73m^2.

2.3.5 Steroid dosage

The transplanted children received prednisolone treatment on alternate days and the dosage range was 0.2 – 0.5 mg/kg/alt day (median 0.2).

2.3.6 Acid- base status

The TCO_2 of the group ranged from 18 – 25 (median 22.5) mmol/l.

Table 2.1: Mean ± SD of patient characteristics

Group	Age (yrs)	Pubertal status	BMI SDS	GFR*	TCO2 (mmol/l)	Ht SDS	Ht Vel SDS	Wt SDS	Creat (umol/l)	albumin (g/l)	Tx duration (month)	Steroid (mg/kg)	Duration dialysis (month)
CRF	11.7±3.6	2.0±1.0	0.0±1.2	22.5±7.4	22.9±3.3	-1.4±1.5	-0.4±3.4	-0.9±1.7	373±215	41.0±3.8	na	na	na
Tx	12.3±2.8	2.0±1.0	0.9±1.2	43.8±22.4	21.6±1.9	-1.6±1.2	0.6±3.7	0.2±1.3	139±89	41.0±2.3	42.6±26.7	0.3±0.2	na
ESRD	10.7±4.5	1.0±1.0	0.1±1.4	na	26.4±2.7	1.8±1.2	-0.3±2.4	-1.0±1.3	822±279	34.5±2.6	na	na	29.3±22.1
NGFR	11.8±1.7	2.0±1.0	0.4±1.8	na	24.3±2.9	-0.2±1.3	-0.9±2.2	0.31.2	54±13	37.4±3.9	na	0.4±0.2	na

* mls/min/1.73m2

Chapter 3

Growth and the Growth-Hormone-Insulin-like Growth Factor axis in children with chronic renal failure, end-stage renal disease and renal transplants

Growth and the growth-hormone-insulin-like growth factor axis

3.1 Introduction

3.1.1 Endocrinology of normal growth

The many factors that influence normal growth are shown in figure 3.1. This chapter will focus on studying the GH-IGF-I axis in children with both normal and abnormal growth patterns.

3.1.1.1 Growth Hormone

GH was isolated in the 1950s. It consists of 181 amino acids and is coded for by a gene located on chromosome 17 within a cluster of five related genes in series over a 66.5 kb span. Different isoforms of human GH (hGH) arising from post-translation modifications can been identified in the circulation, with variations in molecular weight. Twenty and 22 kDa are the main isoforms, of which the 22 kDa form is the most abundant (76). Both the 20 and 22 kDa forms are bioactive and can suppress GH secretion from the pituitary gland (77).

GH has been regarded as important in children and adolescents, and it is now becoming clear that it has a role in foetal growth as GH deficiency leads to reduction in birth length of approximately 1 SDS (78). Its action appears to be most important in mid-childhood, but it is now recognised that it has a role in early childhood (7) and children with GH deficiency now receive replacement therapy in infancy. Nutrition still has a very strong influence on growth in this period (79).

GH is released from the pituitary gland in response to stimulation from GH releasing hormone (GHRH) secreted by the hypothalamus, and its release is inhibited by GH release inhibiting hormone (GHRIH). A diurnal variation in GH secretion exists, with highest levels produced at night. GH is released in a pulsatile manner, but although frequency and amplitude of the pulses are important, it appears that rate of change of serum levels dictates efficacy (80). Random samples, therefore, are often unreliable as GH levels maybe low and provide no information on rate of release of GH.

GH levels are also influenced by other hormones (see figure 3.1), such as insulin, thyroxine, cortisol and the sex steroids (during puberty); and other factors such as starvation, stress, sleep and exercise (7). By opposing the actions of insulin, GH has a "diabetogenic" effect that raises blood sugar levels and therefore leads to increased insulin levels (81). Chronic glucocorticoid therapy leads to decreased GH secretion (82).

GH is transported in the circulation complexed to binding proteins (GHBP), the role of which is unclear. The structure of the GHBP is identical to that of the GH receptor from which it is produced by cleavage of the receptor from the extracellular site (83). GH actions are mediated via growth factors, although there is some evidence that it has direct influences as well on growth (84).

The GH receptor consists of a single polypeptide chain that extends across cell membranes. The extracellular domain is formed by the ligand-binding, folded amino terminal of the polypeptide chain which extends transmembranely to form the intracellular domain from the C-terminal end. GH mediates its actions by binding to two of its receptors (dimerization) and this initiates intracellular signalling (85) by activating tyrosine kinase Janus kinase 2 (JAK2) which phosphorylates itself and the cytoplasmic domain of the GH receptor. Inability to phosphorylate JAK2 leads to lack of response to GH stimulation (86).

3.1.1.2 Growth factors

Growth factors are produced by the stimulatory effects of GH on the liver and other tissues. Previously these were labelled " somatomedins ", but are now recognised as insulin-like growth factors (IGFs) as they are structurally homologous to proinsulin. Two forms - IGF-I and IGF-II, 7.5 kDa molecular weight, 70 amino acids, have been identified. The type 1

IGF receptor is a heterotetrameric glycoprotein and consists of two alpha sub-units containing the binding domain, and two beta sub-units containing the tyrosine kinase domains (87), and mediates the mitogenic actions of IGFs (88).

The type 2 IGF receptor binds IGF-II with high affinity, is identical to the cation independent mannose-6-phosphate receptor, and its role appears to be the degradation of IGF-II (88).

Insulin can bind to IGF receptors with approximately 1% of the affinity of IGF-I. IGF-I and, particularly, IGF-II can cross react with insulin receptors with approximately 2% of the affinity of insulin. The affinity of IGF-II for the IGF type 1 receptor is about 33% of that of IGF-I (89). The insulin receptor is a heterotetrameric transmembrane tyrosine kinase receptor (88).

3.1.1.2.1 IGF-I

In addition to the liver, IGF gene expression has been detected in essentially all organs (84). IGF-I may have autocrine and paracrine (local production) as well as endocrine actions (hepatic production). IGF-I is believed to have a role predominately later, whilst IGF-II is more influential earlier in pregnancy. A correlation of cord blood IGF-I levels with birth size has been demonstrated (78). Levels increase with age

postnatally. Adult levels are attained by the end of puberty, but rise 2-3 fold during puberty.

Nutritional status influences levels of IGF-I. Fasting and malnutrition result in low serum levels of IGF-I, with a sub-optimal response to exogenous GH administration (90). Endogenous GH levels are increased in fasting (91). In rats, fasting leads to reduced hepatic GH receptors, with decreased concentrations of both GH receptor mRNA and IGF-I mRNA (92). Protein malnutrition may result in post receptor defects as neither GH receptors nor GH binding is decreased (90).

Other factors that influence free IGF-I levels are insulin, hypothyroidism and steroid therapy (93). The former influences an increase in levels (94, 95) , whilst reduced IGF-I levels are noted with hypothyroidism (96). Steroids interestingly alter IGF bioactivity by induction of IGF inhibitors (97).

Administration of IGF-I to rats leads to increased body weight and nitrogen retention, indicating protein deposition (98). Carcasses of these animals had reduced body fat and increased organ weights. IGF-I has also been shown to stimulate proliferation of differentiated chondrocytes, acting in synergy with GH to promote longitudinal bone growth (99). IGF-I gene deletion results in profound intrauterine growth retardation (IUGR) (78)

IGF-I is transported in the circulation bound to proteins, the IGF binding proteins (IGFBPs), which increase its half life (usually ~10 min) (100), and prevent fluctuations and diurnal variations in levels. Approximately 99% IGF-I is complexed. Free IGF is bioactive (101,102, 103). Imbalances between the ratio of the proteins to IGF-I can arise as in chronic renal failure (CRF), affecting the proportion of free IGF-I.

3.1.1.2.2 IGF-II

IGF-II levels increase gradually with age throughout childhood and adolescence. Levels are low in GH deficiency (104, 105) and malnutrition (106). IGF-II is secreted by the liver and other tissues, and rises in response to GH therapy in children (107). A role for IGF-II in antenatal growth has been established (108), but not in post-natal life. IGF-II has a higher affinity for the insulin receptor than IGF-I (109).

3.1.1.3 Insulin-like growth hormone binding proteins (IGFBPs)

The IGFs circulate in the plasma bound to binding proteins-(IGFBP) (260 amino acids in length). At least 7 IGFBPs (six "high affinity" IGFBPs (1-

6)) have been identified , found in varying quantities, in most of the biological fluids of the body. Other proteins which have a weaker affinity for the growth factors have also been identified and labelled IGFBP-rP (IGFBP 7). The middle region of each BP is characteristic for each protein (110).

Experimentally it has been shown that the binding proteins can have an inhibitory effect on the actions of the IGFs, presumably by reducing the free amount available to bind to the receptors as IGFBPs have a higher affinity for IGFs. It is postulated that smaller IGFBPs (IGFBP-1 and -2) may inhibit IGF actions at tissue level by binding to IGF in extravascular spaces (64). In addition some evidence indicates that the IGFBPs may have a direct inhibitory role in the absence of IGFs. In certain situations IGFBPs appear to have an enhancing influence by increasing delivery to the target sites, and reducing clearance of the growth factors (110).

3.1.1.3.1 IGFBP-1

IGFBP-1 gene expression occurs primarily in the liver, decidualized uterine endometrium, ovary granulosa cells and kidney (111). Levels are regulated predominantly by insulin which inhibits transcription of IGFBP-1, but have also been shown to decline with age, correlate inversely with increases in IGF-I, and decrease during pubertal development (112). Thyroid hormone (113) and epidermal growth factor (114) stimulate gene

expression, as do glucocorticoids in conditions of low or absent insulin levels (115). Cytokines - interleukin-1β (IL-1β), TNF-α and interleukin-6 (IL-6) stimulate IGFBP-1 production (116).

IGFBP-1 appears to inhibit the influence of IGF-I on somatic and brain growth as observed in transgenic mice that over express IGFBP-1 (117). IGFBP-1 administered intravenously increases blood glucose levels and thus may have a role in carbohydrate metabolism (118). Low concentrations of IGFBP-1 enhance the actions of IGF-I by an increase of its half-life (119). IGFBP-1 levels are raised in intra-uterine growth retardation (78).

3.1.1.3.2 IGFBP-2

This binding protein is the second most abundant binding protein in the circulation and preferentially binds IGF-II over IGF-I. Both IGF-II and IGFBP-2 appear to have important roles in fetal development and growth (120). Transgenic mice overexpressing IGF-II have raised levels of IGFBP-2, suggesting a role for IGF-II control in the synthesis of IGFBP-2 (121). Elevated levels are also found after fasting and in certain pathological conditions such as diabetes mellitus, malignancies, cirrhosis and CRF (122).

IGFBP-2 appears to have a role in inhibiting the actions of IGF-II in animal models, leading to reduction in organ and carcass weights (123). In cell culture studies IGFBP-2 inhibits the autocrine IGF-II dependent proliferation of human colonic carcinoma cells (124).

3.1.1.3.3 IGFBP-3

This is the most abundant IGFBP in the circulation by several fold, and in the adult, highest tissues concentrations occur in the kidney. Its affinity for the IGFs exceeds that of the other IGFBPs. IGFBP-3 associates with an acid-labile unit (ALS ~ 80kDa) after binding IGF-I or -II, forming a 150kDaternary complex that is too large to leave the circulation. Addition of ALS to the IGF-IGFBP-3 binary complex increases IGF half-life from 20-90 min to 12-14 hr (100).

Differences in molecular weight, usually 41 and 38 kDa, arise due to variation in N-terminal glycosylation of IGFBP-3, with a 29 kDa core molecular mass which has a lower affinity for IGF-I (125).

GH stimulates production of IGFBP-3 from kidney, liver, spleen, heart, and muscle, the function of which appears to be to act as a reservoir of IGF-I and -II, thus preventing rapid changes in levels and potentiating their actions (126). IGFBP-3 may also have effects on growth that are

independent of IGF and can be inhibitory (127). Levels increase with age and as nutritional status improves (110).

3.1.1.3.4 IGFBP 4-6

Only a brief mention of these proteins is necessary as these were not included in the study. These binding proteins are predominantly located within the tissues.

IGFBP-4 in adults is involved in bone formation and resorption (128), and in vitro inhibits the actions of IGF-I and -II (129).

IGFBP-5 is the most abundant IGFBP in bone extracts where it appears to be essential for IGF storage, especially IGF-II (130). Recently it has been identified as a component in the ternary complex formed with IGF and ALS in the circulation (131). Levels rise with GH treatment.

IGFBP-6 has a markedly higher affinity for IGF-II than IGF-I. It is found in a variety of tissues and although associated with inhibition of tumour cell growth in vivo and vitro (130), probably via IGF-II inhibition, its exact role remains unclear.

3.1.1.4 Proteases

These are enzymes that disrupt the binding of IGF to IGFBP by proteolysis of the binding protein, thus lowering its affinity for IGF. They are specific for each IGFBP, and so far proteases for IGFBP-2, -3, -4 and -5 have been identified (110).

3.1.1.5 Other hormones

Hormones such as insulin and thyroxine are important in growth. Classically these are not part of the GH-IGF axis. Proinsulin has significant structural homology (~50%) with the IGFs and weak cross-stimulation of the receptors can be observed (110). Receptor structure and stimulatory pathways are similar for IGF-I (IGF-IR) and insulin (IR) (133). Mice lacking both receptors are more growth retarded than those lacking either receptor alone. Absence of IR leads to approximately 10% reduction in size at birth. In humans absence of the IR leads to hypoglycaemia due to overstimulation of IGF-IR by elevated insulin levels (134). Insulin has an important role in protein turnover, fat metabolism and is also involved in the regulation of IGFBP-1 (135)

Deficiency or excess of thyroxine leads to alterations in growth and metabolism. Thyroxine is also important in cerebral function.

Hypothyroidism is associated with a fall in pituitary GH mRNA levels which are restored with treatment with thyroxine (7).

Sex steroids are important in supplementing the actions of GH, most noticeable during puberty. Low levels observed in delayed puberty, or absent puberty as in girls with Turners syndrome, result in short stature that responds to replacement sex steroid therapy (136).

3.1. 2 Endocrinology in CRF and renal transplantation

Abnormalities of the endocrine axis are well recognized in CRF. The kidneys are involved in the clearance of some hormones such as GH (137), levels of which are affected by loss of functional renal tissue. Insufficient nutritional calorie intake in CRF has endocrinological implications as well. Renal osteodystrophy arises secondary to low serum calcium, elevated phosphate and low Vitamin D metabolite levels (27). Parathyroid hormone (PTH) can be markedly elevated.

GH levels are either normal or increased, with preservation of amplitudes and frequency of pulses and a rise in basal levels in CRF (138), whilst the transplanted population, in whom steroids are used as an immunosuppressive agent, tends to have low levels of GH (139). Ferraris showed that the area under the curve (AUC) for GH levels in response

to GHRH was smaller in the transplanted population than in the CRF group (140). He also found that the amplitude of the spontaneous overnight pulses were higher in the CRF group (140).

Levels of IGF-I measured using radioimmunoassay have been found to be in the low - normal range (141) whilst IGF-II may be normal or elevated in children with CRF compared to normal controls (64, 142) despite elevated GH, indicating resistance to the actions of GH. Additionally there may be some inhibition of the action of IGFs with loss of the negative feedback loop which normally suppresses GH production. This maybe due to the elevated IGFBPs (142, 143). The most important of these is IGFBP-3 and raised levels are predominantly caused by high levels of the 29 kDa fragment, although some workers have found elevation of fragments of other molecular weights such as 19 and 14 kDa (125). In healthy controls, it appears that the total molar value of IGF-I and -II approximates that of IGFBP-3, as it appears that one mole of each binds in the 150 kDa complex (144), but in CRF this relationship is lost. IGFBP-1 and –2 levels are also raised (143).

Relationships observed in normal prepubertal controls, such as the correlation of IGF-I or IGFBP-3 with Ht SDS, appear to be absent in CRF (143).

A constellation of endocrine abnormalities have been reported in children

with CRF as a result of numerous studies. This thesis reports on a study conducted to elucidate the effect of CRF and steroid therapy on endocrine function, and in particular on IGF bioactivity, in children with normal and abnormal growth. Studies published previously have focused on children with short stature (107,139,140).

The study was limited by the lack of control samples from healthy children with normal growth. Data from children with normal GFR but on low dose, steroid treatment (alternate day predominantly) was used as controls for statistical purposes. Historical data from normal, healthy children was supplied by the laboratory as a reference range for each assay where available . It has also been assumed that children complied with fasting requirements as requested.

3.2 Hypothesis

The hypothesis for the study was that levels of endocrine factors comprising the GH-IGF-IGFBP axis and their importance to growth are altered by deteriorating GFR and steroid usage and this will influence the Ht SDS and Ht Vel SDS of patients with CRF, ESRD and renal transplants.

3.3 Aim

The aim of the research was to study clinical and endocrinological parameters in children with moderate and severe CRF and in those on steroid therapy, with or without growth retardation, to determine the importance of each variable to Ht SDS in all the children and to Ht Vel SDS in prepubertal children.

3.4 Methods

Auxological measurements and blood samples were obtained from each child on the same morning. Samples were collected following an overnight fast, with only water permitted until collection time. These were obtained at, or as close to, 9.00 am whenever possible. Two children were on overnight tube feeds which were discontinued at least 6 h prior to sampling time. Those on peritoneal dialysis (PD) maintained their normal

exchange pattern, and as the dialysate contains glucose, samples from children on PD cannot strictly be considered fasting as glucose absorption occurs. This could potentially affect some results (GH, IGFBP-1, insulin). Children on haemodialysis (HD) had samples collected prior to the dialysis session by the staff on the Dialysis Unit simultaneously with routine clinical samples.

Samples for endocrine investigations were taken to the laboratory and centrifuged for 5 minutes at 3000 revolutions per minute. The supernatant was pipetted into clean tubes and frozen at -70° Centigrade until the time of analysis.

Families were requested to defer the medication of the subjects until after sample collection. Those on steroids were instructed to leave a minimum interval of 24 hours between dosage and sampling, or if on daily steroids, to take the medication as early as possible on the preceding day.

On their arrival at clinic, auxological measurements were undertaken. An upright Harpenden stadiometer (Harpenden, Crymych, UK) was used to measure height, whilst weight was obtained using an electronic scale with a digital display precision of 10 gm (SECA). Both instruments were calibrated prior to use, and all measurements were obtained by the same individual (researcher). Pubertal status was determined according to the Tanner and Whitehouse criteria (145). A retrospective height recorded wherever possible 12 months (minimum 9 months) prior to the current measurement was also obtained from the notes to calculate height velocity.

Height (Ht), and Ht Velocity (Ht Vel) were converted to standard deviation scores (SDS) using the following equation

$$SDS = (Sp - Sm) / SD$$

whereby Sp is the subject's measurement, Sm is the mean of the population , and SD is one standard deviation of the population from which the mean was calculated. SDSs enable ease of comparison for values with a Gaussian distribution that vary with age and gender. Normal data obtained for derivation of new reference curves was used to calculate SDS (10).

The value of Ht Vel SDS in children of pubertal age is controversial, especially in renal patients in whom puberty can be delayed and the growth spurt abnormal (16). For this reason data from children of an age when healthy children were likely to have commenced puberty (females aged > 11 yrs, males > 12.5 yrs) were excluded when statistical analysis was performed to determine if any relationship was present between the clinical and endocrinological variables and Ht Vel SDS.

BMI was calculated from the height and weight of the children, and SDS were derived using age and sex matched reference data (155).

The aetiology underlying the renal disease was obtained from the notes. Further details of this are provided in Chapter 2.

GFR was obtained from the notes for children with CRF and post-renal transplantation. Creatinine levels were also obtained from routine clinical monitoring. Children on dialysis have ESRD and GFR is not monitored in this group as it is minimal, and creatinine levels are extremely elevated. Group 3 children had no renal impairment, thus normal GFR (>80mls/min/1.73m^2) and creatinine levels for their age. Therefore for both Groups 3 and 4, these values were not available.

Due to the nature of the study which involved blood sampling, ethical approval to obtain samples from healthy children was not sought, and hence where possible, historical normal values provided by the laboratory carrying out the analyses were used for comparison.

3.4.1 Sample analysis

GFR was calculated from the clearance of chromium^{51} EDTA as described previously (146).

Creatinine samples were analysed routinely in the hospital laboratory using an Ortho Clinical Diagnostics Vitros analyzer.

Normal values (median and whole range) were supplied by DSL to the laboratory for samples analysed using kits available commercially. Normal values for IGF bioactivity were obtained from Dr Taylor's thesis

(102) and have been published (101). Normal mean values for ALS on age matched children were obtained from published work (147). Historical normal values for age matched children for IGF-I were available in the laboratory from a previous study from which reference curves for the SDS of IGF-I and IGFBP-3 were derived and these were used to calculate SDS (148).

All samples were batched, and analysed simultaneously by an experienced MLSO, J. Jones, using commercially available kits manufactured by Diagnostic Systems Laboratory (DSL-Texas), except for GH , IGF-I , IGF bioactivity , GH hybritech assay and protease.

3.4.1.1 GH radioimmunoassay

GH was measured by radioimmunoassay (RIA) by J.Jones to determine levels in accordance with the technique of the North Thames Regional Unit. Here both sheep and monoclonal mouse anti- GH antihuman antibodies are raised. The mouse antibody is labelled with radioiodine (^{125}I). The assay provides a sensitivity of 0.2mU/l. This assay does not distinguish between the different molecular weights (Isoforms) of GH.

3.4.1.2 IGF-I radioimmunoassay

IGF-I samples were analysed by RIA, using an in house technique. Mouse monoclonal antibodies against highly purified human IGF-I were raised. Label was prepared from radioactive ^{125}I-Iodine. Standard dilutions are carried out to provide a sensitivity of 0.15 to 6 u/ml. Separation of IGF-I from its binding proteins was achieved by acid-ethanol extraction prior to RIA. There is concern about inadequate removal of IGFBPs by acid-ethanol extraction (149), but results comparative to those obtained by
acid chromatography have been obtained using both techniques on the same samples (150). The assay was performed by J. Jones.

3.4.1.3 IGF bioactivity

In this technique, which has been verified previously with serum from acromegalic and pituitary deficient patients, and was modified by Dr Taylor, porcine costal cartilage discs are pre-incubated in Ham's F-12, IGF free, complete culture medium for 24 hours before a 36 hour incubation with serum added for the uptake of ^{35}S-sulphate and 48 hour incubation for ^{3}H-thymidine. The assay was performed by Dr Taylor.

3.4.1.4 IGF-II

Levels were assessed using a two site radioimmunometric assay (IRMA) principle. Separation of the IGF-II from its binding protein is carried out by acid-ethanol extraction, and then it is "sandwiched" between two antibodies, the first of which is immobilized inside a tube wall, and the second is radiolabelled for detection. Four-fold over estimation of IGF-II by

this assay but 1.5 x by acid-chromatography due to "interference" by IGFBPs in uremic serum has been described (149).

3.4.1.5 IGFBP-1 and -3

As the assay to determine levels of both these binding proteins is similar in technique, only one description is provided. Using a two site immunoradiometric technique whereby the first antibody is bound to the inner wall of the tube, and the second is radiolabelled, enabling total levels to be assessed. The state of the IGFBP, whether free or complexed does not interfere with the assay, and there is no cross-reactivity with other BP.

3.4.1.6 IGFBP-2

Serum IGFBP-2 is measured using the same technique as that described for C-peptide and insulin below.

3.4.1.7 ALS

Total serum ALS (complexed and free) is measured by this immunoassay. Incubation of samples and controls in microtitration wells coated with site specific anti-ALS antibody was carried out. After washing the wells were treated with another anti-ALS antibody labelled with the enzyme horseradish peroxidase (HRP), incubated, washed and then incubated with tetramethylbenzidine, acidified, and enzymatic turnover of the substrate is carried out using dual wavelength absorbance measurement at 450 and 620 nm.

3.4.1.8 C-Peptide and insulin

The analysis of c-peptide and insulin is based on the same principle, and depends on competition between radioactive C-peptide/ Insulin (^{125}I- C-peptide/ insulin), and non-radioactive C-peptide/ insulin for a fixed number of antibody binding sites. The amount of antigen (^{125}I- C-

peptide/ insulin) bound to the antibody is inversely proportional to the concentration of the unlabelled antigen. A double antibody system is used to separate free and bound antigen. The sensitivity of this procedure is 0.01ng/ml.

3.4.2 Statistics

As only ranges were supplied by the laboratory for normal historical values comparison with mean values of samples only was possible but not use of statistics to determine significance.

Stepwise multiregression analysis was used to determine, with the assistance of Professor Tim Cole, which of the variables listed below (see results) significantly influenced Ht SDS in all the children and Ht Vel SDS in the prepubertal children. As stated above, data from healthy children was not available for comparison, and therefore the children with a normal GFR were used as the constant group.

$P \leq 0.05$ was set as the significance level.

3.5 Results (Table 3.1, table 3.2, figure 3.2)

The parameters studied for significant influence on Ht SDS were divided into clinical (age, gender, aetiology of renal disease, BMI SDS, dose of prednisolone, duration of dialysis, duration of transplant, GFR) and endocrinological (IGF-I SDS, IGF-I bioactivity, IGF-II, IGFBP-1, IGFBP-2, IGFBP-3 SDS, ALS, insulin, c-peptide, IGF-I moles, IGF-II moles and IGFBP-3 moles). Mean values ± SD for these are shown in table 3.1.

Mean IGF-I SDS were within the normal range (-2 to 2 SDS) for all groups but were lower in the children with decreased renal function, particularly those with CRF and ESRD. These children had higher GH levels than those with normal GFR (It is important to emphasise that GH levels were obtained from single samples which were taken in the fasting state at about 9.00 am). Conversely the mean values of IGFBP-3 SDS for all 4 groups were raised above the upper end of the normal range (> +2 SDS). This elevation was borderline for those with normal GFR, but markedly increased for the other 3 groups with highest values present in the ESRD group.

IGF-II mean values for all 4 groups were increased above the upper end of the normal range supplied by the laboratory. Highest values were

observed for the ESRD group with slightly lower figures for the transplant group and were lowest in the children with normal GFR.

Both IGFBP-1 and IGFBP-2 mean values were elevated in the group with ESRD but not for those children with a normal GFR. IGFBP-1 levels were within the normal range for the CRF and transplanted patients, whilst IGFBP-2 levels were increased for both groups, but not to the extent observed in the ESRD group.

Using multiple regression analysis, variables were independently analysed to determine their relationship with Ht SDS in the three groups - CRF, ESRD and transplant. The significant parameters were then entered into the equation together to obtain the best fit. This is assessed by adjusted R squared (R^2) which is a measure of the percentage variability in Ht SDS and Ht Vel SDS between individuals explained by the significant parameters. Age, treatment modality (CRF/dialysis/ post renal transplant), duration of dialysis, IGF- II, and ALS all had a significant impact on Ht SDS. These parameters accounted for 38.4% (adjusted R^2) of the variation in Ht SDS (table 3.2). The former three variables had a negative relationship with growth, whilst the latter two were positive in their influence. None of the other factors influenced Ht SDS significantly.

IGF-II had the strongest effect on growth ($P < 0.0001$), and ALS was also

significant ($P < 0.02$). ALS and IGF-II together accounted for 30.4% of the variation in Ht SDS. Figure 3.2 shows a scatter plot of the relationship between the residual IGF-II and residual Ht SDS values obtained from the multiregression analysis.

Both dialysis treatment and IGF-I SDS lost their significance when other variables were introduced into the step wise regression. IGF-I SDS was significant ($P < 0.01$) when analysed independently, but became insignificant in the presence of IGF-II, and so was excluded from the final model. Requirement of dialysis treatment had a significant adverse effect on growth when analysed on its own, but this disappeared when duration of dialysis treatment was added to the equation. The latter variable had a negative influence on Ht SDS, consistent with decreasing Ht SDS due to prolonged dialysis treatment.

Aetiology of renal disease, duration of transplant, steroid dose, gender, BMI SDS, GFR, IGF bioactivity, IGFBP-1, IGFBP-2, IGFBP-3 SDS, IGF-I moles, IGF-II moles , IGFBP-3 moles, c-peptide and insulin levels had no significant influence on Ht SDS.

Apart from a positive benefit from transplantation, where the R^2 value was 26% ($P=0.0004$), none of the parameters had a significant influence on Ht Vel SDS.

3.6 Discussion

Growth retardation is a major complication of both chronic illnesses and their treatments (151), and about 30% of the CRF and transplanted patients in the present study had short stature (Ht SDS = -2 SDS). Many factors influence growth, generally by causing an imbalance of the hormones involved in growth, but sometimes by a direct mechanism (152). By studying parameters shown to be particularly significant to growth in other studies, using multiregression analysis an attempt was made to explain the observed variation in Ht SDS in this study between individuals.

Of all the clinical parameters studied for the purpose of this thesis, treatment modality, duration of dialysis treatment and age were the only ones that had a significant effect on Ht SDS and this was negative in all three cases. Most unexpectedly, of the endocrine parameters, IGF-II had the greatest positive influence on Ht SDS and by comparison the influence of IGF-I SDS became insignificant. Surprisingly, IGF bioactivity and the IGFBPs had no effect on growth. Only one factor (transplantation) had a relationship with Ht Vel SDS that was significant .

Although BMI is conventionally used to determine " fatness (154), a value under the 4^{th} percentile is reflective of undernutrition (155) whilst a

normal BMI in chronically ill patients does not exclude disproportionate fat mass to fat free mass ratio compared with healthy controls (156). In this study, BMI was used to exclude malnutrition as a significant problem in the patients. Only three children (1 CRF, 1 dialysis and 1 normal GFR) had a BMI SDS below -2 SDS and of these two (1 CRF, 1 dialysis) had short stature (see appendix 2.1- 2.4). Two of the dialysis patients received nasogastric tube feeds. BMI SDS did not have any significant effect on the growth of the children. Normal growth requires the provision of an adequate quantity of nutrients, including vitamins, minerals, protein and calories so that tissue synthesis prevails over degradation. Malnutrition can occur in CRF, and especially in ESRD (153, 36), but with regular assessments by an experienced renal dietician and using nasogastric tube feeds if necessary, severe malnutrition can be prevented. Despite these measures growth retardation can still prevail in some children (27).

Growth suppression within the first two years of a child's life with normalisation of the growth rate subsequently will result in short stature due to inadequate catch-up growth to compensate for the loss in height at a time of rapid growth (159). This has been noted most commonly in children with congenital renal disease (eg renal dysplasiawith or without vesicoureteric reflux or obstruction). Such infants may be small even at birth with a weight/height 1 SDS below the mean (159). In the present study, aetiology of renal disease had no significant effect on Ht SDS.

This can probably be attributed to the careful attention paid to the nutritional status of these children in infancy, thus minimising height loss in this period as demonstrated by other studies from this centre (27) and other centres (28, 30).

Increasing age had a significant negative influence on Ht SDS, indicating that children with renal disease may have growth stunting which worsens with time compared to their age and gender matched healthy contemporaries. This can be explained by the progressive deterioration of GFR with time, which can lead to worsening of endocrine (143) and nutritional status (51) contributing to a slowing in the rate of growth which eventually manifests as short stature. Furthermore, patients with CRF and post transplant can have abnormalities of pubertal development and this can be associated with an inadequate growth spurt, resulting in further decrease of Ht SDS with age (16). In the study population here however, puberty had no observed impact on Ht SDS. Twenty one percent of all the children in the study had pubertal delay, evident as either inappropriately prepubertal, or commencement of pubertal development but below the 3rd centile for chronological age. The majority of the children were appropriately prepubertal or in early puberty (pubertal stage < 3) before the pubertal growth spurt is observed in healthy children. In these patients abnormalities of the growth spurt would not be present to contribute to the observed negative relationship between age and Ht SDS.

The renal modality (CRF/ transplant/ ESRD) of the patients influenced their Ht SDS (table 3.2). The presence of ESRD was significantly more detrimental to the growth of children than either CRF or renal transplantation. However this relationship altered and was no longer significant when duration of dialysis of dialysis was taken into consideration. Duration of dialysis had a negative influence on Ht SDS. ESRD is associated with poor growth (150) but some initial improvement maybe noticed after commencing dialysis. However with prolonged dialysis, there is an increased level of morbidity and mortality (36,37), and in children growth is affected adversely (56). Children requiring dialysis treatment respond less well to rhGH (160, 162). In the present patient group, increased duration of dialysis had a significant negative effect on Ht SDS. Of the dialysis patients (appendix 2.3), 5 children had normal height, 4 of whom had an adequate rate of growth. These children had developed CRF later in childhood and had been on dialysis for a shorter period (not statistically proven) than those with Ht SDS <-2 SDS. There were insufficient patients to compare the influence of haemodialysis to that of peritoneal dialysis.

Renal transplantation, of all the parameters measured, had the most negative relationship with Ht SDS with the lowest t - ratio in the best fit equation. This group of children also had the lowest mean Ht SDS. However, interestingly, transplantation was the only parameter that had

any significant (positive) effect on Ht Vel SDS (Data of children who were of an age when the growth spurt may be observed, as explained in the methods section above, were omitted from the equation to reduce the confounding influence of puberty). Multiple factors can contribute to growth retardation pre and post-transplantation, such as the original disease, dialysis, episodes of graft rejection with deteriorating graft function, steroid therapy and retransplantation (3 patients in the present study). (29,32,33). However, in this study receiving a transplant improved the children's rate of growth. This finding has been observed by other workers as well (29) and is attributed to the improved renal function.

In the present study, dose of steroids had no effect on Ht SDS, as this variable just missed being significant (P=0.06). This maybe due to the majority of children receiving low dose, alternate day steroids. Despite this, however the relationship of transplantation with Ht SDS showed a deterioration when steroid therapy was added to the equation, demonstrating an adverse influence of treatment and not dose in the present study. Thus it appears that whilst the children with a transplant had the lowest Ht SDS, growth rate improved with renal function after transplantation. Although improvements in height velocity can be observed, these may be insufficient to attain catch-up growth. It is open to speculation as to whether the unfavourable effect of steroids manifested as some suppression of the height velocity which otherwise

would have been greater, or a slowing in growth rate with prolonged use of steroids. Other studies have also demonstrated a detrimental effect of steroids on growth (24, 156). Steroids can have a direct effect on the growth plate but also modulate the bioactivity of factors involved in growth such as IGF-I (97, 152). Glucocorticoids reduce GH secretion (97), increase protein catabolism (157) and act on the chondrocytes to inhibit mitosis (152). Treatment also lowers IGF bioactivity (97) and has an effect on paracrine activity of IGF-I (152) and observations from the present study showed that mean values of patients' samples were lower than historical values from normal children.

In this study, although dialysis did have an adverse effect on growth, GFR had no direct effect on Ht SDS. The GFR of the CRF patients was lower than that of the transplanted group, but those from the latter group had a diverse range, confirmed by the large standard deviation. The mechanism by which a progressively deteriorating GFR influences growth adversely as shown in other studies involves decreased efficacy of hormones such as GH by a combination of insensitivity at receptor level (138) and accumulation of factors that are inhibitory to its mediators (142). Other factors such as nutritional state also become important . In uraemia, resistance to the actions of GH has been demonstrated, with reductions in tissue expression of IGF-I mRNA (162) and IGF-I secretion demonstrated using a mathematical model in the presence of low IGF-I levels(144). This may be due to down regulation of GH receptors in the

presence of elevated GH levels as demonstrated by reduced GH receptor mRNA expression in rat hepatic tissue (163) and decreased GH binding protein which is homologous to the extracellular domain of the GH receptor (83).

IGF-I is recognised as the most important growth factor postnatally in the normal population (108). However in this study in children with renal impairment, the significance of IGF-I SDS disappeared in the presence of IGF-II in the multiregression analysis. The probable explanation for this surprising finding is IGF-I levels that would be considered comparatively low in a population in whom other endocrine factors (GH, IGF-II and IGFBP levels) are elevated (mean IGF-I SDS <1. 0). Considerable variation in individual values of IGF-I within groups, as demonstrated by the magnitude of the standard deviation of the mean, can be observed. As discussed above, steroid treatment and uraemia have a negative effect on GH levels and efficacy and lead to lower IGF-I levels. Low – normal IGF-I values have been described in other studies (144, 150).

Free IGF is bioactive (102, 165) but no relationship was observed in the current study between Ht SDS and IGF bioactivity. Other studies have also been unable to find such a relationship. There was noticeable variation in bioactivity between individuals within the 3 different groups. Free IGF levels are dependent on IGF and IGFBP levels. A direct correlation between IGF bioactivity and GFR has been demonstrated and

this is retained even when the GFR drops to 30 mls/min/1.73m^2 (166). The assay used to determine bioactivity is an in vitro process performed using cartilage discs and is a very simplistic model for the complex process of growth in vivo and may also account for the lack of a relationship between growth and bioactivity.

IGF-II, which has a recognised role in early antenatal growth (108) interestingly had the strongest positive relationship with Ht SDS of all the parameters studied. The exact significance of IGF-II in postnatal growth remains to be clarified but some evidence suggests that it has quite a prominent role. It has been found to be involved in the proliferation of human colonic carcinoma cells and this action can be blocked by IGFBP-2 (124). Transgenic mice over expressing IGFBP-2 have ~ 13% reduction in carcass weight (122). Furthermore levels of IGF-II rise in response to recombinant GH (rhGH) therapy (139) and are low in GH deficient children (105). In the work carried out for this thesis, levels of IGF-II in the children were elevated above the normal range and were highest levels in the dialysis patients. Findings from other studies have also demonstrated that levels are raised in CRF (64, 144) due to accumulation secondary to the decreased clearance of IGFBPs (144,150). IGF-II had a very significant positive effect on Ht SDS in the population studied here, and part of this maybe due to its ability to stimulate IGF-I receptors particularly if free IGF-I levels are low. The actions of the growth factors are complicated by their interactions with

each other and the IGFBPs. In transgenic mice, high levels of IGF-II resulted in low levels of IGF-I, most probably due to displacement with competition for limited IGFBPs (121).

ALS appears to have a role in growth promotion by increasing the half-life of IGFs further by stabilising the binary complex formed between IGF and IGFBP-3. This may explain the observed effect of ALS on the Ht SDS of the children in the present study. Levels in all patients were within published age matched ranges produced using the same assay (147) but mean values were lowest in the ESRD group who would have high resistance to the actions of GH due to severe uraemia. The CRF group who had moderate uraemia had higher mean levels than the transplant group who had both uraemia and received steroid treatment. It is interesting to speculate that this may be a sensitive reflection of the efficacy of GH in conditions where there is resistance to its actions, particularly as ALS levels do not accumulate in CRF. In knockout models of mice, ALS deficiency is associated with slower postnatal growth (~10% by 21 days of age) and decreased levels of IGF-I (67%) and IGFBP-3 as the mice age (172). Powell studied the effects of rhGH in children with CRF, and noted relationships between improvements in Ht SDS and increases in ALS levels (64).

None of the binding proteins had any significant relationship with Ht SDS. Powell noted an inverse correlation between IGFBP-2 and Ht SDS

in his study (107). However his study was performed only in children with CRF, and did not include ESRD or those with a renal transplant. His patients had IGFBP-2 levels that were marginally elevated (1.2 ± 1.2 SDS) whilst in the present study mean levels were 2-3x higher than the upper end of the normal range in the CRF and ESRD patients. It thus maybe that excessive increases in levels of the IGFBPs distorts relationships, particularly when severe reductions in GFR are observed as in ESRD. Whilst no studies appear to have been published studying the effects of steroids on IGFBP-2, synthesis of IGFBP-1 can be stimulated by cortisol. Levels of IGFBP-1 were within the normal range for both the CRF and transplanted children but elevated for the ESRD group. Evidence of IGFBPs' inhibition of IGF bioactivity was provided by Blum when serum from patients with ESRD was passed through an IGF-II affinity column, thereby removing excess IGFBP, leading to an increase in bioactivity (165). In CRF, IGFBPs are elevated (142), due in part to decreased renal clearance as urine from normal individuals contains low molecular weight BPs (< 60 kDa) (165), but there is also evidence of increased expression of IGFBP-1 and -2 mRNA in CRF (162). Studies with transgenic rabbits indicate that IGFBP-2 also inhibits the actions of IGF-I (169).

IGFBP-3 can have both growth promoting (126) and inhibiting effects (127). As the most important binding protein, it needs to considered separately. IGFBP-3 SDS was elevated in all 3 groups. IGFBP-3 levels

were determined here using IRMA that does not distinguish between the intact molecule or the smaller fragments accumulating in CRF. If levels of the intact molecule had been determined, and used in the multiregression analysis, a relationship with Ht SDS may have been present. The molar sum of IGF-I and –II exceeded that of IGFBP-3 in the current study, contrary to findings in healthy individuals (144). The molar ratio may also reflect the increased relevance of other IGFBPs (IGFBP-1,-2,-4) than IGFBP-3 as IGF binders in CRF (167).

Insulin is an important anabolic hormone and highly relevant to growth and therefore needs to be discussed here. Knockout mice models lacking the insulin receptor (IR) show growth retardation that is mild (134), unlike humans where it is significant (173). In CRF, abnormalities of carbohydrate metabolism have been noted (174), and use of steroids following transplantation leads to elevated insulin levels due to resistance to its actions (175) and this may explain the observed lack of effect on Ht SDS. Post receptor abnormalities, decreased insulin receptors and increased hepatic gluconeogensis also contribute to the insulin resistance observed in CRF (174). No relationship of c-peptide with Ht SDS or Ht Vel SDS was present. C-peptide has a longer half life than insulin in normal individuals and therefore provides information about insulin activity. Levels of both c-peptide and insulin in the children studied here were comparable to the normal ranges supplied.

This study could have, potentially, been improved by addressing shortfalls that became apparent retrospectively. The major limiting factor of this study is the absence of results from healthy children to be used as controls. To compensate for this, the group with normal GFR served as the constant in the multiregression analysis. Conversion of IGF-II, IGFBP-1 and -2 to SDS would have enabled comparison with historical studies performed in similar children to determine if levels are raised to the same magnitude in similar populations studied by different workers. Categorisation of GFR into normal, mild, moderate, and severe may have shown a relationship with growth.

In conclusion it is apparent that growth is a complex process involving the intricate balance of multiple factors and the presence of renal impairment adds to the conundrum. The aetiology underlying the CRF had no relationship with Ht SDS in the present study. However the renal modality of the children had an impact on their Ht SDS and this was initially most marked in the case of the dialysis patients. Interestingly however, after adjusting for duration of dialysis, transplantation had the most negative relationship with Ht SDS which deteriorated further after steroid treatment was entered into the best fit equation. Age also showed an inverse relationship with Ht SDS perhaps as a manifestation of progressive deterioration of GFR adversely affecting growth due to worsening of endocrine abnormalities. Of the endocrine parameters, IGF-II had the strongest positive influence on Ht SDS and this may be partly

due to a direct effect, but also its ability to stimulate IGF-I receptors. Mean levels were elevated in the three groups of patients and highest in the ESRD group. In contrast, IGF-I (SDS) mean levels would be considered lowish for this population although normal for healthy children. ALS also was significant to growth. IGF-II and ALS levels, duration of dialysis, age and renal modality explained 38.4% of the variability in Ht SDS, with approximately 61% unaccounted for, and therefore there are other factors not considered here that contribute to the growth process.

Table 3. 1 Mean ± SD of clinical and endocrinological variables

Parameters	normal levels (mean; range)	CRF (mean±SD)	Tx (mean±SD)	ESRD (mean±SD)	NGFR (mean±SD)
Age (years)	na	11.7 ± 3.6	12.3 ± 2.8	10.7 ± 4.5	11.4 ± 1.7
Puberty	na	2 ± 1	2 ± 1	1 ± 1	2 ± 1
BMI SDS	na	0.0 ± 1.2	0.9 ± 1.2	0.1 ± 1.4	0.4 ± 1.8
GFR (mls/min/1.73m2)	na	22.5 ± 7.4	43.8 ± 22.4	na	na
Ht SDS	na	-1.4 ± 1.5	-1.6 ± 1.2	-1.8 ± 1.2	-0.2 ± 1.3
Ht Vel SDS	na	-0.4 ± 3.4	0.6 ± 3.7	-0.3 ± 2.4	-0.9 ± 2.2
Transplant duration (months)	na	na	42.6 ± 26.7	na	na
Dialysis duration (months)	na	na	na	29.3 ± 22.1	na
steroid dose(mg/kg)	na	na	0.3 ± 0.2	na	0.4 ± 0.2
GH RIA (mU/l)	2.2 (0.5 - 5.4)	13.7 ± 17.8	11.8 ± 16.2	8.8 ± 4.7	6.1 ± 5.4
IGF-I SDS	-2 to 2	0.55±1.48	0.91±1.12	0.79±0.77	1.48±1.26
IGFBP-3 SDS	-2 to 2	3.19±1.09	3.39±0.70	3.99±0.78	2.53±0.62
IGF Bioactivity (u/ml)	0.9 (0.5 - 1.2)	0.41±0.40	0.73±0.56	0.35±0.21	0.80±0.43
IGF-II (ng/ml)	869 (752 - 972)	1189±361	1358±281	1555±243	1036±245
IGFBP-1 (ng/ml)	59 (12 - 27)	96.2±40.7	84.1±36.7	127.1±36.5	33.5±16.9
IGFBP-2 (ng/ml)	410 (100 - 675)	1169±447	863±452	2085±881	409±121
CPEP (pmol/l)	497 (265 - 1324)	323.6±357.1	625.7±1441.0	565.1±762.5	414.9±551.7
Insulin (mU/l)	15 (3 - 35)	14.1±5.6	12.3±7.4	14.5±10.7	17.5±14.8
ALS (mcg/ml)	na	38.9±23.6	32.3±6.2	28.4±4.7	47.7±17.01
IGF-I mol	na	114±72	112±59	54±16	59±24
IGF-II mol	na	154±47	177±37	202±32	135±32
IGFBP-3 mol	na	168±44	178±29	204±32	143±27

Table 3.2 Best fit multi-regression model for Ht SDS

R_2 = 38.4% (Dependent variable Ht SDS)

Variable	Coefficient	s.e	t-ratio	probability
constant	-0.50	0.84	-0.63	0.50
transplant	-1.91	0.49	-3.92	<0.01
CRF	-2.19	0.62	-3.52	<0.01
dialysis	-1.57	0.92	-1.68	0.10
female	-0.03	0.29	-0.10	0.90
age	-0.12	0.04	-2.83	0.01
IGF-II	<0.01	<0.01	3.63	<0.01
ALS	0.02	0.01	2.47	0.02
Steroid dose	-2.08	1.09	-1.90	0.06
dialysis duration	-0.04	0.02	-2.76	0.01

Figure 3.1 Factors influencing levels of Growth Hormone

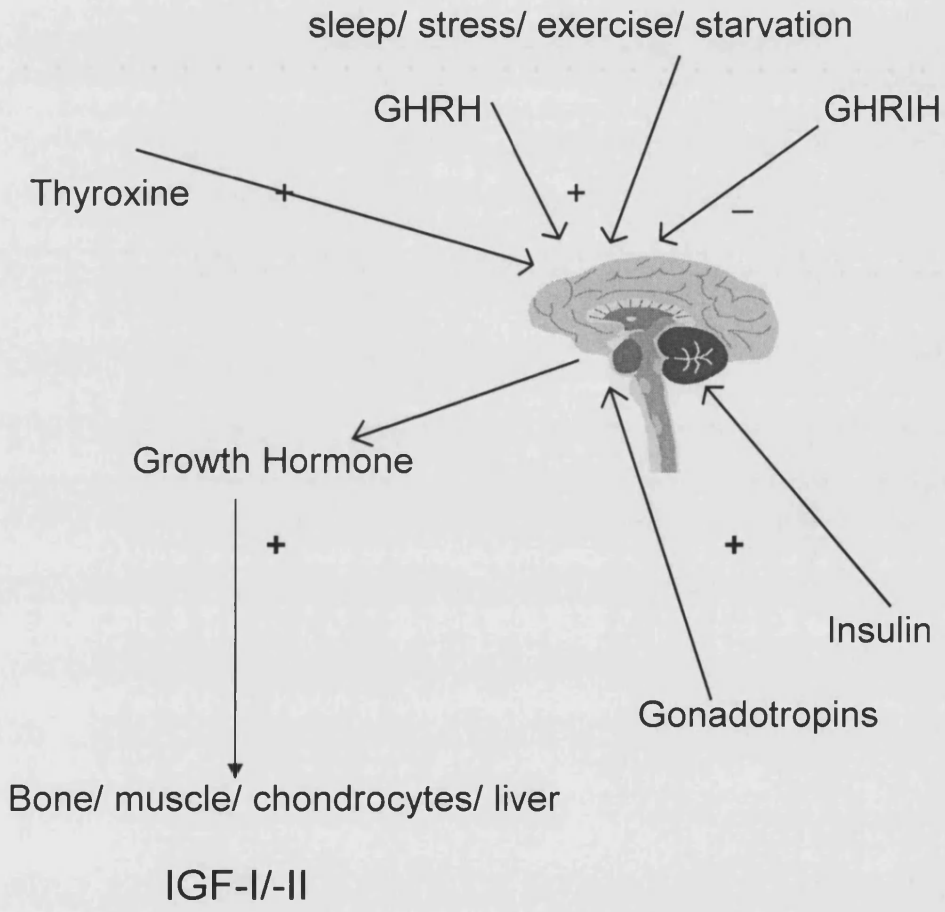
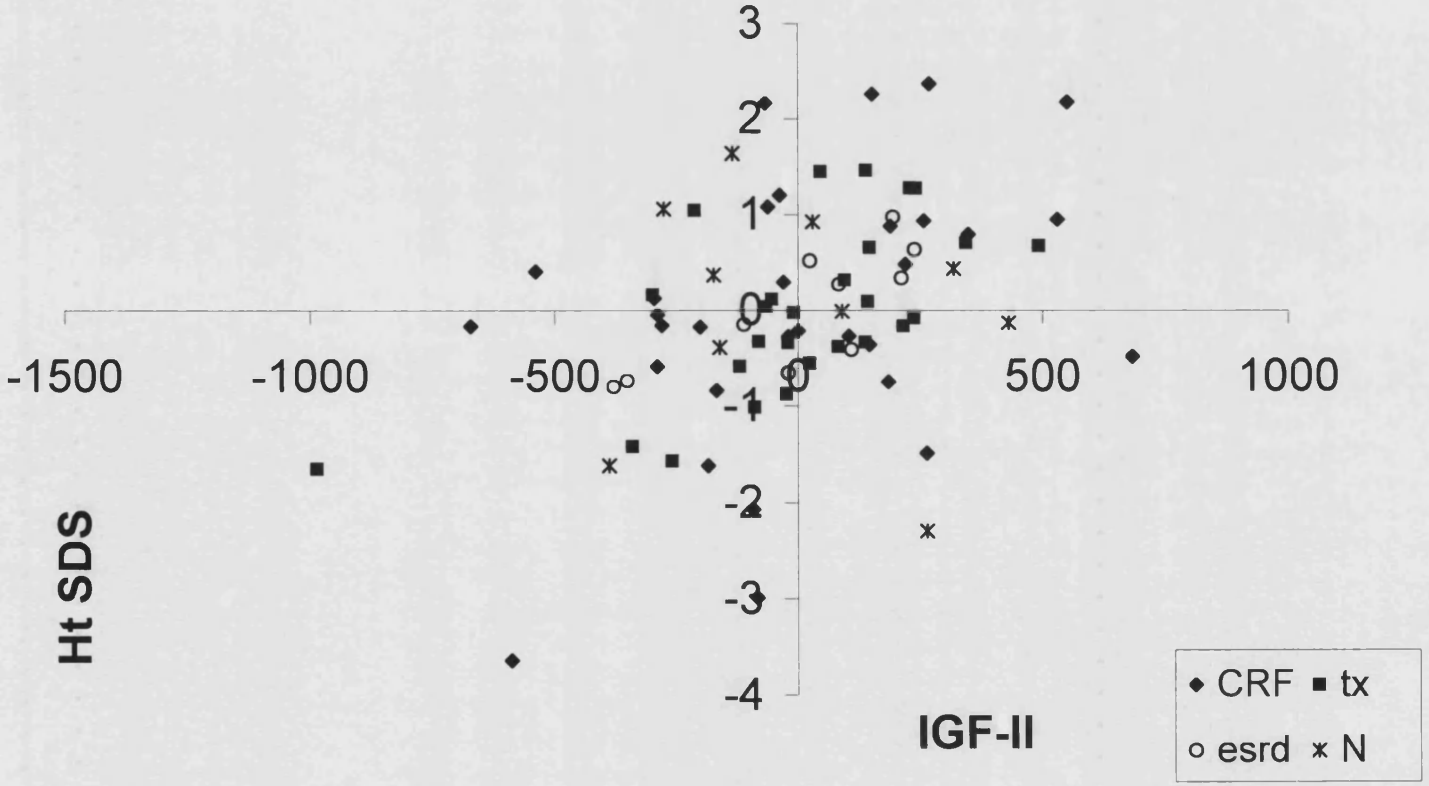


Figure 3.2: Scatterplot of residual of IGF-II and residual of Ht SDS in multiregression analysis



Chapter 4

Effects of recombinant human Growth Hormone on protein turnover, growth and endocrine factors in children with CRF and renal transplants

Protein turnover and growth with rhGH

4.1 Introduction

4.1.1 Protein kinetics

Children with chronic renal failure (CRF) easily become malnourished if inadequate attention is paid to their caloric intake (40). Even in the absence of visible malnutrition their lean body mass tends to be reduced (176). As the renal failure worsens dietary assessment and supplementation may be required. However, protein restriction (which may be necessary in uraemia) decreases lean body mass further (177), whilst excessive dietary protein intake results in decreased flux and synthesis (178).

Body protein is dynamic and is continuously being degraded to, and synthesised from, amino acids with the balance of the two processes determining whether there is net protein deposition (179). Protein kinetics, with or without recombinant human Growth Hormone (rhGH), have been studied in adult patients with CRF and in healthy volunteers (180, 181, 182). In CRF findings have been reported of an increase in the rate of catabolism and oxidation, particularly in the presence of acidosis (183); an increase in catabolism and synthesis with no change in net balance (180); or decreased rate of protein catabolism and

synthesis with a lower synthesis to flux ratio (178) compared to controls. Other studies have examined the effects of restricted nutrition (normal control rats pair-fed to match caloric intake of uremic animals) (184) and acidosis in the absence of CRF. Studies have also been conducted to observe the influence of glucocorticoids on protein turnover in animals (185) or the effects of differing quantities of dietary protein intake on protein turnover (178). Fewer studies have been conducted in children and these have shown that healthy children have a higher protein turnover than adults that decreases with age (186, 187,188, 189). In severe CRF in adults protein turnover is reduced, with some improvement noted once dialysis commences without normal levels being attained (183, 187).

RhGH increases fat free mass (FFM), with a reduction in adipose tissue in growth hormone (GH) deficient adults (190). In adults with CRF (191) findings are similar to observations in healthy adults (157) and consist of a reduction in oxidation of amino acids with increased synthesis and unaltered proteolysis with the use of rhGH. However Garibotto's study measured kinetics in forearm muscle only (180,191).

Factors that influence protein synthesis and oxidation are uraemia (184, 192), acidosis (185), quantity of daily dietary protein intake (178, 187) and corticosteroid therapy (157). These may have a direct influence on amino acid incorporation into protein, but the effect may also be

mediated via hormones, the response to which may be altered (180, 191). Metabolism of branched chain amino acids and their keto acids is abnormal in CRF compared with controls (194).

4.1.2 Endocrine factors involved in protein turnover and growth (as discussed in chapter 3)

4.1.3 Use of rhGH in CRF

Trials of GH therapy in children with CRF were not carried out until 1983 when Mehls and Ritz (195) demonstrated that supraphysiological doses of GH increased growth velocity in 5/6 nephrectomized rats, particularly if they were fed ad libitum (196). Pilot studies over one year confirmed that recombinant GH (rhGH) led to increased height velocity in children with CRF (197), or post renal transplantation (198). These findings were confirmed by a randomised, placebo-controlled crossover study (199) and a two year multicentre trial (200).

Fine, in a prospective study, showed that improved growth due to rhGH was sustained over five years compared with the pre-treatment period, although the growth velocity decreased in each subsequent year of treatment (201). Once children had attained their target height, rhGH

therapy was interrupted. Some children maintained their growth velocity, whilst in others it dropped to pre-treatment levels, necessitating recommencement of rhGH.

4.1.4 The use of stable isotopes

Stable isotopes, by incorporation into organic compounds such as amino acids, have been used in metabolic and nutritional studies since the 1950s. They are naturally occurring variants of isotopes such as carbon (^{12}C, stable isotope ^{13}C) and hydrogen (H, stable isotope ^{2}H) that are present in the environment in very small concentrations. They can therefore be used as "labels" and traced in metabolic reactions. No radioactivity is present, thus they overcome the potential dangers posed by radioactive tracers used previously. Use of stable isotopes has also been enhanced by the development of better mass spectrometers that can analyse very small samples (202).

Whole body protein is constantly being synthesised and degraded, and the process accounts for 15-20% of the resting metabolic rate (179). Many amino acids can be synthesised in man, but some termed "essential" cannot, and therefore need to be ingested in the diet. One amino acid pool is located intracellularly, and amino acid entry into the pool occurs either from protein catabolism, active transport from plasma, synthesis de novo or conversion from ketoacids (figure 4.1). Amino acid

removal from the pool occurs by either synthesis of protein, oxidation or release into the circulation. The first step in oxidation is loss of α-nitrogen by transamination (except lysine) or oxidative deamination to the ketoacid of the amino acid, and in some cases this is a freely reversible reaction. The tissue in which metabolism of different amino acids occurs varies. Leucine can be metabolised in skeletal muscle and liver, whilst phenylalanine can only be metabolised in hepatic tissue and glutamine is metabolised by gut and kidney (202).

Validation of protein turnover studies has been performed by using stable isotope infusions, and comparing protein kinetic results with muscle biopsies (203), or using animals which have then been sacrificed and their muscle homogenized (204). Using two different amino acids simultaneously with separate labels also provides further information about the accuracy of the technique (205).

Studies of protein kinetics using continuous infusions are based on the assumption that there is a single pool involving the labelled amino acid and that the concentration of this amino acid in the pool does not change in the steady state and thus is representative of its turnover. Therefore the rate of entry of the amino acid into the pool must equal its rate of removal from the pool. Entry into the pool (rate of appearance) occurs from either diet, or protein catabolism, whilst removal (rate of disappearance) is by either irreversible oxidation or protein synthesis

(206). The rate of appearance of the amino acid can be restricted to that originating from catabolism only by conducting studies with the patient fasting or postabsorptive, eliminating the ingested component. By using infusions of labelled amino acids, the proportion of the labelled amino acid to unlabelled amino acid remains constant, as the metabolic processes do not distinguish between the two (202). The enrichment, or percentage dilution of the labelled to unlabelled amino acid, is obtained from samples in the steady state and using this calculations of turnover can be made. Using the enrichment of CO_2 in breath samples, the rate of oxidation of the amino acid can be calculated and hence rate of non-oxidative disposal (synthesis) of the amino acid can be determined.

4.1.5 Leucine kinetics (Figure 4.2)

Leucine is frequently used as the amino acid to carry the stable isotope ^{13}C as it an essential amino acid. Its removal from plasma into the intracellular compartment is by an energy dependent process. From here removal is either by incorporation into protein synthesis, or oxidation which will lead to carbon dioxide (CO_2) production. The advantages of using leucine are that it reaches steady state, whereby the rate of entry of leucine into the plasma pool is equal to its rate of removal from the pool, within 1.5 hours of commencing an infusion, thereby shortening the duration of studies. 1-^{13}C-Leucine is easy to infuse intravenously, and can provide information on whole body protein kinetics. It is metabolised

peripherally in skeletal muscle, the body's principal protein reservoir, and leucine appears to have a regulatory role on muscle protein synthesis and degradation (207, 208). Intracellularly leucine equilibrates rapidly with its metabolite ketoisocaproic acid (KIC), and is one of three amino acids that will do this. The short study period minimises the likelihood of recycling of the isotope, which can complicate studies, and urinary losses. Infusing the label avoids the complication of faecal losses. The disadvantage of using leucine is that it is oxidised to release CO_2, thereby necessitating collection of breath samples and calculation of CO_2 volume production using indirect calorimetry.

Oxidation of amino acids like leucine results in CO_2 production, but not all the CO_2 produced is released as breath due to CO_2 fixation and entry into other pools with a slower turnover rate, such as bone (209). In order to determine the percentage of labelled leucine oxidised, the percentage of labelled CO_2 recovered in breath must be determined. Therefore studies using labelled sodium bicarbonate ($H^{13}CO_3^-$) have been performed (210). $H^{13}CO_3^-$ is infused at a constant rate until steady state is reached. Breath samples are then obtained to determine the enrichment of CO_2, and by measuring the volume of CO_2 produced per minute, the percentage of infused $H^{13}CO_3^-$ recovered can be calculated. In short term studies (<12h), in healthy adults about 81% of the label is recovered (210). This figure is then used in leucine oxidation calculations as a corrective factor. Studies of fractional recovery or percentage of ^{13}C recovered in children

are limited, and figures ranging from 63-94 % have been obtained depending on method of administration of label (209). Recovery in children with renal impairment has not been determined, and in these studies the conventionally accepted figure of 0.81 has been used (211), although studies involving other paediatric patients have used their own calculated corrective factor (212).

It is now considered more appropriate to use the enrichment of KIC in calculations of protein turnover (213). Leucine rapidly equilibrates with KIC intracellularly, as the transamination step is reversible. KIC thus provides more accurate values for the intracellular enrichment of leucine at the site of protein synthesis, degradation and leucine oxidation. Intracellular KIC concentrations in turn equilibrate rapidly with the plasma pool of KIC.

4.1.6 Effect of feeding on protein kinetics

Most studies are conducted in the postabsorptive state, as intake does not have to be accounted for. This simplifies the model for protein turnover and calculations. Endogenous substrates are used as fuel sources to provide energy when feeding does not take place and the respiratory quotient provides an indication of the substrate (fat, carbohydrate, protein) predominantly being metabolised (214).

Feeding increases rates of whole body and skeletal muscle protein turnover, with all parameters (except whole body protein breakdown) significantly higher in fed individuals compared to fasted. Although leucine oxidation is increased, the net protein balance is higher in fed individuals (203).

4.1.7 Limitations of stable isotope studies

In stable isotope work, certain assumptions are made:

i) The stable isotope is not differentiated from the usual isotope by the metabolic processes in the body.

ii) Perfect equilibration occurs of intra and extracellular KIC, and between leucine and KIC intracellularly.

iii) Assumptions regarding intracellular events involving leucine and KIC are accurate, and leucine is not metabolised directly without being equilibrated.

iv) Samples obtained accurately reflect intracellular protein kinetics and have not altered between point of release into the circulation and sampling.

v) No alteration of isotope enrichment in the infusion syringe occurs or sampling tubes occurs.

vi) Minute CO_2 production rate measured during indirect calorimetry is representative of that throughout the study.

vii) Recycling of label during the study does not occur.

viii) Protein turnover rates are constant throughout the day with no circadian rhythm in the postaborptive state.

ix) In disease, kinetics of the amino acid still follow those observed in health.

x) The fuel being metabolised during the study does not alter to any extent, as fat has a lower natural enrichment than carbohydrate.

4.1.8 Protein turnover in children with CRF

Studies of protein turnover in children with CRF are limited, with only two previous studies identified (187, 211). Both studies included patients with CRF/ ESRD but not those with a renal transplant. In the former study the amino acid used to carry the stable isotope was lysine, which is metabolised in the liver and not skeletal muscle. The ages of the patients ranged from 4.1 to 18.8 years. The samples were obtained when a steady state had been reached, postabsorptively. Flux was the only parameter assessed, and was compared with historical normal values obtained from literature, and found to be substantially lower, with no significant improvement following a dialysis session. No information on the rate of protein synthesis or oxidation was provided.

The second study compared protein kinetics in children with CRF, who were and were not acidotic, and noted that acidosis leads to increased catabolism and amino acid oxidation (211).

In this thesis studies of protein turnover in children with renal transplants with comparison to findings in those with CRF has been undertaken before and after use of rhGH, and do not appear to have been conducted previously. In adults with CRF, improvements in protein synthesis have been noted with use of rhGH (191), but there is no information on the effects of rhGH on protein turnover in children with CRF and renal transplants.

Performing studies in children is dependent on the age and ability of the child to understand and comply with requirements. In the protein kinetic studies it had to be assumed that all children fasted as requested. The children had to remain on bed rest for the duration of each study but most did not lie still and some times had difficulty staying awake. The protocol entailed the use of rhGH, and patient compliance with daily injections had to be assumed, although this can be verified partially by the results obtained. Physiological differences in follow up studies could not be taken into account, nor the changes in stages of growth which maybe very relevant when some patients are of pubertal age. Equipment was calibrated prior to use, but does not fully exclude artefacts, especially during indirect calorimetry.

4.2 Hypothesis

Use of rhGH in children with CRF and renal transplants will improve growth, lean body mass, reduce adipose tissue and increase protein turnover that maybe reduced compared to healthy individuals.

4.3 Aim

The aim of this study was to determine protein turnover at baseline in children with CRF and those who had had a renal transplant, and study how it altered, along with growth and the GH-IGF axis, with use of rhGH therapy.

4.4 Method

Protein turnover was measured using isotopicaly labelled leucine. However in the first part of the study the fractional recovery rate (FRR) of ^{13}C in carbon dioxide (CO_2) in children with CRF was determined, as a prelude to protein turnover studies. Protein turnover was then measured and the effect of rhGH studied. RhGH was prescribed at 4 iu m^{-2} per day. Synchronous blood samples were also obtained for endocrine analysis.

Patient details are as described in chapter 2, and appendix 2.5 (Table 4.1).

None of the patients were on a protein restricted diet, and their intake was greater than 3 g/kg/day. They underwent regular assessment by a dietician to ensure that their caloric intake was adequate.

Each study was conducted in the fasting state (within 12-18 hours of last meal consumption), with no intake permitted from midnight on the eve of the study, except for water. Patients were instructed not to take any medications until the study had been completed. Children on steroid treatment received alternate day prednisolone and therefore took their medication at least 24 hrs prior to the commencement of the study.

Height was measured using an upright Harpenden stadiometer (Harpenden Ltd, Crymych, UK), whilst weighing was carried out using a digital display scale (SECA, UK). Anthropometry was performed using Holtain calipers (Holtain, UK) to obtain subscapular and triceps skinfold thickness, with measurements checked twice for accuracy, and averaged. Mid-arm circumference (mid-point of shoulder tip and olecrenon) was obtained using a non-distendable tape measure. The left arm was used on all occasions in each individual, with the arm relaxed and hanging down. After their height and weight had been measured, bed rest was commenced.

Height (Ht) standard deviation scores (SDS) were calculated using reference values obtained from normal, age matched children (10).

1-^{13}C-Leucine was the tracer used for the reasons discussed above. It has been used extensively in studies involving adult subjects (healthy, with renal failure or endocrine abnormalities) (180, 203,205, 207) and children (188, 212).

4.4.1 Fractional recovery of ^{13}C-bicarbonate in children with chronic renal disease

Baseline breath samples were obtained, before a priming bolus of 1-^{13}C bicarbonate was administered at 2mg/kg via an indwelling intravenous catheter, immediately after which an infusion was commenced to deliver 2mg/kg/hour of the isotope. The infusion was prepared on the study day using the weight obtained. Stable isotope was obtained in powder form from the Isotope Labs, Cambridge, Andover, USA and converted into sterile solution with a 99% enrichment (confirmed by Gas Chromatography Mass Spectrometry) of 1-^{13}C Bicarbonate by the Stable Isotope Services of Northwick Park Hospital Pharmacy, London. Further

sampling then occurred at 1.0, 2.0, 2.5, 2.75, 3.0, 3.25, 3.5 hours into the study (Table 4.2).

Breath samples were collected by exhalation into a modified one way PVC bag with one inlet and one outlet valve from which 20 ml samples were aspirated and injected into vacutainer tubes for storage until analysis. Both the bag and the syringe were "washed out" twice, immediately before each sample collection, by exhalation into the bag by the subject with disposal of the expired air.

Indirect calorimetry was performed with the Deltatrac II machine (Deltatrac II, Datex, Finland) in an open circuit mode 1-1½ after isotope infusion was commenced. The machine was calibrated on each occasion prior to usage after a "warm up" period of half an hour, with O_2 and CO_2 cylinders (Vickers, UK). The transparent hood and canopy were placed over the child's head and shoulders and secured to permit no leakage. One end of the canopy was attached to the Deltatrac via a plastic elephant tube, whilst the other end was attached to a free ending tube through which air entered the hood and was drawn from here into the machine. Minute readings commenced from when a steady state (approximately after 5 minutes) with less than 10% fluctuation in consecutive readings showed on the monitor. Readings were obtained for a minimum of twenty minutes, ideally 40 min or longer if tolerated without the child becoming restless or falling asleep. Values were

obtained for the minute volume CO_2 produced and O_2 consumed. Using these the respiratory quotient (RQ) and resting energy expenditure (REE) were calculated by the software on the Deltatrac (215), based on the child's age and auxology which was recorded at the beginning of each session on the Deltatrac.

4.4.2 Leucine Studies

The protocol here was designed to determine protein turnover before and after rhGH. Eight patients completed all studies. Patients had a baseline study, which was then repeated after two months of rhGH treatment (figure 4.3). Four patients (two with CRF) selected randomly (by a "blind observer) went on to participate in a further study three months after the second one in order to obtain further information about the "longer" term effects of rhGH. No distinction was made for gender, nor renal status (CRF or renal transplant) in view of the small number of patients.

Practically the study differed from the fractional recovery studies above only in that 1-^{13}C-leucine was administered both as a priming bolus of 1mg/kg and an infusion of 1 mg/kg/hr. ^{13}C-Bicarbonate was administered as a bolus only, of 1mg/kg, to prime the HCO_3^- pool (Table 4.2). Use of priming doses reduces the time taken to reach steady state.

Breath samples, as described above, as well as blood samples to determine the concentration and enrichment of 1-^{13}C-α ketoioscaproic acid (KIC), the metabolite produced by reversible transamination of leucine, were collected simultaneously. A second cannula inserted into the contralateral hand at the same time as that inserted for the infusion was used to obtain blood samples.

Blood samples for endocrine investigations were also collected when baseline enrichment samples (Table 4.2) were obtained, and analysed for GH, IGF-I, IGF-II, IGFBP-1, IGFBP-2 and IGFBP-3, insulin, c-peptide, ALS and IGF bioactivity. Total serum CO_2, a measure of acid-base balance, and cortisol levels were also determined.

All stable isotope blood samples were placed on ice for transportation to the laboratory where they were centrifuged for 5 minutes at 3000 revs/min. The supernatant was pipetted into sterile tubes and frozen at -70° C until analysed.

4.4.3 Calculations

4.4.3.1 Anthropometrics

Triceps skinfold thickness and mid-arm muscle circumference (MAC) (Table 4.3) were used to calculate approximations of cross sectional fat and muscle using the equations (216):

$$\text{muscle } (mm^2) = (MAC - [\pi S])^2 / (4\pi)$$

$$\text{fat } (mm^2) = (S * MAC/2) + (\pi S^2/4)$$

where MAC is the mid-arm circumference in mm, S is the triceps skinfold thickness in mm. and from these equations a ratio of fat: muscle was derived. In adults there can be 15-25% overestimation in arm muscle area and 10% in fat area by anthropometry as confirmed by computerised tomography (217).

Body mass index (BMI) was calculated using the equation

$$BMI = \text{weight (kg)} / \text{height}^2 \text{ (m)}$$

BMI was compared to gender based reference curves derived in 1990 from normal British children (155).

Resting energy expenditure (REE) was calculated during indirect calorimetry by the REE software (215) on the Deltatrac II machine from the minute volume O_2 consumed and CO_2 produced by the children.

4.4.3.2 Stable isotope calculations

Enrichment values were plotted to obtain graphs (figure 4.4) showing variation with time. From these plateaus of enrichment during the steady state period were obtained and the mean of the points was used in calculations of protein kinetics as described below. A plateau was defined as an area on the graph that showed a curve parallel to the axis on which time was plotted, indicating no increase in enrichment with time. There was less than 10% fluctuation of the points in this region about the mean derived from a minimum of 3 adjacent points for ^{13}C KIC, with less than 5% fluctuation about the mean for $^{13}CO_2$.

4.4.3.2.1 Fractional recovery (FRR) of ^{13}C

The minute volume of expired CO_2 (VCO_2) was obtained by indirect calorimetry, and the rate of appearance (Ra) of the label in it can be calculated from the following equation:

$$Ra = F \{(E_i / ECO_2)-1\}$$

where F is the rate of infusion of the labelled sodium bicarbonate (μmol/min); E_i is the enrichment of the infusion (atoms % excess- APE) and E CO_2 the enrichment of expired CO_2 (APE).

4.4.3.2.2 Protein kinetics

During steady state the rate of appearance of the amino acid in the plasma pool is equal to its rate of disappearance and reflects the protein turnover rate or flux (F). Entry into the pool occurs due to protein catabolism (C) and ingestion (I), whilst removal is due to protein synthesis (S) and non reversible oxidation (O). This can be equated:

$$F = C + I = S + O$$

In the postabsorptive state, no ingestion takes place, and therefore

$$F = C = S + O$$

(In some studies but not all, the quantity of leucine infused is subtracted from the flux to calculate catabolism. In present study this amounted to approximately 0.1- 0.12 μmol/kg/min of leucine, or 0.3g/kg/day of protein. This was not subtracted from any of the calculations of flux and synthesis)

Using the enrichment of CO2 in breath, the rate of oxidation can be determined as follows:

$$O = F^{13}CO_2 [(1/Ep)-(1/Ei)]* 100$$

$F^{13}CO_2$ is the CO_2 production rate by ^{13}C-leucine oxidation (μmol/kg/hr); Ei = enrichment of infused leucine (APE); and Ep = enrichment of plasma at steady state.

$F^{13}CO_2$ is calculated from:

$$F^{13}CO_2 = FCO_2E\ CO_2 / W\ (60*41.6/ [\ 100*0.81])$$

E CO_2 is the mean enrichment of expired breath at steady state; FCO_2 is the CO_2 production rate (l/min); W= weight of subject (kg); 60 (min/hr) and 41.6 (μmol/l) converts FCO_2 to μmol/hr; 100 changes APE from a percentage to a fraction and 0.81 is the corrective factor that accounts for the fractional recovery of ^{13}C from the oxidation of ^{13}C-leucine.

The flux/turnover can be determined by the dilution of the ^{13}C-leucine infusion in plasma once the steady state is reached, using the following equation:

$$Q = i [(Ei/ Ep)- 1]$$

where Q= flux/turnover; i = 1-^{13}C-leucine infusion rate (μmol/kg/hr); Ei = enrichment of infused leucine (APE); and Ep = enrichment of plasma at steady state.

Net balance is derived from the subtraction of rate of appearance (protein degradation) of amino acid in plasma from rate of disappearance (synthesis). Therefore

$$\text{Net balance} = \text{synthesis} - \text{flux}$$

(as flux is equal to protein degradation in studies during the fasting state)

The figure 590 was used to convert leucine into protein (590μmol leucine/ g protein).

4.4.4 Sample Analyses

4.4.4.1 Breath

These were analysed in batches by a commercial firm (BSIA, London) using an isotope ratio mass spectrometer (IRMS). The principle of this is that the sample is introduced into the vacuum chamber of the spectrometer, with bombardment by electrons generated from a filament wire which induces a positive charge on the sample molecules. By a combination of repulsion using electrodes, and deflection with magnets, the molecules can be separated on the basis of their mass. Detection plates quantify the different isotopes of the gas.

Calibration of the samples in the IRMS can be carried out using a reference gas with a known enrichment.

4.4.4.2 Leucine / Ketoisocaproic acid

Blood samples were used to determine plasma enrichment. Although 1-^{13}C-leucine was infused, the enrichment of its metabolite, 1-^{13}C-α ketoisocaproic acid (^{13}C-KIC) was determined as this is a more accurate reflection of intracellular events where protein synthesis/ oxidation occurs (213). Enrichment is determined by gas-chromatography mass spectrometry (GCMS). Samples were analysed by the stable isotope unit at Dundee University, UK.

^{13}C-KIC is derivatised to its more volatile forms-O-phenylenediamine (OPD) and tert-butyldimethylsilyl-methylfluoroacetamide (MtBSTFA). This is then injected into the GCMS and vaporised on entry at a temp of 250° C. The vaporised sample is swept by inert gas (helium, hydrogen, nitrogen, methane) through the chamber into a column that is coated with a non-volatile liquid. Here some of the sample will elute before entering the separator at the interface between the gas chromatography and ionisation chamber of the mass spectrometer (MS). The separator reduces the pressure of the "gases", and also ensures that more of the sample and less of the inert gas enters the MS.

Enrichment of the infusates was confirmed by GCMS of the solutions, and compared with internal standards deuterium labelled leucine and deuterium labelled KIC (d-leucine and d-KIC).

4.4.4.3 Endocrine

Samples were batched and analysed by an experienced technician using commercially available kits manufactured by Diagnostic Systems Laboratory (Texas) for all assays except GH (J. Jones), IGF-I (J. Jones), IGF bioactivity (Dr Taylor). The methodology is as described in chapter 3.

Cortisol was analysed by competitive immunoassay procedure by the lab staff using a kit provided by Diagnostic Products Corporation.

TCO_2 was analysed by lab staff as part of the routine clinical monitoring of the children.

4.4.5 Statistics

The Wilcoxon rank test was used to determine significant differences between paired samples, before and after rhGH was commenced. Due to the small number of patients completing all the studies, subdivision into groups on the basis of the interval between the first and second leucine isotope study for statistical analysis was not possible . Spearman's test was used to determine correlations between the different parameters (flux, synthesis, oxidation, arm muscle area, cross

sectional fat area, BMI, weight, Ht SDS, insulin, REE, Gh, cortisol, IGF-I, IGFBP-1, IGFBP-3 and IGF bioactivity) before and after rhGH. Guidance was obtained from Ms J Thompson about the appropriate statistical tests to be used.

4.5 Results

For statistical analysis, results for the CRF and transplanted patients were pooled due the small number of patients. The transplanted children, with the exception of one patient (Patient d) had CRF of their graft thus minimising clinical differences between the groups. It was not possible to recruit more children to the study to which clinical indication for, and consent to use of rhGH by the family and patient was a criteria.

4.5.1 Anthropometric changes

Table 4.5 shows the cross sectional area of fat and muscle, and the ratio of fat: muscle in the individual patients, before and after the use of rhGH. All children showed a decrease in the fat: muscle ratio with the use of rhGH. Patients a,c,f,h are children with CRF, whilst b,d,e,g are those with a renal transplant. The cross sectional fat area in the transplanted children is approximately four times that of the CRF patients, whilst the muscle area is approximately 1.5 times greater in the transplanted group

(based on median values for the groups). The muscle area in the CRF group increased by 35%, whilst that of the transplanted group increased by 11%. Similarly the fat area in the CRF showed a larger decrease of 19% whilst that of the transplanted children dropped by 13%. Statistically the muscle area before and after the use of rhGH differed significantly ($p=0.02$), whilst the fat:muscle ratio also showed a significant difference ($p=0.01$). There was no difference in fat area.

4.5.2 Changes in weight, body mass index (BMI) and resting energy expenditure (REE) with rhGH

There was no significant alteration in the weight (figure 4.5, Table 4.5) of the children before or after the use of rhGH. The transplanted children were heavier than the CRF patients, but it was not possible to confirm this statistically due to the small number of patients.

The BMI of the children did not change significantly with use of rhGH, but was lower in the CRF group than the transplanted group (figure 4.6, Table 4.5). BMI for the former group generally was below the 50^{th} centile with one exception, which was closer to the 90^{th} centile. The BMI for the transplanted group was predominantly located between the 75^{th} and 91^{st} centiles, with that of one patient closer to the 99^{th} centile.

All the children showed an increase in REE with use of rhGH, which was significant (p=0.01) (figure 4.7, Table 4.5). The median REE for the transplanted group was lower than that of the CRF group before rhGH, but this increased to equal that of the CRF group with rhGH therapy.

The respiratory quotient (RQ) of the children was low at 0.78 (median value) and this decreased, but not significantly, to 0.74 with rhGH use (Table 4.6). RQ is the ratio of oxygen consumption to carbon dioxide production and reflects substrate oxidation. Carbohydrate oxidation is indicated by higher RQ than fat oxidation (218). Therefore the patients in this study were metabolising fat more than carbohydrate and this increased with use of rhGH.

4.5.3 Fractional recovery of ^{13}C-bicarbonate

Of the 12 children studied, four dropped out and did not proceed to the protein turnover studies.

The fractional recovery of 1-^{13}C ranged from 53% to 85%, with higher recovery obtained for the children with CRF generally than transplant as shown in Table 4.4. Variability in the fractional recovery arises from CO_2 "fixation" in slowly turning over pools.

4.5.4 Protein turnover, growth and endocrine factors before and after use of rhGH

IGF-I SDS and IGFBP-3 SDS were calculated as described in the previous chapter. IGF-I and IGFBP-3 combined are more representative of GH activity than either peptide individually (148, 219).

Table 4.6 and figures 4.8 and 4.9 show the results obtained for each patient for protein flux and synthesis before and after the use of rhGH. Table 4.7 shows the protein flux and oxidation in μmol/kg/min for comparison with historical work in a similar group of patients (211). Flux and synthesis were higher in those with CRF rather than the renal transplant group, but could not be confirmed by statistical analysis due to the small numbers. The difference between the groups was not as noticeable for oxidation figures. Changes in indices with the use of rhGH differed. Most of the patients showed a rise in flux with use of rhGH, whilst the change in synthesis was more variable as 3 patients (2 CRF) had a decrease in the rate of synthesis with use of rhGH. Similarly the response noted in the endocrine parameters varied.

Figures 4.10 (i)- 4.17 (ii) show the trend in endocrine factors, flux, synthesis and percentage of leucine synthesis : flux at baseline and after use of rhGH. Figures represent data on each patient individually, and

have been divided between parameters with low (i) and high values (ii) to enable clarity. IGF-I, IGFBP-3 and c-peptide values have been divided by 10 to enable better scaling. Children who were monitored over 5 months of rhGH treatment (patients e,f,g,h) show three time points (time 0,2,5) on their figures, permitting monitoring of the effects of continued rhGH. Most children showed a consistent response to rhGH, with a rise in IGF-I levels, IGFBP-3 and ALS. In some insulin and c-peptide also rose, but in two patients the response of insulin and c-peptide was not synchronous, in that whilst c-peptide rose, insulin levels dropped. C-peptide accumulates with reductions in GFR as it is dependent on renal clearance, unlike insulin which is metabolised by the liver.

Changes in percentage of leucine flux being incorporated into leucine seemed to follow the trend of IGF-II more than IGF-I in most cases, except for patient g where the percentage synthesis decreased, whilst IGF-II rose. However, IGFBP-3 also rose noticeably. Interestingly at this point the TCO_2 of the patient had dropped to 17 from 19 mmol/l.

Effects of rhGH with time seemed to wane as the initial post rhGH response produced a steeper slope, which became flatter between the second and third observation for IGF-I, ALS, insulin and IGFBP-3.

Table 4.6(i-v) shows the median values and range of protein turnover, growth parameters and endocrine factors before and after commencement of rhGH.

Median values of all parameters except IGFBP-1 showed an increase with use of rhGH. However this was significant only for IGFBP-3 SDS (p= 0.01), GH (p=0.02) and Ht Vel SDS (p=0.03) and c-peptide (p=0.05).

Figure 4.18 shows the changes in Ht SDS, and figure 4.19 shows the Ht Vel SDS in all children before and after use of rhGH. Although for the majority of patients both parameters appear to increase, no significance can be attached to this as the observation period was short.

4.5.5 Correlations between protein kinetics, body composition, and auxological parameters

4.5.5.1 Pre RhGH (table 4.8)

IGF-II correlated with cross-sectional arm muscle area (r=0.833, p=0.01), BMI (r=0.802, p=0.02), and weight (r=0.833, p=0.01). IGF-II also correlated with the percentage of leucine incorporated into protein synthesis (r=0.708, p=0.05), indicating that that higher levels of IGF-II

may lead to increases in protein synthesis. The exact role of IGF-II in growth in postnatal life remains unclarified, but levels are low in GH deficiency and increase with rhGH treatment (107).

Insulin correlated directly, but just losing significance, with fat (r=0.667, p=0.07).

GH levels had an inverse correlation with REE (r=-0.81, p=0.02). This relationship is complicated by acidosis and the use of steroids, both of which increase REE (220), but decrease GH production (97). GH levels correlated with the ratio of synthesis : flux (r=0.781,p=0.02) A direct correlation between GH and Ht SDS was present (r=0.667, p=0.07), but just lost significance.

Cortisol levels also correlated strongly with Ht SDS (r=0.893, p=0.007), and with GH levels (r=0.750, p=0.05). Physiological cortisol levels are necessary to maintain GH receptor expression and growth (152). The correlation of cortisol with IGF-I was negative (r=-0.786, p=0.04). This may be a reflection of the stimulatory effect of cortisol on IGFBP-1 mRNA during low levels of insulin (115) and in CRF there is decreased insulin action due to resistance, but cortisol also reduces production of local IGF-I (152). A direct correlation of cortisol with IGFBP-1 (r=0.893, p=0.007) was present. A significant inverse correlation of IGFBP-1 with IGF bioactivity (r=-0.829, p=0.04) was present.

4.5.5.2 Post rhGH therapy (table 4.9)

Both flux and synthesis correlated negatively with both IGF-I ($r \geq -0.762$, $p \leq 0.03$) and with GH levels ($r=-0.738$, $p=0.04$). Oxidation also correlated inversely with IGF-I, but just missed being significant ($r=-0.690$, $p=0.06$). GH and IGF-I increase synthesis, but in uraemia, particularly in the presence of acidosis or use of steroids, the normal relationships are attenuated. Differences between pre and post rhGH levels of TCO_2 correlated directly with differences in the synthesis : flux ratio ($r=0.840$, $p=0.009$) showing that acid-base balance influences the amount of leucine released by catabolism incorporated into synthesis.

The correlation of IGF-II with muscle area was no longer significant ($r=0.643$, $p=0.09$). The correlation of IGF-II with REE occurred but just missed being significant ($r=0.690$, $p=0.06$). Weight also correlated with REE ($r=0.714$, $p=0.05$), as did muscle with REE, with loss of significance ($r=0.667$, $p=0.07$).

A negative correlation of muscle area was observed with the difference in Ht SDS after and before rhGH treatment ($r=-0.714$, $p=0.05$). Thus increases in linear length with treatment is associated with lower cross sectional muscle area.

TCO_2, a measure of acid-base balance, correlated negatively with insulin levels, (r=-0.766, p=0.01). Acidosis reduces the efficacy of insulin. Differences in TCO_2 and in ratio of leucine synthesis to flux correlated negatively before and after rhGH (R=-0.840, p=0.009). Increasing acidosis leads to decreased leucine incorporation into protein synthesis.

4.6 Discussion

4.6.1 Protein turnover

Protein kinetics are higher in healthy children than adults (179, 208), but can be altered by illness, drugs, nutritional state and hormones. More information on protein kinetics is available in adults than in children, particularly in chronic illnesses. Skeletal muscle is the largest reservoir of protein in the body, and therefore in patients with CRF where lean body mass can be reduced (181) protein turnover will be affected (182, 191, 221). Information on lean body mass in children with CRF is limited but shows that this can be reduced (176, 221), and growth is suppressed in these patients (71, 72). To some extent this can be due to suboptimal caloric intake (193, 221). However endocrine imbalances (see below)

and metabolic acidosis (180, 183) will also affect growth and protein turnover.

The study of protein turnover in this thesis has been performed in children with both CRF and those post-renal transplant. There are several potentially important limiting factors to interpretation of the results such as the differential time interval between repeat studies. Growth monitoring over a short term (≤ 3 months) can also be inaccurate. Due to the criteria of clinical indication for the use of rhGH for recruitment to the study, numbers were limited and it was not possible to control for age, gender or pubertal development. The age of these children, only one of whom was female, ranged from 10 – 16 yrs. The median pubertal stage for the group was 2 with none of the children at stage 3+. Results from protein turnover studies performed in adolescents are not available, and therefore it is not possible to comment on the effects of puberty, whilst an age related decrease in protein synthesis has been noted. Logically it would be expected that protein turnover would increase in line with the growth spurt.

Thus the group studied here is heterogeneous and although this can potentially influence results, the probability of this is low as discussed above and for the following reasons:

I. Data from other studies (235) suggest that after commencing rhGH, changes in metabolic rate are maximal by 6 weeks with little alteration noted thereafter. The work carried out here corroborates this as no obvious difference was apparent between the studies performed at 2 or 3 months.

II. The transplanted children were on alternate day, low dose steroids which have minimal systemic side effects (59) and thus might not be expected to greatly influence their metabolism, including protein turnover. These patients however would previously have received high dose steroids and this can influence lean body mass and body fat. This has been discussed further below.

III. Children in previous protein turnover studies have not been divided on the basis of gender and there is no data to suggest that in prepubertal females the protein flux will differ from that of age matched males.

IV. The influence of age is discussed further below.

The protein flux of the CRF patients in the study was comparable to that observed in healthy children (187, 188), but higher than that of the children who had undergone a renal transplant and were on steroids. Due to the variation in fractional recovery of ^{13}C in breath samples from the patients, the conventionally accepted figure of 0.81 was used as the correction factor in calculations to enable comparison between the

protein turnover results from this study and those from others. This correction factor has been validated in many studies involving adult subjects (205, 207) and is used in adults with CRF (181), and children with (211) and without CRF (188). The transplanted group had higher BMI than the CRF group, phenotypically had more fat and a greater fat: muscle ratio. Few studies of protein turnover base their results on fat free mass, using body weight (kg) instead, but higher rates of protein turnover have been obtained with adjustments for lean body mass in CRF (211). However in healthy, obese children protein kinetics are higher than in non-obese children, and when adjusted for fat free mass, differences between obese and lean children disappear (222). In these children it appears that although adipose tissue is increased, there is a concomitant increase in lean body mass and REE is higher than in non-obese children (223, 224). Protein metabolism occurs predominantly in skeletal muscle which represents 70% of the lean body mass, but also in organs such as the kidney (225). A contribution of adipose tissue to phenylalanine kinetics has been found in one study, which may indicate some contribution of adipose tissue to protein turnover (226). In the patients here it appears that whilst BMI and adipose tissue were higher in the transplanted children than CRF, this does not appear to apply to their LBM.

Protein turnover decreases with age (179, 186) and infants have high protein turnover (6-7 g/kg/day) (188, 189) compared with adults (179). The results of the transplanted group (per kg body weight), who mostly were older and pubertal compared with the CRF group, are closer to those of healthy adults, whilst that of the CRF group are comparable to those observed in younger children with CRF in other studies (211) and lie between values observed for adults (187) and infants (188). However the results of the transplanted children appear comparable to protein kinetics observed in healthy adolescents admitted for corrective surgery following injury from burns (186).

Even mild acidosis can stimulate activity of branched chain keto-acid dehydrogenase in muscle (208), with increased protein degradation (185, 211) and metabolism of branched chain amino acids in uraemia (194). An inverse correlation between protein degradation, when corrected for lean body mass, and acidosis has been reported by Boirie (211), but was not observed in this study, perhaps as protein turnover was not corrected for lean body mass and patient numbers were small. The TCO_2 levels, a measure of acid-base balance in the patients, were at the lower end of the normal range (20 - 26 mmol/l) as shown in table 4.6. Patients who had levels at or below the normal range showed an increase in oxidation, with either no change in flux and synthesis or an increase in the former and a decrease in the latter with use of rhGH. The influence of acidosis

on protein turnover was confirmed by the direct correlation of differences in TCO_2 with differences in synthesis to flux ratio with use of rhGH. One patient had a 9 am cortisol level that clearly would be considered subnormal, and in this patient synthesis and flux increased whilst oxidation decreased. The effects of acidosis appear to be mediated, to some extent, by glucocorticoids, as protein degradation and oxidation in adrenalectomized animals, when acidotic, do not differ from normal controls until given dexamethasone (185). Steroids increase catabolism and oxidation in adults and animals (157,185, 227) with increased turnover of non-essential amino acids (228) and REE (220). Marked muscle wasting is present in patients with Cushing's Disease when endogenous cortisol levels are excessive (229) and chronic acidosis can elevate cortisol levels (185). There was an inverse correlation of TCO_2 with insulin levels before use of rhGH. Insulin has antiproteolytic actions (94), and elevation of insulin levels in acidosis is consistent with resistance to the actions of insulin.

Use of rhGH produced differing responses in the patients, but the median values for flux, synthesis and oxidation showed an increase of 3.3%, 4.1% and 2.5% respectively from baseline. The median cross sectional arm muscle area of the children increased (8%) significantly and cross sectional fat:muscle ratio decreased significantly with use of rhGH, with a 23 % decrease in cross sectional adipose tissue observed. Greater

increases in lipolysis (21%) than protein synthesis (11%) have been documented by others as well with use of rhGH (190) with more rapid changes in adipose tissue than muscle observed (230). A major reason for the discrepancy between the results of the isotope studies and anthropometric findings is that that the isotope studies were conducted in the fasting state which a not a common physiological state. Anthropometric findings represent prolonged observations over various physiological states. Fasting in normal individuals is associated with increased oxidation, and decreased synthesis, whilst the effect on protein catabolism remains controversial (179). In CRF these protein kinetics are exaggerated and fasting, uremic rats show an attenuated response with greater weight loss, protein degradation and oxidation than control animals (184, 192). It is interesting to speculate that this "exaggerated response" is a manifestation of inefficient metabolism of substrates such as ketones originating from adipose tissue which is the main fuel source as determined from the low RQ of 0.78 (discussed further below), with a detrimental effect on acid-base balance resulting in further catabolism. Use of rhGH, which has lipolytic actions, would exacerbate these findings, and would explain why Garibotto observed increased metabolism of branched chain amino acids (194) as fuels alternative to fat are metabolised. In normal individuals insulin levels are low during fasting and IGF action may enhance substrate metabolism. IGF-I levels are also decreased by fasting. In patients with CRF IGF actions are

inhibited by an excess of IGFBPs and insulin resistance is also present. Feeding results in increased muscle and whole body protein synthesis, and increased oxidation of amino acids (203, 231).

Effects of feeding on protein degradation vary, with either no change (203) or a decrease in catabolism (231). The lack of inter and intra individual consistency in studies here can be attributed to three observations from other studies: i) An inter individual physiological variation of 10% observed even in health (186, 232) and within individuals (3-6% for flux and synthesis) (179); ii) Whole body protein kinetics were measured which shows less change with intervention (203, 205) and are lower than skeletal muscle turnover which accounts for 40% of protein synthesis in the body (203, 225). Other studies conducted in adults with CRF in which rhGH was used measured forearm muscle turnover (191). iii) In CRF factors such as acidosis and uraemia attenuate the response to the anabolic hormones (insulin, GH and IGF-I) (180). RhGH in adults with GH deficiency, uraemia and normal controls causes an increase in protein synthesis, does not alter degradation, reduces oxidation and has a lipolytic effect leading to decreases in adipose tissue (157, 190, 191,233, 234). In the present study, children with normal TCO_2 levels showed a rise in protein synthesis with use of rhGH. GH may have a direct influence on protein turnover (191, 234), although many of its actions are mediated by IGF-I, and use of rhGH

results in increased insulin levels (81). The increase in muscle area was mirrored by significant rises in REE (see figure 4.7) of all children. An increase in energy expenditure with the use of rhGH in children has also been found previously (235).

The change in IGF-I SDS with the use of rhGH was not consistent in all children, and did not correspond to changes in protein turnover. This suggests that IGF-I mediates some of the actions of GH but not all as also observed in animal studies (236). IGF-I has a direct influence on turnover (227, 237) and decreases protein catabolism and oxidation (237). In uraemic animals (238) and healthy adults (237) a dose dependent effect has been shown with no significant effect of low dose IGF-I on protein kinetics, whilst high dose IGF-I increased protein synthesis and decreased oxidation. Addition of prednisolone to IGF-I administration increases proteolysis and oxidation but does not alter synthesis (227). In CRF, IGF-I activity is affected by reduced renal clearance of IGFBPs, and there is some evidence of defects in post receptor phosphorylation with increased IGF-I receptor mRNA expression and numbers compared to normal in animal studies (238).

Insulin also has anabolic actions, consisting mainly of inhibition of protein degradation and lipolysis (239). However there appears to be some conflict between findings in animal and human studies with regard to its

actions on stimulating protein synthesis, which has been demonstrated in the former (240) but not latter (233, 239, 241), and in fact in one study involving diabetics, insulin inhibited protein synthesis, albeit less than degradation and oxidation (205). In the present study no relation was observed between insulin/ c-peptide and the markers of protein turnover either before or after the use of rhGH, despite the significant increase in c-peptide levels. Insulin did however correlate with cross-sectional areas of both muscle and fat before rhGH treatment. Resistance to the actions of insulin is well recognised in CRF (174), with use of steroids (157) and obesity (242) into which category some of the children come close to (BMI> 98th centile), and thus may explain the lack of observed association between proteolysis and insulin. The mechanism of resistance to insulin in CRF appears to be a combination of inhibition of insulin actions by acidosis (174), binding to a relatively newly described peptide (IGFBP-7) (243), binding to the NH_2-terminal fragment of IGFBP-3 (243), both of which accumulate in CRF, and some degree of post receptor abnormality (174). There is an overlap in the actions of IGF-I and insulin which have 50% structural homology with cross-stimulation of receptors observed (132). Dose dependent responses in protein turnover to insulin and IGF-I have been observed (237).

The observed correlations in the present study of IGF-II with muscle area, BMI, weight indicate a role for it in protein turnover and growth,

which has been established in antenatal life but not postnatally. Concentrations increased with rhGH treatment, but did not reach significance. IGF-II can stimulate insulin receptors, with greater affinity than IGF-I, and thus have insulin type actions (134) but also stimulates the IGF type 1 receptor which mediates the actions of IGF-I (134).

In healthy individuals it has been found that 20 % of the REE arises from protein oxidation, compared to 15 % in patients with CRF who are malnourished, but with no observed difference in total REE between the two groups (214). Fat oxidation was higher in these CRF patients than healthy subjects, with a reduction in total body fat. The median respiratory quotient (RQ) obtained approximately 2 hr into the study from our patients was 0.78, which is lower than that in Schneweiss's study (214), and is more indicative of starvation and fat metabolism. The RQ decreased further in most of our patients with rhGH therapy, non significantly to 0.74, as would be expected with increased fat metabolism.

4.6.2 Growth and Endocrine changes with use of rhGH

Short stature is emerging as a major complication in adults with onset of CRF in childhood (34, 59). Use of rhGH shows improvement in Ht SDS (72, 75), and this was evident in 5 study patients rather than all, but maybe confounded by the short interval between observations which

can make measurements unreliable (7). Measurements of height velocity (Ht Vel SDS) suggest that the rate of growth improved significantly in all patients except one (bearing in mind the short term between observations). The response of children with CRF to rhGH is not as marked when compared to short normal children (193), and this blunted response maybe attributed to endocrine, metabolic and nutritional abnormalities. The mode of administration of rhGH, a single bolus compared to the endogenous nocturnal pulses, may also be influential as the rate of increase of levels is more important than duration of exposure to GH in determining the magnitude of the response (80). This may explain to some extent why children with CRF in whom GH levels are elevated, exogenous GH administered once daily show less improvement in growth rate than those with normal levels.

The mechanism by which short stature arises has been discussed in chapter 3. The importance of GH was confirmed by the correlation with Ht SDS in the patients before use of rhGH. In children with CRF GH levels can be elevated (137, 138) or reduced by steroid treatment (139). GH levels were not elevated in the children with CRF, nor reduced in those with a transplant as determined by a single, fasting 9 am sample, but IGF-I SDS were low or in the low - normal range in some patients, perhaps a manifestation of the resistance to the actions of GH observed in CRF. Administration of high dose rhGH (4iu.m^{-2} daily) produced no

significant improvement in IGF-I SDS, unlike other reported studies (71, 75, 199, 201) where the observation period was over 3 months. Unlike IGF-I SDS a consistent increase of IGFBP-3 SDS in all patients to rhGH therapy was observed which was significant. This increment has been shown to be due to increased production of the intact molecule in CRF and thus has a positive role in growth promotion (170). RhGH also enhances growth by decreasing IGFBP-1 levels (191), the mechanism of which is perhaps of suppression by increased insulin and IGF-I levels. However the observed decrease in IGFBP-1 levels in the present study was not significant.

Tolerance to the effects of rhGH appears to occur with long term use, and this is most marked when longitudinal growth studies in children are conducted (201). Changes in body composition and REE have also been shown to be maximal after 6 weeks of treatment, with lower increments noted there after (235). Protein deposition requires a rate of synthesis above that of degradation, and it appears that there is a time lag between increase in synthesis and degradation when a new steady state develops (179). The effect of these observations on patients in the time frame of the present study is difficult to quantify. Four of the children were restudied after a further three months (5 months from commencing rhGH treatment). The changes noted in protein turnover, as seen by the percentage synthesis to flux ratio, did not appear greatly different from

those at 2 months with no consistent pattern between the four children. The changes seen in IGF-I SDS and IGFBP-3 SDS graphically appeared less marked during this time period, whilst there appeared to be no overall change in IGFBP-1.

In summary, protein turnover, expressed as whole body weight, and REE were higher in patients with CRF than in those children with a renal transplant, and were reflective of the amount of lean body mass. Protein kinetics determined using stable isotopes are altered by the physiological state prevailing during the time of the study only, and during fasting the rate of catabolism observed was higher than that of synthesis. The acid-base status also influences the amount of leucine being incorporated into synthesis as confirmed by the strong correlation of the differences in TCO_2 levels and synthesis to flux ratio. The anabolic actions of insulin are also altered by acidosis, with a negative correlation of TCO_2 and insulin present, and to what extent this influences the previous observation is difficult to quantify. The RQ reflects the use of fat predominantly as the endogenous substrate during the studies, and this particularly in the presence of insulin resistance observed in CRF, will contribute to acidosis with increased ketone production resulting in further catabolism which may account for the "exaggerated response observed in CRF" described in other studies. Use of rhGH did not consistently increase the rate of flux and synthesis in individuals due in

part to the acid-base balance and to variable levels of resistance to the differing actions of GH within and between individuals as observed by the changes in IGF-I SDS and IGFBP-3 SDS. The effect of rhGH over a prolonged period provides more accurate information about changes in body composition as both fed and fasting states are incorporated into the observation period. REE, muscle area (LBM) and Ht Vel SDS all increased significantly, whilst fat area (adipose tissue) decreased with use of rhGH. It is important to point out that the time interval between repeat studies is short and this limits the significance of the the observations. There is evidence over 5 months of tolerance to the actions of rhGH with lower responses in endocrine factors. IGF-II appears to have a role in increase in lean body mass and growth in the present study, and this has not been reported previously.

Table 4.1: Demographics of patients in fractional recovery of 13CO2 study.

Patient	Patient	Age (yrs)	Gender	GFR+	Pubertal stage	Steroids (mg/kg/alt d)
a	1	11.6	m	22	1	na
k	2	16.7	m	*	2	na
j	3	7.5	m	34	1	na
f	4	11.6	m	12	1	na
c	5	14.8	m	34	3	na
h	6	12.5	m	18	1	na
l	7	15.2	m	20	1	na
m	-8	12.0	m	25	1	0.5
e	-9	16.0	m	37	3	0.2
g	-10	13.8	m	7	3	0.2
d	-11	10.8	f	85	1	0.3
b	-12	17.1	m	27	3	0.2
	median	**13.15**		**25**	**1**	**0.2**

Patients a-h proceeded to protein turnover studies

(-Transplant patients) * on dialysis. +mls/min/1.73m-2

Table 4.2: Format of each stable isotope (1-^{13}C-leucine) study

***Time* (hr)**	**0**	**1**	**2**	**2½**	**2¾**	**3**	**3¼**	**3½**
Bolus ($H^{13}CO_3^-$ ± 1-^{13}C-leucine)	√	X	X	X	X	X	X	X
Infusion ($H^{13}CO_3^-$ or 1-^{13}C- leucine)	→							
Blood sample (enrichment)	√	√	√	√	√	√	√	√
Breath sample	√	√	√	√	√	√	√	√
Blood sample (endocrine)	√	X	X	X	X	X	X	X

Table 4.3: Triceps skinfold thickness and mid-arm muscle circumference (MAC) pre and post rhGH

Patient	Triceps (mm)		MAC (mm)	
	pre rhGH	(post rhGH)	pre rhGH	(post rhGH)
a	10.0	(8.0)	175.0	(180.0)
c	12.2	911.6)	275.0	(278.0)
f	7.0	(6.0)	174.0	(197.0)
h	7.8	(5.8)	190.0	(210.0)
b	23.0	(17.0)	272.0	(252.0)
d	24.0	(16.0)	249.0	(240.0)
e	32.0	(30.0)	306.0	(334.0)
g	21.0	(22.2)	268.0	(294.0)
median	**16.6**	**(13.8)**	**258.5**	**(246.0)**

Table 4.4 : Fractional recovery (%) of 1-^{13}C during $H^{13}CO_3$- infusion

Patient	FRR (CRF)	patient	FRR (transplant)
a	82		
c	53	b	#85
f	72	d	64
h	57	e	61
i	67	g	61
k	*66	m	73
l	79		
median	**62**		**61**

*patient on peritoneal dialysis(PD)

#patient had been on rhGH for 6months

Table 4.5 : Anthropometry before and after use of rhGH (post rhGH results *)

	BMI (kg/m)	BMI* (kg/m)	Weight (kg)	Weight* (kg)	Fat (mm^2)	Fat* (mm^2)	Muscle (mm^2)	Muscle* (mm^2)	REE (kcal/day)	REE* (kcal/day)
	15.2	15.6	26.1	28.2	953.5	770.0	1641.8	1909.9	944	1336
	23.0	23.7	50.0	54.0	1794.3	1718.0	4460.4	4646.4	1017	1384
	17.3	16.7	28.5	28.0	647.5	619.3	1840.0	2527.2	1362	1537
	17.3	17.7	30.8	33.4	788.8	635.4	2181.0	2928.6	1096	1404
	22.3	21.3	48.8	47.4	3543.0	2399.0	3177.7	3121.1	1235	1471
	23.2	21.6	34.1	33.4	3440.2	2121.0	2400.6	2867.0	1118	1135
	28.7	29.3	61.1	65.3	5699.8	5716.5	3362.9	4578.4	960	1651
	24.9	25.6	52.0	54.6	3160.2	3650.3	3250.7	4005.3	1433	1607
Median	**22.7**	**21.5**	**41.5**	**40.5**	**2477.3**	**1919.5**	**2789.1**	**3024.8**	**1107**	**1438**

Table 4.5 cont: Anthropometry before and after use of rhGH
(post rhGH results *)

Patient	Fat:Muscle ratio	Fat:Muscle* ratio	REE (kcal/day)	REE* (kcal/day)
a	0.60	0.40	944	1336
c	0.40	0.37	1362	1537
f	0.35	0.25	1118	1135
h	0.36	0.21	1433	1607
b	1.11	0.77	1017	1384
d	1.43	0.74	1096	1404
e	1.69	1.25	1235	1471
g	0.97	0.91	960	1651
median	**0.79**	**0.57**	**1107**	**1438**

Table 4.6(i): Age and protein turnover before and after use of rhGH (post rhGH results *)

Patient	Age (yrs)	Age* (yrs)	Flux g/kg/day	Flux* g/kg/day	Synthesis (g/kg/day)	Synthesis* (g/kg/day)	Oxidation (g/kg/day)	Oxidation* (g/kg/day)	S:F (%) (leucine synthesis: flux)	S:F* (%)
a	12.0	12.4	6.05	6.16	5.45	5.36	0.60	0.80	90.0	87.0
c	14.8	15.4	4.95	4.95	4.44	4.40	0.51	0.55	90.0	89.0
f	11.6	11.9	4.89	5.28	4.67	4.67	0.56	0.61	89.0	88.0
h	12.5	12.9	6.65	6.88	6.00	6.16	0.66	0.73	90.0	89.0
b	16.7	17.0	3.69	4.34	3.25	3.81	0.44	0.53	88.0	88.0
d	10.9	11.3	4.34	4.24	3.55	3.66	0.79	0.58	82.0	86.0
e	16.0	16.3	4.36	4.60	3.67	3.93	0.69	0.67	84.0	78.0
g	14.1	14.3	3.77	3.76	3.47	3.31	0.30	0.45	92.0	88.0
median	**13.3**	**13.6**	**4.63**	**4.78**	**4.00**	**4.17**	**0.58**	**0.60**	**90.0**	**88.0**
% change with use of rhGH				**3.2%**		**4.3%**		**3.4%**		

Table 4.6(ii): Endocrine factors before and after use of rhGH (post rhGH results *)

Patient	GH (mu/l)	GH* (mu/l)	ALS (mcg/ml)	ALS* (mcg/ml)	Cortisol (nmol/l)	Cortisol* (nmol/l)
a	6.8	6.0	23.6	26.8	353	123
c	1.4	3.3	23.0	24.0	178	403
f	1.0	3.1	20.9	20.6	na	185
h	1.8	5.1	20.8	30.8	282	439
b	1.5	7.1	26.8	33.9	155	263
d	0.5	11.6	24.2	20.8	196	282
e	1.1	14.5	29.1	30.8	13	54
g	3.7	14.9	25.0	37.6	293	108
median	**1.5**	**6.6**	**23.9**	**28.8**	**196**	**224**

Table 4.6(iii): Endocrine factors before and after use of rhGH (post rhGH results *)

patients	IGF bioactivity (u/ml)	IGF* bioactivity (u/ml)	IGFBP-1 (ng/ml)	IGFBP-1* (ng/ml)	IGF-II (ng/ml)	IGF-II* (ng/ml)	IGFBP-2 (ng/ml)	IGFBP-2* (ng/ml)
a	0.07	0.08	165.9	113.4	1082	1113	1220	978
c	na	0.89	20.2	47.7	1231	1033	966	790
f	0.25	0.31	94.7	84.2	1230	1290	1028	1086
h	0.59	0.55	93.2	93.7	1191	1251	838	1164
b	na	0.51	65.7	43.5	374	719	688	494
d	0.44	na	64.7	96.1	971	1217	310	838
e	0.49	0.84	12.3	5.7	1108	868	364	402
g	0.13	0.10	148.3	55.9	1344	1376	1180	1170
median	**0.35**	**0.51**	**79.5**	**70.1**	**1150**	**1165**	**902**	**908**

Table 4.6(iv): Endocrine factors before and after use of rhGH (post rhGH results *)

Patients	Insulin (mU/l)	Insulin* (mU/l)	C-peptide (pmol/l)	C-peptide* (pmol/l)	TCO_2 (mmol/l)	TCO_2* (mmol/l)	ΔTCO_2 (mmol/l)	Δ S:F (%)
a	7.6	14.5	343	756	24	21	-3	-3
c	28.1	17.6	131	585	19	18	-1	-1
f	10.5	7.7	589	393	23	23	0	-1
h	10.8	20.4	530	630	21	23	2	-1
b	5.6	12.3	245	720	20	24	4	0
d	9.3	7.3	134	67	24	26	2	4
e	35.2	55.6	63	1027	21	19	-2	-6
g	8.2	29.3	859	1382	19	17	-2	-4
median	**9.9**	**16.1**	**294**	**675**	**21**	**22**	**-1**	**-1**

Table 4.6(v): IGF-I SDS, IGFBP-3 SDS and Ht SDS (post rhGH results *)

Patient	IGF-I SDS	IGF-I* SDS	IGFBP-3 SDS	IGFBP-3* SDS	Ht SDS	Ht* SDS
a	-1.28	-3.32	2.93	4.80	-2.37	-2.16
c	0.67	-0.11	2.94	3.10	-2.45	-3.65
f	0.08	-0.51	3.14	3.21	-2.58	-2.50
h	-2.23	0.56	3.21	3.54	-2.41	-3.03
b	0.02	3.29	2.59	5.96	-3.74	-3.33
d	-0.98	-0.87	1.26	2.12	-3.26	-2.60
e	0.79	2.68	3.26	3.63	-3.52	-2.25
g	-0.82	8.12	3.19	5.14	-2.21	-2.11
median	**-0.45**	**0.72**	**3.04**	**3.59**	**-2.52**	**-2.55**

Table 4.7: Flux and oxidation in study patients compared with published results

Patient	Flux µmol/kg/ min	Oxidation µmol/kg/ min	Flux* µmol/kg/ min	Oxidation* µmol/kg/ min	*flux µmol/kg/ min*	*oxidation µmol/kg/ min*
a	2.48	0.25	2.52	0.33	**3.34**	**0.49**
b	1.51	0.18	1.78	0.22	**4.96**	**0.62**
c	2.03	0.21	2.03	0..23	***3.36***	**1.06**
d	1.78	0.32	1.74	0.24	**3.07**	***0.59***
e	1.79	0.28	1.88	0.27	**6.42**	**1.26**
f	2.00	0.23	2.16	0.25	**2.98**	**0.84**
g	1.54	0.12	1.54	0.18	**1.92**	**0.28**
h	2.72	0.27	2.82	0.30	**2.20**	**0.20**
					2.50	**0.61**
* post rhGH					**2.99**	**0.59**

Figures in ***bold, italics*** – (right hand columns) obtained from literature (ref 211) first 5 children were acidotic.

Table 4.8: Correlations between protein kinetics, body composition, and auxological and endocrine parameters pre rhGH.

		R value	P value
Flux:	synthesis	0.952	0.001
IGF-II:	arm muscle area	0.833	0.01
	BMI	0.802	0.02
	Weight	0.833	0.01
	Ht SDS	0.643	0.09
	% leucine synthesis : flux	0.708	0.05
Insulin:	muscle	0.643	0.09
	fat	0.667	0.07
GH:	REE	-0.810	0.02
	Ratio of synthesis : flux	0.781	0.02
	Ht SDS	0.667	0.07
Cortisol:	Ht SDS	0.893	0.007
	GH	0.750	0.05

		R value	P value
Cortisol:	IGF-I	-0.786	0.04
	IGFBP-1	0.893	0.007
	C-peptide	0.786	0.04
BMI :	Cross sectional fat area	0.738	0.04
	Cross sectional muscle area	0.802	0.02
Weight:	Cross sectional fat area	0.738	0.04
	Cross sectional muscle area	0.929	0.001
IGFBP-1:	IGF-I	-0.667	0.07
	insulin	-0.643	0.09
	IGF bioactivity	-0.829	0.04
Insulin:	IGFBP-3	0.643	0.09
	IGF bioactivity	0.886	0.02

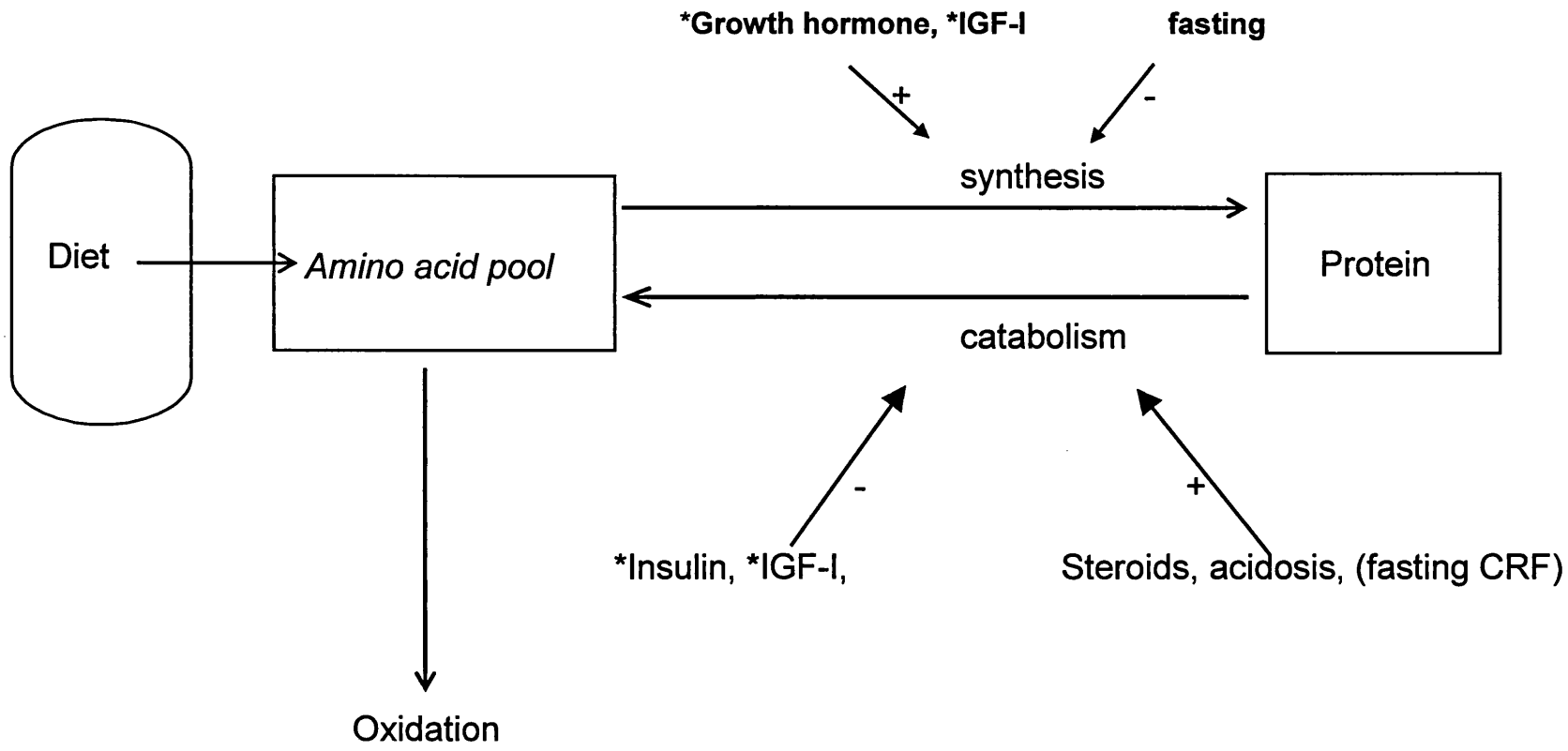
Table 4.9: Correlations between protein kinetics, body composition, and auxological and endocrine parameters with rhGH treatment.

		R value	P value
Flux :	Synthesis	0.976	0.001
	Oxidation	0.810	0.02
	IGF-I	-0.786	0.02
	GH	-0.738	0.04
Synthesis:	IGF-I	-0.762	0.03
	GH	-0.738	0.04
Oxidation:	IGF-I	-0.690	0.06
IGF-II:	Cross sectional muscle area	0.643	0.09
	REE	0.690	0.06
BMI:	Cross sectional fat area	0.833	0.01
	Cross sectional muscle area	0.857	0.007

		R value	P value
Weight:	Cross sectional fat area	0.833	0.01
	Cross sectional muscle area	0.857	0.007
	REE	0.714	0.05
REE:	Cross sectional muscle area	0.667	0.07
GH:	IGF-I levels	0.786	0.02
	ALS	0.649	0.08.
ALS:	IGF-I	0.743	0.04
	IGFBP-3	0.826	0.01
IGF-II:	IGFBP-2	0.929	0.001
TCO_2 :	Insulin	-0.766	0.01
IGFBP-3:	C-peptide	0.810	0.02
⊿Ht SDS:	Cross sectional muscle area	-0.714	0.05
ΔTCO2 :	Δ percentage synthesis:flux	0.840	0.009

(Δ represents difference between post and pre rhGH value)

Figure 4.1: Amino acid turnover and influencing factors :



(increased with steroids, acidosis, fasting)

*resistance to actions of hormones in CRF

Figure 4.2 ^{13}C Leucine Kinetics

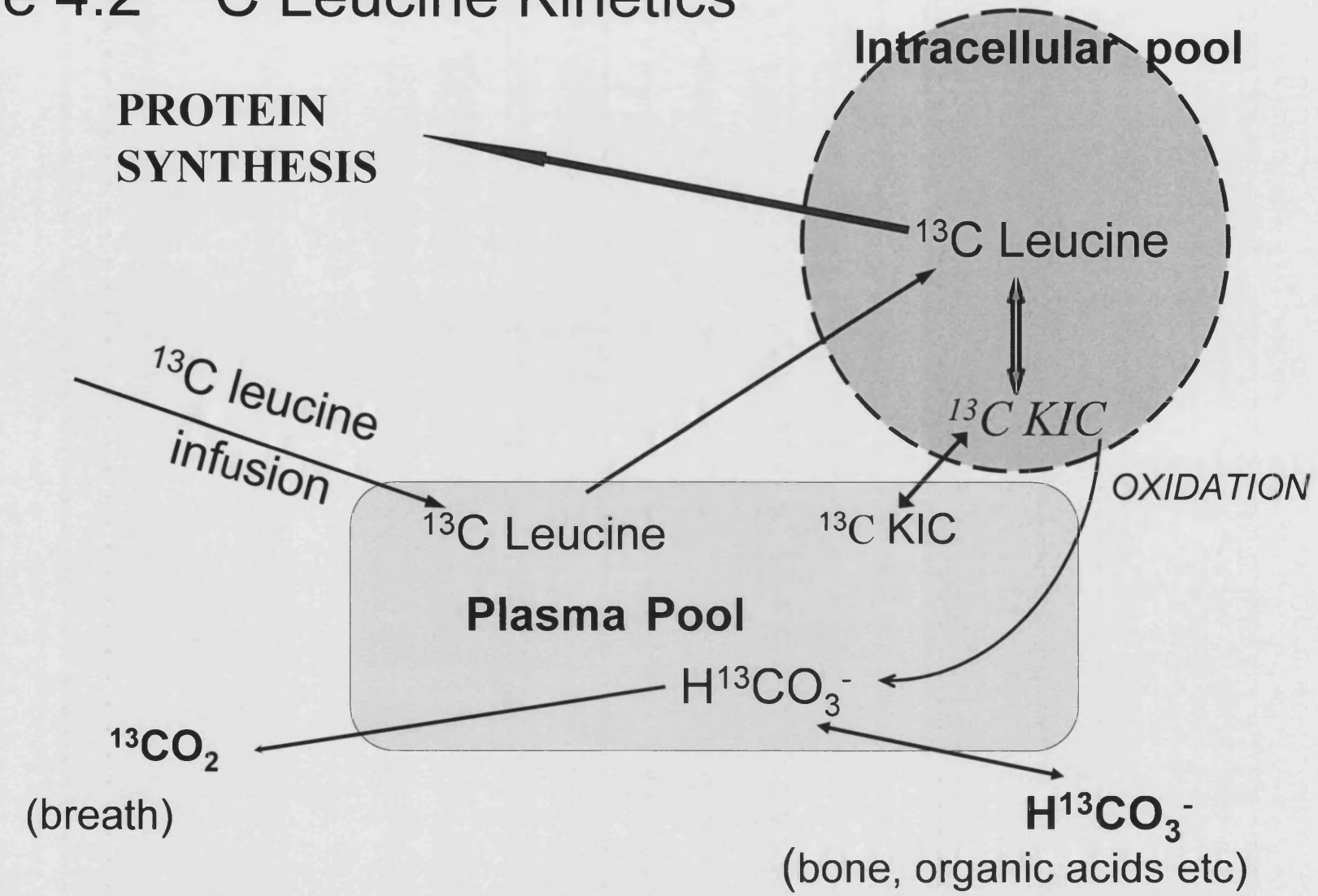


Figure 4.3: Outline of rhGH protocol

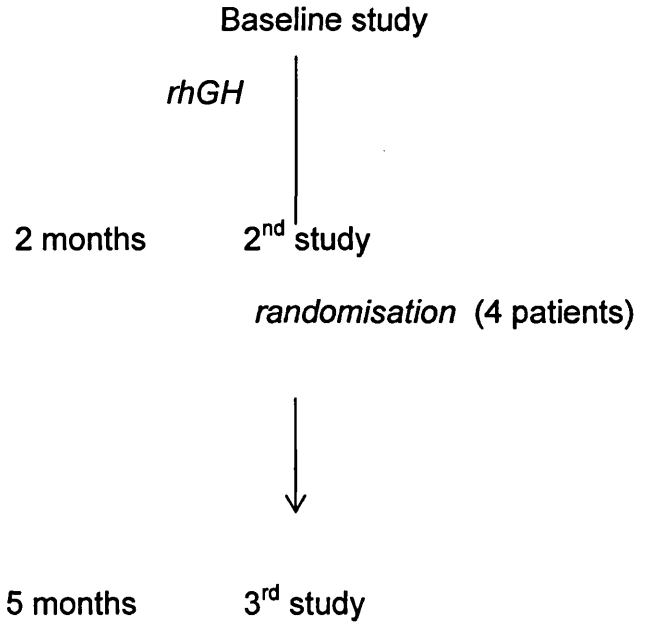


Figure 4.4 Enrichment of breath and KIC (atoms %)

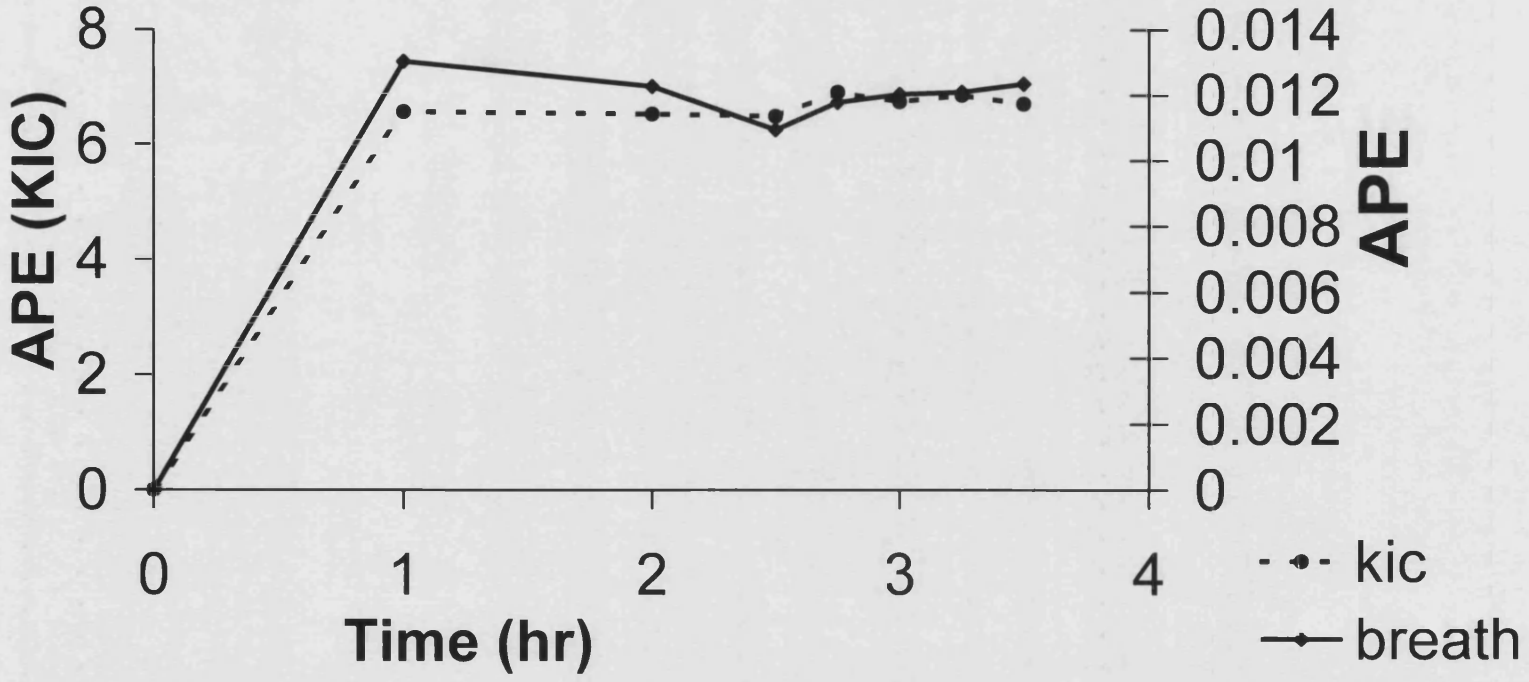


Figure 4.5: Changes in weight with use of rhGH

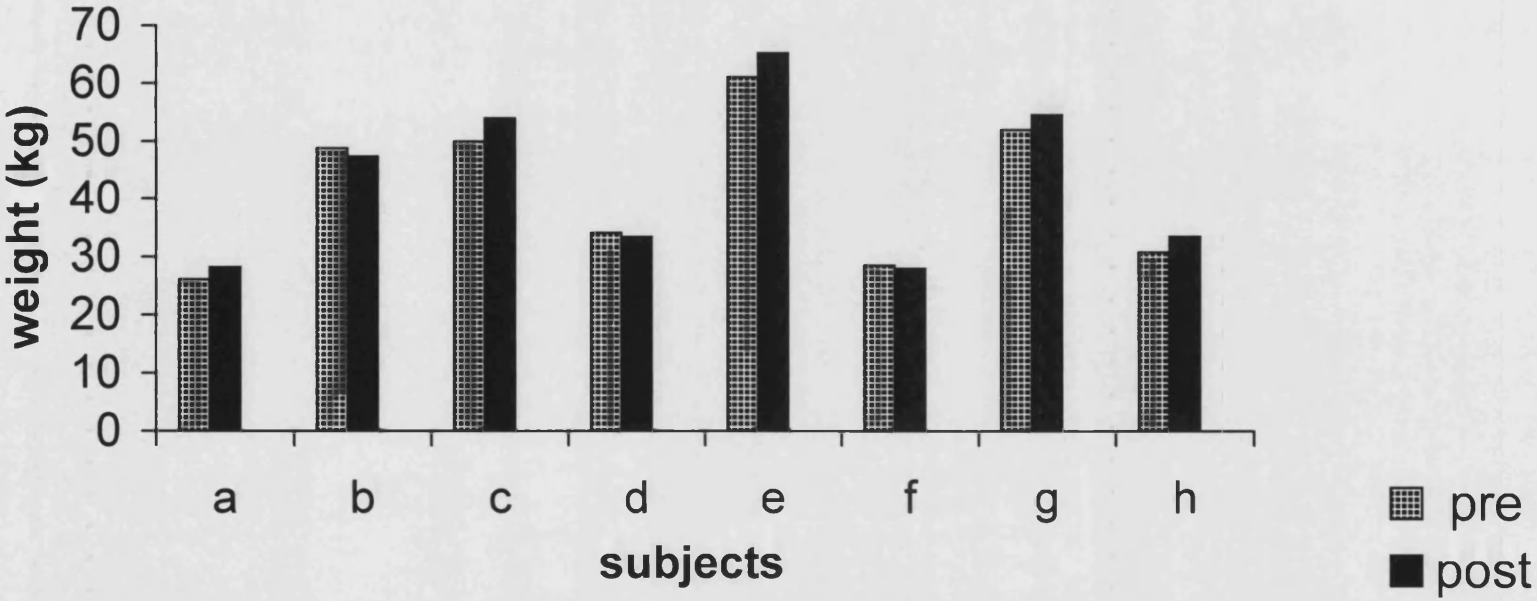


Figure 4.6: Changes in BMI with use of rhGH

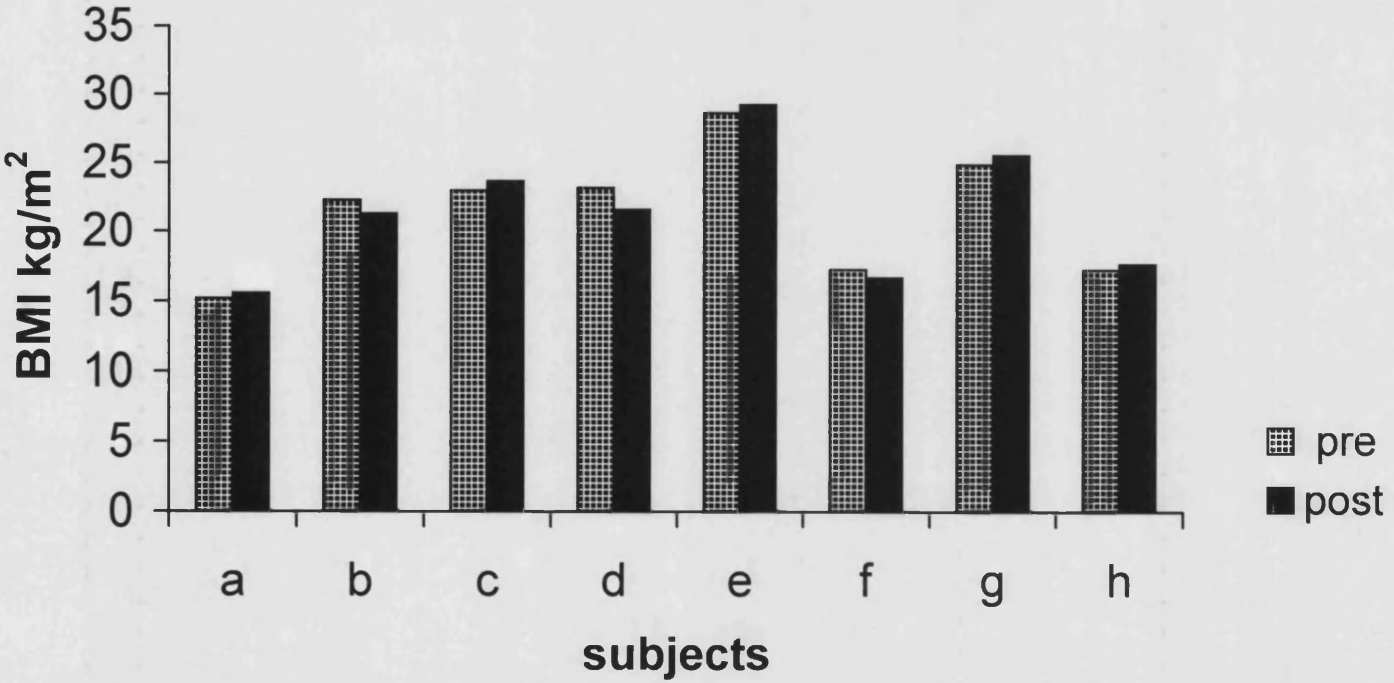


Figure 4.7: Changes in REE with use of rhGH

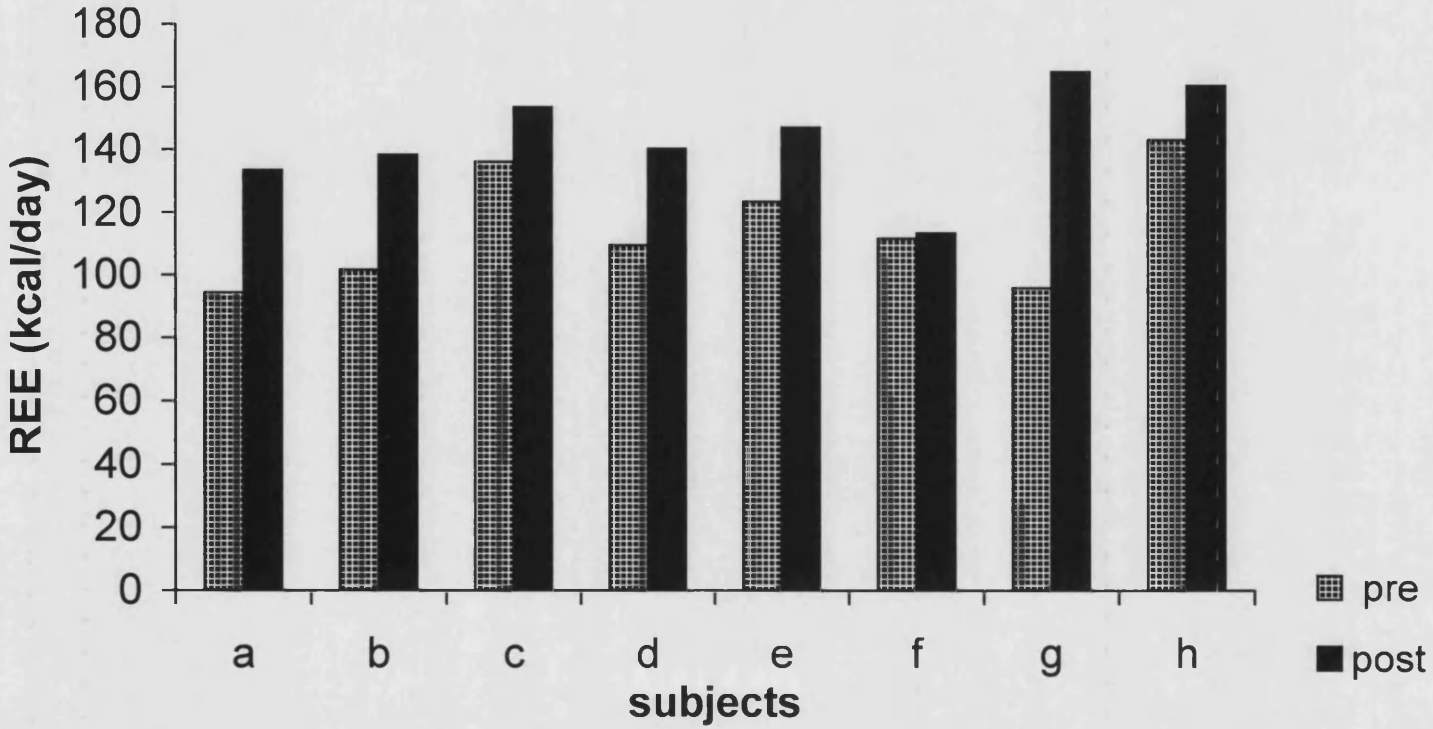


Figure 4.8: Pre and post rhGH flux in patients a-h

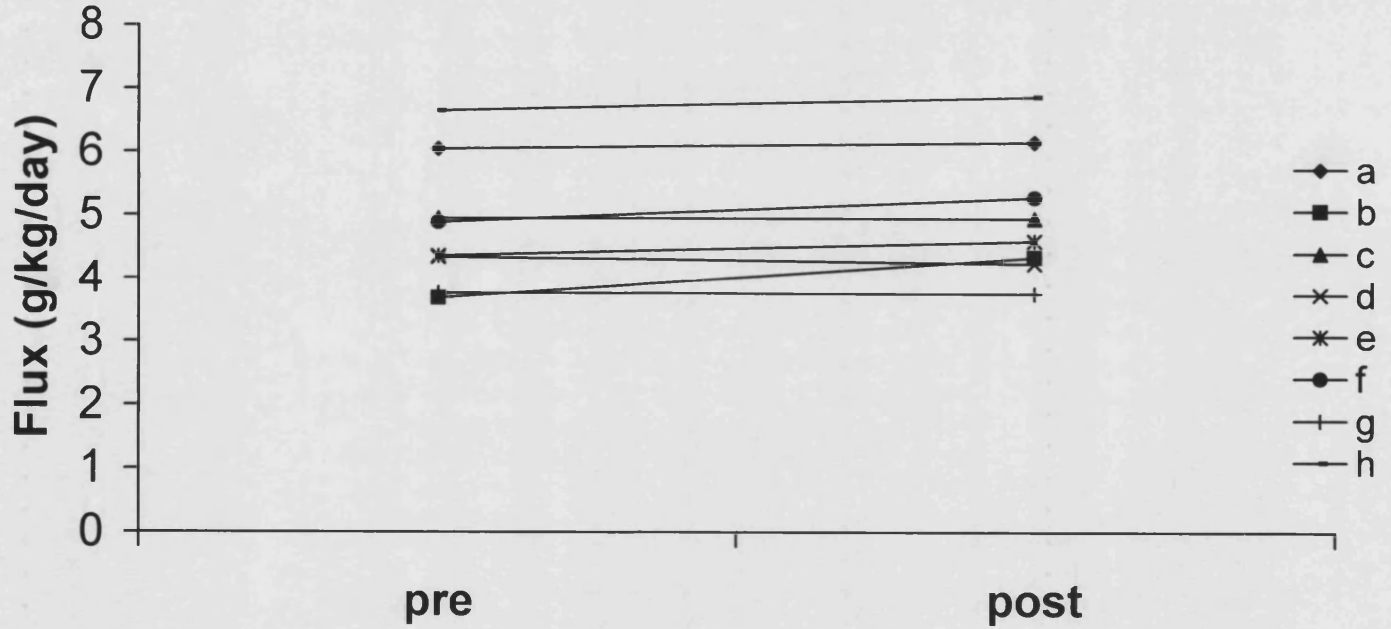


Figure 4.9 Synthesis pre and post rhGH in patients a-h

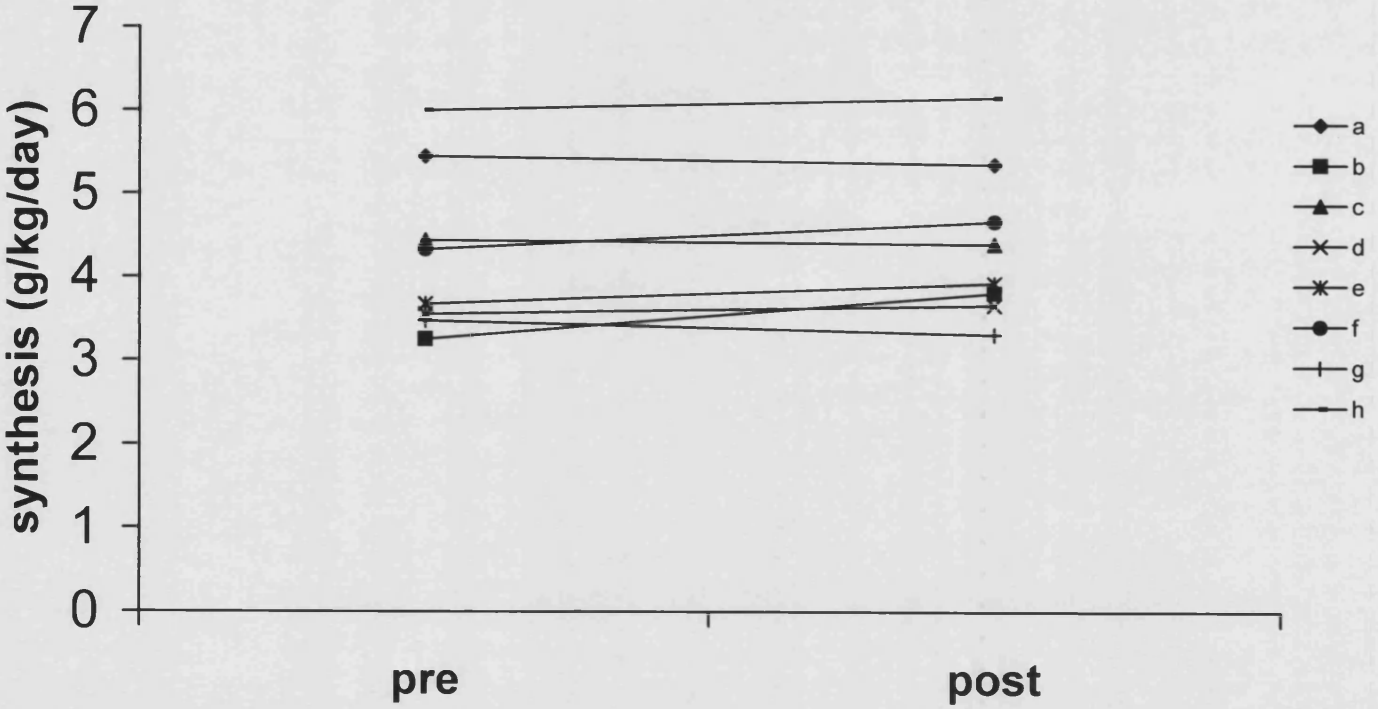


Figure 4.10(i): Endocrine changes pre and post rhGH in patient a

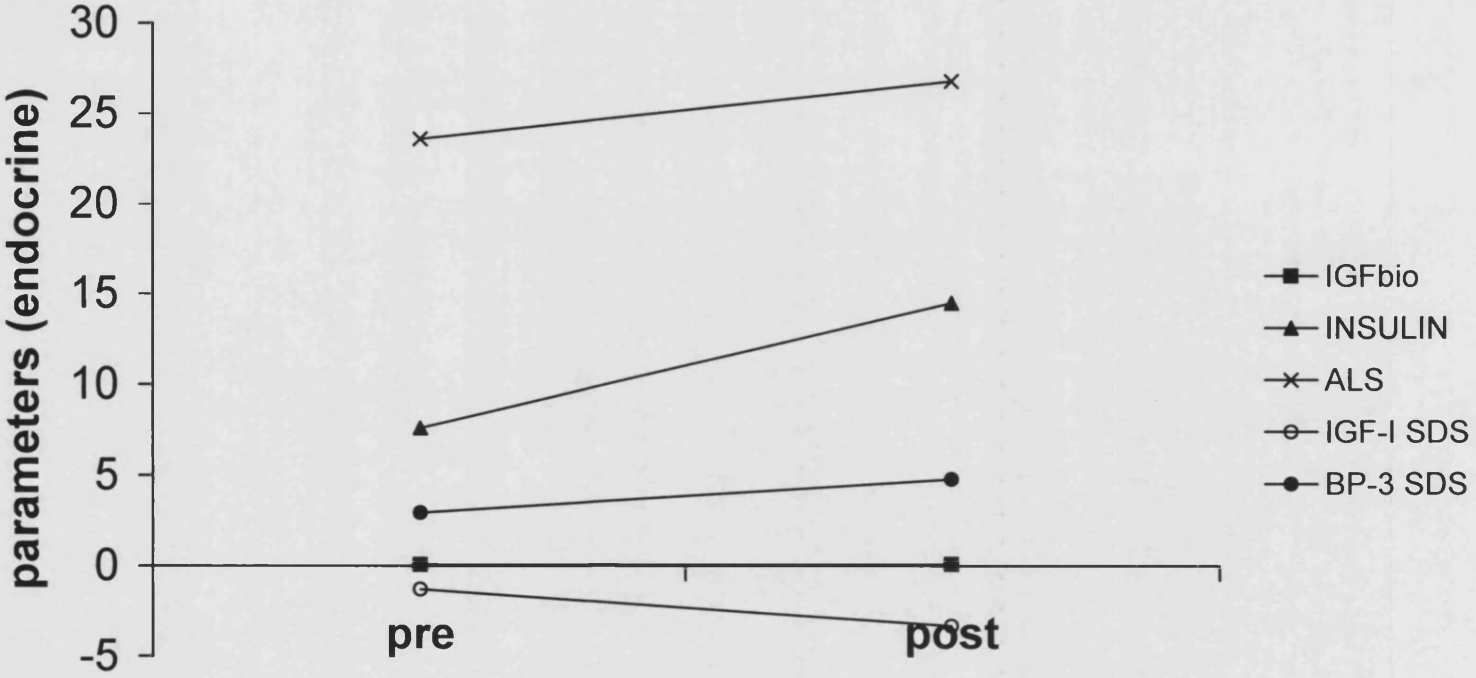


Figure 4.10(ii): Endocrine and leucine changes pre and post rhGH in patient a

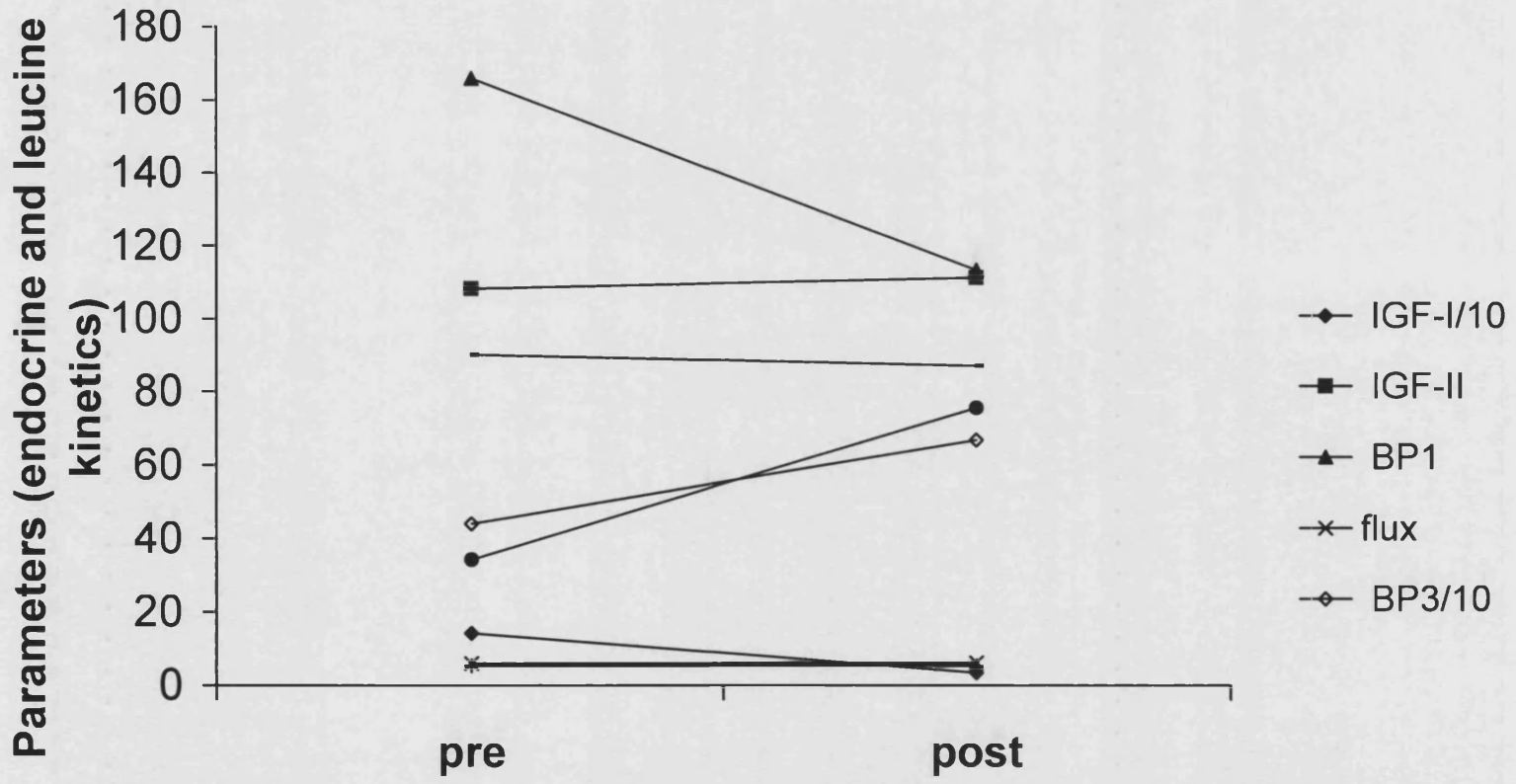


Figure 4.11(i): Endocrine changes pre and post rhGH in patient b

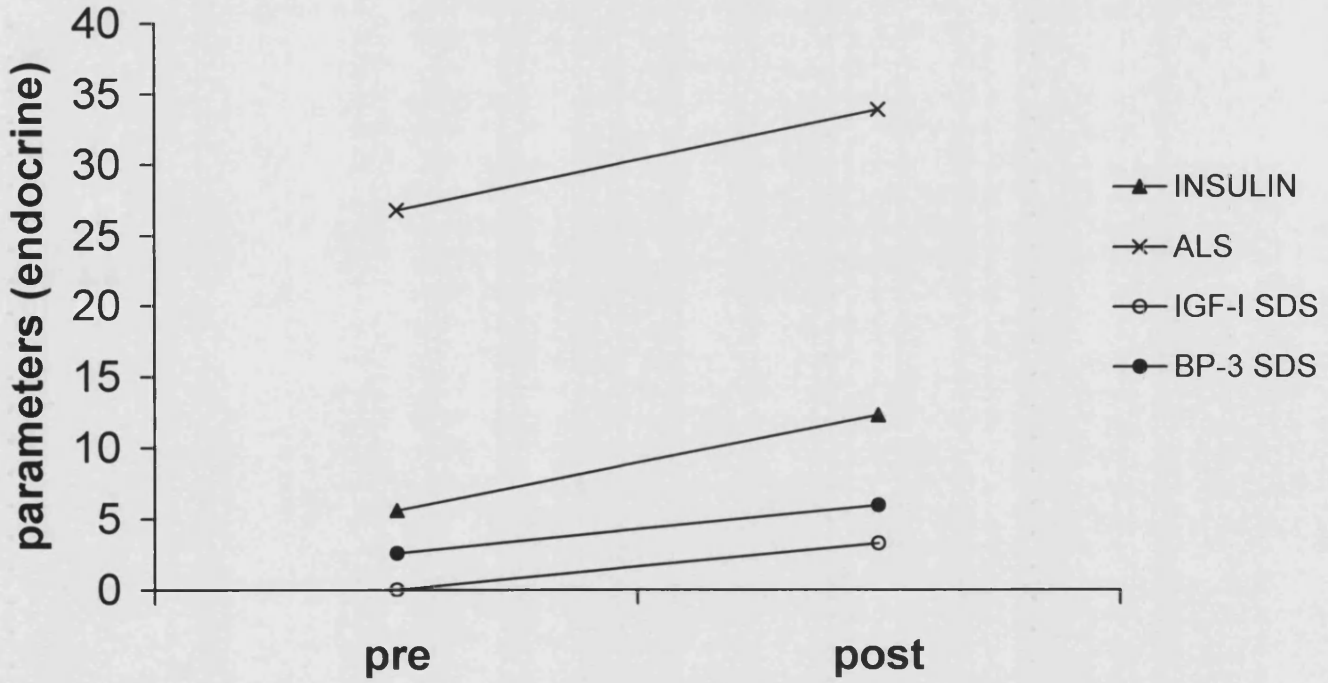


Figure 4.11(ii): Endocrine and leucine changes pre and post rhGH in patient b

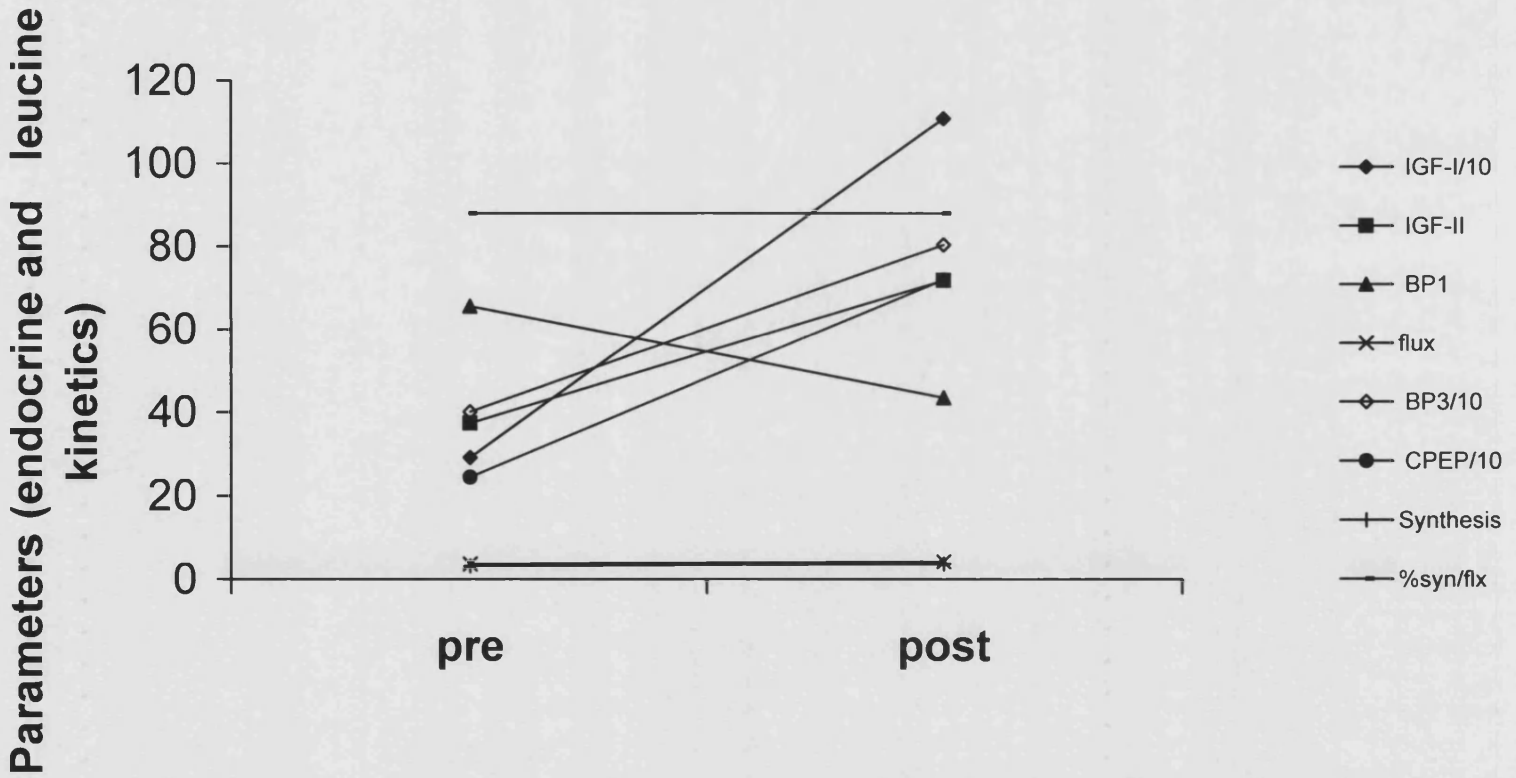


Figure 4.12(i): Endocrine changes pre and post rhGH in patient c

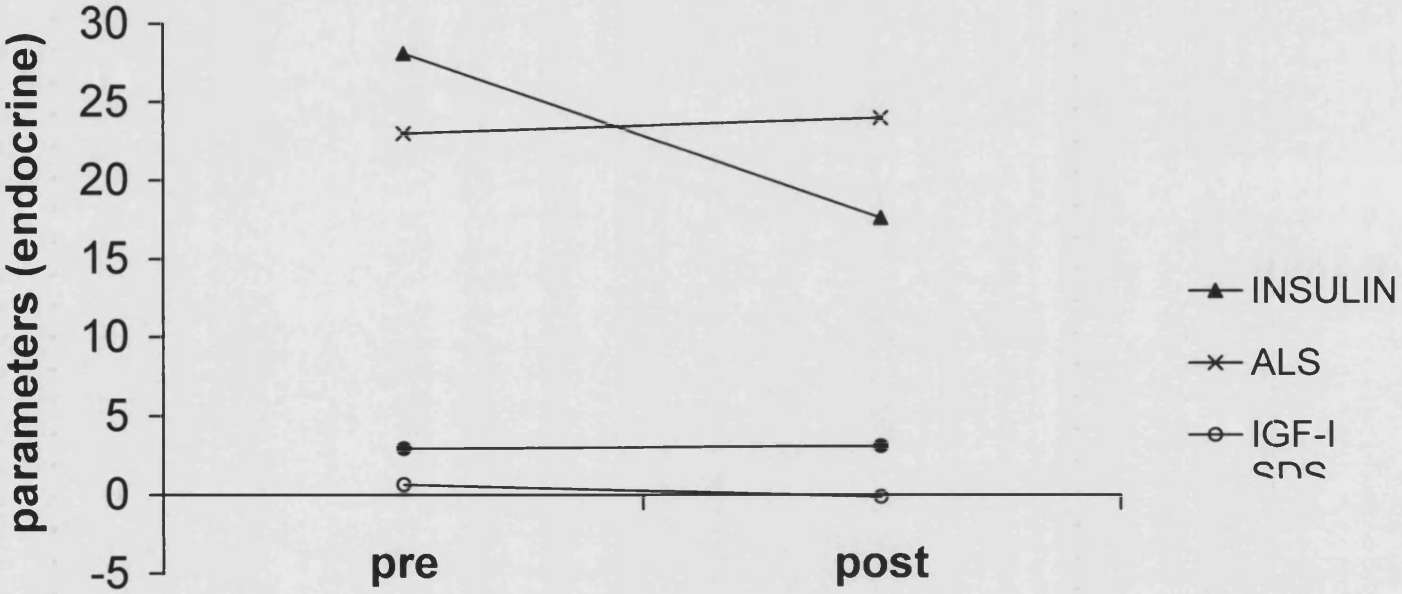


Figure 4.12(ii): Endocrine and leucine changes pre and post rhGH in patient c

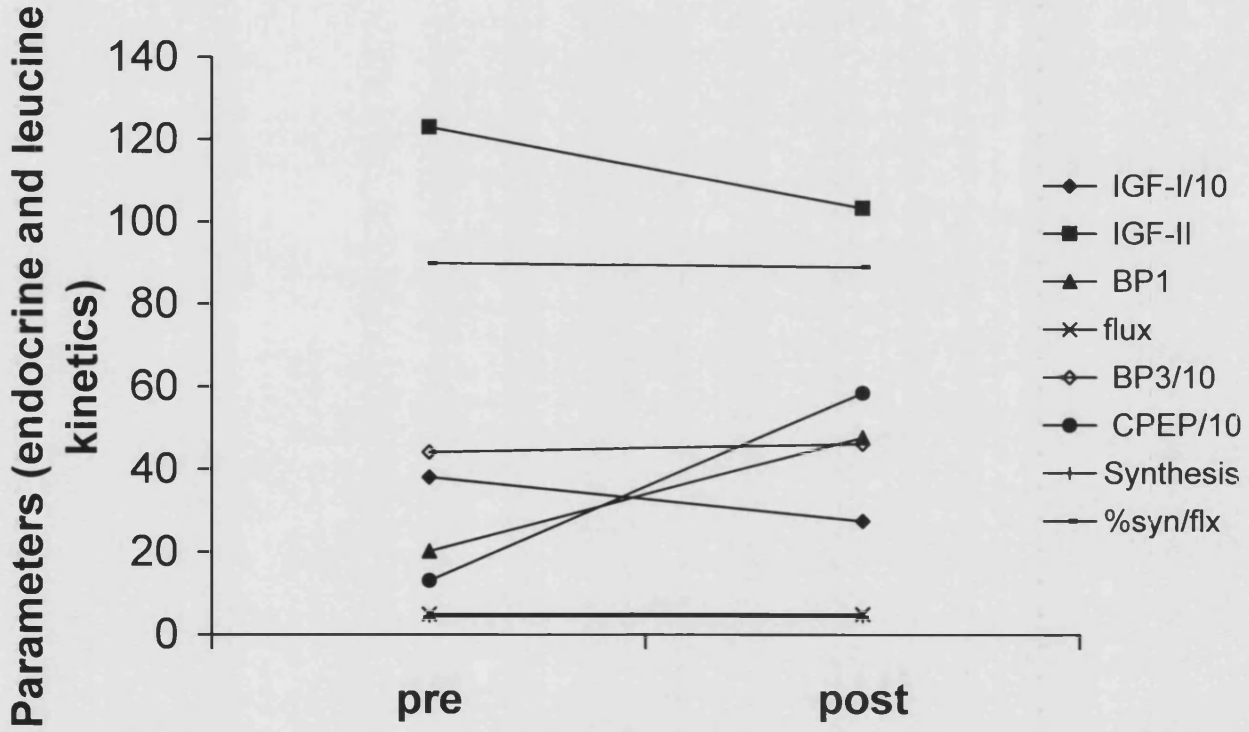


Figure 4.13(i): Endocrine changes pre and post rhGH in patient d

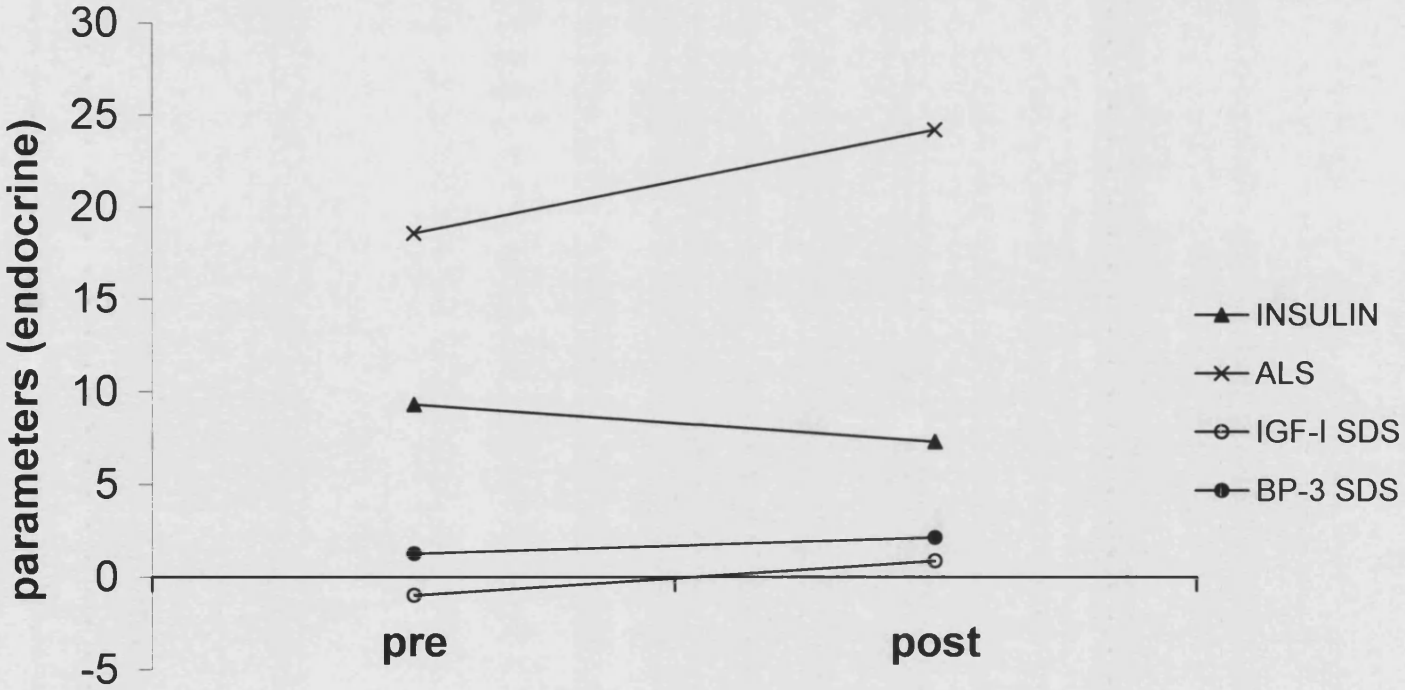


Figure 4.13(ii): Endocrine and leucine changes pre and post rhGH in patient d

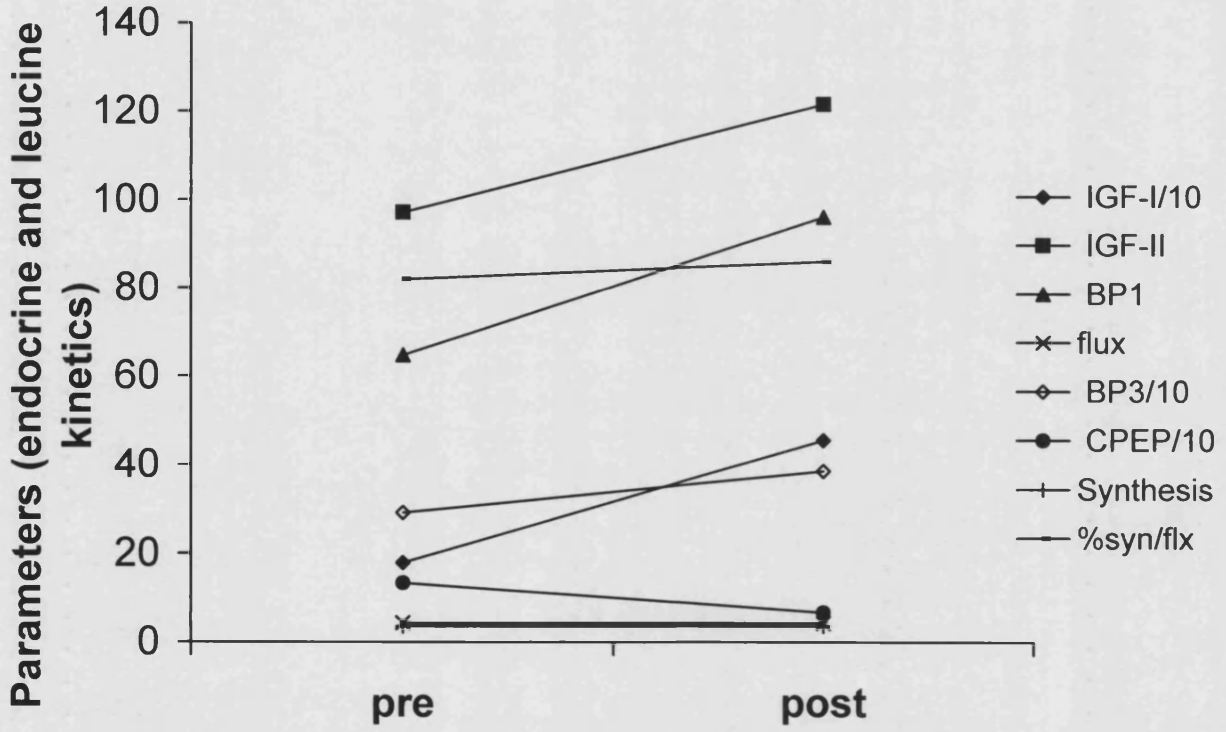


Figure 4.14(i): Endocrine changes pre and post rhGH in patient e

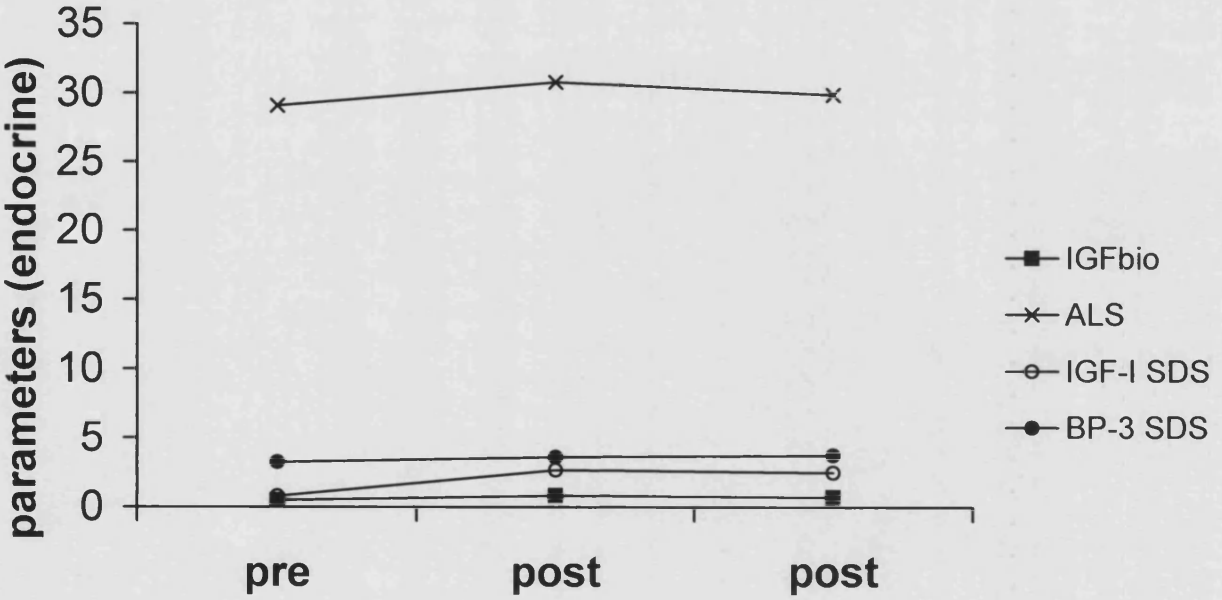


Figure 4.14(ii): Endocrine and leucine changes pre and post rhGH in patient e

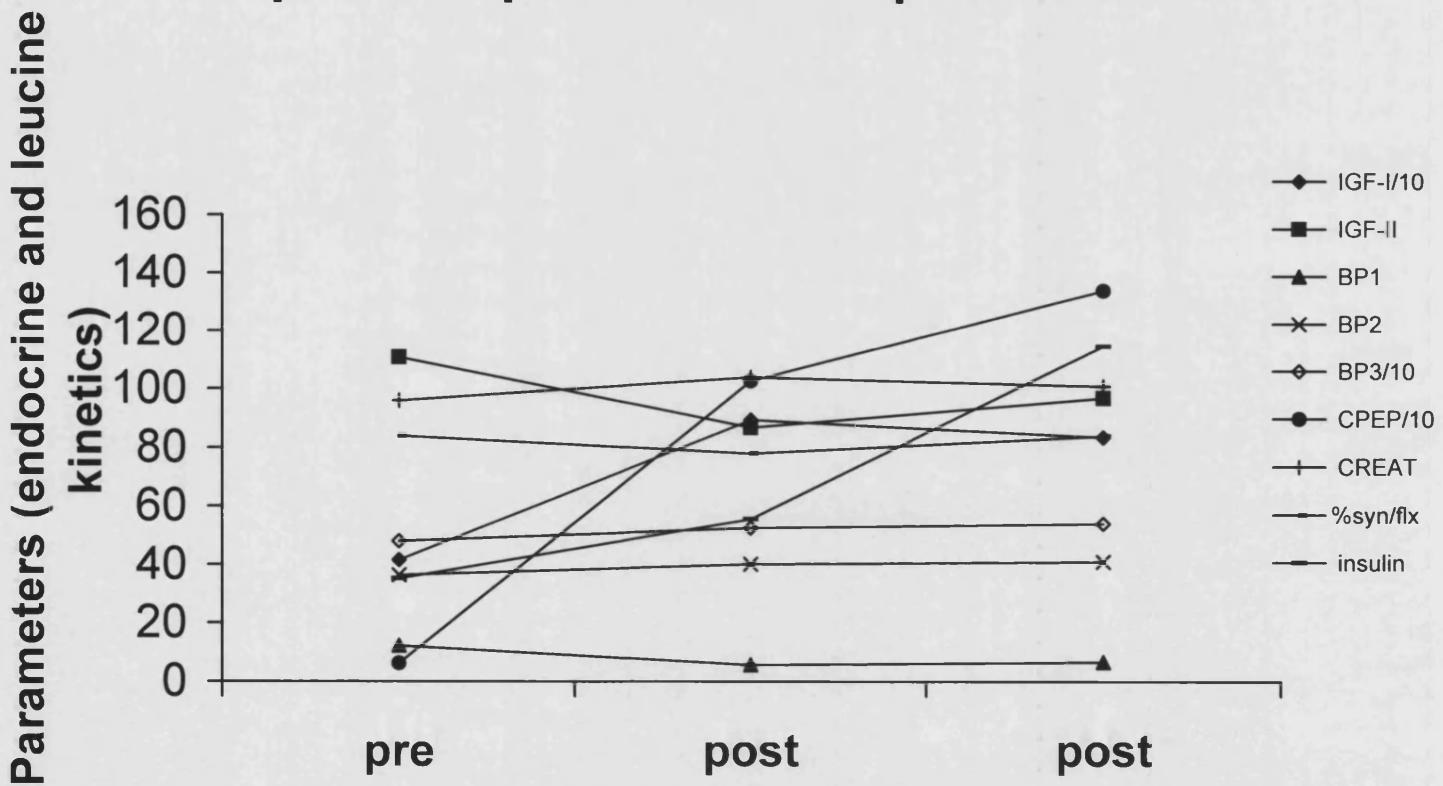


Figure 4.15(i): Endocrine changes pre and post rhGH in patient f

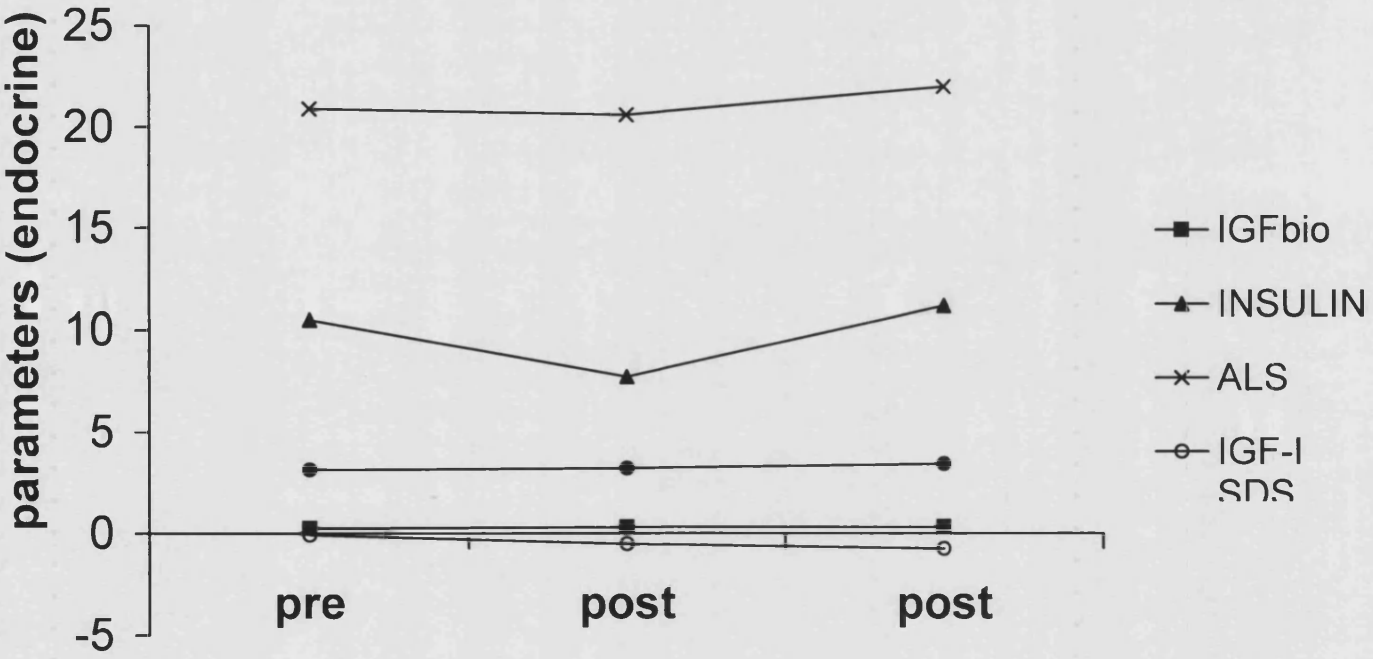


Figure 4.15(ii): Endocrine and leucine changes pre and post rhGH in patient f

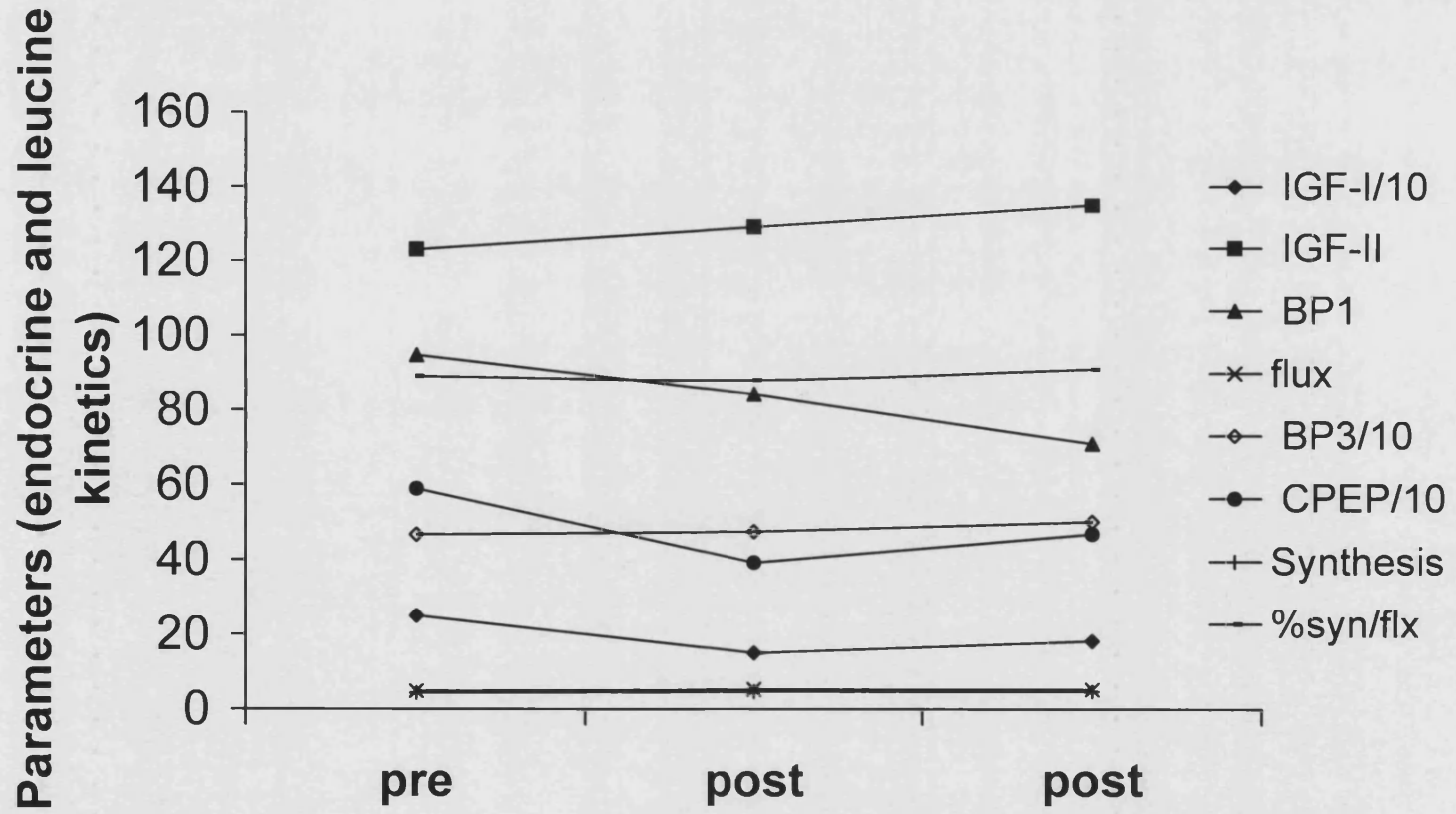


Figure 4.16(i): Endocrine changes pre and post rhGH in patient g

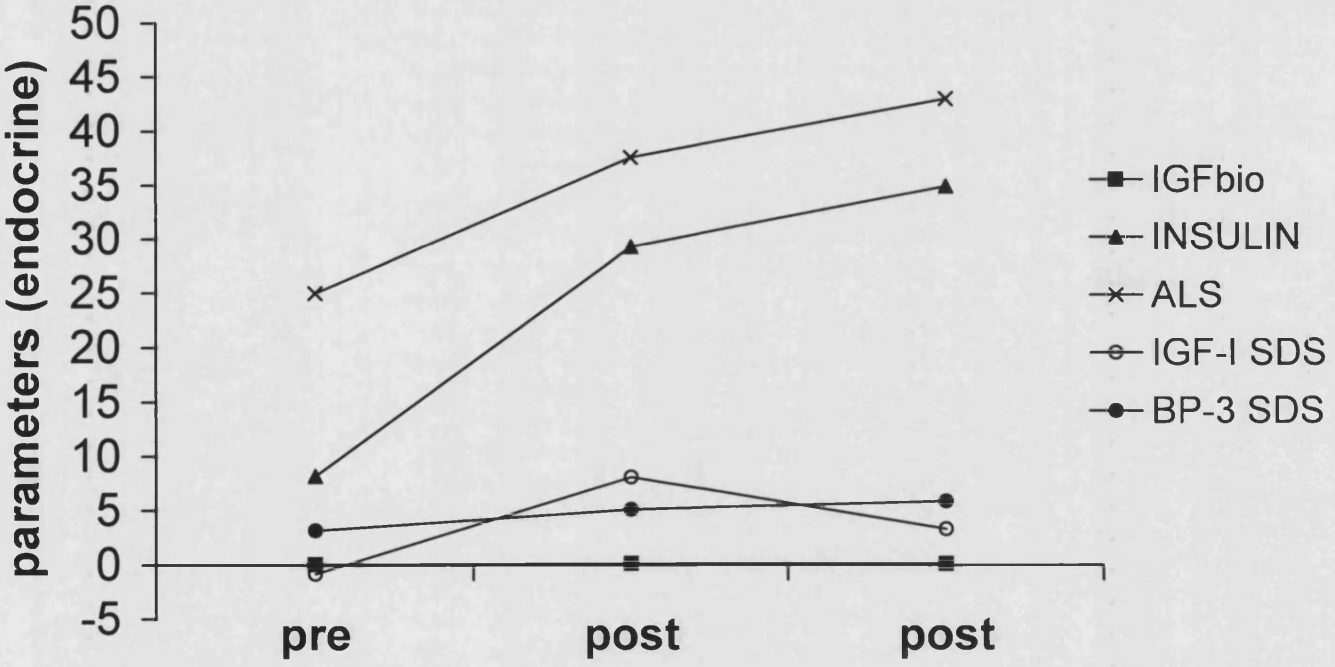


Figure 4.16(ii): Endocrine and leucine changes and post rhGH in patient g

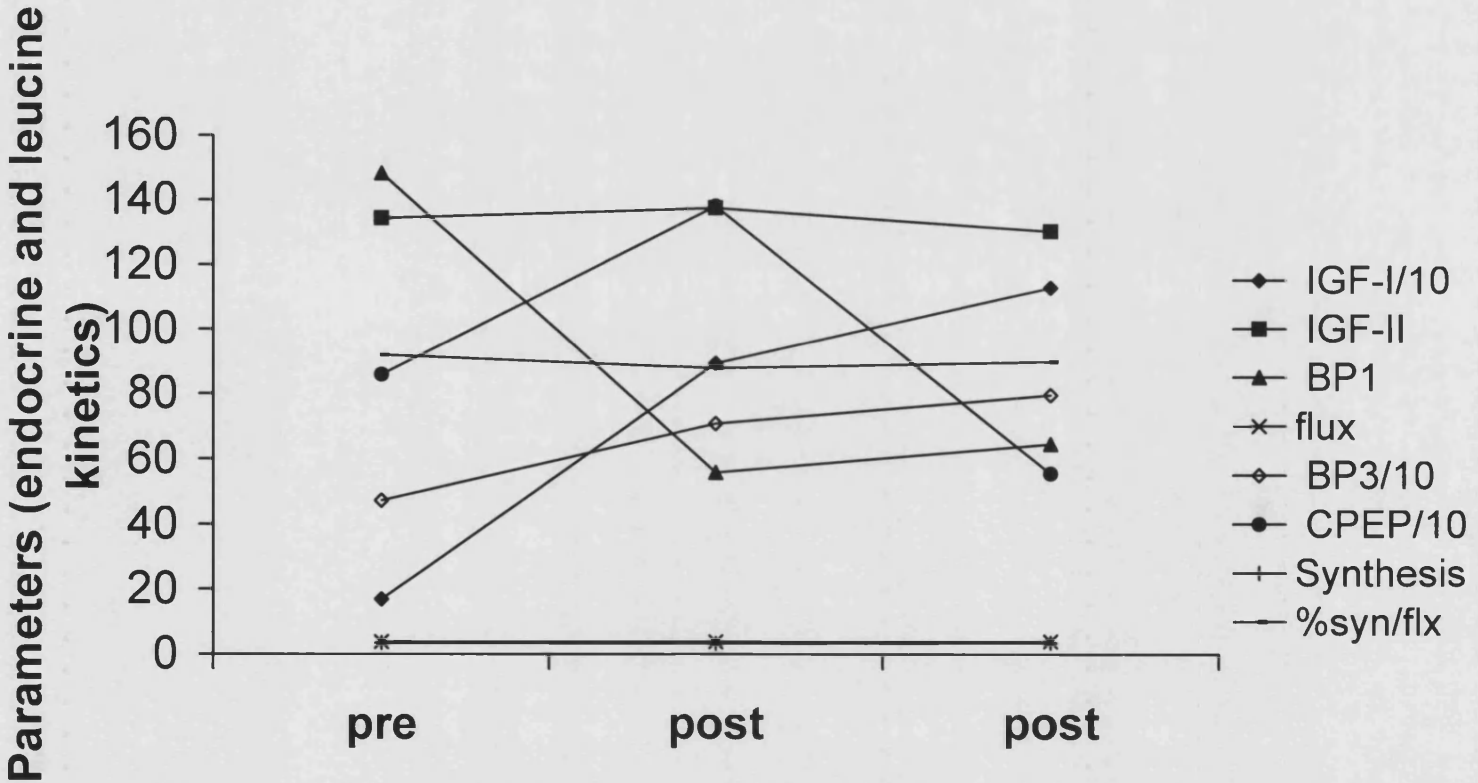


Figure 4.17(i): Endocrine changes pre and post rhGH in patient h

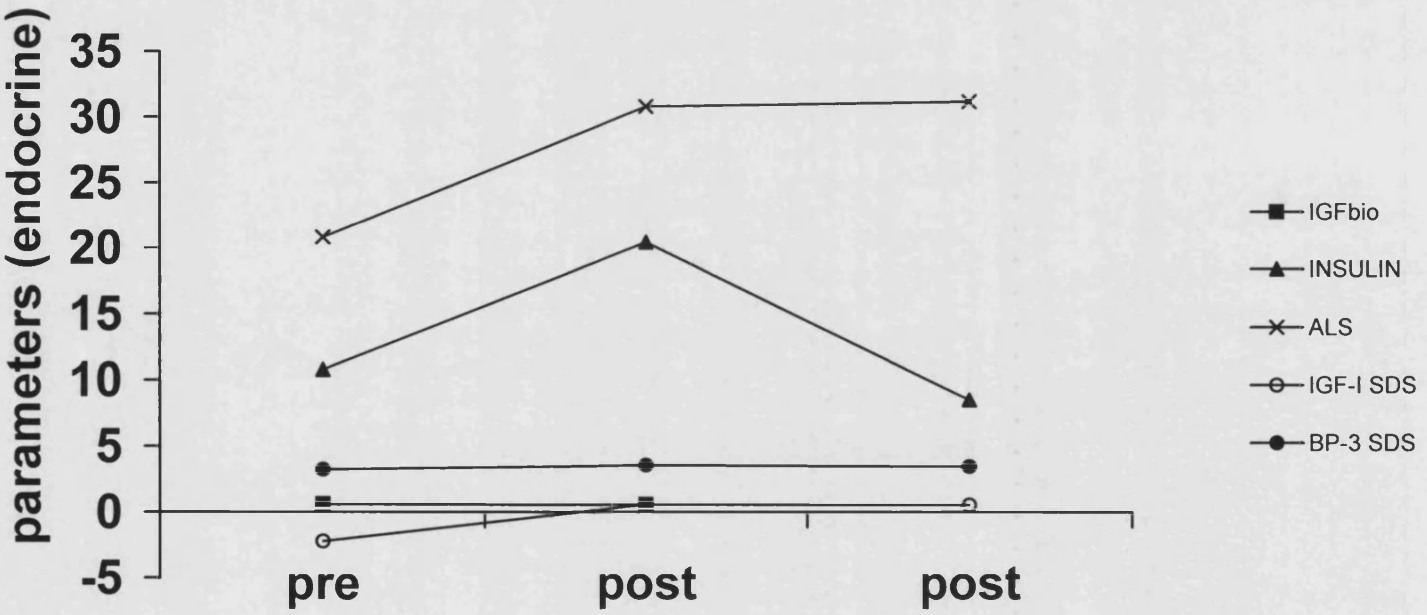


Figure 4.17(ii): Endocrine and leucine changes pre and post rhGH in patient h

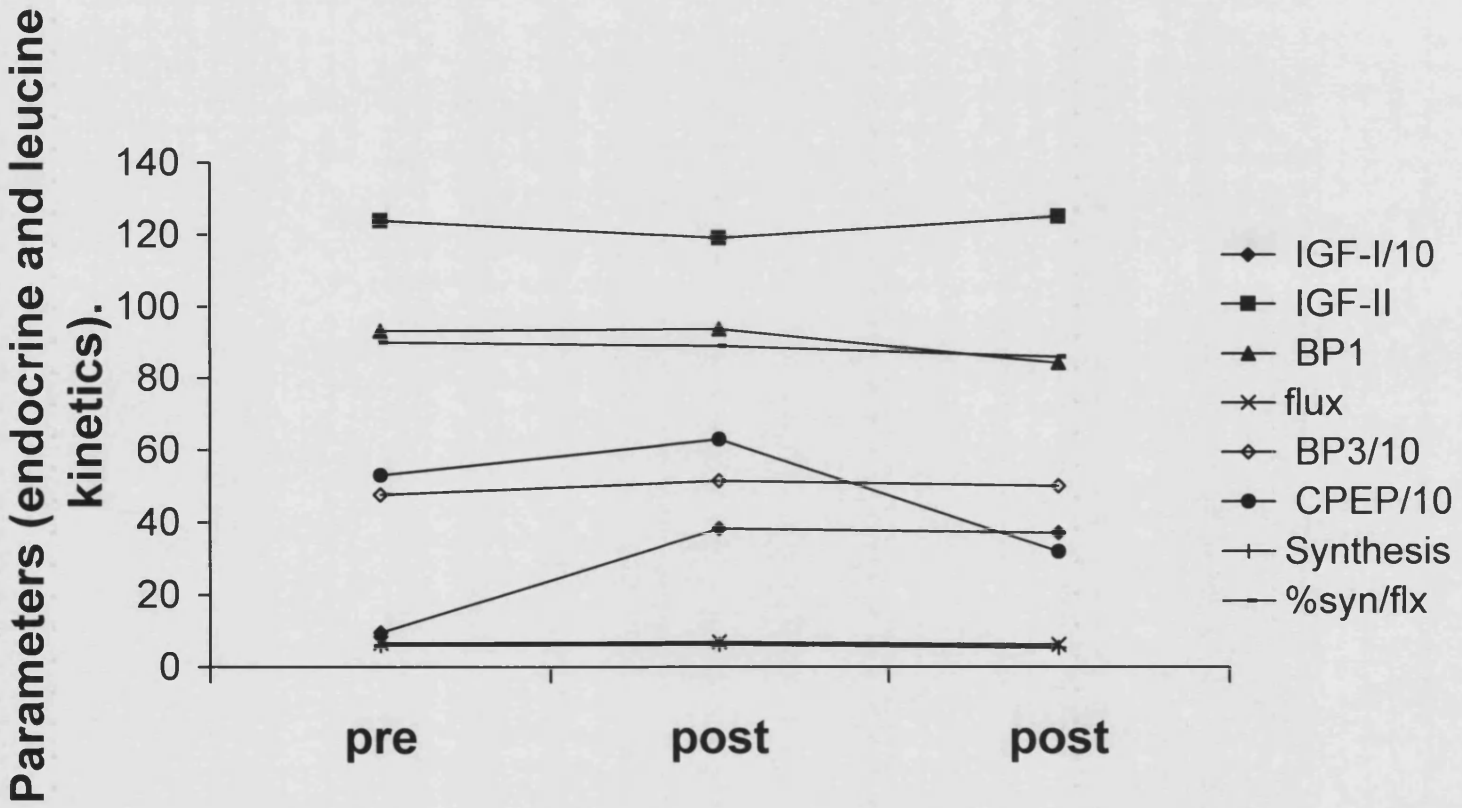


Figure 4.18: Ht SDS Pre and post rhGH (patients a-h)

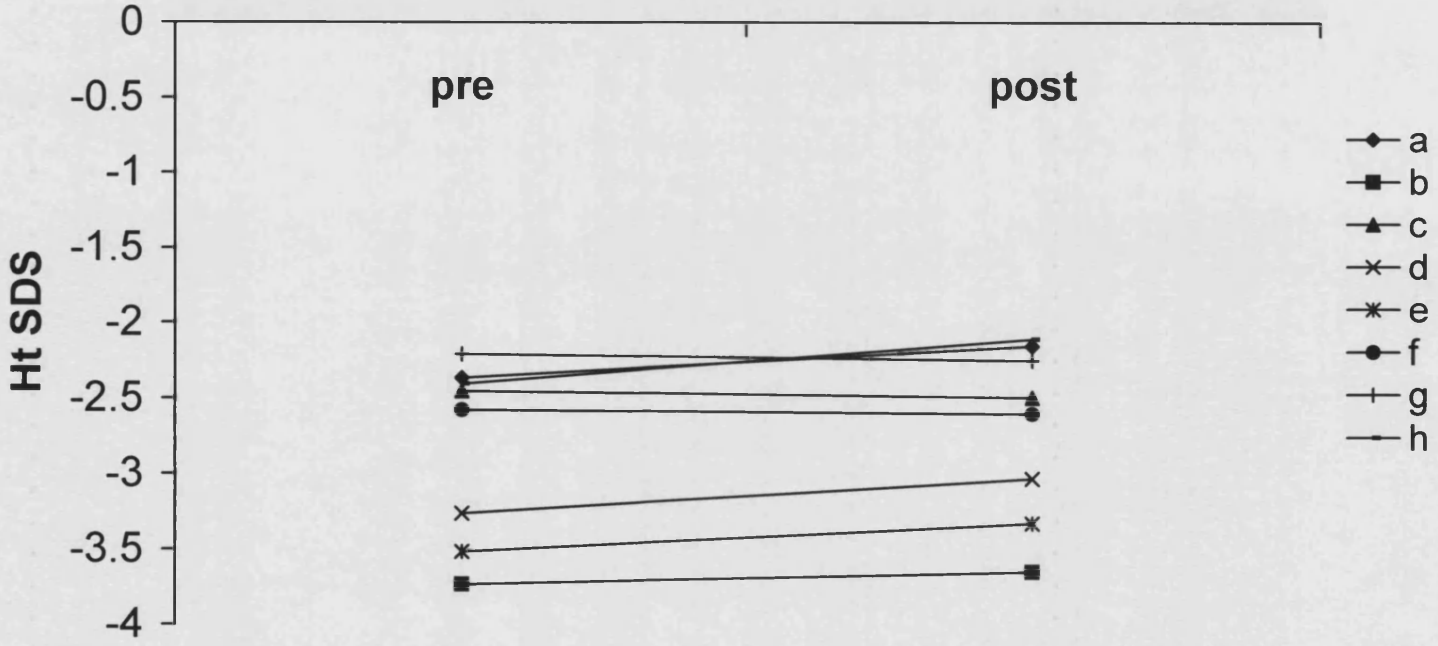
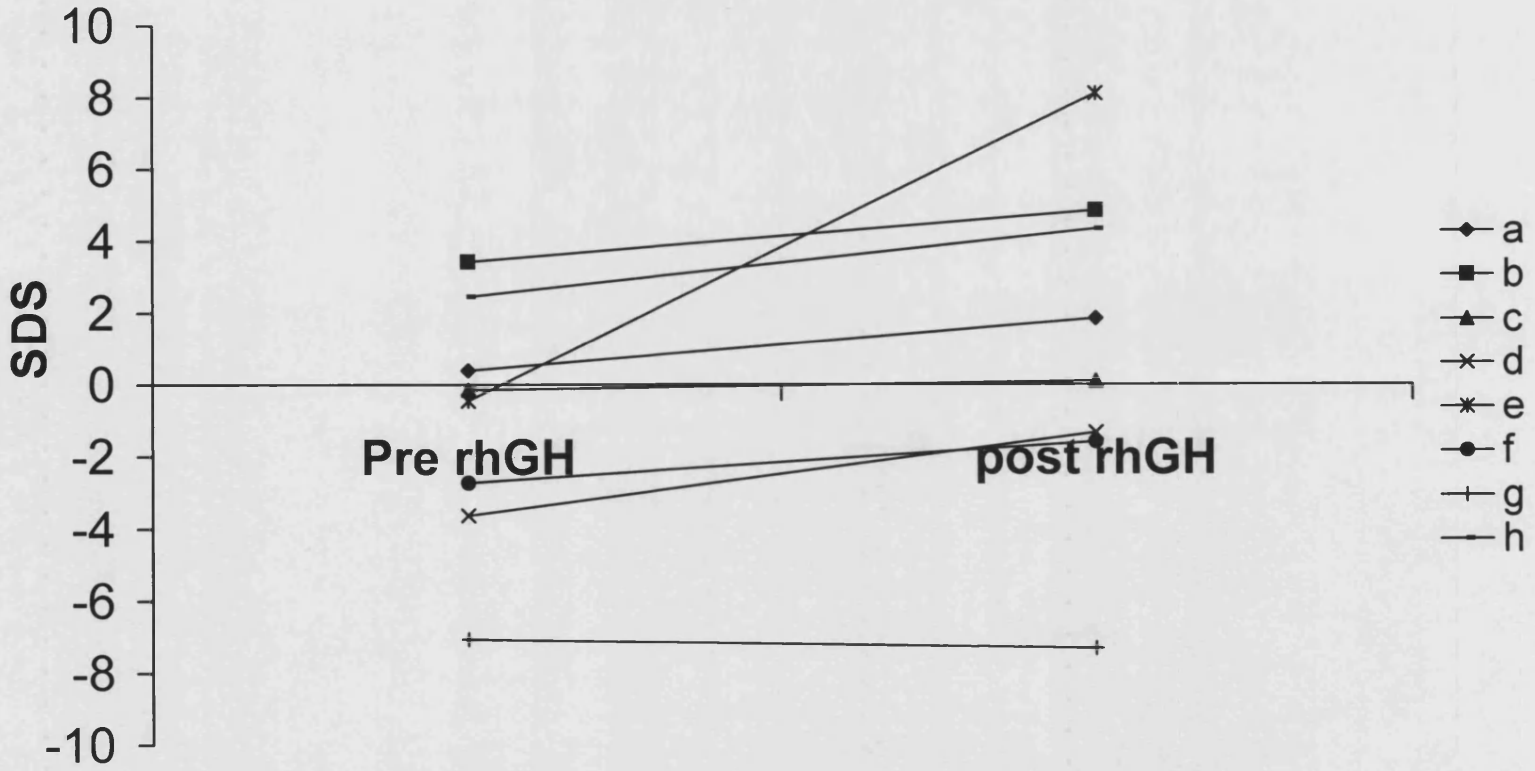


Figure 4.19 Ht Vel SDS pre and post rhGH (patients a-h)



Chapter 5

Final discussion and conclusion

Discussion

5.1 Principal observations

In this study 30% of children with chronic renal failure (CRF) (glomerular filtration rate (GFR) under 40 mls/min/1.73m^2) and 30% of children with a renal transplant, aged between 2 and 17 years had short stature. This is defined as height (Ht) standard deviation score (SDS) ≤ -2 SDS. Short stature was also present in children with end stage renal disease (ESRD) (150) and affected 50% of those recruited to the study. Of the children with CRF, 20% had a delay in their pubertal development, whilst 26% of those with a transplant had pubertal delay. In the ESRD group this figure rose to 30% and dropped to 10% in those with normal GFR. The percentage of all with pubertal delay from the children recruited to the study was 22%.

The reason for the variation in Ht SDS, allowing for genetic and overt environmental factors such as nutrition, is not entirely clear and this study was designed to attempt to determine which parameters of clinical (age, renal status, BMI, pubertal status, aetiology of renal failure, duration of dialysis/ transplant and dose of steroids) and endocrinological origin

(IGF-I, IGF-II, IGF bioactivity, IGFBPs, insulin, ALS) were important in influencing Ht SDS. As data from healthy children were not available for comparison, those with normal renal function but requiring steroids for either nephrotic syndrome or vasculitis were used as the constant in the statistical analysis and potentially this could be a limiting factor in the study (These children were initially recruited to be a control for only the transplant group). However these children were on low dose steroids as maintenance which all but two received on an alternate day basis and this regimen has been associated with minimal systemic side effects including growth suppression (59).

Ht SDS can be particularly influenced by inadequate growth during times of rapid growth i.e. infancy and puberty. The latter is complicated by the variation in age of onset of puberty, particularly with CRF where puberty can be delayed and the growth spurt attenuated. However, the majority of children in the study were either appropriately prepubertal or in early puberty before the growth spurt occurs. Pubertal staging had no relationship with Ht SDS.

Growth retardation is most noticeable in children with congenital renal disease but aetiology of renal disease had no impact on the Ht SDS of

the population studied, perhaps because of optimisation of conservative management during infancy which influences growth positively (30).

BMI, which is normally used more as an assessment of obesity, can also provide information about nutritional status if very low. It however had no association with Ht SDS. The BMI SDS of most of the patients was above
-1 SDS.

The level of renal function (CRF, post renal transplant or receiving dialysis treatment) of the children related negatively to Ht SDS. Requiring dialysis therapy had the worst effect on Ht SDS, but interestingly this relationship became less detrimental when duration of dialysis was taken into account, leaving transplantation as the factor with the most negative influence on Ht SDS. Commencing dialysis improves the quality of life for the patients with terminal CRF, but increased morbidity and growth failure can be noted with prolonged dialysis (40). The relationship of transplantation with Ht SDS became more negative when use of steroid medication was entered into the multiregression analysis. However the dose of steroids used had no effect on Ht SDS, perhaps as the children were on low dose treatment. Steroid treatment has also been reported by other workers to cause growth retardation(97).

Surprisingly, transplantation was the only variable of all the clinical factors studied that had any correlation with Ht Vel SDS and this was positive. As confirmed by other studies, an increase in growth rate is observed with improved renal function following transplantation (32).

Age had a negative association with Ht SDS, even though the majority of children were prepubertal. Nutritional and endocrine abnormalities increase with age, as renal function generally deteriorates and this can lead to growth suppression.

Of all the endocrine variables studied, IGF-II surprisingly, and not IGF-I (SDS), had the strongest relationship with Ht SDS. The latter lost its significance when both IGF-I SDS and IGF-II were added into the best fit analysis equation. IGF-II levels increase with decreasing GFR most probably secondary to accumulation of the IGFBPs which bind the growth factors and decrease the rate of their clearance from the circluation. IGF-II has been shown to have an influence on growth in the antenatal period. In postnatal life it's role is more uncertain but low levels can be present when GH deficiency occurs (105), and in vitro mitotic effects can be observed (109). IGF-I levels can be lowered by IGF-II (121). The children in the study had mean IGF-I SDS that were <1 SDS and would be considered low in a population with elevated GH and IGFBPs (144, 150).

Neither IGF bioactivity nor IGFBP -1, -2 or -3 had a relationship with Ht SDS. IGF bioactivity depends on levels of IGF-I, and –II and those of IGFBP-1, -2, and -3. The range within the different groups was wide and this would weaken any correlation with Ht SDS. It is also important to remember that bioactivity is determined in vitro using cartilage discs and may not be an accurate reflection of in vivo events where different tissues are involved in the growth process.

Accumulation of IGFBPs would distort relationships with growth observed in healthy individuals. In some CRF patients, an association between IGFBP-2 and Ht SDS has been shown (107). This was not present in the children studied here, partly perhaps because only some children had CRF, but also may be because levels are more elevated (comparatively) than in the study mentioned previously . IGFBP-3 SDS was greater than 3 SDS in all three groups, consistent with marked increase in levels. This occurs due to decreased clearance of molecular fragments (125).

Acid-labile subunit (ALS) also related significantly to Ht SDS. ALS stabilises the binary complex formed between IGF and IGFBP-3, thus increasing the half-life of IGFs and thus is involved in growth promotion. ALS deficiency results in decreased growth (172) and lower IGF and IGFBP-3 levels.

Recombinant human growth hormone (rhGH) improves growth in children with CRF and renal transplants (71, 72, 75). Patients with CRF and those on steroid treatment can have reduced lean body mass (LBM) (176, 181, 221). In order to determine whether LBM is reduced due to increased catabolism or decreased synthesis, whole body protein kinetics were determined using stable isotopes of carbon (^{13}C) incorporated into leucine and bicarbonate. Whilst results (table 4.6 and 4.7, appendix 4.1) were comparable to those observed in other studies as shown in table 4.7 (211), protein turnover and resting energy expenditure (REE) appeared to be higher in children with CRF than in those with a transplant (this could be not confirmed statistically due to the small number of patients in each group), despite the higher BMI and use of steroids in the latter group both of which have been associated with increased turnover in normal individuals (220, 223). This indicates that the LBM of the transplanted group may be lower than that of the CRF children. Use of rhGH did not show consistent changes in the children, with increased turnover observed in some children only, although median values of flux, synthesis and oxidation all increased. For protein deposition to occur synthesis has to exceed breakdown (179), but in the present study, catabolism exceeded synthesis whilst anthropometric data revealed that there was a significant increase in LBM. The inconsistency between the two findings highlights the discrepancies that can arise from short term observations made in states such as fasting which are not commonly

encountered in the population being studied but are used in an attempt to standardise studies. Observations carried out over a longer period are therefore more physiological. Fasting in CRF leads to increased catabolism, which is exacerbated by acidosis partly by increased resistance to the actions of anabolic hormones such insulin and GH. TCO_2, a measure of acid-base status in the children, correlated negatively with insulin and leucine incorporation into protein synthesis. Variable resistance to GH, and tolerance to prolonged rhGH use was evident from the inconsistent increases in IGF-I SDS, unlike IGFBP-3 SDS which had risen significantly at 2 months but was slightly lower at 5 months. The rise in LBM was confirmed by the significant increase in REE with use of rhGH, and adipose tissue decreased. The rate of growth also increased significantly with use of rhGH although measurement over the short term can be unreliable.

Interestingly IGF-II, but not IGF-I, was associated significantly with the cross-sectional muscle area, ratio of synthesis to flux, weight and BMI before use of rhGH but not after. Thus IGF-II appears to have a significant role in anthropometric parameters in children with CRF. This has been demonstrated in both the study of the GH-IGF axis and that of protein turnover in children with CRF.

5.2 Therapeutic possibilities for the management of the growth of children with CRF and renal transplants

5.2.1 Titrated dose of rhGH

Resistance to the actions of hormones in CRF has been clearly documented by other studies (137, 138). The degree of this appears to vary in patients and thus alters the response to rhGH. Whilst changes in height, body fat and LBM are reliable indications of the efficacy of rhGH treatment in the long term, measuring IGF-I, IGF-II and IGFBP-3 levels in combination (105, 148, 219) might provide more immediate information about the appropriateness of the rhGH dose being used. The dose of rhGH could then be adjusted to produce the desired response in children. However the majority of children with CRF and renal transplants show a good response to the recommended dose of 4 iu/m^2/day over a prolonged period (72). Careful work is required to ensure that any increase in dosage would not be associated with either additional adverse effects, or increased frequency of those recognised at present (slipped femoral epiphyses, hypothyroidism, benign intracranial hypertension).

5.2.2 Recombinant human IGF-I (rhIGF-I)

IGF-I is produced in response to GH stimulation and mediates the majority of its actions (88). Recombinant IGF-I is used in patients with severe GH resistance, either due to receptor insensitivity or deficiency (5) and improves growth in these individuals. It has also been shown to be effective in animal models with CRF (236). In normal adults IGF-I appears to improve renal function and size (244), reduces insulin levels by direct action on the pancreas (245) and in uremic animals reduces cholesterol levels (236).

In children with Laron Syndrome (LS) IGF-I treatment reduces excess adipose tissue, lowers serum cholesterol, reduces serum lipoprotein (a), increases GFR, induces head growth and increases linear velocity (5).

Isolated use of rhIGF-I in infants with LS is less efficacious than rhGH in patients with normal GH receptors (5) as the contribution from the direct action of GH on epiphyseal cartilage progenitor cells is absent (99).

Use of rhIGF-I is limited by cost and concerns about side effects including suppression of thyroid stimulating hormone (5). Dose related effects include papilloedema (5), jaw pain, tachycardia and gingivial hypertrophy

in patients with ESRF (246). Hypoglycaemia in response to IGF-I therapy (245) has not been observed in all studies (246, 247)

At present insufficient information is available to recommend its usage in children with CRF.

5.2.3 Combined rhGH and rhIGF-I

The combination of GH and IGF-I in animal studies has a greater effect on weight gain and growth than either agent on its own (236, 248). Both agents are administered by injection and therefore hypothetically could be administered together.

GH increases (219), but IGF-I decreases (246) levels of IGFBP-3, the major carrier protein of IGF-I with an important role in increasing the half-life of IGF-I (and IGF-II) by decreasing clearance from the circulation (100). Combined administration in CRF may thus be beneficial in that resistance to the action of GH is overcome by administration of IGF-I, but levels of intact IGFBP-3 are increased (248) and it may be possible to use lower doses of each so decreasing the risk of potential side effects .

The major benefit of combined GH and IGF-I treatment in patients with CRF would potentially be of their differential effects on insulin and lipoprotein (Lp) a levels, both of which are raised with GH treatment (81,

249), but lowered by IGF-I administration (249). Elevated levels of insulin and Lp (a) are associated with increased atherosclerosis (250), and cardiovascular disease is the commonest cause of morbidity in CRF (251).

5.2.4 Oral GH secretagogues

In children, one of the difficulties with rhGH treatment is that it has to be administered by injection. Oral agents such as MK-677 that stimulate GH release are effective in adults in increasing serum GH concentrations, IGF-I levels, fat free mass and energy expenditure (252, 253). In short children GH secretagogues increase IGF-I levels and whilst they have a synergistic action with GH releasing hormone (GHRH), they are partially refractory to inhibitory influences that abolish the actions of GHRH (254). Data on long term studies using these secretagogues have not yet been published.

Currently rhGH is administered by a single nocturnal injection. However, endogenous GH secretion is pulsatile and frequent and there is some evidence that the rapidity with which GH levels increase may modulate its response (80). In animals intermittent infusion is more effective than continuous infusion (255). Theoretically rhGH can be administered in a pulsatile manner but this would entail repeated injections, or boluses of an infusion under manual operation as no device is available (to the best

of the author's knowledge) that can be programmed to administer boluses. Using variable release preparations of oral secretagogues, it maybe possible to manipulate medication to mimic pulsatility of endogenous GH.

5.2.5 IGFBP inhibitors

Elevation of insulin-like growth factor binding proteins (IGFBPs) in CRF leads to inhibition of IGF activity as the affinity of the IGFBPs for IGF-I and –II is greater than that of their receptors (88). Analogues of recombinant human IGF-I that bind to the IGFBPs, but do not stimulate IGF receptors, in animals models without renal failure, improve bone growth and weight gain by displacement of bound endogenous IGF-I particularly when given in combination with IGF-I (256). Non-peptide molecules based on peptide structures that will specifically bind to only one IGFBP (257) and thus displace IGF-I are being considered, and, if orally active, will make administration much easier(256).

5.2.6 Protein intake

Even with adequate caloric intake, LBM in patients with CRF can be reduced (181). Studies have demonstrated that in CRF and ESRD

patients, excessive (178) or insufficient protein intake (187) leads to inefficient protein turnover with reduced synthesis. Protein restriction, particularly with advanced CRF, reduces urea production and may slow progression of nephron loss (177). In children protein intake, especially of essential amino acids, has to be closely monitored to enable growth, and it maybe possible to optimise protein intake by using techniques such as DEXA, that provide information on body composition (258), to enable accurate assessment of LBM on various protein intakes.

5.2.7 Exercise

Children with CRF and renal transplants probably have lower exercise tolerance than their healthy peers due to reduced lean body mass and complications such as anaemia and acidosis. However exercise increases endogenous GH secretion (259), IGF-I levels (260), and LBM (261), which in turn improves BMD (262). In renal patients, individually tailored exercise programs maybe of benefit.

5.2.8 Nutrition

The importance of nutrition in growth is now universally accepted, particularly in infants (30, 38). Strict conservative management with close attention to diet and energy supplements has been effective in preventing decreases in Ht SDS (79). Use of overnight nasogastric tube feeds or percutaneous gastrostomy feeds if necessary are widely implemented to ensure adequate caloric intake (79).

In older patients, especially adults, inadequate nutrition is associated with increased morbidity and mortality (153).

5.3 Final Conclusion

Short stature with a Ht SDS ≤ -2 SDS can be observed in children with CRF, post renal transplantation or those with ESRD on dialysis treatment. Many factors, both clinical and endocrinological, influence Ht SDS. IGF-II was the single most important variable as determined by multiregression analysis. Modality of renal treatment, age, duration of dialysis treatment and ALS were also significant. Surprisingly IGF-I (SDS) and IGFBPs had

no significance. The value of rhGH therapy in improving the growth of short children with CRF and renal transplants is widely acknowledged. Increase in lean body mass is an important consequence of rhGH therapy despite the lack of significant alterations in protein kinetics.

Appendices

Appendix 2.1 Clinical and laboratory features of children with CRF

Patient	Gender	Age (yrs)	Pubertal status	Diagnosis	BMI* (kg/m2)	GFR	TCO2 (mmol/l)	BMISDS	Height (cm)	Weight (cm)	Ht SDS	Ht Vel SDS	Ht Vel (cm/yr)	Creat (umol/l)	albumin (g/l)
1c	f	10.4	1	c	17(50th)	34	23	-0.23	135.2	30.4	-0.68	-0.76	6.5	97	44
2c	m	12.0	2	c+vur	15(9th)	17	na	-1.32	144.2	31.7	-0.80	-0.04	5.4	na	45
3c	m	14.0	3	alports	20(75-91st)	10	na	0.43	163.9	52.9	0.13	-1.69	7.1	616	38
4c	m	11.0	1	c+vur	17(50-75th)	13	20	-0.06	124.0	25.8	-2.99	-2.47	3.3	823	43
5c	m	17.0	3	puv	18(25-50th)	25	25	-1.46	142.5	35.8	-4.48	7.60	5.9	706	41
6c	f	12.5	1	c	28(98-99.6th)	16	22	2.42	138.1	52.5	-2.09	-2.99	5.2	598	46
7c	f	8.8	1	acq	14(2-9th)	20	22	-1.07	112.7	18.4	0.03	-3.86	6.0	167	40
8c	m	12.5	2	c	17(25-50th)	18	21	-0.23	133.5	30.8	-2.49	2.46	na	191	45
9c	m	14.8	3	puv	22(91-98th)	34	18	1.32	147.6	50.0	-2.44	-0.15	8.4	323	42
10c	m	11.7	1	vaters	17(50th)	12	23	-0.13	129.0	28.3	-2.62	-2.20	5.1	542	42
11c	m	7.5	1	cyst	15(9-25th)	34	25	-0.54	95.2	13.5	-5.65	-0.64	4.6	119	33
12c	m	12	1	arpkd	15(9th)	22	21	-1.41	131.4	26.1	-2.35	0.39	6.8	325	43
13c	m	17.0	5	c+vur	23(91st)	20	17	0.79	172.4	67.4	-0.45	10.86	8.3	503	42
14c	m	13.5	4	unknown	16(9th)	28	na	-1.30	152.1	37.1	-1.07	-4.10	4.5	476	40
15c	m	11.5	1	c	15(9-25th)	29	22	-1.38	139.8	29.2	-0.82	1.48	6.0	289	44
16c	f	10.3	1	c	15(9-25th)	16	23	-0.84	134.4	27.9	-0.82	-0.75	4.8	474	44

for key to diagnosis refer to list of abbreviations *centile GFR-mls/min/1.73m2

Appendix 2.1 (cont) Clinical and laboratory features of children with CRF

Patient	Gender	Age (yrs)	Pubertal status	Diagnosis	BMI* (kg/m2)	BMISDS	Height (cm)	Weight (cm)	Ht SDS	Ht Vel SDS	Ht Vel (cm/yr)	Creat (umol/l)	albumin (g/l)
17c	m	13.3	2	wilms	17(25-50th)	-0.49	150.1	38.9	-0.77	-3.47	9.3	369	37
18c	m	14.3	3	fsgs	20(75-91st)	0.36	157.0	48.6	-1.00	-3.61	3.0	810	44
19c	m	15.0	3	c+vur	17(25-50th)	-1.10	149.5	38.4	-1.65	0.56	10.0	312	44
20c	f	13.8	4	fsgs	19(50-75th)	-0.22	167.2	52.4	1.11	-1.54	0.8	617	42
21c	m	11.5	1	arpkd	25(99.6th)	2.31	140.8	48.6	-0.77	2.05	6.4	531	44
22c	m	9.5	1	acq	13(0.4th)	-2.36	123.1	20.0	-2.74	2.69	7.2	216	43
23c	f	13.0	4	c+vur	22(91-98th)	1.23	159.6	57.1	0.51	0.09	5.2	153	35
24c	m	16.3	4	c+vur	21(75-91st)	0.44	165.0	57.8	-1.16	2.20	4.2	184	38
25c	m	3.3	1	arpkd	15(9th)	-0.59	93.2	13.3	-2.24	-3.49	2.7	125	35
26c	m	4.50	1	arpkd	16(50th)	0.11	97.9	15.1	-1.90	-0.98	8.4	na	30
27c	m	4.0	1	puv	17(75-91st)	0.78	97.4	15.9	-1.46	-1.89	4.9	212	39
28c	f	13.0	1	c+vur	25(98th)	1.79	146.0	52.6	-1.23	-4.14	3.4	185	42
29c	m	7.0	1	puv	19(98th)	2.03	119.7	27.8	0.08	-3.86	6.3	461	41
30c	m	15.5	4	c	19(50-75th)	-0.42	175.5	57.7	0.51	na	9.0	228	42
31c	m	12.3	2	c+vur	20(91st)	0.91	153.5	46.6	0.51	0.60	4.9	167	42

for key to diagnosis refer to list of abbreviations *centile GFR-mls/min/1.73m2

Appendix 2.2 Clinical and laboratory features of children with renal transplant

Patient	Gender	Age	Pubertal Status	Diagnosis	BMI* (kg/m2)	BMISDS	GFR	TCO2 (mmol/l)
1t	m	10.3	1	c+vur	25.6(99.6)	2.71	81	20
2t	m	16.3	1	oto-brach	19.9(25-50)	-0.10	60	na
3t	m	11.3	1	c	17.2(50-75)	0.10	14	24
4t	m	12.0	1	c	20.7(91-98)	0.93	25	21
5t	m	17.3	2	c+vur	19.1(25-50)	-0.72	70	22
6t	m	16.7	3	fsgs	22.3(75-91)	0.72	27	20
7t	m	14.1	3	c	25.2(98-99.6)	1.94	7	na
8t	f	10.7	1	fsgs	23.4(98-99.6)	1.96	85	25
9t	m	16.0	3	fsgs	28.4(98-99.6)	2.32	37	21
10t	m	11.3	1	c+vur	26.2(99.6)	2.62	59	21
11t	m	14.0	1	puv	24.0(91-98)	1.74	13	21
12t	m	13.0	1	c+vur	23.4(91-98)	1.82	33	19
13t	m	12.5	1	c	19.4(91)	0.74	16	na
14t	m	15.0	4	c	17.4(9)	-0.96	23	na

for key to diagnosis refer to list of abbreviations *centile GFR-mls/min/1.73m2

Appendix 2.2(cont) Clinical and laboratory features of children with renal transplant

Patient	Height (cm)	Weight (cm)	Ht SDS	Ht Vel SDS	Ht Vel (cm/yr)	Creat (umol/l)	albumin (g/l)	Transplan duration (month)	Steroids*
1t	141.6	51.3	0.26	-0.35	5.0	85	43	3	0.2
2t	157.1	49.1	-2.32	4.72	12.4	109	39	9	0.2
3t	142.7	35.1	-0.27	4.56	8.2	258	43	24	0.3
4t	129.5	32.9	-2.79	-0.97	4.2	na	44	16+	0.5
5t	150.7	43.4	-3.56	5.61	0.4	133	42	5+	1.0
6t	148.0	48.8	-3.67	3.43	10.2	152	41	36	0.2
7t	144.4	52.0	-2.2	-7.32	0	246	43	30	0.2
8t	120.3	33.9	-3.15	-3.48	0	57	42	48	0.3
9t	146.6	61.1	-3.48	-0.46	na	96	47	72	0.2
10t	145.5	55.4	0.12	0.48	5.3	158	40	48	0.2
11t	147.4	52.1	-1.74	-3.52	4.4	78	40	36	0.2
12t	139.6	45.8	-2.01	-2.34	3.4	125	39	36	0.2
13t	146.6	41.8	-0.61	6.92	10.4	318	42	96+	0.2
14t	154.9	41.8	-1.77	-1.92	5.1	155	41	84	0.2

+ retransplanted *mg/kg/alt da

Appendix 2.2(cont) Clinical and laboratory features of children with renal transplant

Patient	Gender	Age	Pubertal Status	Diagnosis	BMI* (kg/m2)	BMISDS	GFR	TCO2 (mmol/l)
15t	m	6.5	1	c	18.3(91-98)	1.66	66	22
16t	m	10.3	1	puv	19.5(91-98)	1.27	53	25
17t	f	9.0	1	c	24.1(>99.6)	2.49	62	21
18t	f	11.8	1	c	20.6(91-98)	0.96	40	25
19t	m	10.0	1	c	19.5(98)	1.34	40	22
20t	m	14.5	4	unknown	22.7(98)	1.32	na	na
21t	m	6.1	1	c	15.8(50)	0.25	76	19
22t	m	13.5	2	mesangio	23.3(98-99.6)	1.68	55	22
23t	m	12.5	2	puv	18.2(50-75)	0.20	49	21
24t	f	10.0	1	c	15.6(25-50)	-0.66	48	20
25t	m	11.5	1	c	17.9(50-75)	0.36	53	19
26t	f	12.5	5	fsgs	17.9(50-75)	-0.24	20	23
27t	f	13.5	1	fsgs	15.4(9-25)	-1.93	28	na

for key to diagnosis refer to list of abbreviations *centile GFR-mls/min/1.73m2

Appendix 2.2(cont) Clinical and laboratory features of children with renal transplant

Patient	Height (cm)	Weight (cm)	Ht SDS	Ht Vel SDS	Ht Vel (cm/yr)	Creat (umol/l)	albumin (g/l)	Transplant duration (month)	Steroids*
15t	121.0	26.8	0.58	2.62	8.8	76	40	40	0.3
16t	130.0	32.9	-1.54	1.5	6.2	120	40	72	0.3
17t	123.7	37.0	-1.66	3.47	8.2	73	43	38	0.2
18t	135.5	37.8	-1.75	2.24	10.4	67	36	16	0.2
19t	134.1	35.0	-0.59	4.38	8.3	139	39	na	0.3
20t	154.7	54.4	-1.29	-5.04	3.4	78	37	4	0.2
21t	108.1	18.5	-1.73	2.86	9.2	62	41	32	0.4
22t	149.1	51.8	-1.26	0.19	5.4	87	40	36	0.2
23t	146.0	38.7	-0.71	4.47	8.5	94	41	36	0.3
24t	134.1	28.1	-0.80	0.46	5.9	151	38	74	0.4
25t	136.8	33.5	-1.32	0.72	5.5	127	40	72	0.3
26t	135.4	32.8	-2.30	-5.37	2.3	453	42	72	0.2
27t	141.1	30.6	-2.34	-2.22	3.4	127	43	72	0.2

*mg/kg/alt day

Appendix 2.3 Characteristics of children with ESRD

Patient	Gender	Age (yrs)	Pubertal status	Diagnosis	BMI* (kg/m2)	BMISDS	TCO2 (mmol/l)
1d	m	10.8	1	c	22.8(98-99.6)	2.08	25
2d	m	14.5	2	puv	17.4(50th)	-0.80	26
3d	f	7.0	1	cns	17.2(50-75)	0.80	na
4d	m	16.8	3	c+vur	15.9(9-25)	-2.19	na
5d	m	13.0	1	fsgs	14.7(2nd)	-2.11	28
6d	f	9.0	1	acq	17.7(75-91)	0.59	30
7d	f	6.0	1	c	15.9(50th)	0.27	23
8d	f	14.3	1	fsgs	18.4(50th)	-0.48	na
9d	m	2.5	1	c	18.5(91-98)	1.48	na
10d	f	12.8	2	c+vur	21.2(91-98)	0.92	na

for key to diagnosis refer to list of abbreviations *centile

Appendix 2.3 (cont) Characteristics of children with ESRD

Patient	Height (cm)	Weight (cm)	Ht SDS	Ht Vel SDS	Ht Vel (cm/yr)	Creat (umol/l)	albumin (g/l)	Duration dialysis (month)
1d	124.2	35.1	-3.00	-3.15	2.9	598	35	60*
2d	159.5	44.3	-0.86	1.86	11.5	1278	36	12
3d	106.5	19.5	-2.97	-2.54	3.6	na	35	60*
4d	146.7	35.4	-3.79	na	na	na	33	48
5d	153.8	34.8	0.03	0.12	5.7	1203	39	6
6d	119.6	25.3	-2.14	-1.43	4.4	832	37	72
7d	107.8	18.5	-1.20	-1.60	4.6	586	32	36
8d	158.6	46.4	-0.23	4.44	5.2	850	33	12
9d	87.0	14.0	-0.75	0.42	8.7	600	30	24
10d	136.8	39.7	-2.44	-0.86	7.0	633	35	24

* haemodialysis

Appendix 2.4 Characteristics of children with normal GFR on steroid treatment

Patient	Gender	Age (yrs)	Pubertal status	Diagnosis	BMI* (kg/m2)	BMISDS	TCO2 (mmol/l)
1n	m	13.3	3	ns	24(98-99.6)	1.90	na
2n	m	10.8	1	vas	24(98-99.6)	2.32	21
3n	m	9.9	1	ns	14(2-9)	-1.86	23
4n	m	10.0	1	ns	21(98-99.6)	-3.47	22
5n	m	14.3	3	ns	18(50-75)	-0.38	25
6n	m	11.2	1	ns	21(98-99.6)	1.61	23
7n	f	9.8	1	ns	(91-98)	1.43	26
8n	f	11.5	1	vas	20(91)	1.00	27
9n	f	13.3	2	ns	22(75-91)	1.09	22
10n	m	13.5	3	ns	20(75-91)	0.83	30

for key to diagnosis refer to list of abbreviations *centile

Appendix 2.4 (cont) Characteristics of children with normal GFR on steroid treatment

Patient	Height (cm)	Weight (kg)	Ht SDS	Ht Vel SDS	Ht Vel (cm/yr)	Creat (umol/l)	albumin (g/l)	Steroid mg/kg/alt day)
1n	154.2	57.3	-0.41	-1.64	3.0	76	43	0.3
2n	139.8	46.7	-0.60	-4.90	1.6	52	42	0.4*
3n	138.3	26.4	0.09	0.01	5.3	35	36	0.6
4n	146.3	26.4	1.30	-0.78	4.7	65	34	0.3
5n	163.1	48.1	-0.09	-0.79	6.6	71	39	0.2
6n	159.2	53.7	1.53	1.09	5.7	44	39	0.1
7n	132.0	35.9	-0.79	-0.80	4.8	40	34	0.6
8n	145.2	43.2	-0.08	na	na	61	33	0.6*
9n	155.3	53.4	0.74	2.60	8.7	44	33	0.6
10n	135.0	37.2	-2.91	-2.99	3.5	56	41	0.5

*daily steroids

Appendix 2.5 Characteristics of patients in study of effects of rhGH on protein turnover and growth

Patient	Age (yrs)	Gender	Pubertal stage	GFR	Steroid (mg/kg)	Ht SDS	Ht Vel SDS	BMI (centile) (kg/m2)	TCO2 (mmol/l)	albumin (g/l)	Bone age (yrs)
a(12c)	11.6	m	1	22	na	-2.50	1.67	15.1(9th)	21	43	10
c(9c)	14.8	m	3	34	na	-2.44	1.56	21.7(91-98th)	18	42	12
f(10c)	11.6	m	1	12	na	-2.62	-2.73	17.3(50th)	23	42	na
h(8c)	12.5	m	2	18	na	-2.49	2.46	17.3(25-50th)	21	45	9.2
j(11c)	7.5	m	1	34	na	-5.65	-0.64	14.9(9-25th)	25	35	na
k+(4d)	16.7	m	3	na	na	-3.79	8.84	15.9(0.4th)	23	33	na
l*(2t)	15.2	m	1	14	na	-2.59	1.90	17.0(9-25th)	22	43	na
b*(6t)	17.1	m	3	27	0.2	-3.40	7.12	22.6(75th)	24	41	13
d*(8t)	10.8	f	1	85	0.3	-3.14	-3.71	23.2(98th)	24	42	na
e*(9t)	16.0	m	3	37	0.2	-3.48	-0.46	28.7(98th)	24	47	14
g*(7t)	13.8	m	3	7	0.2	-1.99	-7.06	24.9(98th)	19	43	14
m*(4t)	12.0	m	1	25	0.5	-2.79	-0.97	20.1(91st)	21	44	na

*Transplant / +ESRD / CRF GFR mls/min/1.73m2

a-m patients in protein turnover study, (x) patient identity in GH-IGF study.

Appendix 3.1 Results of children with CRF

Patient	IGF-I SDS	IGFBP-3 SDS	IGF Bioactiv (U/ml)	IGF-II (ng/ml)	IGFBP-1 (ng/ml)	IGFBP-2 (ng/ml)	GHRIA (mU/l)	GHH (ng/l)
1c	0.25	3.44	0.34	967	29.5	1300	1.6	1.0
2c	1.56	3.69	0.57	1168	90.9	760	12.5	5.9
3c	1.09	3.85	0.62	1284	87.3	1600	0.9	0.5
4c	0.28	3.77	0.10	1424	66.3	880	5.3	1.5
5c	0.26	3.65	0.15	1207	107.0	2180	0.8	0.5
6c	2.37	4.27	0.20	1414	50.8	540	2.0	0.5
7c	-1.23	1.96	0.35	896	125.0	1790	12.6	6.5
8c	-2.23	3.22	0.59	1239	93.2	838	1.8	0.5
9c	0.67	2.94	na	1231	20.2	966	1.4	0.5
10c	-0.15	4.33	0.44	1299	72.0	772	22.0	17.2
11c	0.19	1.24	0.33	374	160.0	1432	6.5	2.1
12c	0.3	3.15	0.36	1004	96.1	900	1.2	0.5
13c	4.72	4.91	0.16	1227	26.6	720	7.7	3.5
14c	0.81	4.00	0.20	1085	81.2	1200	1.3	0.5
15c	-0.55	3.09	0.19	1030	160.0	1620	15.4	6.7
16c	0.62	3.18	0.14	1189	128.0	1780	70.6	34.4
17c	1.06	3.21	0.34	1684	112.4	1466	70.4	32.7
18c	1.36	4.12	0.27	1552	92.7	1270	10.2	4.1
19c	1.69	3.62	0.60	1134	79.1	936	8.8	2.6
20c	na	na	0.25	1302	66.2	1600	31.7	15.2
21c	1.57	3.22	0.20	1339	66.9	74.4	3.6	1.9
22c	na	na	0.23	818	131.7	1020	7.3	4.8
23c	0.92	3.24	2.24	1363	77.1	636	31.8	17.5
24c	1.36	3.23	0.79	1369	64.4	1130	10.6	5.9
25c	-2.54	0.07	0.11	370	160.0	1520	13.5	5.9
26c	-2.02	0.61	0.14	250	160.0	1150	13.8	7.3
27c	2.19	3.99	0.28	1769	160.0	1826	6.2	2.9
28c	-0.42	2.49	0.63	1395	84.7	1092	0.6	0.5
29c	0.07	2.97	0.18	1316	146.0	1302	4.0	1.4
30c	1.74	4.33	0.74	1478	74.4	890	33.4	14.5
31c	-0.13	2.62	0.67	1694	112.3	1050	13.8	6.2

Appendix 3.1 Results of children with CRF

Patient	CPEP (pmol/l)	INSULIN (mU/l)	ALS (mcg/ml)	PROTEASE (%)	CREAT (umol/l)	IGF-Imol	IGF-IImol	IGFBP-3mol
1c	33	7.1	48.2	52.8	97	43	126	176
2c	33	10.5	88.0	49.3	na	74	152	187
3c	33	12.6	60.0	57.0	616	61	167	193
4c	630	17.2	35.7	45.1	823	46	185	190
5c	33	15.3	49.0	47.8	706	45	157	185
6c	111	9.5	89.0	66.0	598	111	184	224
7c	33	10.1	18.0	72.0	167	17	116	129
8c	530	10.8	20.8	63.4	191	12	161	167
9c	131	28.1	23.0	26.2	323	57	160	155
10c	514	9.1	26.0	42.6	542	35	169	214
11c	33	7.7	5.8	52.0	119	25	49	89
12c	88	13.5	36.0	39.8	325	44	131	164
13c	123	23.6	90.0	58.2	503	198	160	238
14c	117	10.3	29.0	41.4	476	57	141	200
15c	33	4.2	62.0	62.2	289	134	134	161
16c	33	11.5	40.6	52.3	474	155	155	179
17c	95	22.9	30.6	13.2	369	219	219	166
18c	1263	17.2	38.0	19.5	810	202	202	205
19c	451	10.7	24.0	48.5	312	147	147	184
20c	33	13.2	62.0	57.2	617	169	169	216
21c	235	25.1	71.0	46.8	531	174	174	167
22c	324	10.0	12.5	60.2	216	106	106	108
23c	797	16.5	34.5	46.6	153	177	177	181
24c	800	12.1	30.2	17.4	184	178	178	167
25c	94	20.0	3.1	51.6	125	48	48	51
26c	878	10.4	3.5	30.4	na	33	33	67
27c	48	12.2	34.2	39.5	212	230	230	200
28c	96	13.7	28.0	37.1	185	181	181	150
29c	1041	14.0	24.0	17.3	461	171	171	156
30c	675	20.3	36.9	40.0	228	192	192	214
31c	694	17.7	30.4	27.2	167	220	220	142

Appendix 3.2 Results of children with renal transplant

Patient	IGF-I SDS	IGFBP-3 SDS	IGF Bioactiv (U/ml)	IGF-II (ng/ml)	IGFBP-1 (ng/ml)	IGFBP-2 (ng/ml)	GHRIA (mU/l)	GHHYBRIT (ng/l)
1t	1.83	3.49	1.29	1484	45.6	266	1.4	0.5
2t	1.96	3.21	0.59	1299	59.3	1108	1.0	0.5
3t	1.00	3.80	0.62	1569	112.2	606	22.0	9.8
4t	0.73	2.98	0.06	1223	98.9	864	3.7	34.1
5t	0.19	4.12	na	1071	45.1	754	26.7	14.1
6t	0.02	2.59	na	374	65.7	688	1.5	0.5
7t	-3.22	3.49	0.11	1300	126.9	866	1.2	0.5
8t	-1.27	1.29	1.32	963	71.3	262	1.3	na
9t	0.79	3.26	0.49	1108	12.3	364	1.1	5.0
10t	1.56	3.57	0.84	1393	87.1	1084	27.6	8.3
11t	0.38	2.40	na	1333	90.0	1102	5.4	2.1
12t	0.92	3.26	0.48	1531	163.5	756	2.0	0.5
13t	2.22	5.01	0.73	1880	122.3	2432	1.6	0.7
14t	1.45	4.08	na	1633	114.6	1592	25.8	10.5
15t	1.55	3.43	na	1580	67.7	648	3.2	0.5
16t	0.86	2.95	na	1240	113.0	1014	2.2	0.5
17t	1.33	3.39	na	1400	57.4	412	4.6	1.5
18t	0.85	2.87	2.19	1453	92.0	928	65.6	29.6
19t	1.00	3.86	0.55	1681	135.1	938	2.8	0.5
20t	0.81	4.00	na	1361	47.4	890	1.4	0.5
21t	0.36	2.93	na	1404	142.1	1426	24.4	12.8
22t	1.90	3.45	na	1569	57.0	504	9.2	2.9
23t	1.94	3.73	na	1561	94.1	674	5.2	1.4
24t	1.76	2.95	na	1134	21.4	426	28.8	14.6
25t	1.56	3.38	1.10	1435	83.5	1140	1.6	0.5
26t	1.65	4.32	0.13	1310	68.7	672	44.5	22.1
27t	0.52	3.59	0.49	1374	76.7	896	3.2	1.1

Appendix 3.2 Results of children with renal transplant

Patient	CPEP (pmol/l)	INSULIN (mU/l)	ALS (mcg/ml)	PROTEASE (%)	CREAT (umol/l)	IGF-Imol	IGF-IImol	IGFBP-3mol
1t	751	15.3	34.9	32.8	85	193	178	193
2t	52	14.4	38.1	19.3	89	169	166	169
3t	967	14.5	32.4	36.2	59	204	191	204
4t	397	9.3	25.0	54.6	81	159	156	159
5t	570	11.4	28.7	50.1	45	139	205	139
6t	245	5.6	26.8	53.3	38	49	140	49
7t	1043	13.7	24.0	57.8	6	169	178	169
8t	67	6.6	19.9	66.2	20	125	103	125
9t	63	35.2	29.1	40.7	54	144	168	144
10t	41	20.7	33.5	40.3	76	181	181	181
11t	50	14.0	28.1	39.3	35	173	133	173
12t	42	5.2	24.9	39.3	50	199	168	199
13t	86	13.0	39.0	47.0	86	244	243	244
14t	49	4.3	36.1	49.7	71	212	203	212
15t	43	8.6	40.4	35.6	70	205	176	205
16t	54	7.2	28.0	34.3	56	161	155	161
17t	39	8.6	35.9	30.1	67	182	187	182
18t	37	7.4	30.0	42.9	59	189	165	189
19t	7575	7.8	34.2	56.8	61	219	194	219
20t	61	19.7	40.5	29.4	108	177	206	177
21t	20	4.5	28.0	51.8	38	183	154	183
22t	799	17.9	44.2	16.1	94	204	190	204
23t	617	15.7	44.0	18.3	86	203	188	203
24t	1115	27.8	29.1	22.0	83	147	169	147
25t	880	11.8	32.1	14.9	55	187	173	187
26t	944	6.6	34.0	55.8	84	170	226	170
27t	286	4.4	32.1	35.8	53	179	196	179

Appendix 3.3 Results of children with ESRD

Patient	IGF-I SDS	IGFBP-3 SDS	IGF Bioactiv (U/ml)	IGF-II (ng/ml)	IGFBP-1 (ng/ml)	IGFBP-2 (ng/ml)	GHRIA (mU/l)	3HHYBRIT (ng/l)
1d	0.82	3.38	0.58	1585	89.2	1874	2.0	0.5
2d	1.18	4.78	0.35	1817	144.1	2448	14.8	7.0
3d	1.67	4.91	0.16	1649	58.2	1164	5.0	1.6
4d	-0.60	3.29	0.08	1181	118.0	1154	6.0	2.2
5d	1.86	4.68	0.25	1628	164.2	2124	9.0	3.3
6d	1.09	4.21	na	1734	160.0	2808	8.0	2.9
7d	0.76	3.04	0.44	1412	160.0	1818	7.6	1.4
8d	1.06	4.91	0.69	1902	116.2	2530	7.2	1.8
9d	0.08	3.62	na	1431	160.0	3882	18.0	9.2
10d	0.03	3.12	0.23	1208	100.6	1050	9.9	3.8

Appendix 3.3 Results of children with ESRD

Patient	CPEP (pmol/l)	INSULIN (mU/l)	ALS (mcg/ml)	PROTEASE (%)	IGF-Imol	IGF-IImol	IGFBP-3mol
1d	2000	18.7	25.2	15.2	49	206	174
2d	94	7.3	31.5	44.9	59	236	233
3d	2000	43.2	31.6	32.5	70	214	232
4d	355	10.8	24.0	51.7	32	154	169
5d	229	13.9	33.1	41.9	80	212	229
6d	142	6.1	29.8	50.2	60	225	222
7d	94	11.1	27.3	55.4	42	184	173
8d	144	13.5	35.5	23.6	68	247	251
9d	218	12.4	25.5	99.6	41	186	183
10d	375	8.2	20.6	52.3	42	157	176

Appendix 3.4 Results of children with normal GFR on steroids

Patient	IGF-I SDS	IGFBP-3 SDS	IGF Bioactiv (U/ml)	IGF-II (ng/ml)	IGFBP-1 (ng/ml)	IGFBP-2 (ng/ml)	GHRIA (mU/l)	GHHYBRIT (ng/l)
1n	0.88	2.768	1.18	900	13.3	350	3.3	1.5
2n	1.61	3.028	0.90	860	25.1	324	0.9	0.5
3n	1.55	1.768	0.39	841	65.9	470	0.6	0.5
4n	4.03	1.850	0.40	724	25.5	230	12.3	6.5
5n	2.67	2.945	0.81	1104	35.4	440	13.2	2.6
6n	-0.40	2.173	0.67	889	57.9	360	1.2	1.2
7n	1.25	2.890	0.54	1398	21.5	306	14.4	7.0
8n	-0.05	1.638	0.43	960	37.7	592	6.8	1.6
9n	1.72	3.360	1.77	1396	19.3	416	3.0	0.8
10n	1.55	2.911	0.87	1287	33.7	604	4.8	1.0

Appendix 3.4 Results of children with normal GFR on steroids

Patient	CPEP (pmol/l)	INSULIN (mU/l)	ALS (mcg/ml)	PROTEASE (%)	CREAT (umol/l)	IGF-Imol	IGF-IImol	IGFBP-3mol
1n	33	13.9	51.5	53.0	76	58	117	148
2n	33	11.0	90.0	49.4	52	73	112	159
3n	33	5.9	49.4	48.0	35	21	109	108
4n	33	7.0	46.0	50.2	65	46	94	111
5n	33	12.1	41.0	63.5	71	96	144	155
6n	33	7.8	48.0	54.3	44	35	116	124
7n	809	24.3	33.9	15.5	40	66	182	166
8n	732	12.2	24.3	53.5	61	39	125	116
9n	1629	55.3	48.2	18.0	44	87	181	186
10n	781	25.0	44.5	17.3	56	70	167	154

Appendix 4. 1 Protein turnover indices and anthropometric data from protein turnover study

Rhgh	PATIENT	FLUX* g/kg/day	SYNTH* g/kg/day	oxidation* g/kg/day	RQ	REE kcal/day	IGFI SDS	IGFBP-3 SDS	fat *mm2*	muscle *mm2*
pre	a	6.05	5.45	0.60	0.81	944	-1.28	2.93	*954*	*1642*
post	a	6.16	5.36	0.80	0.75	1336	-3.32	4.80	*770*	*1910*
pre	b	3.69	3.25	0.44	0.74	1017	0.02	2.59	*3543*	*3178*
post	b	4.34	3.81	0.53	0.72	1384	3.29	5.96	*2399*	*3121*
pre	f	4.89	4.33	0.56	0.78	1118	-0.08	3.14	*648*	*1840*
post	f	5.28	4.67	0.61	0.73	1135	-0.51	3.21	*619*	*2527*
post	f	5.31	4.82	0.49	0.68	1423	-0.74	3.43	*371*	*1958*
pre	e	4.36	3.67	0.69	0.81	1235	0.79	3.26	*5700*	*3363*
post	e	4.60	3.61	0.66	0.76	1471	2.68	3.63	*5717*	*4578*
post	e	3.83	3.21	0.62	0.82	1576	2.50	3.76	*5717*	*4578*
pre	c	4.95	4.44	0.51	0.87	1362	0.67	2.94	*1794*	*4460*
post	c	4.95	4.40	0.55	0.73	1537	-0.11	3.10	*1718*	*4646*
pre	g	3.77	3.47	0.30	0.78	960	-0.82	3.19	*3160*	*3251*
post	g	3.76	3.31	0.45	0.77	1652	8.12	5.14	*3650*	*4005*
post	g	3.99	3.59	0.40	0.72	1502	3.34	5.89	*3566*	*3242*
pre	d	4.34	3.55	0.79	0.72	1096	-0.98	1.26	*3440*	*2401*
post	d	4.24	3.66	0.58	0.87	1404	0.87	2.12	*2121*	*2867*
pre	h	6.65	6.00	0.66	0.72	1433	-2.23	3.21	*788*	*2181*
post	h	6.89	6.16	0.73	0.79	1607	0.56	3.54	*635*	*2928*
post	h	6.20	5.34	0.86	0.80	1509	0.51	3.43	*412*	*2797*

Appendix 4. 1 Endocrine parameters from protein turnover study

RhGH	PATIENT	IGF1 ng/ml	IGFbio U/l	IGFII ng/ml	IGFBP-1 ng/ml	IGFBP-2 ng/ml	IGFBP-3 ng/ml	GHRIA mU/l	CPEP pmol/l	INSULIN mU/l	ALS mcg/m	CORTISOL nmol/l
pre	a	142	0.07	1082	165.9	1220	4410	6.8	343	7.6	23.6	353
post	a	34.7	0.08	1113	113.4	978	6690	6.0	756	14.5	26.8	123
pre	b	292	na	374	65.7	688	4010	1.5	245	5.6	26.8	155
post	b	1109	0.51	719	43.5	494	8050	7.1	720	12.3	33.9	263
pre	f	250	0.25	1230	94.7	1028	4670	1.0	589	10.5	20.9	232
post	f	150	0.31	1290	84.2	1086	4750	3.1	393	7.7	20.6	185
post	f	182	0.33	1351	71.0	1264	5020	8.0	469	11.2	22.0	209
pre	e	416	0.49	1108	12.3	364	4810	1.1	63	35.2	29.1	13
post	e	894	0.84	868	5.7	402	5260	14.5	1027	55.6	30.8	54
post	e	836	0.74	970	6.7	410	5410	9.9	1340	114.7	29.9	92
pre	c	381	na	1231	20.2	966	4420	1.4	131	28.1	23.0	178
post	c	275	0.89	1033	47.7	790	4620	3.3	585	17.6	24.0	403
pre	g	169	0.06	1344	148.3	1180	4730	3.7	859	8.2	25.0	293
post	g	894	0.13	1376	55.9	1170	7090	14.9	1382	29.3	37.6	108
post	g	1130	0.10	1304	64.7	1082	7970	14.4	556	34.9	43.0	112
pre	d	179	0.44	971	64.7	390	2920	0.5	134	9.3	18.6	80
post	d	456	na	1217	96.1	310	3860	11.6	67	7.3	24.2	196
pre	h	95	0.59	1239	93.2	838	4760	1.8	530	10.8	20.8	282
post	h	383	0.55	1191	93.7	1164	5150	5.1	630	20.4	30.8	439
post	h	372	na	1251	84.3	842	5010	1.8	319	8.5	31.2	281

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