GROWTH PROMOTION

IN THE

SHORT NORMAL CHILD

"Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Medicine by Heather Fiona Stirling".

September 1994

DECLARATION

This thesis is the result of my own work. The material contained in the thesis has not been presented, nor is currently been presented, either wholly or in part for any other degree or qualification.

The research/clinical work was carried out at The Royal Hospital for Sick Children, Edinburgh, and The Department of Child Life and Health, University of Edinburgh.

The growth and endocrine studies were carried out by myself, under the supervision of Dr CJH Kelnar, Royal Hospital for Sick Children. The psychological studies were carried out in collaboration with Dr L Scarth, Department of Child and Family Psychiatry, Royal Hospital for Sick Children, Edinburgh.

GROWTH PROMOTION IN THE SHORT NORMAL CHILD

Heather Fiona Stirling

Short stature and puberty delay can cause problems, both physical and psychological. Until relatively recently growth hormone was only available for children who met the criteria of "classical" growth hormone deficiency. Recombinant human growth hormone (rhGH) is now available in "unlimited" supply. Detailed studies are required to evaluate its use in short children who are not growth hormone insufficient in the traditional sense, but who may benefit from treatment. This thesis presents three studies in short normal children to evaluate the physical and psychological effects of growth promoting agents over the first two years of treatment.

1) a double blind placebo controlled study of rhGH in 37 pre-pubertal children (mean age 8.0 yrs) with familial short stature. This unequivocally demonstrates the short-term growth promoting effects of rhGH - over the first year the children treated with rhGH grew at a mean rate of 7.67 cm/yr, compared to 4.76 cm/yr for those who received placebo and 4.83 cm/yr for those who received no treatment. The onset and rate of puberty, especially in the girls, tended to be advanced.

2) in a randomised study in 43 peri-pubertal boys (mean age 11.6 yrs) with familial short stature, the growth promoting effects of rhGH were compared with the anabolic steroid oxandrolone, a combination of rhGH with oxandrolone, and a control group who received no active treatment. In the short term growth improved in the three actively treated groups compared to the control group - over the first study year the boys who received rhGH grew at a rate of 7.58 cm/yr, compared to 8.08 cm/yr for oxandrolone alone, 9.92 cm/yr in those who received rhGH plus oxandrolone, and 4.73cm/yr in the control group. In the groups who received oxandrolone, either singly or in combination with rhGH, onset of puberty was earlier and skeletal maturation more rapid. Caution is required in using oxandrolone to promote growth in younger boys without significant growth delay.

3) in a randomised study in 33 boys with puberty delay (mean age 14.9 yrs) the growth promoting effects of rhGH were compared with oral testosterone undecanoate, and a combination of the two drugs. There were no significant differences in the growth promoting effects (rhGH 8.59, testosterone undecanoate 8.48, combination 9.91 cm/yr) or rate of pubertal progression between the three groups There is no advantage of rhGH therapy in boys with puberty delay, compared to oral testosterone undecanoate.

Children of short stature are often thought to suffer from psychological or behavioural problems. A range of self report questionnaires was undertaken in these children prior to entry into the studies and at yearly intervals. They were not as a group clinically disturbed, but tended to score highly on hyperactivity. In those who received active treatements, especially rhGH, the reported behaviour and self esteem tended to improve, but the effects were not marked.

It is possible to accelerate the growth of short normal children, at least in the short term, though it is less likely there will be a significant improvement in final height. There are psychological effects of growth promotion but they are subtle.
It is difficult to justify the use of rhGH in young children with familial short stature, or in boys with puberty delay. Growth hormone must not be used indiscriminately in the short normal child.

TABLE OF CONTENTS

Introduction

Growth hormone was first used to treat a "pituitary dwarf" by Raben (Raben 1958, Raben 1959). His patient, a 17 year old male, was treated with thrice weekly intramuscular growth hormone extracted from human pituitary glands. This produced a significant improvement in height velocity from 0.5 inches/year to 2.6 inches/year. A similar preparation was introduced in the United Kingdom in 1959 under the auspices of a Medical Research Council working party.

Early studies (Melvin et al 1967, Tanner et al 1971) suggested hypophysectomised individuals appeared to be more sensitive to growth hormone than subjects with an intact pituitary (Melvin et al 1967). In the United Kingdom the "Working Party of the Pituitary Hormone Committee of the Medical Research Council" designed and carried out a clinical trial of human growth hormone as a growth promoting agent. The Working Party not only investigated the efficacy of growth hormone, but also the best utilisation of the limited supplies available. The initial studies had suggested that growth hormone "deficiency" was essential for benefit to result from growth hormone therapy and thus certain requirements had to be met for inclusion into the MRC trial and treatment with growth hormone, namely :

1) stature >2.5 SD below the mean for age and sex

2) growth velocity <25th centile for age and sex

3) impaired growth hormone secretion during insulin tolerance test

4) satisfactory general paediatric, auxological and endocrine evidence to exclude or define any complicating factors (Milner et al 1979).

The majority of children treated had either idiopathic growth hormone deficiency, or intracranial disorders with growth hormone deficiency. However a group of children with miscellaneous conditions was treated - Russell Silver syndrome, growth delay, Turner syndrome and psychosocial short stature. The children were all treated with growth hormone extracted from cadaveric human pituitary glands by the Raben or Wilhelmi methods. Of the 131 patients with idiopathic "isolated" growth hormone deficiency, all but six responded to treatment with a marked increase in height velocity from 3.2 to 9.9 cm/year. Predictors of response seemed to be bone age, skinfold thickness and stature at the start or treatment - a greater response occurring with the younger the bone age, greater the skinfold and shorter the child (in terms of SDS for age). In the miscellaneous group of children there was generally a poor response particularly in those with Russell Silver syndrome, growth delay or psychosocial short stature (Milner et al 1979). Tanner et al had included four children with growth delay in their cohort of

growth hormone treated children reported in 1971. There was no definite response to therapy but psychosocial factors complicated the picture in at least one of the children. In these early studies there was little evidence of benefit for non-GH deficient children, but their numbers were small and they were being treated with a sub-optimal regimen by today's standards. Despite these earlier studies, by 1985 there was beginning to be a reassessment of the use of growth hormone for other causes of short stature than classical growth hormone deficiency (Buchanan et al 1987).

In 1985 reports began to appear of the neurodegenerative Creutfzeldt-Jakob Disease (CJD) occurring in young adults who had been the recipients of cadaveric human growth hormone, (Koch TK et al 1985, Powell-Jackson et al 1985), and it was presumed that the cause was contamination of one or more batches of the pituitary derived growth hormone with the CJD agent. Following these reports, pituitary derived human growth hormone was withdrawn in the United Kingdom and USA. It was recommended that treatment with growth hormone should only be commenced in "essential" cases eg. GH-deficient patients with hypoglycaemia, and that only the newly developed biosynthetic growth hormone should be used (Report of the Committee on Growth Hormone Use of the Lawson Wilkins Pediatric Endocrine Society, May 1985). At least 23 young adults who had been treated with pituitary derived growth hormone have been victims of CJD (Brown et al 1992). More cases are still being reported.

By the mid 1980's biosynthetic human growth hormone was becoming increasingly available. By means of recombinant DNA techniques, the gene for hGH had been expressed in E. Coli and the methionyl analogue (met-rhGH) was derived and purified. This had a similar biopotency to pituitary derived hGH in animal studies and was shown to be biologically active in normal adult male volunteers (Hintz et al 1982). Clinical trials were begun in the USA in 1981 in hypopituitary children. The results from 46 children reported in 1986 (Kaplan 1986) showed that met-rhGH was equipotent to pituitary derived hGH in promoting linear growth in hypopituitary children. Antibodies against met-rhGH developed in a substantial proportion of patients treated with met-rhGH (Kaplan 1986). Further refinement of techniques enabled the production of biosynthetic growth hormone with a sequence identical to the naturally occurring molecule ie. without the methionyl group, and in 1987 "authentic" natural sequence growth hormone became available. The recombinant natural sequence human growth hormone (rhGH) used in the studies we are

reporting was first synthesised in 1983 by Novo Nordisk (Dalboge H et al 1987) and the first clinical trials reporting its use were published in 1988 (Rasmussen 1988). Authentic rhGH is much less antigenic than met-rhGH and its growth promoting efficacy in growth hormone deficient patients is equal to that of met-rhGH and pituitary derived growth hormone (Rasmussen 1988).

Once growth hormone became available by biosynthetic production its supply became relatively unlimited, though a major constraint is its cost (£5,000 -£10,000 per patient per year). It is not now in such short supply as to restrict its use only to those patients with growth hormone insufficiency as defined by the MRC working party. Its efficacy in other causes of poor growth and short stature can now be explored more fully, thus raising the question "Which children should receive growth hormone treatment ?"

At the same time that growth hormone treatment was evolving, other agents, for example anabolic steroids, were being explored for their potential as growth promoting treatments. We have therefore now reached the situation where we can compare varying methods of growth promotion, not just growth hormone alone, in groups of short children who previously would not have been eligible for treatment.

Normal Variant Short Stature - the "Short Normal Child"

There are two main explanations (or a combination of both) as to why a child may be normal, but small.

A child who is small but otherwise normal and healthy with no significant past medical history, may be small because he (or she) has small parents. It is important to ensure that the parents themselves do not have a disorder which has affected their growth and in which in turn they could have passed onto their child eg. hypochondroplasia. Assuming the parents are normal, one would expect their children also to be small - ie the child has "familial short stature", a type of normal variant short stature. The child will grow along a centile appropriate for his or her parental heights (eg. along or below but parallel to the 3rd centile), and will reach a final adult height appropriate for the parents.

Other children are small, healthy and with no significant past medical history but have growth delay. They will ultimately reach an acceptable final height which is appropriate for their parents, but will take longer than average to complete their growth. They are likely to enter puberty later than their peers. These children also have a type of normal variant short stature, described as "constitutional delay of growth and puberty".

A further group of children will have a combination of familial short stature with constitutional delay of growth and puberty as cause of their normal variant short stature.

Normal variant short stature is the commonest cause of short stature presenting to paediatricians and paediatric endocrinologists. At the Growth and Endocrine clinic at the Royal Hospital for Sick Children, Edinburgh approximately 500 children are seen because of "normal variant " short stature, compared to 25 children with idiopathic growth hormone deficiency or panhypopituitarism, 20 children who are GH deficient as a result of radiotherapy, and 32 girls with Turner syndrome. The extremely high number of children with normal variant short stature may be biased due to the clinic's interest in the condition, but most paediatricians and paediatric endocrinologists find that it accounts for the majority of referrals to a growth clinic. A good understanding of the growth process and its normal variations is vital to help make a correct diagnosis, but "normal variant short stature" is a diagnosis that must only made after a full history, examination and necessary investigations to exclude other pathological causes of short stature and poor growth.

Although the child has no pathological cause for his or her short stature he/she may suffer as a consequence of it just as much as if he/she has an underlying disorder causing the problem. The priorities in trying to help a child with normal variant short stature are:

1) determining that the cause of the short stature is a normal variant by excluding underlying pathology

2) a thorough explanation to the child and the parent, with a prediction of expected height and pattern of growth that will follow

3) an attempt to determine how the child is coping or suffering because of his/her lack of growth or development. It is important to differentiate the parents expectations from the child's hopes and fears and to treat the child and not the parents.

Growth Promotion in the Child with Normal Variant Short Stature

If it is clear that the child has no underlying pathology for his/her short stature (which may at least entail following growth for 6-12 months), and is suffering because of it, then it is worth considering whether treatment may be of help. Normal variant short stature is by definition a variant of normal growth and not pathological, so one could reasonably ask why one would even consider promoting growth in such children.

Disease can be defined as a deviation from the biologic norm of health, and medical interventions ethically acceptable only if they preserve health or restore it by preventing or treating a disease or illness (Lantos et al 1989). Thus treating a short child with "classical" growth hormone deficiency is undoubtedly ethically acceptable. The definition of growth hormone deficiency is not, however, black and white - growth hormone secretion is a continuous spectrum and is not an all or nothing phenomenon. Tall children secrete more growth hormone physiologically than their shorter peers (Albertsson-Wikland and Rosberg 1988), and within a group of children there is a relationship between growth hormone secretion and height velocity (Hindmarsh et al 1987). The definition of a growth hormone secretory problem becomes more difficult to define, and cannot now simply be classed as failure to reach an arbitrary value after a pharmacological stimulation test. Identifying the child with a growth hormone secretory problem is more complex than previously thought, and some children with apparent normal variant short stature do have subtle abnormalities of growth hormone secretion (Spilliotis et al 1984).

Although normal variant short stature is not itself a disease it may cause psychological morbidity - thus there may be an argument for treating it. However, if this is the case one must show that improved growth will relieve this morbidity (Lantos et al 1989). Psychological parameters must therefore be included as outcome measures.

There are two questions to be considered in the treatment of normal variant short children - that of efficacy and safety of any proposed treatment, and the ethical issue of whether medical treatment for normal short children is justified even if it is safe and acceptable.

The majority of children with normal variant short stature will be aware that they are small compared to their peers, but many will cope with this adequately and not come to medical attention. However, a proportion will suffer and seek help - for these children one must know if there is any benefit (physically and psychologically) in trying to help them with a growth promoting therapy. Of prime importance is the fact that these are normal

healthy children and when considering any form of growth promotion the possible benefits in the short and long-term must be balanced with the risks of potential side-effects. The child will want to know "Will the treatment work?", the parents will ask " Will the treatment work, and is it safe?". There is a need for answers to these questions and the studies presented in this thesis are an attempt to provide some of the answers to these clinical questions.

Clear outcome measures must be defined before embarking on any trials of treatment. In these studies on growth promotion in normal variant small children there are both short term and long term outcome measures to be considered. The short term measures are the effects on growth rate, pubertal timing and progress, safety of treatments, and immediate psychological changes. Long term outcome measures are final height, and ultimate psychological outcome. The two must be clearly differentiated, as even if there are no significant long term effects, short term benefits of improved growth and in psychological well being may be of vital importance to a young child. If there are long term improvements in final height one must still answer the question "Is taller really better?" (Diekama 1990) in terms of ultimate psychological functioning.

It is also necessary to compare rhGH with other forms of growth-promoting therapy to determine which is most effective and safe. The best form of treatment may vary at different ages and stages of childhood and puberty. Some growth-promoting therapies already have a recognised role in some groups of children eg. oxandrolone in boys with puberty delay. It does not follow that their use can safely be extrapolated to other groups of normal short children as one may see differing effects.

This thesis presents three studies in children with normal variant short stature examining the effects of growth promoting treatments:

1) a double blind placebo controlled study of rhGH in the treatment of pre-pubertal children with familial short stature.

2) a comparison of rhGH, the anabolic steroid oxandrolone, and a combination of rhGH plus oxandrolone in peri-pubertal boys with familial short stature.

3) a comparison of rhGH, the orally active androgen testosterone undecanoate, and a combination of rhGH plus testosterone undecanoate in boys with puberty delay.

The work presented focuses on the short term effects of growth-promoting therapy. These children are being followed to adulthood in order to determine the long-term outcome of such treatments.

SECTION 1: GROWTH PROMOTION IN PRE-PUBERTAL CHILDREN WITH FAMILIAL SHORT STATURE

1.1. Introduction

This study was designed to investigate the effects of recombinant human growth hormone (rhGH) on pre-pubertal children with familial short stature who do not have significant growth delay. The design of the study was a double-blind placebo controlled therapeutic trial. Although the children will be followed to adult height, I am presenting the short-term results ie. the first two years.

1.2. Definition of familial short stature

The children included in this study have familial short stature. They have small parents, and hence one would predict that the child's final adult height will be low. The children in this study do not have significant growth delay - their bone age is less than two years behind their chronological age - thus skeletal maturation is proceeding at an appropriate rate and they would be expected to reach their final height at an average age. It is vital in this situation to ensure that the parents are normal and that the child is not small because they have an inherited a pathological cause for their short stature eg. hypochondroplasia. Where necessary we have investigated the families to ensure we are not missing any of the more subtle forms of skeletal dysplasia.

1.3. Effects of short stature in the pre-pubertal child

Early studies on mixed groups of short children suggested that they function poorly with under-achievement at school (Pollitt and Money 1964), immaturity (Holmes et al 1985, Holmes et al 1986) and inadequate coping (Steinhausen and Stankhe 1976). These studies are difficult to interpret as they contain very heterogeneous groups of children, some with major pathological causes of their short stature which would produce other problems in themselves eg. significant dysmorphic features or learning difficulties. It is difficult to be sure how the findings relate to the short child who is otherwise normal.

There is a mixed picture in the literature of the psychological effects of normal variant short stature in the pre-pubertal child with some studies suggesting an increased incidence of behaviour problems and decreased self esteem (Gordon et al 1982) and a higher incidence of learning problems (Gold 1978). Not all such short children appear to have such significant problems. In the Wessex growth study (Voss et al 1991) the short children appeared to have unimpaired self esteem and normal patterns of behaviour, but a tendency towards hyperactivity and poor concentration.

There may well be differences in the self esteem of children who present to a paediatrician because of concerns (either their own or parental) about their short stature than in those who are unselected.

For more details of the psychological effects of short stature, please see Section 4.2.

1.4. Treatment of normal variant short stature

- background literature review

Tanner et al included four children with growth delay in their cohort of children treated with human growth hormone reported in 1971. There was no definite response to therapy, but psychosocial factors complicated the picture in at least one of the children. In these early studies there was little evidence of benefit for non-GH deficient children, but their numbers were small and they were being treated with a sub-optimal regimen by today's standards. Despite these results, by the early 1980's there was beginning to be a reassessment of the use of pituitary-derived growth hormone for causes of short stature other than classical growth hormone deficiency. Kowarski et al (1978) had described two children with growth failure but normal growth hormone levels who were treated with growth hormone with improvement in their growth rates. Rudman et al (1979, 1980, 1981) gave children with normal variant short stature a ten day course of human growth hormone and then suggested that the children who were more likely to respond to a six month period of daily growth hormone injections were characterised by an increase in the anabolic responses of soft tissues and IGF1 (somatomedin C) at ten days. Van Vliet et al (1983) treated fifteen short normal children aged 4 to 15 years with thrice weekly intramuscular growth hormone for six months - not all responded but those who did best were younger, had a greater delay in bone age and a slower pre-treatment growth rate than the non-responders. Plotnick et al (1983) and Gertner et al (1984) used similar regimes to treat short children who were capable of normal growth hormone secretion - their subjects showed an increase in growth rate over six months from a mean of 3.6 to 7.4 cm/yr and 4.3 to 7.4 cm/yr respectively, but the response was not predictable by the acute rise in IGF1 after five to ten days of treatment. Albertsson-Wikland (1986) investigated a group of 31 short normal children treated with a daily injection of pituitary-derived growth hormone with good growth responses in the majority of the children. Those who responded best had the lowest levels of endogenous pulsatile growth hormone secretion. These studies were encouraging, but such trials were stopped after the appearance of Creutzfeldt-Jakob disease and the

withdrawal of pituitary-derived human growth hormone (Buchanan et al 1987).

The development of biosynthetic human growth hormone allowed more clinical trials of growth hormone in the short normal child to develop. Wit et al from the Dutch Growth Hormone Working Group (1989) reported thirty short, slowly growing children with normal growth hormone responses to standard provocation tests, randomly assigned to methionyl growth hormone or no treatment for a year, with a significant improvement in growth rate in the growth hormone treated group. Hindmarsh and Brook (1987) reported 26 short normal pre-pubertal children treated for two years with methionyl growth hormone. Height velocity improved from a pre-treatment mean of 5.3 cm/yr to 7.4 cm/yr. Several studies have shown benefit in the short term from rhGH treatment in the short normal child (Lin et al 1989, Zadik et al 1992) but good placebo controlled studies are understandably more rare. Short placebo controlled studies (Wales and Milner 1989, Ackland et al 1990, Cowell 1990) suggested that there may be some placebo effect from the injection, but these have only included giving placebo for a maximum of six months treatment, which is probably not long enough to be confident of the response. A placebo controlled study for at least a year was needed to fully evaluate the placebo effect of daily "rhGH" injections.

It is vital that any child included in a rhGH study is followed to adult height. As biosynthetic growth hormone has only been available since 1985, there are as yet no long term studies published on the effect on adolescent growth and adult height. There are increasing concerns about the effcts of rhGH on the timing and tempo of puberty (Darendeliler et al 1990), and good prospective studies are needed in pre-pubertal children being treated with rhGH to ellucidate the effects on the short normal child.

Some medium-term studies are now in the literature. Hindmarsh et al (1990) have reported three year follow up of their cohort of children. By the end of three years there did seem to be an improvement in predicted final height, but to maintain good growth rates it was necessary to increase the dose of rhGH given, and the children had not yet gone through puberty. Moore et al (1992) concluded that most short normal children will show an initial improvement in growth in response to rhGH, with about half maintaining accelerated growth into the third year of treatment. Hopwood et al (1993) also reported three years of rhGH treatment in a large cohort of children, and again showed the best growth rate was acheived during the first year of treatment. Although timing of pubertal onset appeared to be

normal, boys seemed to be progressing through puberty faster than would be expected.

The objectives in treating the short normal child with growth-promoting agents is not just to increase final height. Indeed we do not yet have the studies to prove or refute this. Improving growth in the short term may make the child more like his peers, but we must show that this leads to psychological benefit to justify treatment. Good studies looking at both the growth responses and the psychological effects of treatment are needed (for more details please see Section 4: The psychological effects of short stature and its treatment).

1.5. The effects of biosynthetic human growth hormone treatment in the management of pre-pubertal children with familial short stature : a double-blind placebo controlled therapeutic trial

1.5.1. The aims of this study were to determine in pre-pubertal children with familial short stature:

1) whether treatment with rhGH accelerates growth velocity compared to placebo or "no treatment"
2) whether treatment with rhGH has effects on the timing of puberty and rate of pubertal progression.
3) whether treatment with rhGH will improve final height.
4) the benefits (physical and psychological) of rhGH treatment.
5) the complications (if any) of rhGH treatment.
6) whether there are any parameters that are predictive of the response to rhGH treatment.

1.5.2. Patient Recruitment

Children were recruited from the growth and endocrine clinics at the Royal Hospital for Sick Children, Edinburgh, and attached peripheral clinics in Scotland. Children are referred to the growth clinic either by their general practitioners or the school medical service, or are tertiary referrals from other paediatricians.

The following inclusion criteria had to be met :

Height at, or below, 3rd centile for chronological age (HSDS < -1.88)
Height velocity standard deviation score (HVSDS) at or below 0
Peak serum GH concentration of 20mU/l or more during insulin-induced hypoglycaemia or clonidine stimulation
Clinically and biochemically pre-pubertal
Bone age < 8 years
Bone age delay of < 2 years
No other cause found for their short stature.

Ethical approval for the study was obtained from Lothian Health Board Committee on Medical Ethics. Parents and children were given detailed information about the study, and signed appropriate consent forms prior to entry.

1.5.3. Study protocol

Pre-treatment Assessments:

1. Auxology

Prior to entry into the study the children were assessed at the growth clinic for a minimum of 6 months, though the majority had measurements performed over at least one year.

Standing height was measured by a single observer (HS) using a fixed wall mounted Harpenden stadiometer. Sitting height was measured using a sitting stadiometer by a single observer (HS). Weight was measured using a standard balance. Measurements were compared to the standards of Tanner et al (1966, 1976). Triceps and subscapular skinfold thicknesses were measured using Holtain calipers by a single observer (HS) and compared to the standards of Tanner and Whitehouse (1975). Pubertal assessment was performed by a single observer (HS) using the standard ratings of Tanner (Tanner 1962, Marshall and Tanner 1969, Marshall and Tanner 1970). Testicular volume was estimated by comparison with the standard ovoids of a Prader orchidometer (Zachmann et al 1974).

Bone age was assessed by X-ray of left hand and wrist, and the Tanner Whitehouse II 20 bone method (Tanner et al 1983) of analysis performed by a single observer (HS).

Blood pressure was measured in the right upper limb by a single observer (HS) using an appropriate sized sphygmomanometer cuff for each child (de Swiet et al 1992).

2. Endocrine assessment

a) Overnight profiles

Prior to entry into the study all the children underwent overnight blood sampling form 20.00 hrs to 08.00 hrs, with samples collected at 20 minute intervals to measure plasma growth hormone. The children were admitted to hospital in the early evening following a normal days activities and eating pattern. Topical anaesthetic cream (EMLA) was applied to an ante-cubital fossa and subsequently an indwelling intravenous cannula was inserted at least 45 minutes before blood sampling was commenced. The children were allowed to be freely active, and eat and drink normally during the evening. They were strongly encouraged to be in bed by 10.00pm with "lights out" at 10.30pm. In practice the majority slept soundly until 7.30-8.00am the following morning. 2 ml samples of blood were taken every 20 minutes, collected into lithium heparin tubes, immediately centrifuged at -4C, then

separated and the plasma stored at -20C until assayed. All samples from a given child were assayed in the same batch.

The samples were assayed in the Regional Hormone Laboratory, Edinburgh using an immunoradiometric assay (IRMA). The growth hormone profiles obtained were evaluated using the Munro modification of PULSAR program on an Apple Macintosh personal computer.

For an additional study, timed 12 hour urine collections (20.00 hrs to 08.00 hrs) were also obtained from the children. Urinary growth hormone was measured in these samples at the Regional Hormone Laboratory, Edinburgh using an amplified enzyme immunoassay (Novo Nordisk).

Insulin like growth factor 1 (IGF1) levels were measured at 08.00 hrs in all children. IGF1 was assayed by Novo Nordisk using a radio-immunoassay.

Testosterone and oestradiol levels were measured in boys and girls respectively at 08.00 hrs (Wu et al 1993).

b) Dynamic pituitary function tests

The following morning the children underwent combined pituitary function tests, using either insulin-induced hypoglycaemia (0.15iu/kg) or clonidine (0.15mg per sq.m body surface area) together with TRH (7 micrograms/kg to a maximum of 200 micrograms) and LHRH (0.25 micrograms/kg). 29 of the children underwent insulin induced hypoglycaemia, with the remainder receiving clonidine for the growth hormone provocation test. The tests were performed in a recognised growth centre, on a ward where the staff were well acquainted with the potential hazards of such tests and their management, and full facilities for resuscitation were available (Shah et al 1992). The tests were supervised by the same person (HS) on all occasions. There were no significant adverse events as a consequence of these tests.

The pituitary function tests were performed without the child being "primed" with the appropriate sex steroid, as we were trying to fully evaluate the physiological status of the child prior to treatment. The LHRH test used is a very low dose one and is likely to be more physiological than the supra-maximal stimulus of the conventional doses of 100 microgms or 2.5 microgms/kg (Hughes 1989). This study in combination with the studies in older boys allowed us to evaluate the low dose LHRH test as to whether it is a more useful way of assessing the imminence of puberty than the conventional dose.

3. Psychological assessment

The psychological studies were performed using a series of questionnaires involving parent, child and teacher reports. Those children under 8 years of age were excluded as the questionnaires are not valid for use in younger children.

For details please see Section 4.3.

Randomisation of treatment

For the first year the study was a double-blind placebo controlled trial. At entry the children were randomised into one of three groups receiving :

1) rhGH 24iu/sq.m/week, given as a daily sub-cutaneous injection
or 2) placebo 24iu/sq.m/week, given as a daily sub-cutaneous injection
or 3) no treatment.

The placebo consisted of glycine, sodium bicarbonate and mannitol (each 12 iu placebo vial containing 60.0 gm glycine, 7.5 gm sodium bicarbonate, and 6.0 gm mannitol) and was identical in appearance and reconstitution to the active rhGH.

After twelve months the children in group 2 were changed to active rhGH in a dose of 15iu/sq.m/wk, given as a daily sub-cutaneous injection. After 24 months the children in group 3 were started on rhGH 15iu/sq.m/wk. Thus, after 24 months all children entering the study were receiving active rhGH.

The study design not only allowed us to assess the effect of a placebo versus active treatment, but also to compare the effects of two doses of rhGH in pre-pubertal children with familial short stature.

Assessment of response

After entry into the study the children were reviewed at three monthly intervals. Detailed auxological measurements (standing height, sitting height, weight, triceps and subscapular skinfold thicknesses, pubertal staging and blood pressure) were made every three months by a single observer (HS). Left hand and wrist X-ray was performed every six months, and bone age assessed by a single observer (HS) using the Tanner Whitehouse TW II method of analysis.

Haematological (full blood count with differential white cell count, and in a subgroup T and B cell counts) and biochemical (liver function, renal function, glucose, glycosylated haemoglobin (HbA1), cholesterol, triglycerides, thyroid function) parameters were measured at entry and at three months, six months and at six monthly intervals thereafter. IGF1 was measured at entry and six monthly. Bone derived alkaline phosphatase was measured at entry, three months, six months and at six monthly intervals.

LH, FSH and testosterone or oestradiol (as appropriate depending on sex of the child) levels were measured six monthly. Although these were random samples, all were taken between 09.00 and 12.00 in the morning.

1.5.4. Pre-treatment Growth Status

37 children (23 boys and 14 girls) entered this study, all with full written informed consent of their parents and themselves.

The mean age of the children was 8.00 years (range 4.70 to 10.37)
The mean HSDS was -2.62 (range -3.60 to -1.79)
The mean height velocity was 4.69 cm/year (range 3.02 to 5.84)
The mean HVSDS was -1.22 (range -3.14 to +0.26)
The mean bone age was 6.54 years (range 3.80 to 8.90)
The mean bone age delay was 1.45 years (range -2.75 to +0.28)
All the girls included in the study have a normal 46XX karyotype
All children were clinically pre-pubertal on entry into the study.

The mean height of the fathers was 165.7 cms (SD 5.24) = 3-10th centile
The mean height of the mothers was 153.9 cms (SD 6.10) = 3-10th centile
The mean target height of the boys was 167.0 cms (SD 3.59) = 10th centile
The mean target height of the girls was 152.3 cms (SD 4.54) = 3-10th centile

The mean predicted height of the boys at entry was 159.6 cms (SD 2.41) = <3rd centile
The mean predicted height of the girls at entry was 152.4 cms (SD 2.41) = 3-10th centile

For the boys, the mean predicted height at entry (calculated using the formula of Tanner 1983 based on height, bone age, growth rate and rate of bone maturation) at entry was significantly less than the target height (calculated as mid parental centile height) by 7.40 cms ($p = <0.001$). For the girls, the mean predicted height was not significantly different to the target height.

The mean birth weight of these children was 2.76 kg (SD 0.56, range 1.55 - 3.94 kg). All but one child was born at term. This child was born at 33 weeks gestation and had no major neonatal problems. Using a definition of small for gestational age (SGA) as <10th centile weight for gestational age, 23 of the children were SGA. Unfortunately accurate birth lengths were not available. The high percentage of SGA infants in the cohort is a probably a reflection of the relatively small maternal size.

1.5.5. Pre-treatment Endocrine Status

a) Growth hormone

Spontaneous growth hormone (GH) secretion is in a pulsatile fashion, and thus the GH profiles we obtained are a measure of the child's physiological GH status. The overnight growth hormone profiles we obtained from each child were analysed using the Munro modification of the PULSAR program. This program detects pulses of GH, and the overnight GH secretion can be described in terms of pulse amplitude (PA), sum of pulse amplitude, pulse interval, area under the curve and mean GH level. It has been found that there is an asymptotic relationship between HVSDS and pulse amplitude (Hindmarsh et al 1987), and that pulse amplitude is the best feature of the GH profile to relate to growth.

To summarise the results from the overnight GH profiles I have expressed the data as the means (SD) of mean PA, sum PA, and mean GH level, all expressed in mU/l, for the children in the groups of the study. Also included in the table are the mean responses to the provocation test and mean IGF1 levels.

	All children	No treatment	Placebo	rhGH
Overnight GH				
Mean PA	14.1 (5.7)	13.6 (5.4)	13.0 (7.5)	15.4 (3.9)
Sum PA	71.0 (25.1)	65.9 (17.0)	63.8 (24.9)	83.2 (29.6)
Mean GH level	7.4 (2.7)	7.0 (2.3)	6.8 (2.7)	8.3 (2.8)
Peak stim. GH (mU/l)	35.0 (12.7)	34.2 (11.1)	30.0 (7.8)	40.7 (16.3)
IGF1 (U/ml)	0.47 (0.26)	0.55 (0.23)	0.39 (0.26)	0.48 (0.26)

There were no significant differences in the mean GH parameters measured overnight in the three groups, namely mean pulse amplitude, sum of pulse amplitude or mean overnight GH level.

In order to enter the study all children were required to reach 20 mU/l or more following a standard provocation test, which is conventionally recognised as a "normal" response to a growth hormone stimulation test. There were no significant differences in the mean peak GH response to the provocation tests between the three groups.

IGF1 results are expressed as units/ml (1 Unit/ml = 37.4 nmol/l). The reference range quoted by the laboratory is :

0-5 years 0.1-0.96 Units/ml

6-10 yrs 0.1- 2.41 Units/ml

The mean levels found in our children are well within the expected range, though at the lower end. There was no significant differences between the no treatment and the rhGH groups at entry, but the mean IGF1 level in the placebo group was significantly lower (p=0.01) than the no treatment group but not the rhGH group.

b) LHRH, testosterone and oestradiol levels

Measurement of overnight pulsatile LH secretion using highly sensitive assays has been shown to be the best way of detecting the hormonal onset of puberty in younger children (Wu et al 1990, 1991). However this was not practical to do in all our children and so we used the LH response to a low dose of LHRH (0.25 microgms/kg) as a sensitive way to assess the pubertal activity of the hypothalamo-pituitary axis. Pre-pubertal children normally have baseline LH levels of <1.0 units/l, and will only show a small increment in the concentration of LH in response to this dose of LHRH. We have found that the pre-pubertal response of LH to LHRH is to a level of 3.8 units/l or less, whereas once puberty is underway level of >4.5 units/l or more are achieved (see results for older boys in Sections 2 and 3).

	All children	No Treatment	Placebo	rhGH
Mean (SD) peak LH units/l	3.30 (1.47)	3.20 (1.37)	3.55 (1.94)	3.14 (1.03)
Median 08.00 testost. nmol/l	<0.5 n=23	<0.5 n=10	<0.5 n= 7	<0.5 n= 6
Median 08.00 oestradiol pmol/l	<57 n= 14	<57 n= 3	<57 n= 5	<57 n= 6

The mean peak LH response of all the children in the study was pre-pubertal, with no significant differences seen between the groups. Individually three children produced peak LH responses to LHRH of >4.5 units/l.

Early morning testosterone levels have been shown to be a useful marker of the imminence of puberty. Boys in whom early morning testosterone is <0.7 nmol/l are unlikely to enter puberty within the next 12

months (Wu et al 1993). All but one of the boys included in this study had a 08.00 testosterone of <0.7 nmol/l (the remaining boy having a level of 0.7 nmol/l) with median levels being <0.5 nmol/l. Median early morning oestradiol levels in the girls in the study were all <57 pmol/l ie. pre-pubertal.

Thus, biochemically these children appeared to be pre-pubertal at entry and there were no significant differences between the groups.

1.5.6. Growth Results

See Table : Summary of growth data.

There were no significant differences between the three groups at entry into the study in terms of : chronological age, height, height SDS, height velocity, height velocity SDS, bone age, bone age delay, and predicted adult height.

1 year growth results

37 children entered the study

13 received no treatment

12 received placebo injections

12 received rhGH

One girl in the placebo group and one girl in the rhGH group entered puberty during the first year of treatment - their growth data are not included in the following analysis, as in this study we wished to determine the effects of growth promotion in pre-pubertal children without the compounding factor of the pubertal growth spurt. One boy in the "no treatment" group defaulted from follow up during the first study year. Thus data from 34 children are presented.

"No treatment" group

The twelve children who received "no treatment" over the first year of the study, and who completed follow up, grew at a mean rate of 4.83 cm/yr (mean HVSDS -0.66) compared to a mean rate of 4.37 cm/yr (mean HVSDS -1.42) over the pre-study year. This difference was not significant. Mean height SDS did not change significantly over the year. Mean bone age advance/chronological age advance was 0.92 years. There was no significant change in mean HSDS for BA (-1.18 cf -1.19 pre-entry) nor was there any significant change in mean predicted adult height compared to that prior to randomisation (157.2 cms cf 157.4 cms).

Study 1: Summary of Growth Data

	No treatment	Placebo	rhGH
At entry:			
No. children	11	11	11
Age (yrs)	8.51 (1.51)	7.22 (1.34)	7.69 (1.64)
HSDS	-2.58 (0.49)	-2.75 (0.61)	-2.50 (0.47)
HV (cm/yr)	4.37 (0.60)	4.70 (0.57)	4.96 (0.81)
HVSDS	-1.42 (0.65)	-1.32 (0.73)	-0.90 (0.86)
Bone age (yrs)	7.02 (1.35)	5.78 (1.18)	6.28 (1.36)
HSDS for BA	-1.19 (0.72)	-1.31 (0.97)	-1.07 (0.90)
PAH (cms)	157.4 (4.33)	155.8 (5.29)	156.9 (4.73)
At one year:			
No. children	12	11	11
HSDS	-2.60 (0.46)	-2.75 (0.57)	-2.02 (0.61)
HV (cm/yr)	4.83 (0.65)	4.76 (0.47)	7.67 (1.43)
HVSDS	-0.66 (0.87)	-1.01 (0.46)	+2.48 (1.82)
dBA/dCA	0.92 (0.45)	1.11 (0.44)	1.37 (0.42)
HSDS for BA	-1.18 (0.71)	-1.62 (0.77)	-1.16 (0.77)
PAH (cms)	157.2 (4.14)	155.9 (5.27)	159.0 (5.00)
At two years:			
No. children	11	10	11
HSDS	-2.69 (0.52)	-2.34 (0.67)	-1.85 (0.70)
HV (cm/yr)	4.48 (0.58)	7.37 (1.53)	6.23 (1.41)
HVSDS	-0.97 (0.68)	+2.33 (1.84)	+1.10 (1.87)
dBA/dCA	1.18 (0.52)	1.36 (0.67)	1.20 (0.58)
HSDS for BA	-1.40 (0.73)	-1.45 (1.09)	-1.14 (0.63)
PAH (cms)	157.5 (4.86)	157.9 (6.69)	159.3 (5.45)

Placebo group

Eleven children received placebo injections for the first year. Over the first year of the study they grew at a mean rate of 4.76 cm/yr (mean HVSDS -1.01) compared to a mean rate of 4.70 cm/yr (mean HVSDS -1.32) over the pre-study year. This difference did not reach significance. In order to determine whether there was a placebo effect apparent early in the study year the mean height velocity over the first six months was compared to the mean height velocity over the second six months of treatment. Mean height velocity at six months was 4.75 cm/yr (mean HVSDS -1.06) compared to mean height velocity between six and twelve months of 4.83 cm/yr (mean HVSDS -0.85) - this does not reach significance, and the rates are comparable to those children who received no treatment at all.

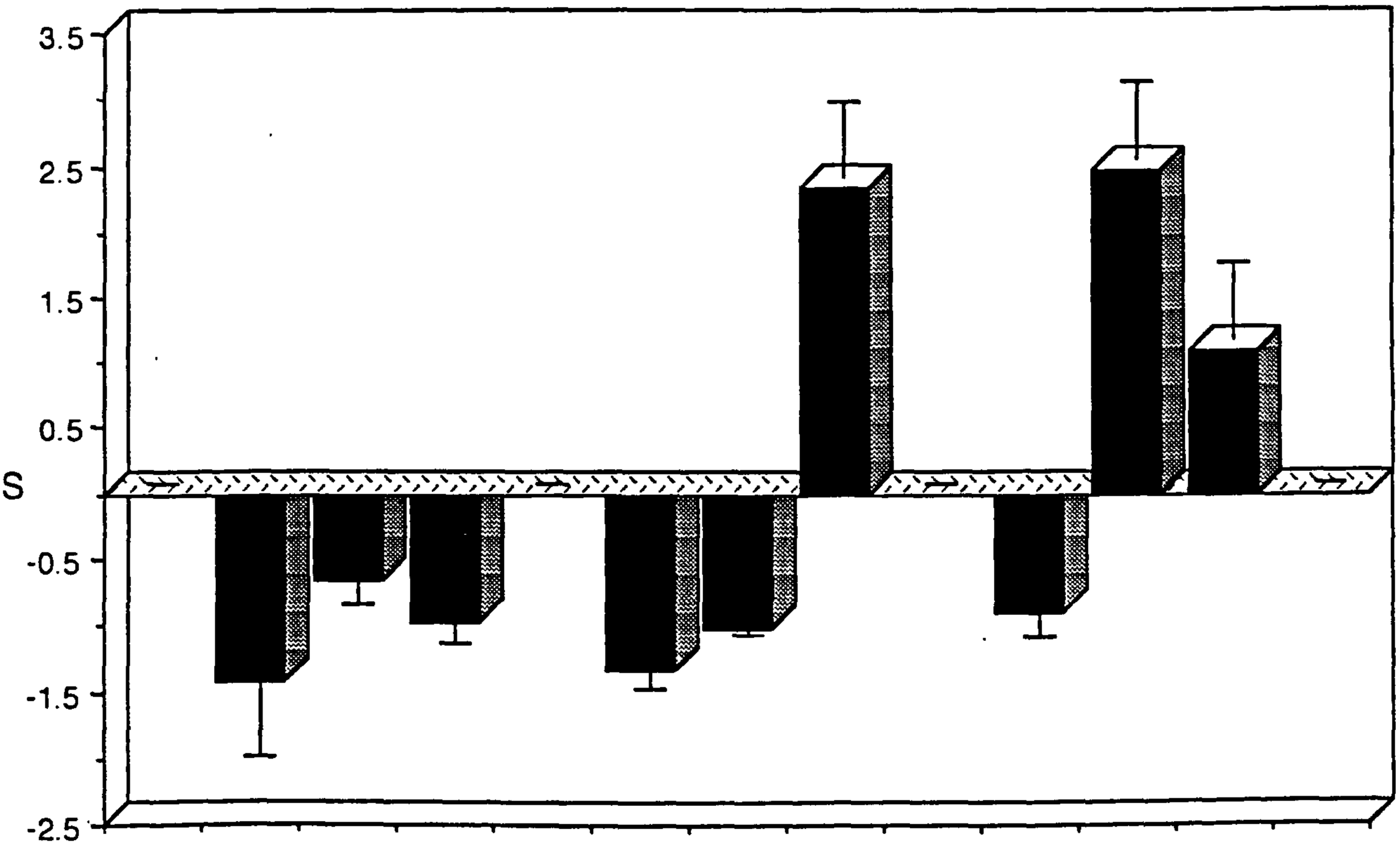
	0-6 months	6-12 months
No. children	11	11
HV	4.75 (0.78)	4.83 (0.70)
HVSDS	-1.06 (0.95)	-0.85 (0.77)

Thus there was no discernible placebo effect. Mean height SDS did not change significantly over the year. Mean bone age advance/ chronological age advance was 1.11 years. There was no significant change in mean HSDS for BA (-1.62 cf -1.31 pre-entry) nor was there any significant change in mean predicted adult height compared to that pre-randomisation (155.9 cms cf 155.8 cms)

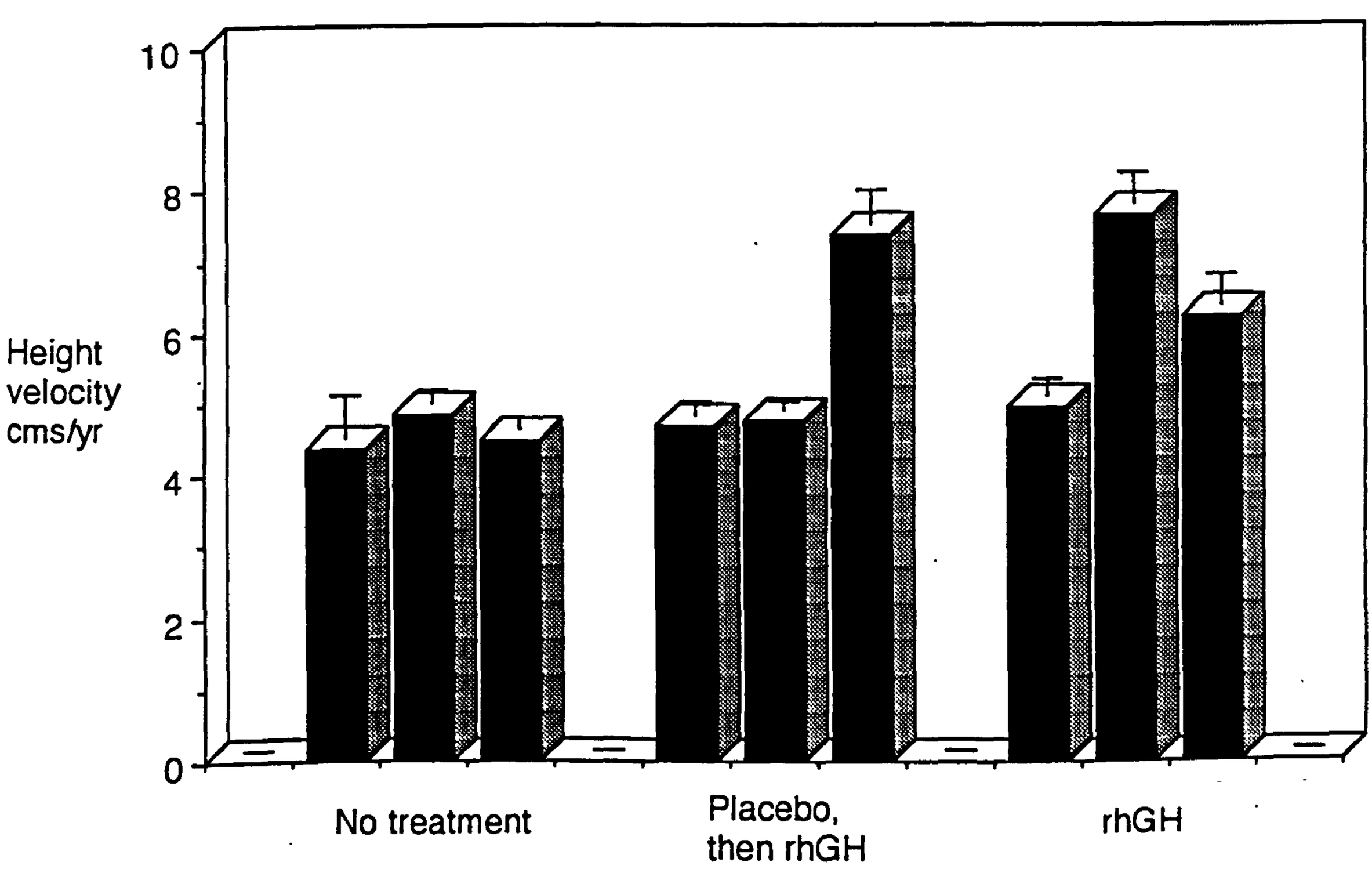
rhGH group

Eleven children received treatment with active recombinant human growth hormone (rhGH) 24 iu/sq.m/wk over the first twelve months of the study. Mean height velocity improved from 4.96 cm/yr (mean HVSDS -0.90) pre-treatment to 7.67 cm/yr (mean HVSDS +2.48) over the study year. This difference is highly significant ($p = <0.001$). These children grew significantly faster than those who received "no-treatment" or placebo injections ($p = 0.001$). Mean height SDS improved significantly over the year (-2.50 pre-treatment compared to -2.02 at one year, $p = <0.001$). Mean bone age advance/chronological age advance was 1.37 years. There was no significant change in mean HSDS for BA (-1.16 compared to -1.07 pre-entry) but there was a significant improvement in predicted adult height from a mean of 156.9 cms pre-randomisation to 159.0 cms at twelve months ($p = <0.001$).

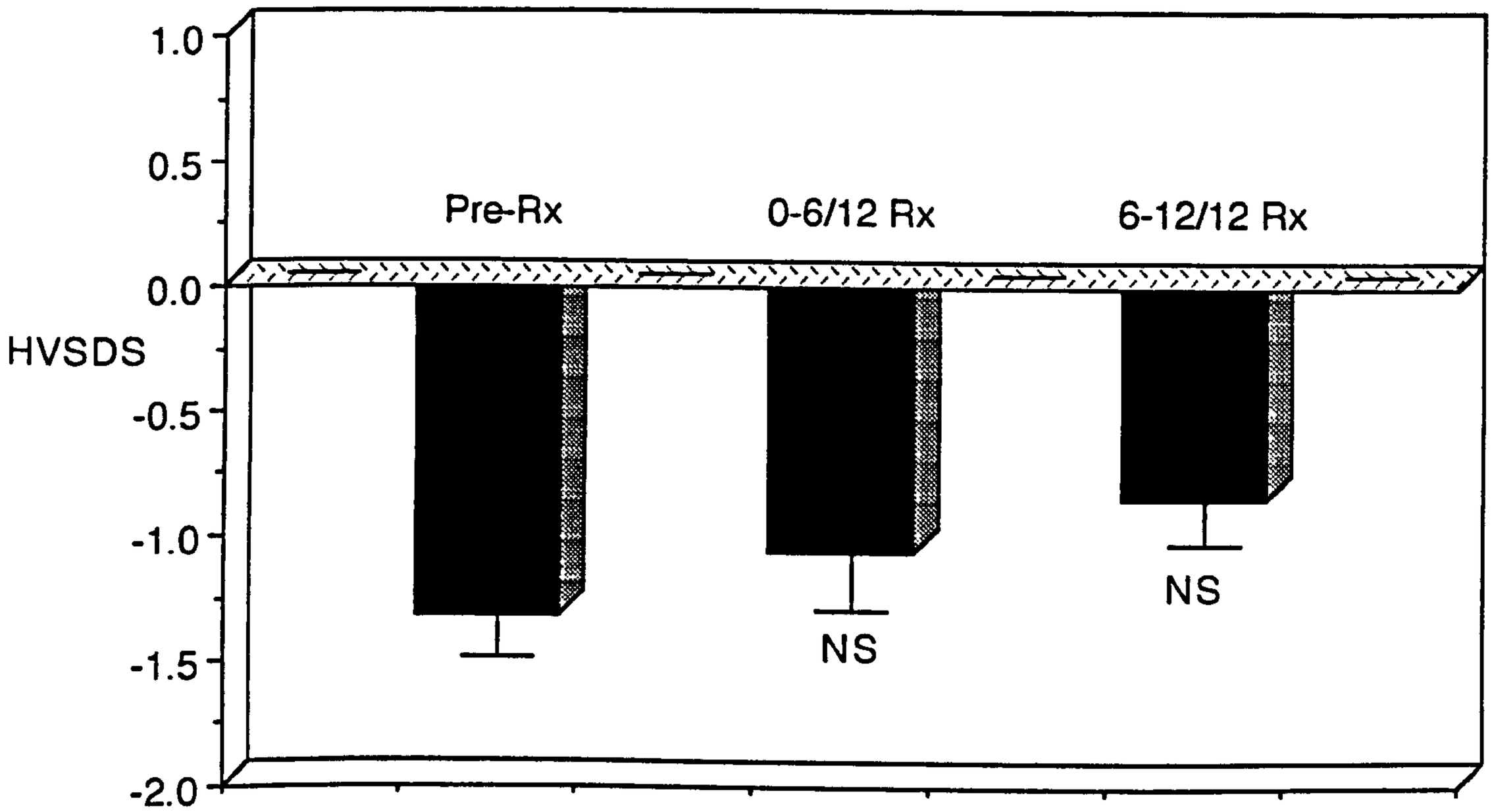
HVSDS (SE) at entry, 12 months and 24 months



Height velocity (SE) at 12 months and 24 months



Effect of placebo on growth : HVSDS (SE)



2 Year Growth Results

"No treatment" group

Eleven of the "no-treatment" group of children had completed 24 months of follow-up from the time of entry into the study. Over the second study year they received "no treatment" again. They grew at a mean rate of 4.48 cm/yr (mean HVSDS -0.97) over this second twelve months, which was not significantly different to their growth rate in the pre-treatment year or the first study year. Mean HSDS gradually fell over the two years and was -2.69 at the end of 24 months. Mean bone age advance/chronological age advance was 1.18 years over the second year, so that skeletal maturation was proceeding at a normal rate over the two year period. There was no significant change in mean HSDS for BA or in mean predicted adult height over the second year.

Placebo/15iu rhGH group

Ten of the "placebo" group of children had completed 24 months of the study. At the end of the first twelve months they had been changed onto active rhGH in a dose 15iu/sq.m/wk - ie. a smaller dose than the children who were treated with rhGH from the outset. Over the second study year they grew at a mean rate of 7.37 cm/yr (mean HVSDS +2.33). This was significantly faster than the growth rate over the year when they received placebo injections ($p = <0.001$). Mean HSDS improved over this second year from -2.75 to -2.34 ($p = 0.001$). Mean bone age advance/chronological age advance was 1.36 years over this second twelve month period. There was no significant change in mean HSDS for BA but mean predicted adult height improved from 155.9 cms to 157.9 cms ($p = 0.01$).

It is important to note that these children's growth rate over the second study year was not significantly different to the first year growth rate in the children who received 24 iu/sq.m/wk from the outset of the study (7.37 compared to 7.67 cm/yr).

rhGH group

All of the original rhGH treated group have completed a second year of treatment. Over this second year their mean height velocity was 6.23 cm/yr (mean HVSDS +1.10) ie. slower than that over the first treatment year. They showed the waning of effect that is characteristically seen in any group of children treated with rhGH with the best growth response early in treatment. However their mean growth rate was still improved compared to their pre-treatment rate ($p = 0.02$). Mean HSDS continued to improve to -1.85 ($p = 0.05$ compared to one year HSDS). Mean bone age advance/chronological age advance was 1.20 years over this second twelve month period, giving a mean total bone age advance of 2.57 years over the two study years. There was no significant change in mean HSDS for BA or predicted adult height over this second year.

Further growth data

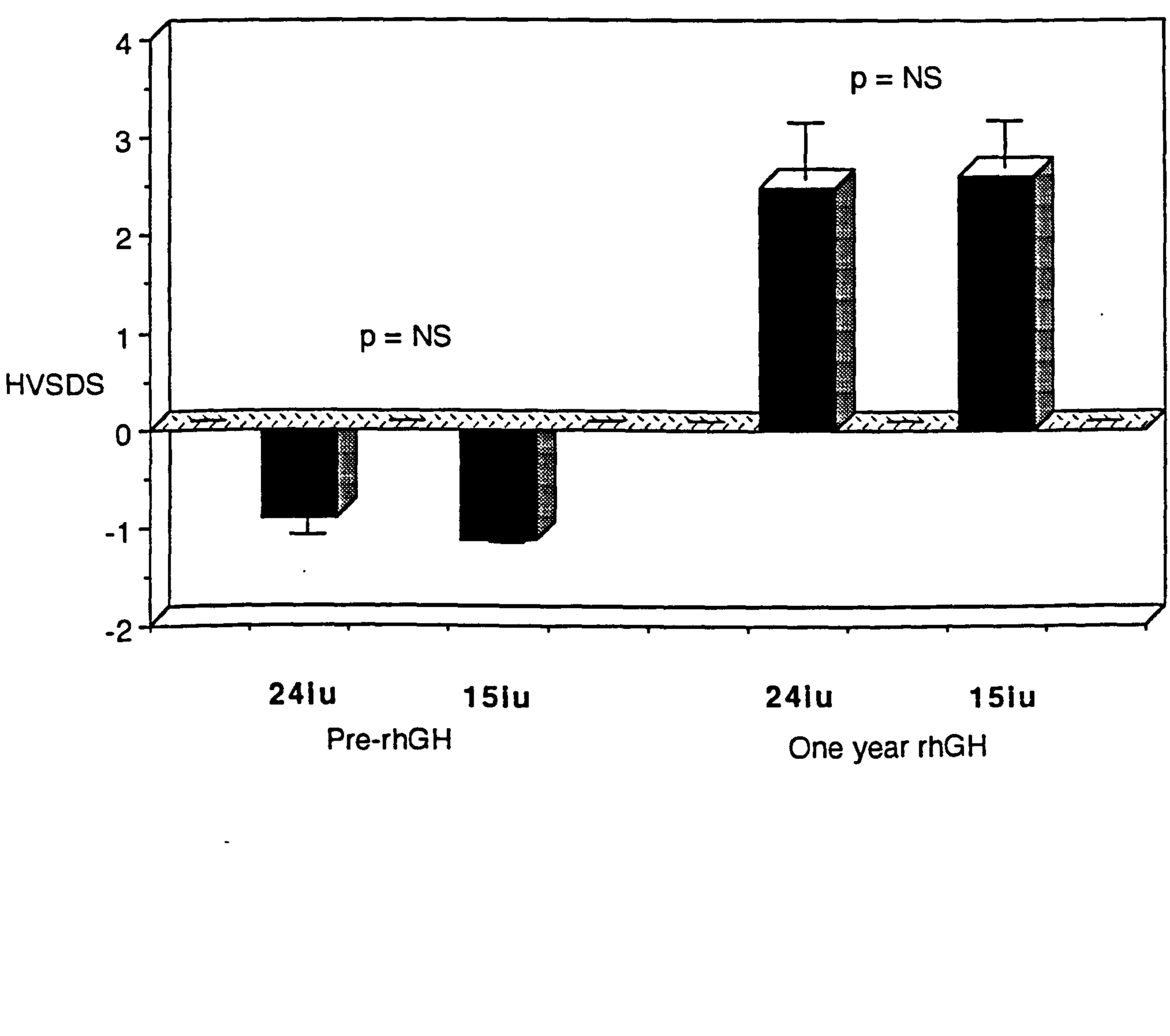
Growth data are available from the two girls who entered puberty during the first study year - these two girls grew at rates of 8.44 and 7.40 cm/yr respectively. The first received placebo, whilst the second received active rhGH. Both girls had a considerable increase in height velocity over their pre-treatment growth rates but this is only what one would expect for girls in early puberty. Thus, their results have been excluded from the above analyses.

Smaller numbers of children have now completed three or more years of the study, but the numbers are too small to analyse in separate groups.

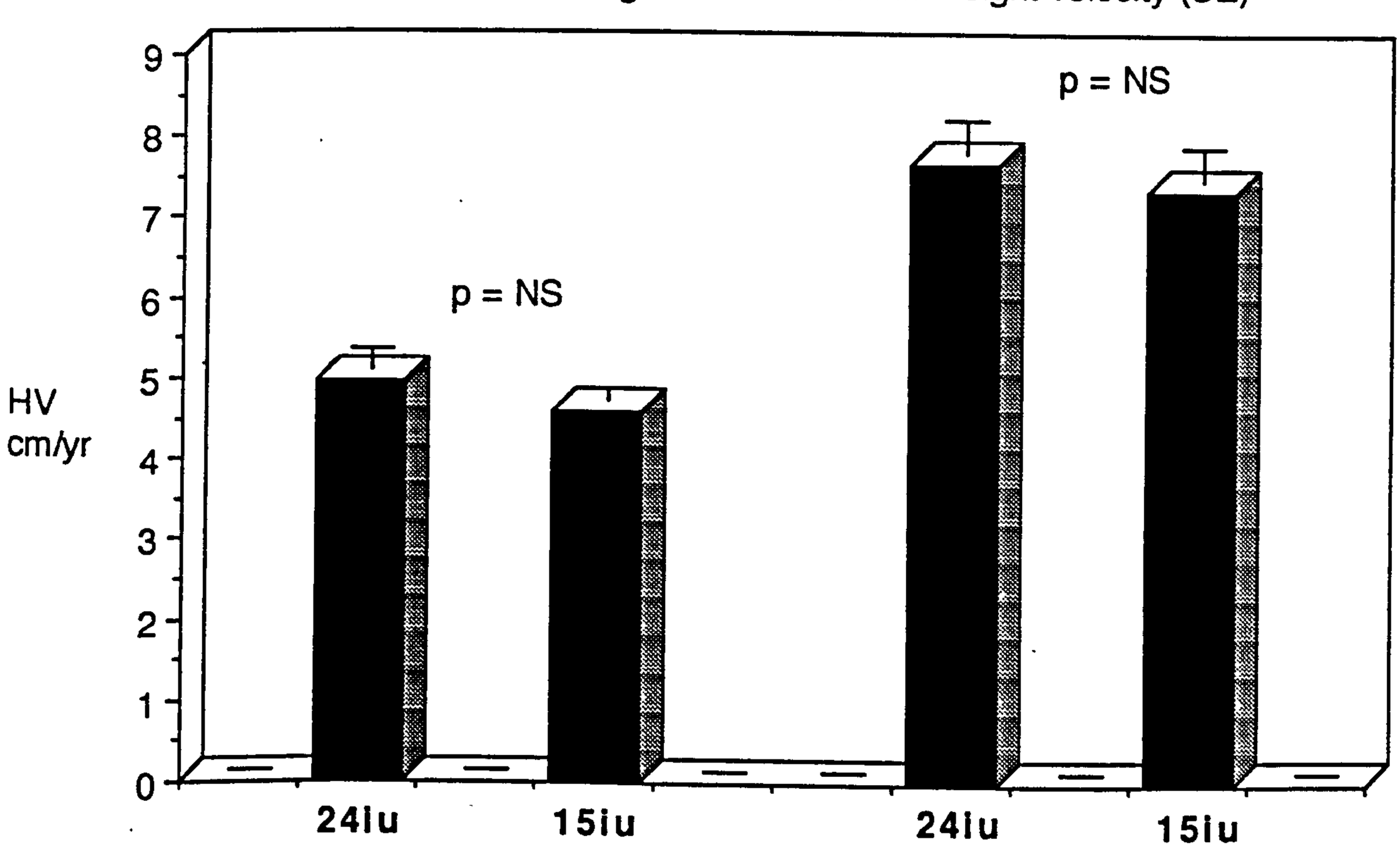
Comparison of two doses of rhGH

By combining the data from all the children treated with 15iu rhGH (either having had a year of placebo or two years of no-treatment) and who remained pre-pubertal during their first year of active treatment ($n = 15$), we can obtain a comparison of the effects of two doses of rhGH in treating pre-pubertal children with familial short stature.

Effects of differing doses of rhGH on HVSDS (SE)



Effect of differing doses of rhGH on height velocity (SE)



	24 iu/sq.m/week	15 iu/sq.m/week	
No. children	11	15	
Age at start of rhGH (SD)	7.69 (1.64)	8.61 (1.42)	p = NS
Pre Rx HV (SD) cm/yr	4.96 (0.81)	4.59 (0.57)	p = NS
Pre Rx HVSDS (SD)	-0.90 (0.86)	-1.12 (0.51)	p = NS
One year HV (SD) cm/yr	7.67 (1.43)	7.35 (1.50)	p = NS
One year HVSDS (SD)	+2.48 (1.82)	+2.62 (1.75)	p = NS

It is clearly apparent that there is no significant difference in pre-pubertal children's growth responses to 15 or 24 iu rhGH/sq.m/wk, and in this context there is no benefit in prescribing the larger dose of rhGH.

In the majority of children treated with rhGH (either 24iu or 15iu/sq.m/wk) there is a good response over the first 12-24 months of treatment with acceleration of growth. There is then waning of the acceleratory effect with the children assuming a more normal growth rate - they cross centiles upwards on their growth charts for approx 12-24 months, and then grow along their "new centile" - see examples of growth charts in appendix.

1.5.7. Effects on puberty

In children with isolated idiopathic GH insufficiency it has been suggested that treatment with growth hormone significantly shortened the duration of puberty (Darendeliler et al 1990). Therefore in this group of children who were pre-pubertal at the onset of treatment it is especially important to follow them prospectively with regard to the timing of pubertal onset and the speed of progression through puberty.

At randomisation and entry into the study all the children were clinically prepubertal (defined as girls at Tanner breast stage 1, boys at Tanner genitalia stage 1 and testes <4ml). This was also demonstrated by their response to low dose LHRH and early morning testosterone and oestradiol measurements (see above). There were no significant differences between the groups in terms of the mean peak LH response to low dose LHRH, nor in the median testosterone and oestradiol levels measured at 08.00 hrs.

The first sign of male puberty is testicular enlargement, most accurately determined with ultrasound, but more practically in comparison with standard ovoids, the "Prader" orchidometer (Zachmann et al 1974)).

Testicular volumes of 4 ml or more indicate that puberty is underway. According to Marshall and Tanner (1970) the average age at which genitalia stage 2 is reached is 11.6 years, with 95% of boys reaching this between the ages of 9.5 and 13.5 years. In a further study of normal adolescent growth and development patterns, Buckler documented the 50th centile for the attainment of genitalia 2+ to be 12.5 years (Buckler 1990).

The onset of puberty in girls is defined as the appearance of breast buds (Tanner breast stage 2) with the average age for initial breast development being 11.2 years with 95% girls developing breast buds between 8 and 13 years of age (Marshall and Tanner 1969). In a Scottish cohort the average age of breast stage 2 is a little earlier at 10.5 years (unpublished data), and is at 11.0 years in Buckler's study (Buckler 1990).

In the ten girls in our study who have so far reached puberty, the mean age of attaining breast stage 2 was 10.39 (SD 0.88) years. Two of these girls were not on active rhGH when they entered puberty (one receiving placebo and one receiving the second year of "no treatment"). When their data are discounted the mean age for girls to achieve breast stage 2 was 10.21 (SD 0.80) years at a mean bone age of 9.1 (SD 0.80) years. The mean duration of rhGH treatment (either 24iu or 15iu/sq.m/wk) prior to the onset of puberty was 1.35 years (range 0.3 to 2.9 years). Only two girls have so far reached menarche at an average age of 13.25 years (not very different to the Tanner standard of 13 years), but the numbers are too small to provide any meaningful results.

In the nine boys who have so far reached puberty the mean age at attainment of 4 ml testes was 11.78 (SD 0.69) years. One boy was in his second year of "no treatment", and when his data are discounted the mean age for boys to achieve 4 ml testes was 11.78 (SD 0.74) years, at a mean bone age of 10.7 (SD 0.89) years. The mean duration of rhGH therapy prior to pubertal onset was 1.58 years (range 0.6 to 2.7 years). Four of these boys have now reached 12 ml testes, at mean age of 13.45 years.

Compared to the Tanner and Buckler standards, our study girls who have received rhGH are tending to enter puberty a year earlier on average than one might expect, whereas in the boys the timing of pubertal onset appears to be more average. rhGH may have a more marked effect on the timing of puberty in girls, in whom it is well recognised there is an increased tendency for precocious puberty to occur. In the boys who have attained 12 ml testes this has been reached somewhat earlier than the median Tanner standard of 14.5 years, suggesting that perhaps the rate of pubertal

progression is faster than would have been expected in the boys. The numbers so far are too small to be conclusive.

Any effects on pubertal timing and rate of progression will affect final height outcome. If puberty is reached earlier, and hence final height achieved earlier than average, then any net increase in height obtained pre-pubertally may be lost. It is imperative that the children included in this study are followed through puberty to final height to determine whether or not this is the case.

1.5.8. Effects on biochemical markers of growth

Two biochemical markers of growth have been measured in these children. Insulin-like growth factor 1 (IGF1) is predominantly produced by the liver, and is known to rise in GH-deficient children treated with growth hormone, but the pre-treatment IGF1 level is not an accurate predictor of the response to treatment (Dean et al 1982).

Bone-derived alkaline phosphatase (bALP) is a more specific marker of bone turnover. Few studies have specifically looked at this marker in response to growth-promoting treatments, or as a predictor of response to GH therapy.

1. IGF1

IGF1 was measured at entry into the study and at six months. The results are expressed as Units/ml (1 Unit/ml = 37.4 nmol/l). The mean levels at entry in our children are at the lower end of the expected range.
There were no significant differences between the no treatment and the rhGH groups at entry, but the mean IGF1 level in the placebo group was significantly lower (p = 0.01) than the no treatment group but not the rhGH group.

	IGF 1 at entry Mean (SD)	Increase in IGF1 at 6 months
No treatment	0.55 (0.23)	0.04 (0.08)
Placebo	0.32 (0.39)	0.08 (0.15)
rhGH 24 iu	0.48 (0.26)	0.63 (0.52)
rhGH 15 iu	0.50 (0.18)	0.45 (0.22)
(all pre-pubertal children combined n = 15)		

Mean IGF1 levels did not change significantly in the children who received "no treatment". In the children who received placebo for the first year, there was no significant change in mean IGF1 during the placebo period. Mean IGF1 levels increase in response to rhGH. In the children who were treated with rhGH 24iu/sq.m/week from the outset, there was a highly significant rise in IGF1 levels at six months (p = 0.003). In the children who were treated with 15iu/sq.m/week rhGH after either placebo or no treatment, and who remained pre-pubertal, there was a highly significant increase in IGF1 at six months (p = 0.001). The magnitude of this response was not different from that seen in the 24iu treated group of children.

There was no correlation between the pre-treatment IGF1 level and the height velocity over the first year of active treatment, therefore we cannot

use pre-treatment IGF1 levels as a predictor of which short normal child may benefit from rhGH treatment. There was a weak correlation (r = 0.53) between increment in IGF1 at 6 months and height velocity over the first year of active treatment.

2. Bone-derived alkaline phosphatase (bALP)

This was measured at 3 months in children receiving a treatment (either placebo or growth hormone) and at 6 months in those receiving no intervention. bALP was measured by an in-house lectin affinity electrophoresis method at the Royal Hospital for Sick Children (Crofton 1992). The results are expressed as the mean increase in bALP at three (or six) months over the baseline measurement.

Mean (SD) increase in bALP (units/l) at three months :

	Increase in bALP
No treatment	+ 34.2 (44.5)
Placebo	- 8.3 (55.9)
rhGH 24 iu	+112.9 (59.6)
rhGH 15 iu	+112.1 (79.0)
(all pre-pubertal children combined n = 15)	

There was no significant change in mean bALP in the children who received either placebo or no treatment at three months. Children treated with active rhGH, either 24iu/sq.m/week form the outset or 15iu/sq.m/week following a no-treatment or placebo period, show a highly significant rise in bALP by 3 months compared to those children who received no treatment ($p = >0.01$). The increment in bALP in the two groups who received differing doses of rhGH was very similar.

The effect on the biochemical markers of growth (IGF1 and bALP) is similar in the two groups of children treated with the different doses of rhGH. The growth promoting effects were also similar (see section 1.5.6.). The biochemical markers provide further evidence that the effects of the two doses are similar, and in this context there is no advantage in prescribing a larger dose of rhGH.

1.5.9. Side effects of treatment

1. Effects on body fat

Growth hormone has well known lipolytic and anabolic effects. It has long been recognised that growth hormone deficient children have increased body fat compared to normal children, and that when they are treated with replacement growth hormone they become leaner (Tanner et al 1977). The greatest effect appeared to be within the first three months of treatment.

Skinfold thicknesses are a practical way to estimate body fat (Brook 1971, Durnin and Womersley 1974). In order to determine the effects of rhGH on the body fat of short normal children, we measured triceps and subscapular skinfold thicknesses at entry into the study and at three monthly intervals. The measurements were made by a single observer (HS) using Holtain calipers. The individual measurements were log transformed and then expressed as the mean sum (SD) of log transformed triceps and subscapular skinfold measurements.

	No treatment	Placebo	rhGH
At entry	333.7	339.0	331.5
3 months	326.3	332.4	318.4 **
6 months	325.1	332.8	317.4 **
12 months	331.7	340.6	322.3
18 months	329.4	317.1 *	323.9
24 months	332.3	320.1	331.8
		* p = 0.03	** p = 0.02

The children who received active rhGH (24iu/sq.m/wk) during the first year of the study had a significant decline in skinfold thickness, and thus body fat. This was highly significant by three months and reached a maximum at six months. However, by twelve months skinfold thicknesses were not significantly different to the pre-treatment measurements. The children who received placebo for the first year had no significant changes in skinfold thicknesses during the first twelve months of the study, nor did the children who received "no treatment".

The children who received placebo for the first year of the study, went on to receive active rhGH (15iu/sq.m/wk) for the second year of the study. They showed an almost identical fall in skinfold thickness to the ones who had received the larger rhGH dose over their first six months of active rhGH therapy. Thus, normal children treated with growth hormone become transiently leaner.

2. Effects on cholesterol and triglyceride levels :

McCaughey et al (1993) have suggested that growth hormone treatment in normal short children alters plasma cholesterol and triglyceride levels.

In our study, plasma cholesterol and triglyceride levels were measured at entry into the study and at three months, six months and then at six monthly intervals. The baseline values were often fasting (taken at the time of the child's GH provocation test) and the subsequent levels were non-fasting, as it was not practical to bring the children to clinic fasting. This will not affect the cholesterol level but may affect the triglyceride levels, making them subject to wider variation. .

Mean (SD) cholesterol levels (mmol/l):

	No treatment	Placebo	rhGH
At entry	4.56 (0.53)	4.73 (0.75)	4.17 (0.94)
3 months	NA	4.76 (0.84)	4.61 (1.45)*
6 months	4.54 (0.73)	4.54 (0.72)	4.23 (0.75)
12 months	4.72 (0.71)	4.56 (0.61)	4.04 (0.73)
18 months	NA	4.49 (0.54)	4.07 (0.60)
24 months	4.61 (0.49)	4.66 (0.75)	4.10 (0.80)
			* p = 0.05

Reference range = 2.5-6.3 mmol/l

The pre-treatment mean cholesterol levels we measured were very similar to a control group of 7 year old children reported by Larsson et al (1992). In the rhGH treated group one child had a very high cholesterol level at three months (8.2 mmol/l). This was repeated at six months and was normal and has since remained normal. If this child is excluded from the analysis the mean cholesterol level at three months in the rhGH treated children (4.25 mmol/l SD 0.86) did not show a significant difference to the baseline value.

Mean (SD) triglyceride levels (mmol/l):

	No treatment	Placebo	rhGH
At entry	0.84 (0.37)	1.23 (0.79)	0.99 (0.45)
3 months	NA	1.37 (0.91)	0.95 (0.30)
6 months	1.27 (0.31)	1.01 (0.42)	0.98 (0.29)
12 months	1.23 (0.93)	0.82 (0.37)	0.57 (0.21)*
18 months	NA	0.89 (0.41)	0.80 (0.25)
24 months	0.90 (0.30)	0.76 (0.44)	0.67 (0.16)
			* p = 0.04

Reference range = 0.1-1.5 mmol/l

Mean plasma triglyceride levels were within the normal range for childhood, and did not increase significantly during treatment.

There were no significant changes in cholesterol or triglyceride levels in the children treated with active rhGH compared with those treated with placebo or who received no treatment. This is different to the effects described by McCaughey et al who noted a rise in cholesterol and triglyceride levels after twelve months of rhGH treatment although it was in a higher dosage (30iu/sq.m/week) than we used.

3. Carbohydrate metabolism

- effects on blood glucose and glycosylated haemoglobin

It would not be unexpected if the growth hormone treatment of short normal children led to impaired glucose tolerance (see discussion). To determine whether there were any major effects on glucose metabolism, plasma glucose and HbA1 levels were measured at entry into the study and at 3 months, 6 months and then at 6 monthly intervals. We did not have the opportunity to measure serial fasting plasma insulin levels - as it was impractical for our children to attend the clinics fasting. Urine was checked for glycosuria at 3 monthly intervals.

Mean (SD) plasma glucose mmol/l:

	No treatment	Placebo	rhGH
At entry	4.42 (0.40)	5.38 (0.93)	4.79 (0.45)
3 months	NA	4.57 (0.85)	5.03 (0.65)
6 months	4.79 (0.80)	4.72 (0.64)	5.10 (0.93)
12 months	4.72 (1.16)	4.38 (0.75)	4.34 (0.53)
18 months	NA	4.21 (0.82)	4.63 (1.33)
24 months	4.52 (0.45)	4.54 (0.73)	4.83 (0.79)

Mean (SD) Glycosylated Haemoglobin (HbA1) % of total Hb:

	No treatment	Placebo	rhGH
At entry	6.15 (0.80)	5.99 (0.95)	5.88 (0.67)
3 months	NA	6.32 (0.89)	6.57 (1.03) *
6 months	6.29 (0.75)	6.05 (0.55)	6.35 (0.84)
12 months	6.33 (1.14)	6.51 (0.65)	6.16 (1.01)
18 months	NA	6.22 (0.50)	6.16 (0.70)
24 months	6.29 (0.99)	6.42 (0.85)	6.28 (0.57)
			* p = 0.02

Reference range = 4.7-7.9%

There were no significant changes in plasma glucose level in the growth hormone treated groups, although it is interesting that there is a trend for random glucose levels to increase at three and six months in the group treated with 24 iu/sq.m/week of rhGH. The only significant change in glycosylated haemoglobin was at three months in this group when there was a rise in HbA1 level compared to the pre-treatment value, but the HbA1 remained within the normal range. This occurred at the same time of the trend in blood glucose to rise. HbA1 levels were not persistently elevated. This trend was not demonstrable in the children treated with 15iu/sq.m/week of rhGH. No child in this study developed glycosuria.

We did not demonstrate any convincing evidence of glucose intolerance. However this does not mean that there was no development of insulin resistance and this may well have occurred. It was not practical to bring our children to the clinic fasted and therefore there was no opportunity to assess fasting insulin levels. See comments in Section 2.5.9.

4. Effects on thyroid function

There are few published data about the effects of growth hormone on thyroid function in normal children, though it has been shown to affect the thyroid function of GH deficient children (Pirazzoli et al 1992). We followed thyroid function in our children with measurements of total thyroxine, free thyroxine and thyroid binding globulin.

Mean (SD) Total T4 nmol/l:

	No treatment	Placebo	rhGH
At entry	112.2 (22.2)	120.2 (20.4)	109.3 (18.3)
3 months	NA	121.0 (20.3)	109.1 (18.3)
6 months	114.9 (17.3)	126.9 (27.6)	114.9 (23.4)
12 months	112.3 (22.9)	126.0 (27.2)	111.3 (26.1)
18 months	NA	116.0 (13.8)	105.9 (13.8)
24 months	109.0 (16.0)	117.0 (13.8)	114.5 (18.6)

Reference range 70-180 nmol/l

Mean (SD) Free T4:

	No treatment	Placebo	rhGH
At entry	12.9 (2.5)	17.2 (3.5)	15.6 (2.3)
3 months	NA	13.8 (3.1)	13.1 (3.7)
6 months	12.0 (2.7)	14.9 (3.4)	13.6 (2.6)
12 months	15.4 (2.7)	15.9 (4.7)	15.2 (2.5)
18 months	NA	15.2 (2.8)	13.0 (1.4)
24 months	15.3 (3.3)	14.4 (3.0)	13.6 (2.2)

Reference range 9-23 pmol/l

Mean (SD) Thyroid binding globulin:

	No treatment	Placebo	rhGH
At entry	18.3 (0.6)	26.2 (2.4)	22.2 (2.9)
3 months	NA	24.3 (4.7)	24.1 (3.6)
6 months	23.0 (6.4)	22.6 (3.9)	23.4 (5.1)
12 months	20.9 (3.7)	23.7 (3.8)	23.5 (5.0)
18 months	NA	26.4 (6.2)	21.7 (5.5)
24 months	20.3 (2.0)	22.0 (3.4)	21.8 (4.3)

Reference range 12-30 mg/l

There were no significant changes in total T4, free T4 or thyroid binding globulin during treatment with active rhGH.

5. Production of Growth Hormone Antibodies

Methionyl hGH (the first synthetically produced hGH) was identical to pituitary derived hGH except for an additional methionine residue at the N-terminal. A high incidence of GH antibodies soon became apparent in patients treated with met-rhGH (Kaplan 1986). The rhGH used in our study had an identical amino acid sequence to naturally occurring hGH, and is less antigenic (Rasmussen 1988).

GH antibodies were measured in all children at entry into the study and at six monthly intervals. 2 children developed rhGH antibodies whilst they were receiving active rhGH. One had a positive titre after 12 months rhGH treatment, which was undetectable at 18 months. The second child had a weakly positive titre detected after six months of rhGH treatment which was undetectable at twelve months. In both cases the antibodies were detected transiently, and in the second case it is debatable whether they were present to a significant degree. In neither case were there any apparent deleterious effects on growth at the time the antibodies were detectable.

6. Effects on liver function

Liver function was followed serially by measuring the transaminases ALT and GGT at entry, three months, six months, and subsequently six monthly.

Mean (SD) ALT:

	No treatment	Placebo	rhGH
At entry	15.7 (3.6)	15.4 (3.0)	14.1 (2.9)
3 months	NA	16.0 (4.9)	18.1 (4.7)
6 months	16.4 (4.3)	18.1 (5.9)	17.7 (4.6)
12 months	17.7 (5.0)	21.7 (12.1)	17.1 (3.7)
18 months	NA	17.4 (5.4)	18.7 (5.3)
24 months	16.9 (4.9)	17.1 (4.2)	15.8 (4.5)

Reference range 10-40 units/l

Mean (SD) GGT:

	No treatment	Placebo	rhGH
At entry	10.7 (1.8)	11.3 (2.1)	9.8 (1.5)
3 months	NA	14.1 (5.2)	10.6 (1.6)
6 months	11.3 (2.6)	10.7 (2.1)	11.0 (2.0)
12 months	12.4 (3.1)	13.0 (2.9)	10.6 (1.8)
18 months	NA	11.6 (2,6)	10.9 (3.4)
24 months	11.7 (1.2)	12.5 (4.3)	10.7 (1.5)

Reference range <35 units/l

There were no significant changes in the mean transaminase levels measured.

However there was one child who developed major hepatic dysfunction during rhGH treatment. He received placebo injections for the first twelve months of the study without incident. He then received active rhGH for 7 months, during which time serial transaminase levels measured at his routine checks were not elevated. He then refused rhGH injections for ten months, following which both he and his mother requested that he restart them. After six weeks of restarting rhGH treatment he developed fulminant hepatic failure, and required a liver transplant. He is currently making good progress. The hepatic failure appeared to be due to non-A non-B non-C viral hepatitis though the infecting organism was never identified. The histology of his liver at surgery was compatible with this diagnosis (Kelly, personal communication). There are no reports in the literature of any other child who has developed fulminant hepatic failure whilst receiving growth hormone (either pituitary derived or recombinant).

There is no particular theoretical reason to implicate the rhGH in the causation of this boys liver failure, and indeed the use of rhGH in post liver transplant children to improve their growth has been under clinical trial. However this was a major adverse event occurring in a previously healthy child, and as such must be taken seriously. We have notified the Committee on Safety of Medicine and KIGS (Kabi International Growth Study) as it will be vital to recognise if such problems are being increasingly seen as the use of rhGH becomes more widespread.

1.6. Discussion

Many of the studies on the short normal child have included both children with familial short stature and growth delay in the same cohort. Although it is often impossible to separate the two conditions entirely, we have tried to keep our group of children as clearly defined as possible. The ultimate aims and effect of growth promotion may be different if the underlying problem is familial short stature rather than growth delay. Our children had short parents with no pathological cause for their short stature, and bone age delay was less than two years. Thus they were identified as a group whose main problem was familial short stature.

The first aim of the study was to detemine whether treatment with rhGH accelerates growth velocity during treatment for twelve months. This double-blind study shows that in pre-pubertal "short normal" children, rhGH does produce a significant acceleration in height velocity compared with placebo or no-treatment. The mean height velocity of the rhGH treated group over the first 12 months of treatment was significantly better than the pre-treatment height velocity, and was also significantly better than the height velocity of the placebo or no-treatment groups of similar children. These results are similar to those shown in earlier studies: Wit et al from the Dutch Growth Hormone Working Group (1989) reported thirty short, slowly growing children with normal growth hormone responses to standard provocation tests, randomly assigned to methionyl growth hormone or no treatment for a year, with a significant improvement in growth rate in the growth hormone treated group. Hindmarsh and Brook (1987) reported 26 short normal pre-pubertal children treated for two years with methionyl growth hormone. Height velocity improved from a pre-treatment mean of 5.3 cm/yr to 7.4 cm/yr. In our study the children treated with growth hormone grew faster than their control counterparts, and at similar rates over the first treatment year to that seen in other studies of both pituitary derived growth hormone (Van Vliet et al 1983, Plotnick et al 1983, Gertner et al 1984, Albertsson-Wikland 1986) and biosynthetic growth hormone (Hindmarsh and Brook 1987, Wit et al 1989).

Skeletal maturation in the rhGH treated children was faster than in the placebo or no-treatment groups. Although HSDS for chronological age improved significantly in the rhGH-treated group, HSDS for bone age did not. Predicted final height improved over the first study year in the rhGH group. These children continued on the same dosage regime (24iu/sq.m/wk) over the second year of the study. Although height velocity remained significantly better than it was prior to treatment, there was some waning of

effect - similar findings have occurred in other studies that have looked at the medium-term response to rhGH treatment (Hindmarsh et al 1990, Hopwood et al 1993). However as both HSDS for chronological age and HSDS for bone age continued to improve, there may be a small improvement in final height. This group of children need to be followed until adult height is achieved to determine whether or not this is the case. The potential effect of growth hormone therapy upon other physiological events, particularly puberty, will come into play before final height is reached (see later in discussion).

In our study, one of the groups of children received placebo growth hormone for one year before commencing active therapy. This was done on a double-blind basis ie. neither the child, the parents, nor the medical staff knew whether the patient was receiving the active preparation or the placebo. The placebo itself was indistinguishable in appearance and presentation from the active growth hormone and was reconstituted and administered in an identical fashion by daily subcutaneous injections.

It is a difficult ethical question whether is is justifiable to give children a daily placebo injection (Editorial, Lancet 1992). A six-month placebo limb was included in the multicentre study of the use of pituitary-derived hormone in Turner syndrome and normal variant short stature (Buchanan et al 1987). This study was stopped in 1985 because of the occurrence of Creutzfeldt-Jakob disease. In the 19 normal variant short children no placebo response was seen but as the study had been terminated early it is difficult to draw firm conclusions. Early studies using recombinant hGH in short normal children (Hindmarsh and Brook 1987) did not include a placebo group.

Receiving a daily injection may have a powerful placebo effect, and thus it is vital to determine whether it is the active rhGH or the injection itself that is affecting growth. It is well recognised that psychosocial well-being is very important for normal growth, and the psychological effects of a placebo may improve growth. Other studies have tried to examine this but have only given placebo for relatively short periods of time (Wales and Milner 1989, Ackland et al 1990, Cowell 1990, Boulton et al 1992). In the study by Ackland et al (1990) the placebo treated children showed an improvement in height velocity over six months ($p=0.03$), though it was not as dramatic as in the growth hormone treated children. By only considering six month height velocity, one may be detecting seasonal variation in growth rates, therefore a full year's data are really the minimum that can be fully analysed. To date there are no published studies looking at the placebo effect over a period of time greater than six months. The group of children in our study are unique.

It would be scientifically desirable to continue treatment with placebo until final height is achieved as a proper "control" for growth hormone treatment. However we are not studying laboratory animals but children, and placebo treatment for many years until adult height is reached is not ethically justifiable. Twelve months of placebo injections is the maximum that can be accepted ethically. We feel that a balance was obtained in this study between what is scientifically desirable and what is ethically acceptable.

In this study we did not see a significant improvement in height velocity in the placebo treated group either at six months or over the entire year. There was no difference in height velocity during the first six months of placebo treatment and the second six months. Skeletal maturation proceeded normally during placebo therapy. Thus, in the group of children we studied, no placebo effect was detected.

The third group of children in the study acted as "controls" and received no treatment at all for two years. These children received the same support as the other two groups in terms of number of clinic visits and contact with medical staff offering explanations of their problem. There was no significant change in their height velocity at 12 or 24 months, suggesting that intensive clinic visits with reassurance and explanation do not in themselves improve growth rate. We have therefore shown that, in the short term, rhGH treatment produces significant improvement in height velocity compared to no treatment or placebo.

All the children in the study eventually receive treatment with active rhGH. The children who initially received placebo or "no treatment" commenced rhGH treatment in a dose of 15iu/sq.m/week compared to a dose of 24iu/sq.m/wk in the children who received growth hormone from the outset. This allows us to compare the long-term efficacy of two differing doses of rhGH in similar children.

Data are available from 15 children who have completed a year of lower dose growth hormone (either having previously received placebo or "no treatment") and who are still pre-pubertal. Their mean height velocity over the first twelve months of active rhGH therapy was 7.35 cm/yr and is not significantly different to the mean height velocity of 7.67 cm/year in the 24iu/sq.m/wk treated group over their first year of treatment. The mean age of onset of active rhGH treatment in the 15iu/sq.m/wk group was slightly older than in th 24iu/sq.m/wk group - 8.61 years cf 7.69 years but this is not significant. Not only were the growth rates similar in the two groups, but the effects on the biochemical markers of growth studied (increment in IGF1 at 6 months and increment in bALP at 3 months) were also similar.

There is a reciprocal relationship between GH secretory status and growth response (Hindmarsh et al 1988), and this has led to the suggestion that short children who do not have "classical GH-deficiency" may require higher doses of growth hormone to achieve a good growth response. Our results did not support this, as the children grew well on the dose of 15iu/sq.m/wk - a conventional dose for GH deficiency. We do not feel that doses of 30iu/sq.m/wk as used in some studies of short normal children (Walker et al 1990) are necessary to produce a significant growth response in the pre-pubertal child. In a group of children with treated with high dose rhGH there has only been an approximately 35% increment in comparative height velocities for a fourfold increase in GH dose (Kelnar and Tanaka 1994). The response of GH-deficient patients to human growth hormone is dose dependent (Preece et al 1976, Darendeliler et al 1989). We did not demonstrate a dose-related response in our pre-pubertal children. The results we saw are different to those seen by Cowell (1990), who in a study of pre-pubertal short normal children showed a dose dependent growth response. The regimes he used, however, involved higher doses of growth hormone than in our study.

There appears to be little benefit in prescribing the larger dose of growth hormone, at least over the first year of therapy in short normal pre-pubertal children. Our results have obvious cost implications if one is considering using rhGH for such children. It is unknown whether even lower doses of rhGH or intermittent courses (Kelnar and Tanaka 1994) would be equally as effective, and merit further investigation.

It is interesting to note that the growth rates we acheived with a daily subcutaneous injection were very similar to those reported in the earlier studies of the pituitary derived preparation in which the growth hormone was given in thrice weekly intramuscular injections. Hopwood et al (1993) demonstrated a better growth response in short normal children when the injections were given on a daily basis rather than thrice weekly. GH-deficient children respond better to the same weekly dosage of growth hormone if it is divided into daily injections rather than being given thrice weekly (Smith et al 1988). In the current state of knowledge we should be aiming for the lowest total weekly dose of growth hormone that is effective, but divided into daily doses to obtain the best response.

We aimed to determine whether treatment with rhGH has effects on the rate of pubertal progression. In children with isolated idiopathic GH insufficiency it has been suggested that treatment with growth hormone significantly shortened the duration of puberty (Darendeliler et al 1990).

There is some evidence that progression through puberty may be more rapid in normal children treated with growth hormone (Hindmarsh and Brook 1992, Hopwood et al 1993).

We have noticed a tendency for the girls in our study to enter puberty at a slightly earlier mean age than average - attainment of Tanner breast stage 2 at 10.39 years. Girls on the whole are much more likely to develop idiopathic central precocious puberty than boys, appearing to have a more easily activated hypothalamo-pituitary-gonadal axis. In infertility work growth hormone has been shown to facilitate ovulation induction by gonadotrophins (Homburg et al 1988), and, in vivo, growth hormone increases ovarian levels of IGF1 (Davoren and Hsueh 1986), and so it is possible the rhGH is having direct effects on the gonadotrophin axis and/or the ovaries of the girls in our study.

The boys to date have entered puberty at an average time but their rate of pubertal progression does appear to be hastened which correlates with other reports published to date (Hindmarsh and Brook 1992, Hopwood et al 1993). The numbers of children in our study currently progressing through puberty are still relatively small and it will be vital to follow all the children before firm conclusions can be drawn.

If a child enters puberty early he or she is likely to complete their growth and reach final height at an earlier age than average. Any gain that has been made in height in the pre-pubertal years will be off-set by the early attainment of full skeletal maturation. For example, see the growth chart of girl 1.16 in the appendix. She showed an excellent response to rhGH over the first 24 months of treatment, with subsequent plateau of effect. However she entered puberty early (B2 at 9.37 years), and the second acceleration in growth is her pubertal growth spurt. She is likely to complete her growth earlier than average and hence final height is unlikely to be any greater than one would have predicted at the onset of treatment.

We aimed to determine whether treatment with rhGH will improve final height in short normal children. It is too early to comment on the final height of the children in this study but for the reasons described above on the effects on puberty, it is very difficult to predict. The improvements in HSDS for bone age and the calculated predicted adult heights detailed in the results section are encouraging and suggest that the children treated with active rhGH show a trend to improve their final height. However this may well be offset in due course by a tendency to enter puberty early or progress through puberty more rapidly. Full auxological data to final height is necessary to comment fully. There is no firm evidence to date to suggest that

a significant increase in final height will be obtained. Our data accord with that of Hindmarsh and Brook (1992).

Although we may not achieve a significant improvement in final height, we have shown without doubt that we can promote growth in the short term ie. over 12-24 months. We can now recognise a typical response pattern to rhGH, with an acceleration in growth rate over 12-24 months followed by plateauing along the new centile (see growth charts in appendix). This short term increase in height velocity allows children to become amongst the smaller in the peer group rather than the most conspicuously small. A short term improvement in growth may boost the child's morale and benefit them psychologically, but is it worth the inconvenience of daily injections, and the remote but possible risk of significant side effects? We examined the psychological effects of treatment and these are reported in Section 4.

By recognising this pattern of response of the short normal child to rhGH we can now begin to develop a more constructive plan for rhGH treatment. We need to know what happens when rhGH treatment is stopped - will the child continue along his/her new centile - if so, then it becomes logical to treat for just a short period of time. In the study by Ackland et al (1990) there was a significant fall in height velocity after stopping growth hormone treatment and the children returned to pre-treatment height velocity. However this was after just six months of active treatment, when the children would still have been responding well to the growth hormone, and the pattern may be different if the rhGH is stopped after 12-24 months treatment (Kelnar and Tanaka 1994). Further use of the biochemical markers of growth will be of help in these studies.

We must always remember that the children in our studies are normal healthy children, and therefore any treatment we prescribe must be safe. Studies such as ours our vital to determine whether treatment with rhGH leads to adverse events. We were able to compare the effects on the growth hormone treated children with control groups receiving no-treatment and placebo. It is important to have good control groups as normal data on some of the parameters studied are not easily available in normal children.

With regard to fat mass, the children who received active rhGH (either 15iu or 24iu/sq.m/wk) had a significant decline in skinfold thickness, and thus body fat. This was highly significant by three months and reached a maximum at six months, but was transient as by twelve months skinfold thicknesses were not significantly different to the pre-treatment measurements. This effect has been noted previously (Hindmarsh and

Brook 1987, Walker at al 1990, Gregory et al 1993). Hindmarsh and Brook (1987) showed similar results to our children, with significant fall in triceps and subscapular skinfold measurement at six months with return to baseline values by twelve months. Walker et al (Lancet 1990) showed that six months of rhGH therapy in a larger dosage than we used (30iu/sq.m/week) produced upto 76% loss of fat mass and up to 25% increase in lean body mass in normal short children. It is difficult to know whether there will be any long term effects from the alteration of body composition in this way, however transient it appears. The children merit long term follow up with regard to this.

We did not detect any significant effects on plasma cholesterol or triglyceride levels in the children treated with active rhGH compared with those treated with placebo or who received no treatment. This is different to the effects described by McCaughey et al (1993) who noted a rise in cholesterol levels after twelve months of rhGH treatment although they gave a higher dose (30iu/sq.m/week).

Theoretically one might be concerned that growth hormone treatment of short normal children may lead to impaired glucose tolerance. Acromegaly is frequently associated with hyperinsulinaemia and carbohydrate intolerance. Continuous growth hormone infusions induce acute insulin resistance characterised by impaired suppression of hepatic glucose and decreased insulin dependent glucose disposal. In animal studies B-cell mass increased in the pancreatic islets in rats treated with GH. Walker at al (1989) demonstrated that the growth hormone treatment of children with short stature increases insulin secretion but does not impair glucose disposal. 10 pre-pubertal children with normal variant short stature were studied. They were given hGH in a dose of 0.3 units/kg/day (ie. a relatively large dose). Fasting and post-prandial glucose levels were normal before and after 12 months of treatment. HbA1C levels were also similar before and after treatment. However fasting plasma insulin increased during treatment associated with parallel changes in C-peptide. The insulin and C-peptide responses to post-prandial glucagon stimulation were enhanced by hGH treatment. Insulin secretion after an oral glucose load was also higher after 12 months hGH treatment. Other groups have also shown this effect on fasting plasma insulin levels (Hindmarsh and Brook 1987). We did not detect any significant effects on glucose metabolism, but our markers were relatively crude - random blood glucose and glycosylated haemoglobin. No child in this study developed overt glucose intolerance (see section 2.5.9.).

One child in our study developed fulminating hepatic failure, though we have no evidence to causally relate it to the rhGH. No other child in this study showed any derangement of hepatic transaminases. However it was a very serious event occurring during the course of treatment. It is vital that good data bases are kept of children receiving growth hormone treatment (eg. KIGS - Kabi International Growth Study), as it is only with large numbers of children that rare side-effects will be detected.

We followed thyroid function in our study to detect whether there was any affect of rhGH on the thyroid function on normal children. More has been written about the effect on the thyroid function of children with growth hormone deficiency. Pirazzoli et al (1992) described 57 children with isolated GH deficiency in whom thyroid function was measured regularly during treatment, after a months withdrawal from treatment and after a further six months therapy. During rhGH treatment T3 levels rose with an increase in the T3 : T4 ratio. There was a fall in both total T4 and free T4 levels. This suggests rhGH leads to an enhancement of the conversion of T4 to T3. We did not detect any significant changes in total T4, free T4 or thyroid binding globulin during treatment with active rhGH in our children (cf. section 2.5.9. - the effects of anabolic steroids on thyroid function).

The rhGH used in our study had an identical amino acid sequence to naturally occurring hGH, and is less antigenic (Rasmussen 1988). Two children developed transient antibodies to rhGH whilst they were receiving active treatment. In neither case were there any apparent deleterious effects on growth at the time the antibodies were detectable.

When considering adverse effects of treatment one must not only think about physical effects but also the psychological. We were very aware that these children were receiving active intervention for a "normal" variant and that they and their families had expectations of the treatment. Adverse psychological effects of being included in such studies, and in particular receiving placebo injections, will be considered in Section 4.

We aimed to determine whether there were any features about our children that predicted their response to treatment. The pre-treatment growth rate did not predict the response to treatment, probably because the group was very homogeneous and we were looking at subtle variations in growth rate. Neither pre-treatment growth hormone measurements nor IGF1 levels were good predictors of the response to treatment. Spiliotis et al (1984) described the condition of neurosecretory dysfunction in which children have normal responses to GH provocation tests but abnormal spontaneous GH secretory patterns. Three children had patterns of spontaneous GH

secretion that could be classed as neurosecretory dysfunction (see examples of GH profiles). They responded very well to rhGH treatment, but not particularly better than those without neurosecretory dysfunction. Without measurement of overnight GH secreton it would have been impossible to identify these children from their growth patterns (although urine GH levels tended to be amongst the lowest of all the children studied).

The increase in bALP at three months was a good predictor of the growth response over the first year of active treatment. One expects to see a rise in bALP by three months of rhGH treatment, though in practice it probably occurs much earlier. If this rise does not occur, the child is not responding to treatment - compliance may be a problem which should be checked before the dose of rhGH is increased unnesecarily.

In summary we were able to show that we could improve the growth rate of pre-pubertal children with familial short stature with rhGH. This was not due to a placebo effect. The best growth response occurred over the first 12-24 months of treatment, with subsequent plateauing of growth along a new centile. The growth response was similar for two doses of growth hormone, and there is no indication to use large doses of growth hormone in the normal short child. Because of the effects on pubertal timing, it is unlikely that final height of these children will be significantly improved. Side effcts of treatment include a recoverable decrease in body fat. One boy in our series developed fulminant hepatic failure, but it is unlikely that this was directly attributable to rhGH. The psychological effects of treatment will be discussed in Section 4.

SECTION 2 : GROWTH PROMOTION IN PERI-PUBERTAL BOYS WITH FAMILIAL SHORT STATURE WITHOUT GROWTH DELAY

2.1. Introduction
2.2. Definition of Familial Short Stature
2.3. Effects of short stature in the peri-pubertal child
2.4. Treatment of short stature in the peri-pubertal child - background literature review with particular reference to the use of anabolic steroids.
2.5. Study: Growth promotion in peri-pubertal boys with familial short stature - growth hormone or oxandrolone, singly or in combination
    2.5.1. Aims of the study
    2.5.2. Patient recruitment
    2.5.3. Study protocol
    2.5.4. Pre-treatment growth status
    2.5.5. Pre-treatment endocrine status
    2.5.6. Growth Results
    2.5.7. Effects on puberty
    2.5.8. Effects on biochemical markers of growth
    2.5.9. Side effects
2.6. Discussion

2.1. Introduction

This study was designed to determine the optimum method to promote growth in peri-pubertal boys with familial short stature who do not have significant growth delay. In particular we wished to investigate the role of the anabolic steroid, oxandrolone. Its use has traditionally been in slightly older boys with delay of growth and maturation. We wished to compare the effects of oxandrolone, rhGH and both agents in combination in our group of boys. Although the boys will be followed to adult height, I am presenting the short-term results ie. the first two years.

2.2. Definitions of familial short stature

The boys included in this study have familial short stature. They have small parents, and hence one would predict that the boy's final adult height will be low. Children with growth delay will reach an acceptable final height, though will take longer than average to reach it. They are likely to go through puberty after their peers and skeletal maturation lags behind chronological age. The boys in this study do not have significant growth delay - their bone age is less than two years behind their chronological age - thus skeletal maturation is proceeding at an appropriate rate and they would be expected to reach their final height at an average age. This study differs from many of the published studies evaluating the use of oxandrolone which predominantly explore its use in boys with growth delay.

2.3. Effects of short stature in the peri-pubertal child

The boys included in this study are at a sensitive and vulnerable stage in their psychological development, particularly with regard to self-esteem. They will be becoming much more aware of being different to their peers. As they begin to approach puberty any difficulties they may experience due to their short stature may be a combination of those seen in pre-pubertal children together with those seen in adolescents. For more details please see Section 4.2.

2.4. Treatment of short stature in the peri-pubertal child - background literature review with particular reference to the use of anabolic steroids.

The anabolic steroid oxandrolone (17a-methyl-2-oxa-5a-androstan-17b-ol-3-one, Searle SA, Switzerland), first synthesised in 1961, is an analogue of testosterone. The anabolic potency of oxandrolone, measured by diminished urinary nitrogen excretion, was shown to be approximately 6 times that of methyltestosterone (Fox et al 1962), whereas it had only 24% of the androgenic affects (Lennon and Saunders 1964). Because of its greater anabolic to androgenic ratio than other synthetic anabolic steroids, oxandrolone has been postulated to be a suitable agent for growth promotion in children in whom significant androgenic affects are largely undesirable.

Oxandrolone has been used as a growth-promoting agent in childhood for over 25 years. Early studies included children with varying diagnoses for their short stature (including such conditions as panhypopituitarism and hypogonadism), and varying ages and pubertal stages (Danowski et al 1965), and hence the results are difficult to interpret. Raiti et al (1973) compared oxandrolone and growth hormone, alone and in combination, in six children with idiopathic hypopituitarism, one with Hand-Schuller-Christian syndrome and one with Prader-Willi syndrome. Surprisingly oxandrolone appeared to be an effective growth stimulus in idiopathic hypopituitarism although large doses (0.25mg/kg/day) were given. Early studies on children with constitutional delay of growth or "primordial" short stature (Zangeneh and Steiner 1967, Limbeck et al 1971) demonstrated an improvement in height velocity during treatment. The children included in these studies were heterogeneous with ages varying from 3-16 years, and varying pubertal stages, and the dose of oxandrolone varied considerably from 0.1 to 0.25 mg/kg/day.

More recently Stanhope and Brook (1985) described 24 boys with puberty delay with mean age 14.8 years who were given 2.5 mg oxandrolone daily for 3-6 months. They showed an increase in mean height velocity form 3.7 to 8.1 cms/year. However, some of these boys were well into mid-puberty with a mean testicular volume of 8.4 ml at onset of treatment, and it is difficult to tell how much of the improved growth was oxandrolone-induced or spontaneous. A further study by the same group (Stanhope et al 1988) involved 19 boys with puberty delay and a mean age of 14.4 years, in a double-blind placebo controlled study. Treatment was for

3 months. Height velocity in those treated with oxandrolone rose from 4.5 to 9.6 cm/year, compared to a change from 5.1 to 5.2 cm/year in those who received placebo. A testicular volume of >4 ml prior to treatment was perceived as necessary for an oxandrolone-induced sustained growth spurt to occur.

A comparison of oxandrolone treatment with rhGH in the treatment of boys with constitutional delay of growth and puberty was made by Buyukgebiz et al (1990). The mean age of the 26 boys was 13.8 years, mean testicular volume 5.3 ml, and 3 months treatment with oxandrolone (2.5 mg daily) was compared with 12 months treatment with rhGH (20 units/sq.m/week). Both groups showed a significant improvement in height velocity, though it was better in the oxandrolone treated boys.

These studies all involved boys whose main problem was maturational delay. The major problem of the children included in our study is that of familial short stature. They are a younger group of boys who are pre- or peri-pubertal at the commencement of treatment, and treatment is continued for a longer period of time (until testicular volumes of 6-8 ml are reached and we can be more confident that their own endogenous growth spurt is imminent).

Fewer studies have looked at the use of anabolic steroids in younger children. Marti-Hennenberg et al (1975) treated 9 pre-pubertal boys, mean age 12 years and 5 months, with oxandrolone until peak height velocity had been reached. He did not demonstrate a significant increase in height velocity in treated boys compared to a control group, nor was there any difference in skeletal maturation. Joss et al (1989) treated 36 pre-pubertal children with growth delay with oxandrolone for 12 months. Height velocity did increase significantly, but bone age also advanced significantly compared to chronological age. This group have been followed to final height, and there has been no effect on their adult height. Papadimitriou et al (1991) treated 46 pre-pubertal boys (mean age 11.9 years) with oxandrolone for a mean duration of 0.9 years. Again these children had growth delay, with mean bone age delay of 1.9 years. An increase in mean height velocity from 4.0 to 7.5 cms/year was seen and sustained if testicular volume was >4 ml at the end of the treatment period. Skeletal maturation, as judged by HSDS for bone age was not unduly rapid but it is not clear how long follow up was after treatment was discontinued.

It is still unclear as to exactly how oxandrolone promotes growth and its mechanisms may differ in pre-pubertal and pubertal boys. If it exerts its effects by enhancing growth hormone secretion, one should be able to

detect this directly by physiological or stimulated growth hormone measurements, or less directly by the effects on IGF1. Link et al (1986) treated 10 pre-pubertal boys with oxandrolone and showed no significant changes in the 24 hour growth hormone profile or IGF1 levels despite improvement in growth rate. Clayton et al (1988) demonstrated that growth hormone responses to growth hormone releasing factor (GRF), arginine and sleep were unchanged in pre-pubertal boys during treatment with oxandrolone, whereas in pubertal subjects there was a significant increase in GH secretion during sleep. IGF1 levels increased significantly in the pubertal boys but not in the pre-pubertal children. However Loche et al (1986) reported an increase in the GH response to GRF during oxandrolone treatment in five pre-pubertal subjects. In the double-blind study by Stanhope et al (1988) IGF1 levels increased from 1.01 to 1.23 U/ml ($p=<0.05$) in the anabolic steroid treated group, but there was no significant change in the placebo group. These boys were pubertal, and this may explain the rise seen in IGF1. It is clear that the GH and IGF1 responses of boys to oxandrolone need to be evaluated with the knowledge of their pubertal status. It has been postulated that oxandrolone does not affect the GH status of pre-pubertal boys and promotes growth by a direct action at the growth plate. In vitro studies on rabbit chondrocytes have demonstrated that sex steroid hormones have a direct metabolic effect on skeletal tissue by stimulation of cartilage cell proteoglycan synthesis, and that this effect varies with the age of the animal (Corvol et al 1987).

Anabolic steroids are not without potential side effects - a very important consideration when we are considering their use in young otherwise normal boys.

The children we have included in our study have familial short stature without significant growth delay and prior to treatment have predicted adult heights at or around the 3rd centile. It is vital that any treatment does not compromise final height. There have been conflicting reports on the effects of anabolic agents on skeletal maturation.

Sobel (1968) clearly described the problems there are in assessing the skeletal response to anabolic steroids. The full effects of these agents on bone maturation may not be evident for six to twelve months, and skeletal maturation may continue to advance for up to a year after treatment is discontinued. Thus when interpreting the effects of courses of anabolic steroids on growth and in particular on predicted height follow up data in the post-treatment period is required. Tse et al (1990) have followed 40 boys with constitutional delay of growth and puberty, who had received

oxandrolone for 3-12 months at a median age of 14.2 years, until they reached final height. Reassuringly there was a small increase in final height compared to predicted height. However the boys in this study had been treated in early or mid-puberty and less is known about the prolonged use of anabolic steroids in pre-pubertal boys, which is of more relevance for the boys included in our study.

Anabolic steroids appear to have a greater effect on skeletal maturation in younger children (Zangeneh and Steiner 1967, Jackson et al 1973) than in those whose bone age is approaching that of puberty. This may reflect a difference in sensitivity of the epiphyseal cartilage at an age when it is not normally subjected to androgens, or a greater resistance of more mature epiphyseal cartilage to exogenous influence since it is already under the influence of endogenous gonadal steroids. Bettman et al (1971) also showed that bone age acceleration may continue for 6-12 months after discontinuing oxandrolone therapy, and in 5 out of 14 children with a bone age of less than nine years at the start of oxandrolone treatment demonstrated a fall in predicted adult height of up to 5 cms. Many of these earlier studies used larger doses of oxandrolone (eg. 0.25 mg/kg/day) than we have employed in our study and so interpretation of the effects on skeletal maturation need to be interpreted with caution. Sobel (1968) showed no evidence that the growth response to anabolic steroids was dose related, thus one should be using the smallest effective dose.

Anabolic agents have been postulated to have effects on both the hypothalamo-pituitary-gonadal axis and testicular function. Hopwood et al (1979) treated two groups of children with the anabolic steroids fluoxymesterone and oxandrolone respectively. In those treated with fluoxymesterone there was a fall in mean testosterone levels, a decreased LH response to GnRH and a fall in basal FSH levels, suggesting the anabolic steroid had an effect on the hypothalamo-pituitary-testicular axis. In the oxandrolone treated boys, testosterone levels fell but there was a variable response to GnRH and no suppression of the clinical onset of puberty. Malhotra et al (1993) reported ten boys with constitutional delay of growth and puberty, mean age 13.8 years, treated with oxandrolone for three months. Oxandrolone had androgenic effects suppressing mean serum LH and testosterone concentrations, with a rebound after treatment was stopped.

With regard to testicular function, Marti-Hennenberg et al (1975) showed a decrease in testicular volume index in his oxandrolone treated pre-pubertal boys compared to the control group suggesting a decrease in

the rate of testicular growth in those boys receiving the anabolic steroid. Testicular effects may be dependent on the anabolic steroid used, dosage and duration of treatment, and the age and pubertal status of the child. It is recognised that in athletes abusing these agents there are reversible changes including clinically apparent testicular atrophy, and azoospermia or oligospermia with depression of serum gonadotrophins (Knuth et al 1989). In immature rats treated with large doses of oxandrolone (10mg/kg/day sub-cutaneous injections) there was a decrease in the weights of the testes, prostate glands and seminal vesicles (Grokett et al 1992). It was postulated this was due to effects on the hypothalamus, pituitary and Leydig cells. Thus we must follow with care the pubertal development of our relatively young group of boys who are treated with oxandrolone.

Much of the information on the metabolic effects of oxandrolone and other anabolic steroids comes from young adults who misuse these agents to improve athletic performance or enhance their appearance (Lamb 1984). Significant effects have been seen on carbohydrate and lipid metabolism, liver function and thyroid function. The effects of anabolic steroids on these parameters will be discussed with the relevant data from our study (see section 2.5.9)

2.5. Growth promotion in peri-pubertal boys with familial short stature - growth hormone or oxandrolone, singly or in combination

2.5.1. The aims of this study are to determine in peri-pubertal boys with familial short stature:

1) whether treatment with rhGH or oxandrolone or rhGH plus oxandrolone improves height velocity in the short-term compared to no treatment.
2) whether treatment with rhGH or oxandrolone or rhGH plus oxandrolone has effects on the timing of puberty and rate of pubertal progression.
3) whether treatment with rhGH or oxandrolone or rhGH plus oxandrolone will improve final height in these boys.
4) the benefits (physical and psychological) of these treatment modalities.
5) the complications (if any) of these treatment modalities.
6) whether there any parameters that are predictive of the response to treatment.

2.5.2. Patient Recruitment

Children were recruited from the growth and endocrine clinics at the Royal Hospital for Sick Children, Edinburgh, and attached peripheral clinics in Scotland. Children were referred to the growth clinic either by their general practitioners or the school medical service, or are tertiary referrals from other paediatricians.

The following inclusion criteria had to be met :

Height at, or below 3rd centile for chronological age (HSDS < -1.88)
Bone age >10 years
Bone age delay of <2 years
Pre- or peri-pubertal (testicular volume </= 4ml)
No other cause found for their short stature.

Ethical approval for the study was obtained from Lothian Health Board Committee on Medical Ethics. Parents and children were given detailed information about the study, and signed appropriate consent forms prior to entry.

2.5.3.Study protocol

Pre-treatment Assessments:

1. Auxology

Prior to entry into the study the boys were assessed at the growth clinic for a minimum of 6 months, though the majority had measurements performed over at least one year.

Standing height was measured by a single observer (HS) using a fixed wall mounted Harpenden stadiometer. Sitting height was measured using a sitting stadiometer by a single observer (HS). Weight was measured using a standard balance. Measurements were compared to the standards of Tanner et al (1966, 1976). Triceps and subscapular skinfold thicknesses were measured using Holtain calipers by a single observer (HS) and compared to the standards of Tanner and Whitehouse (1975). Pubertal assessment was performed by a single observer (HS) using the standard ratings of Tanner (Tanner 1962, Marshall and Tanner 1969, Marshall and Tanner 1970). Testicular volume was estimated by comparison with the standard ovoids of a Prader orchidometer (Zachmann et al 1974).

Bone age was assessed by X-ray of left hand and wrist, and the Tanner Whitehouse II 20 bone method (Tanner et al 1983) of analysis performed by a single observer (HS).

Blood pressure was measured in the right upper limb by a single observer (HS) using an appropriate sized sphygmomanometer cuff for each child (de Swiet et al 1992).

2. Endocrine assessment

a) Overnight profiles

Prior to entry into the study all the boys underwent overnight blood sampling form 20.00 hrs to 08.00 hrs, with samples collected at 20 minute intervals to measure plasma growth hormone. The boys were admitted to hospital in the early evening following a normal days activities and eating pattern. Topical anaesthetic cream (EMLA) was applied to an ante-cubital fossa and subsequently an indwelling intravenous cannula was inserted at least 45 minutes before blood sampling was commenced. The boys were allowed to be freely active, and eat and drink normally during the evening. They were strongly encouraged to be in bed by 10.00 pm with "lights out" at 10.30pm. In practice the majority slept soundly until 7.30-8.00am the following morning. 2 mls samples of blood were taken every 20 minutes, collected into lithium heparin tubes, immediately centrifuged at -4C, then

separated and the plasma stored at -20C until assayed. All samples from a given child were assayed in the same batch.

The samples were assayed in the Regional Hormone Laboratory, Edinburgh using an immunoradiometric assay (IRMA). The growth hormone profiles obtained were evaluated using the Munro modification of PULSAR program on an Apple Macintosh personal computer.

For an additional study, timed 12 hour urine collections (20.00 hrs to 08.00 hrs) were also obtained from the children. Urinary growth hormone was measured in these samples at the Regional Hormone Laboratory, Edinburgh using an amplified enzyme immunoassay (Novo Nordisk).

Insulin like growth factor 1 (IGF1) levels were measured at 08.00 hrs in all children. IGF1 was assayed by Novo Nordisk using a radio-immunoassay.

Testosterone level was measured in all boys at 08.00 hrs (Wu et al 1993).

b) Dynamic pituitary function tests

The following morning the boys underwent combined pituitary function tests, using either insulin-induced hypoglycaemia (0.15iu/kg) or clonidine (0.15mg per sq.m body surface area) together with TRH (7 micrograms/kg to a maximum of 200 micrograms) and LHRH (0.25 micrograms/kg). 35 of the boys underwent an insulin tolerance test, with the remainder having clonidine for the growth hormone provocation test. The tests were performed in a recognised growth centre, on a ward where the staff were well acquainted with the potential hazards of such tests and their management, and full facilities for resuscitation were available (Shah et al 1992). The tests were supervised by the same person (HS) on all occasions. There were no significant adverse events as a consequence of these tests.

The pituitary function tests were performed without the boys being "primed" with testosterone, as we were trying to fully evaluate the physiological status of the child prior to treatment. The LHRH test used is a very low dose one and is likely to be more physiological than the supra-maximal stimulus of the conventional doses of 100 microgms or 2.5 microgms/kg (Hughes 1989). This study in combination with the studies in older boys allowed us to evaluate the low dose LHRH test as to whether it is a more useful way of assessing the imminence of puberty than the conventional dose.

3. Psychological assessment

The psychological studies were performed using a series of questionnaires involving parent, child and teacher reports.

For details please see Section 4.3.

Randomisation of treatment

On entry the boys were randomised into one of four groups receiving :

1) rhGH 24iu/sq.m/week, given as a daily sub-cutaneous injection
or 2) oxandrolone 2.5 mg orally once daily
or 3) rhGH plus oxandrolone in the above doses
or 4) no treatment for one year to be followed by rhGH 15iu/sq.m/wk, given as a daily sub-cutaneous injection

Thus after 12 months all the boys were receiving an active treatment . The study design allows us to compare the effects of two doses of rhGH in peripubertal boys.

Assessment of response

After entry into the study the boys were reviewed at three monthly intervals. Detailed auxological measurements (standing height, sitting height, weight, triceps and subscapular skinfold thicknesses, pubertal staging and blood pressure) were made every three months by a single observer (HS). Left hand and wrist X-ray was performed every six months, and bone age assessed by a single observer (HS) using the Tanner Whitehouse TW II method of analysis.

Haematological (full blood count with differential white cell count, and in a subgroup T and B cell counts) and biochemical (liver function, renal function, glucose, HbA1, cholesterol, triglycerides, thyroid function) parameters were measured at entry and at three months, six months and at six monthly intervals thereafter. IGF1 was measured at entry and six monthly. Bone derived alkaline phosphatase was measured at entry, three months, six months and at six monthly intervals.

LH, FSH and testosterone levels were measured six monthly. Although these were random samples, all were taken between 09.00 and 12.00 in the morning.

2.5.4. Pre-treatment Growth Status

Children entered into the study

43 boys entered this study, all with full written informed consent of their parents and themselves.

The mean age of the boys was 11.64 years (SD 1.19, range 9.16 - 13.69)
The mean HSDS was -2.50 (SD 0.56, range -3.91 to -1.63)
The mean height velocity was 4.10 cm/yr (SD 0.91, range 2.29 - 5.98)
All had testicular volume of 4 ml or less
The mean bone age was 10.63 years (SD 1.48, 8.70 - 13.70)
The mean bone age delay was 1.02 years (SD 1.05, range -2.87 to +1.14)

The mean height of the mothers was 153.2 cms (SD 6.20) = 3-10th centile
The mean height of the fathers was 170.5 cms (SD 6.52) = 25th centile
The mean target height of the boys was 168.4 cms (SD 5.03) = 10-25th centile
The mean predicted height at entry was 160.5 cms (SD 4.28) = < 3rd centile

The mean predicted height (calculated using the formula of Tanner 1983 based on height, bone age, growth rate and rate of bone maturation) at entry was significantly less than the target height (calculated as mid parental centile height) by 7.90 cms ($p = <0.001$).

The mean birth weight of these children was 2.95 kg (SD 0.47, range 1.50 - 3.63 kg). Three boys were born pre-term (one at 32 weeks, and the other two at 36 weeks gestation). Using a definition of small for gestational age (SGA) as <10th centile weight for gestational age, 22 of the boys were SGA. Unfortunately accurate birth lengths were not available. The high percentage of SGA infants in the cohort is a probably a reflection of relatively small maternal size.

2.5.5. Pre-treatment endocrine status

a) Growth hormone

Spontaneous growth hormone (GH) secretion is in a pulsatile fashion, and thus the GH profiles we obtained are a measure of the child's physiological GH status. The overnight growth hormone profiles we obtained from each boy were analysed using the Munro modification of the PULSAR program. This program detects pulses of GH, and the overnight GH secretion can be described in terms of pulse amplitude (PA), sum of pulse amplitude, pulse interval, area under the curve and mean GH level. It has been found that there is an asymptotic relationship between HVSDS and pulse amplitude (Hindmarsh et al 1987), and that pulse amplitude is the best feature of the GH profile to relate to growth.

The results from the overnight GH profiles are summarised as the means (SD) of mean PA, sum PA, and mean GH level, all expressed in mU/l, for the boys in the groups of the study. Also included in the table are the mean responses to the provocation test and mean IGF1 levels.

See table : Summary of growth hormone secretory data

As can be seen in the table, the boys in the combination treatment group tended to have lower measured parameters of growth hormone secretion whereas the rhGH treated group had the highest measured parameters. In terms of mean pulse amplitude and sum of pulse amplitude this did not reach significance, but mean overnight GH level was significantly lower in the combination treatment group than the mean for the rhGH treated boys ($p = 0.03$). Mean peak stimulated GH response was lower in the combination group compared to the rhGH treated group ($p = 0.02$), and mean IGF1 level was lower in this group compared to the no treatment group ($p = 0.03$). There were no other significant differences between the groups.

The boys were not required to reach a pre-determined standard on the stimulation test, in contrast to the pre-pubertal children in Section 1. It is well recognised that in peri-pubertal boys who undergo an " unprimed" stimulation test, the growth hormone level may be low as there is physiological blunting of growth hormone secretion pre-pubertally.

b) LHRH test and testosterone levels

Measurement of overnight pulsatile LH secretion using highly sensitive assays has been shown to be the best way of detecting the hormonal onset of puberty in younger children (Wu et al 1990, 1991).

	All boys	No Treatment	rhGH	Oxandrolone	rhGH + Oxand.
Overnight GH :					
Mean PA	16.02 (8.49)	17.07 (7.11)	17.66 (6.64)	17.76 (12.91)	11.79 (5.53)
Sum PA	74.24 (29.78)	79.67 (26.75)	83.10 (24.44)	72.89 (37.78)	61.49 (28.64)
Mean GH level	7.73 (3.56)	7.58 (2.82)	8.84 (2.72)	8.73 (4.28)	6.09 (2.55)
Peak stim. GH (mU/l)	27.98 (15.39)	30.75 (19.38)	35.61 (15.70)	24.62 (13.38)	21.32 (9.29)
IGF1 (Units/ml)	0.65 (0.26)	0.74 (0.26)	0.67 (0.22)	0.65 (0.32)	0.52 (0.20)

Study 2: Summary of GH secretion

	All boys	No Treatment	rhGH	Oxandrolone	rhGH + Oxand.
Mean (SD) Peak LH (U/l)	5.18 (3.26)	4.68 (2.83)	6.45 (4.39)	4.64 (2.84)	5.02 (2.96)
Median 08.00 Testost. (nmol/l)	0.7	0.7	0.7	<0.6	0.7

Study 2: Summary of LH and testosterone levels pre-treatment

However this was not practical to do in all our boys and so we used the LH response to a low dose of LHRH (0.25 microgms/kg) as a sensitive way to assess the pubertal activity of the hypothalamo-pituitary axis. Pre-pubertal children normally have baseline LH levels of <1.0 units/l, and will only show a small increment in the concentration of LH in response to this dose of LHRH. We have found that the pre-pubertal response of LH to LHRH is to a level of 3.8 units/l or less, whereas once puberty is underway level of >4.5 units/l or more are achieved (see results for younger and older children in sections 1 and 3).

See table : Pre-treatment LH and testosterone levels

The mean peak LH response of all the boys was 5.18 units/l in the study suggested that biochemically puberty was just under way in the boys as a whole. However 21 of the boys had responses that we term "pre-pubertal" ie. a peak LH responses to LHRH of <3.8 units/l. These boys were clinically indistinguishable from their hormonally more advanced peers. No significant differences were seen in mean LH response between the treatment groups.

Early morning testosterone levels have been shown to be a useful marker of the imminence of puberty. Boys in whom early morning testosterone is <0.7 nmol/l are unlikely to enter puberty within the next 12 months (Wu et al 1993). Median testosterone levels were 0.7 nmol/l in all treatment groups apart from oxandrolone alone, in which median testosterone levels were <0.6 nmol/l. 14 boys individually had early morning testosterone levels of 0.7 nmol/l or more - again they were clinically indistinguishable from their counterparts, but tended to be the boys who had the most active LH response to LHRH.

These results suggest that clinical examination alone is not wholly reliable in predicting pubertal onset in peri-pubertal boys, and that simple biochemical tests (eg. early morning testosterone and/or peak LH response to low dose LHRH) may be helpful.

2.5.6. Growth Results

See table : Summary of growth data

1 year growth results

43 boys entered the study:

10 received rhGH

10 received oxandrolone

11 received rhGH plus oxandrolone

12 received no treatment

There were no significant differences between the four groups at entry into the study in terms of: chronological age, height, height SDS, height velocity, pubertal staging, bone age, bone age delay, and predicted adult height.

All the boys have completed at least one year of follow up with the majority completing two or more years.

"No treatment" group

The twelve boys who received "no treatment" over the first year grew at a mean rate of 4.73 cm/yr compared to a pre-treatment mean rate of 4.07 cm/yr. This improvement was only just significant ($p = 0.04$), but may be physiologically more significant as one would expect these boys' growth rate to be decelerating as they exhibit normal pre-pubertal slowing. Height SDS did not change significantly over the year. Mean bone age advance/ chronological age advance was 1.11 years. There was no significant change in HSDS for bone age nor was there any significant change in predicted height compared to pre-randomisation.

rhGH group

Ten boys received daily rhGH injections in a dose of 24iu/sq.m/wk. Over the first year of treatment they grew at a mean rate of 7.58 cm/yr compared to a mean rate of 4.24 cm/yr over the pre-study year. This improvement is highly significant ($p = <0.001$), and is also significantly better than the growth of the boys who received "no-treatment" ($p = <0.001$). HSDS improved from -2.43 to -2.15 over the year ($p = 0.03$). Mean bone age advance/chronological age advance was 1.03 years and was not significantly different to those who received "no treatment". HSDS for bone age improved from -1.71 to -1.33 ($p = 0.03$), however predicted adult height did not increase significantly.

	No treatment	rhGH	Oxandrolone	rhGH + Oxan
At entry:				
No. children	12	10	10	11
Age (yrs)	11.52 (1.25)	11.52 (1.29)	11.74 (1.17)	11.80 (1.20)
HSDS	-2.49 (0.48)	-2.43 (0.57)	-2.30 (0.53)	-2.77 (0.61)
HV (cm/yr)	4.07 (0.99)	4.24 (0.97)	4.18 (0.93)	3.94 (0.83)
Bone Age (yrs)	10.1 (1.29)	10.7 (1.36)	11.0 (1.93)	10.8 (1.36)
HSDS for BA	-1.32 (0.75)	-1.71 (0.83)	-1.65 (0.81)	-1.88 (0.86)
PAH (cms)	161.3 (4.50)	161.0 (3.28)	160.4 (4.07)	159.2 (5.23)
At one year:				
No. children	12	10	10	11
HSDS	-2.64 (0.53)	-2.15 (0.85)	-1.96 (0.83)	-2.11 (0.87)
HV (cm/yr)	4.73 (1.02)	7.58 (1.28)	8.08 (2.14)	9.92 (2.10)
dBA/dCA	1.11 (0.63)	1.03 (0.45)	1.54 (0.49)	1.41 (0.61)
HSDS for BA	-1.36 (0.77)	-1.33 (0.92)	-1.68 (0.75)	-1.42 (0.71)
PAH (cms)	161.9 (5.21)	161.7 (4.62)	160.7 (3.52)	160.4 (4.87)
At two years:				
No. children	10	9	8	8
HSDS	-2.53 (0.78)	-2.28 (1.02)	-1.50 (0.77)	-1.50 (0.89)
HV (cm/yr)	7.25 (2.07)	6.59 (1.28)	8.23 (1.62)	9.43 (1.98)
dBA/dCA	1.29 (0.76)	1.21 (0.65)	1.71 (0.80)	1.93 (0.90)
HSDS for BA	-1.22 (0.69)	-1.43 (0.65)	-1.88 (0.73)	-1.65 (0.48)
PAH (cms)	163.0 (4.90)	164.4 (3.89)	162.4 (3.98)	162.0 (5.56)

Study 2: Summary of Growth Data

Oxandrolone group

Ten boys received oxandrolone in a dose of 2.5mg daily, equivalent to a mean of 0.09 mg/kg/day. They also had a significant improvement in height velocity over the first year of treatment, from 4.18 cm/year to 8.08 cm/year ($p = <0.001$). This is not significantly different to the improvement in growth rate seen in the boys treated with growth hormone alone. Mean bone age advance/chronological age advance was 1.54 years, which was considerably more than in the boys who received growth hormone alone ($p = 0.02$). HSDS improved from -2.30 to -1.96 ($p = 0.04$), but as skeletal maturation was rapid HSDS for bone age and predicted adult height did not improve.

rhGH and oxandrolone group

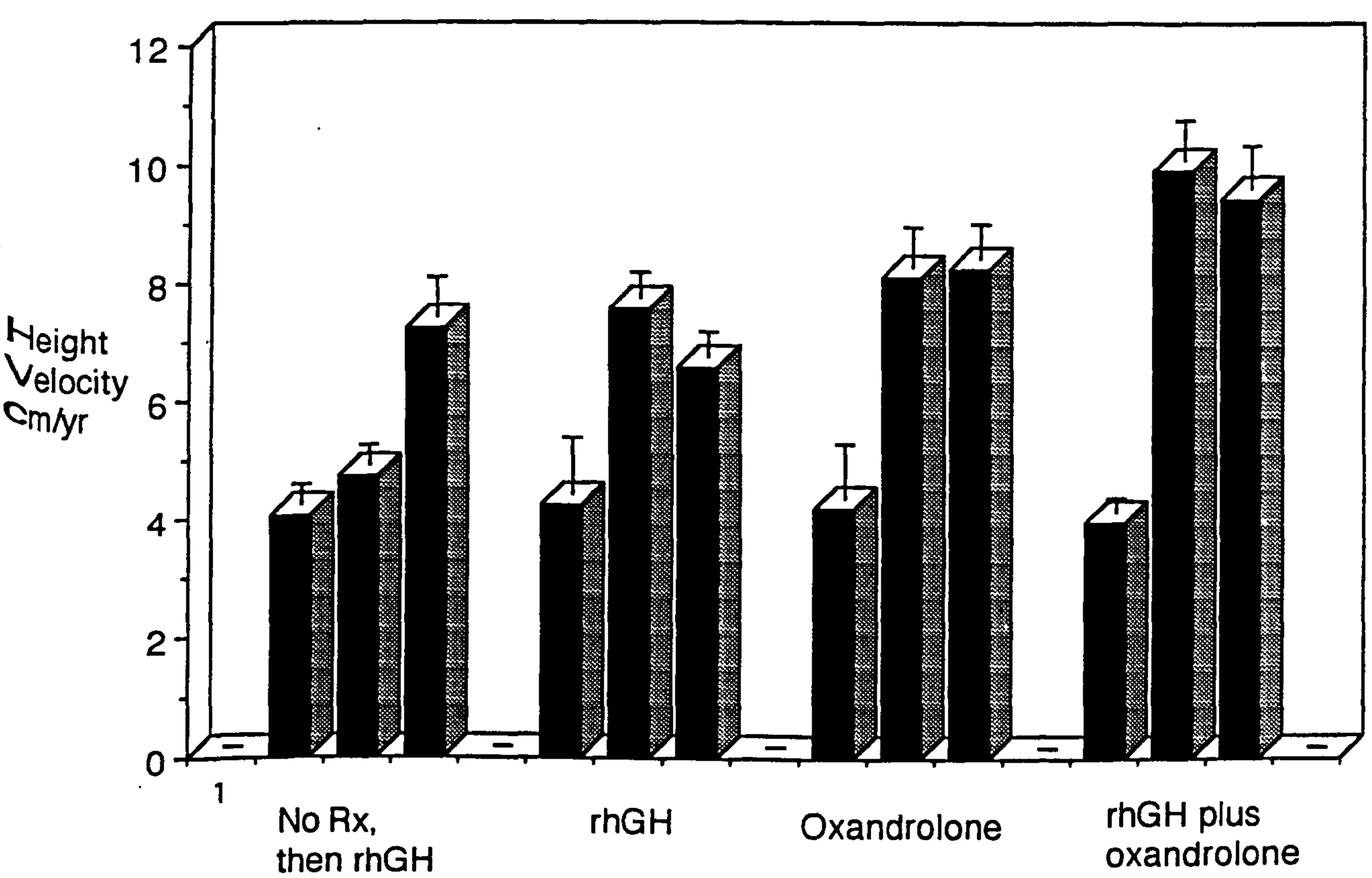
The eleven boys who received a combination of rhGH (24iu/sq.m/week) and oxandrolone (2.5mg daily, equivalent to a mean of 0.09 mg/kg/day) grew fastest over the first year of treatment, with an improvement in height velocity from 3.94 cm/year to 9.92 cm/year ($p = <0.001$). This is significantly better than the growth rate achieved with rhGH alone ($p = 0.01$), but not from that achieved with oxandrolone alone ($p = 0.06$). Mean bone age advance/chronological age advance was 1.41 years. HSDS improved from -2.77 to -2.11 ($p = 0.001$), with an improvement in HSDS for BA from -1.88 to -1.42 ($p = 0.001$), though predicted adult height did not alter significantly.

Second Year Growth Results

No treatment group

During the second year of the study the boys who had received "no treatment" in the first year now commenced growth hormone, but in the dose of 15 iu/sq.m/wk. Their growth rate improved to 7.25 cm/year ($p = 0.001$). Compared to the boys who received growth hormone alone from the onset the improvement in height velocity over the first treated year was not different in the two groups (7.58 cm/yr for the 24iu group compared to 7.25 for the 15iu group, p = NS). The mean age at onset of growth hormone therapy in the two groups was of course different (11.52 years in the 24iu group compared to 12.22 years, p = NS), but even so there appears to be little benefit in prescribing the larger dose of rhGH in this situation.

Height velocity (SE) at entry, 12 months and 24 months



rhGH group

The boys who received 24iu/sq.m/wk of rhGH from the start of the study continued to grow well over the second year of treatment, with a mean height velocity of 6.59 cm/year. This growth rate is slower than that over the first year but is still significantly faster than pre-treatment. These boys show the usual response to growth hormone treatment: the best response in the first year of treatment with subsequent waning of effect. Neither HSDS or HSDS for bone age changed significantly over the second treatment year, but predicted adult height continued to improve. Mean bone age advance/ chronological age advance was 1.21 years over the second study year, giving a total mean BA advance of 2.24 years over the 2 years of treatment.

Oxandrolone group

The boys who received oxandrolone alone from the onset of the study continued this treatment until testicular volume reached 6-8 mls, at which time they should confidently be able to maintain a good height velocity. The boys on average received the anabolic steroid for 1.55 years, thus the majority stopped treatment during their second study year. Their growth rate during the second study year was 8.23 cm/year, not significantly different to that achieved during the first year. Mean bone age advance/chronological age advance was 1.71 years, giving a total mean bone age advance of 3.25 years over the two study years. HSDS for bone age decreased though not significantly, nor was there any change in predicted adult height. However this group is the only one in whom HSDS for bone age was worse at two years than at entry into the study.

rhGH and oxandrolone group

The boys who received a combination of rhGH and oxandrolone form the start of the study continued treatment with the anabolic steroid until testicular volume reached 6-8 mls. The duration of oxandrolone treatment was on average 1.44 years, thus again most of the boys stopped the oxandrolone during their second study year. Growth hormone was continued even after the oxandrolone was stopped. The mean height velocity of these boys during the second study year was 9.43 cm/year (not significantly different to that seen during the first year). Mean bone age advance/chronological age advance over the second year was 1.93 years, giving a total mean bone age advance of 3.34 years over the two study years. Neither HSDS for BA or predicted adult height changed significantly during the second study year.

Over the two study years, bone age advanced much more rapidly in the boys who received oxandrolone alone or in combination with growth hormone, than in the boys who received growth hormone alone ($p = 0.006$ for oxandrolone alone, $p = 0.04$ for growth hormone and oxandrolone in combination). This is of concern and may have implications for final height.

2.5.7. Effects on Puberty

The boys were peri-pubertal when they entered the study, and it is very important to examine the effects of the different treatment modalities on the timing and rate of progression through puberty, as this may lead us to understand more fully the effects on height velocity and ultimately final height.

See table: Pubertal status at 0, 12 and 24 months

There were no significant differences between the groups in terms of clinical pubertal staging, LH response to low dose LHRH or 08.00 testosterone levels at the beginning of the study. Significant differences between the groups were emerging by the end of the first year of the study in the timing of pubertal onset and the rate of progression through puberty.

The boys treated with oxandrolone, either singly or in combination with growth hormone were significantly more advanced in puberty after twelve months of the study than the boys who received rhGH alone or no treatment. Median testicular volume in the oxandrolone treated group at 12 months was 4 ml, and in the combination group was 5 ml. This compared to 2 ml in the no treatment group and 3 ml in the rhGH alone group. The differences were even more marked by 24 months of the study even though oxandrolone was stopped in all boys at testicular volume 6-8 ml, the mean duration of treatment being 1.49 years. The mean age at attainment of 04 mls testes - the traditional marker for the onset of puberty in boys - was earlier in the boys treated with oxandrolone either alone (12.54 years) or in combination with rhGH (12.68 years), compared to the boys who initially received either no treatment (13.30 years) or rhGH alone (13.12 years).

These results suggest that oxandrolone treatment in these boys hastened the onset of puberty and that puberty progresses more rapidly. The effects were seen even after discontinuation of the oxandrolone. These effects on puberty are likely to contribute to the more rapid advancement of

	No treatment	rhGH	Oxandrolone	rhGH + Oxan.
At entry : No. children Age (yrs) Median test. vol (mls) Median G stage Median PH stage Median AH stage	 12 11.52 02 (range 02-04) G1 (range G1-G2) PH1 (all PH1) AH1 (all AH1)	 10 11.52 02 (range 02-03) G1 (range G1-G1+) PH1 (all PH1) AH1 (all AH1)	 10 11.74 02 (range 02-04) G1 (range G1-G2) PH1 (range PH1-PH2) AH1 (all AH1)	 11 11.80 02 (range 02-03) G1 (range G1-G2) PH1 (all PH1) AH1 (all AH1)
At one year : No. children Median test. vol (mls) Median G stage Median PH stage Median AH stage	 12 02 (range 02-08) G1 (range G1-G3) PH1 (range PH1-PH2) AH1 (all AH1)	 10 03 (range 02-08) G2 (range G1-G3) PH1 (range PH1-PH2) AH1 (all AH1)	 10 04 (range 02-08) G2 (range G2-G3) PH2 range PH1-PH3) AH1 (range AH1-AH2)	 11 05 (range 02-12) G2+ (range G1-G3) PH2 (range PH1-PH2) AH1 (all AH1)
At two years : No. children Median test. vol (mls) Median G stage Median PH stage Median AH stage	 10 05 (range 02-20) G2 (range G2-G4) PH2 (range PH1-PH3) AH1 (range AH1-AH2)	 9 06 (range 02-15) G2+ (range G1+ - G4) PH2 (range PH1-PH3) AH1 (range AH1-AH3)	 8 10 (range 06-20) G3 (range G2-G4) PH3 (range PH2-PH3) AH2 (range AH1-AH3)	 8 10 (range 02-15) G3+ (range G1+ - G4) PH2+ (range PH1-PH3) AH1 (range AH1-AH2)
Mean age at 4ml testes (yrs)	13.3 (n=9)	13.12 (n=9)	12.54 (n=10)	12.68 (n=11)

Study 2: Summary of Puberty Data

bone age compared to chronological age in the oxandrolone and oxandrolone plus rhGH groups. The bone age continues to advance faster than chronological age even after discontinuing the oxandrolone and is presumably due to the increased amounts of endogenous androgens from the relatively more advanced puberty.

Malhotra et al (1993) reported ten boys, mean age 13.8 years, treated with oxandrolone for constitutional delay of growth and puberty for three months. Oxandrolone had androgenic effects suppressing mean 24 hour serum LH and testosterone concentrations, with a rebound after treatment was stopped. Our boys were not studied in as much detail, but we do have serial random LH and testosterone levels, measured three monthly during treatment.

Median LH levels (U/l) during treatment :

	No treatment	rhGH	Oxand.	rhGH+ Oxand.
At entry	1.0	1.2	<0.8	0.8
6 months	<0.8	0.8	1.1	0.9
12 months	<0.9	<0.8	1.0	1.4
18 months	1.0	1.0	1.1	1.2
24 months	1.1	1.2	1.4	2.0

Random LH levels are not a particularly good marker of hypothalamo-pituitary function as LH is secreted in a pulsatile manner and it is impossible to know where in a pulse a random level has been taken. However, as puberty progresses one would expect median random levels in a group of boys to gradually increase as the chances of sampling from a pulse of LH increases. Thus median levels across groups are a crude way of looking at the progression of LH secretion. There were no significant differences between the groups until 24 months, when median LH levels were higher in the groups treated with oxandrolone. As the average time of stopping oxandrolone was around eighteen months this may represent some rebound in LH after ceasing treatment. Alternatively as, clinically, puberty had progressed further in the boys treated with oxandrolone, one might have expected LH levels to be higher than in the rhGH and no treatment groups - oxandrolone may have been suppressing LH secretion during treatment. A similar pattern was observed in the random testosterone levels with the highest levels seen at 24 months in the two groups treated with oxandrolone.

Median Testosterone levels (nmol/l) during treatment :

	No treatment	rhGH	Oxand.	rhGH + Oxand.
At entry	0.6	<0.6	<0.6	0.6
6 months	<0.6	<0.6	0.7	<0.6
12 months	0.7	<0.6	0.8	0.8
18 months	0.8	1.1	1.6	1.6
24 months	1.6	1.9	6.0	3.7

Our results complement those of Malhotra et al (1993), but in a younger group of boys.

Interestingly self esteem improved more in the oxandrolone treated group than in the boys treated with rhGH alone although the growth rates achieved were similar - this is possibly due to the more obvious virilising effects being more readily apparent to the boys than the growth-promoting effects - see Section 4 for more details.

Oxandrolone has been used successfully to treat boys with puberty delay and is effective at bringing forward the timing of the pubertal growth spurt (Stanhope and Brook 1985, Stanhope et al 1988). We have now shown that it has marked effects on the timing of puberty and its rate of progression in boys without puberty delay. This anabolic agent must be used with caution, particularly for periods greater than six to twelve months, and the results of studies in boys with puberty delay must not be extrapolated to other groups of short boys without care. We would not at this stage recommend its use in a boy without growth and maturational delay. Although it promotes growth well in the short-term it appears to have significant effects on pubertal timing and may therefore limit the time available for growth. Boys with familial short stature without growth delay already have a height prognosis at the lower end of the adult male range. Any intervention that might compromise this is not justified, however good the short-term gains. It is imperative that the boys in our studies are followed to final height to give more definitive answers on these issues.

2.5.8. Effects on biochemical markers of growth

Two biochemical markers of growth have been measured in these boys. Insulin-like growth factor 1 (IGF1), predominantly produced by liver rises in GH-deficient children treated with rhGH. Bone-derived alkaline phosphatase (bALP) is a more specific marker of bone turnover. Few studies have looked at this marker in response to growth-promoting treatments, or as a predictor of response to growth promoting therapies. It has been postulated that oxandrolone does not affect the GH (and hence IGF1) status of pre-pubertal boys and promotes growth by a direct action at the growth plate, whereas rhGH acts predominantly via IGF1. In order to examine this we compared the effects of rhGH and oxandrolone on IGF1 levels and bALP.

1. IGF1

IGF1 was measured at entry into the study and at six months. The results are expressed as units/ml (1 Unit/ml = 37.4 nmol/l). The reference range quoted by the laboratory is 0.28 -1.36 Units/ml, or more usefully divided into age ranges. :

6-10 yrs 0.1- 2.41 Units/ml

11-16 yrs 0.3 -3.34 Units/ml

Mean (SD) IGF1 Units/ml:

	IGF1 at entry Mean (SD)	Increase in IGF1 at 6 months
No treatment (0-12 mths)	0.74 (0.26)	0.25 (0.31)
rhGH 15iu (12-24 mths)	1.02 (0.36)	0.72 (0.72)
rhGH 24iu	0.67 (0.22)	0.73 (0.17)
Oxandrolone	0.65 (0.32)	0.40 (0.54)
rhGH + Oxandrolone	0.52 (0.20)	0.73 (0.50)

The mean levels at entry in our children are well within the expected range for their age. There were no significant differences in mean IGF1 levels at entry into the study between the four groups at 0 months. Mean IGF1 levels in the "no treatment" group at 12 months (ie. prior to starting 15iu rhGH) were higher than in the other groups when they started treatment.

All groups showed a rise in mean IGF1 at 6 months into the study, but this was most marked in the groups receiving growth hormone either alone or in combination with oxandrolone. The rise in IGF1 in these groups was significantly better than that in the "no treatment" group ($p = 0.001$). Although the oxandrolone alone group did show a rise in IGF1 at six months, this was

not significantly different to the mean rise in the boys who received "no treatment".

2. Bone-derived alkaline phosphatase (bALP)

This was measured at entry into the study and at three monthly intervals in the boys receiving treatment (either growth hormone alone or with oxandrolone) and at entry and six monthly intervals in those receiving no treatment.

Mean (SD) increase in bALP above the baseline measurement :

	Increase in bALP (units/l)
No treatment (0-12 mths)	+ 36.2 (90.9)
rhGH 15iu (12-24 mths)	+104.1 (82.1)
rhGH 24iu	+125.3 (93.2)
Oxandrolone	+116.0 (70.8)
rhGH + Oxandrolone	+274.4 (179.2)

There was no significant change in bALP in the boys who received "no treatment". All the groups who received an active treatment showed an increase in bALP by three months, which was most marked in those who received both rhGH and oxandrolone in combination. It is of note that oxandrolone alone caused a rise in bALP not dissimilar to that in the rhGH groups, and that the rise in bALP in the combination group appeared cumulative.

This is further evidence that oxandrolone is exerting its effect directly at the growth plate rather than being mediated via IGF1.

2.5.9. Side effects of treatment

1. Effects on body fat

Growth hormone has well known lipolytic and anabolic effects. It has long been recognised that growth hormone deficient children have increased body fat compared to normal children, and that when they are treated with replacement growth hormone they become leaner (Tanner et al 1977). The greatest effect appeared to be within the first three months of treatment.

Skinfold thicknesses are a practical way to estimate body fat (Brook 1971, Durnin and Womersley 1974). In order to determine the effects of rhGH on the body fat of short normal children, we measured triceps and subscapular skinfold thicknesses at entry into the study and at three monthly intervals. The measurements were made by a single observer (HS) using Holtain calipers. The individual measurements were log transformed and then expressed as the mean sum (SD) of log transformed triceps and subscapular skinfold measurements.

	No treatment	rhGH	Oxandrolone	rhGH + Oxan.
At entry	330.8 (30.1)	322.1 (31.6)	349.1 (32.9)	346.0 (29.6)
3 months	325.1 (23.5)	306.3 (29.7) *	353.3 (19.5)	314.2 (31.1) **
6 months	342.0 (44.7)	294.0 (34.5) **	333.3 (28.7) *	322.5 (34.8) *
12 months	336.8 (33.0)	305.4 (38.6) *	334.6 (22.9)	325.3 (36.6) *
18 months	312.2 (41.6) *	304.1 (34.5) *	324.0 (24.3)	326.8 (39.3)
24 months	313.7 (44.3)	309.9 (34.0) *	331.6 (23.3)	310.4 (31.4) *
	* p = 0.03	* p = 0.01 ** p = <0.001	* p = 0.03	* p = <0.005 ** p = <0.001

The boys who received rhGH alone during the first year of the study had a significant decline in skinfold thickness, and thus body fat. This was highly significant by three months and reached a maximum at six months. This effect was similar to the effect seen in the pre-pubertal children (section 1.5.7). However, these boys remained leaner for longer as judged by their skinfold measurements. The boys who received rhGH (15iu) in their second study year having completed a year of no treatment also showed this effect - skinfold thicknesses falling significantly by six months of active therapy. They showed an almost identical fall in skinfold thickness to the ones who had received the larger rhGH dose. Normal peri-pubertal boys treated with growth hormone become leaner.

The boys treated with oxandrolone alone also became transiently leaner, though not to such a significant degree. As puberty progresses in normal untreated boys the value for biceps and triceps skinfolds show a

steady decline and subscapular a gradual rise, indicating a changing distribution of body fat (Buckler 1989). The effects we saw with oxandrolone were transient and may be reflecting this change in body fat distribution as puberty is progressing. In young adults who misuse anabolic agents to enhance their appearance the desire is to become "well muscled" and it may be that at the higher doses used in such abuse that more marked effects are seen due to a combination of decrease in body fat and an increase in lean body mass.

The boys treated with combination therapy also became significantly leaner, with the maximum effect being noted at three months into treatment. It was clinically apparent to us that some of the boys treated with a combination of rhGH and oxandrolone did indeed appear "well muscled", more so than in either of the single treatment groups. This effect itself may have psychological effects.

This effect of rhGH on body fat has been noted previously. Hindmarsh and Brook (1987) showed similar results to our children, with significant fall in triceps and subscapular skinfold measurement at six months with return to baseline values by twelve months. Walker et al (1990) showed that six months of rhGH therapy in a larger dosage than we used (30iu/sq.m/week) produced upto 76% loss of fat mass and upto 25% increase in lean body mass in normal short children. It is difficult to know whether there will be any long term effects from the alteration of body composition in this way, and the boys merit long term follow up with regard to this.

2. Effects on cholesterol and triglyceride levels :

There have been concerns about the effects of anabolic agents on plasma lipid levels. Changes in cholesterol distribution (a decrease in high density lipoprotein cholesterol and apoprotein levels and rise in low density lipoprotein cholesterol) have been reported in athletes who abuse anabolic steroids (Lamb 1984, Webb et al 1984). This might relate to an increased risk of atherosclerosis and may be relevant to myocardial infarction in these individuals (Bowman 1990). Little is known about the effects of therapeutic doses on the cholesterol distribution in normal adolescents.

In addition to the alterations in high density and low density cholesterols potentially increasing the risks of atherosclerosis, there have been several reports of acute thrombotic events occurring in patients who have misused anabolic agents eg. massive pulmonary haemorrhage and acute myocardial infarction. Anabolic steroids may enhance platelet aggregation, alter coagulation or fibrinolytic proteins, or increase vascular reactivity producing a hypercoagulable state (Ferenchick 1991).

McCaughey et al (1993) have suggested that growth hormone treatment in normal short children alters plasma cholesterol and triglyceride levels.

In our study, plasma cholesterol and triglyceride levels were measured at entry into the study and at three months, six months and then at six monthly intervals. The baseline values were often fasting (taken at the time of the child's GH provocation test) and the subsequent levels were non-fasting, as it was not practical to bring the children to clinic fasting. This will not affect the cholesterol level but may affect the triglyceride levels, making them subject to wider variation. .

Mean (SD) cholesterol mmol/l

	No treatment	rhGH	Oxandrolone	rhGH + Oxan.
At entry	4.26 (0.61)	4.56 (0.66)	4.42 (0.94)	4.42 (0.43)
3 months	NA	4.75 (0.47)	4.01 (0.79) *	3.88 (0.40) *
6 months	4.68 (0.40)	4.97 (0.84) *	4.11 (0.99)	4.07 (0.50)
12 months	4.55 (0.40)	4.73 (0.64)	4.26 (1.03)	3.78 (0.71) *
18 months	4.37 (0.34)	4.64 (0.56)	4.13 (0.58)	3.52 (0.47) *
24 months	4.65 (0.59)	4.56 (0.80)	3.93 (0.65) *	3.47 (0.33) *
		* p = 0.008	* p = 0.02	* p = <0.01

Normal range = 2.5-6.3 mmol/l

In the boys treated with rhGH alone, either from the outset of the study or after a year of no treatment, there appeared to be no serious effects on

mean plasma cholesterol levels. Mean plasma cholesterol increased transiently in the rhGH group at six months but then settled back to pre-treatment levels. No child treated with rhGH had a plasma cholesterol level above the laboratory reference range at any time. This is different to the effects described by McCaughey et al (1993) who noted a significant rise in cholesterol levels after twelve months of rhGH treatment although they gave a higher dose (30iu/sq.m/week) than we used.

It is of note that in both our groups treated with oxandrolone mean plasma cholesterol levels fell. We were only measuring total cholesterol levels and are unable to comment whether or not this fall in total cholesterol is due to an alteration in the ratio of high density to low density lipoprotein cholesterol. This merits further investigation.

Mean (SD) triglyceride levels

	No treatment	rhGH	Oxandrolone	rhGH + Oxan.
At entry	0.66 (0.14)	0.92 (0.35)	0.76 (0.40)	0.76 (0.27)
3 months	NA	1.29 (0.49) *	0.81 (0.51)	0.80 (0.34)
6 months	1.12 (0.53) *	0.94 (0.44)	0.92 (0.44)	0.90 (0.31)
12 months	0.92 (0.25)	0.94 (0.35)	0.83 (0.43)	0.74 (0.26)
18 months	1.15 (0.54)	0.93 (0.28)	0.85 (0.42)	0.82 (0.46)
24 months	0.73 (0.23)	0.85 (0.47)	0.90 (0.34)	0.91 (0.39)
	* p = 0.03	* p = 0.02		

Normal range = 0.1-1.5 mmol/l

There were no significant changes in triglyceride levels in the children treated with oxandrolone or combination treatment. In the rhGH group there was a transient increase in mean triglyceride level at three months, but a similar rise was also seen in the no-treatment group at six months and is probably a reflection of the non-fasting nature of the blood samples and is unlikely to be of clinical significance.

3. Carbohydrate metabolism

- effects on blood glucose and glycosylated haemoglobin

There are theoretical concerns that the growth hormone treatment of normal short children may lead to impaired glucose tolerance (see section 1.5.9.) Similarly there are concerns about the effects of anabolic steroids on glucose metabolism.

It has long been recognised that anabolic steroid eg. methandienone have effects on carbohydrate metabolism (Landon et al 1962, Landon et al 1963) with a fall in fasting blood sugar but impaired tolerance to oral and intravenous glucose, possibly due to decreased insulin sensitivity. Endogenous hyperandrogenism (eg. polycystic ovarian disease) is also associated with insulin resistance and impaired glucose tolerance. More recently there has been concern about the effects of oxandrolone on carbohydrate metabolism particularly in girls with Turner syndrome (Wilson et al 1988). Impaired glucose tolerance was present in upto 50% of girls receiving oxandrolone either alone or in combination with growth hormone, as judged by oral glucose tolerance tests after one year of treatment, although fasting glucose and HbA1 remained within the normal range. Girls with Turner syndrome are well recognised to have abnormalities of carbohydrate metabolism but in this study treatment with growth hormone alone did not affect this whereas treatment involving oxandrolone did. It has been suggested that oxandrolone may decrease insulin sensitivity. One must not be complacent about the effects of this anabolic steroid on carbohydrate metabolism.

In our study plasma glucose and HbA1 levels were measured at entry into the study and at 3 months, 6 months and then at 6 monthly intervals. We did not have the opportunity to measure serial fasting plasma insulin levels. Urine was checked for glycosuria at 3 monthly intervals.

Mean (SD) plasma glucose mmol/l:

	No treatment	rhGH	Oxandrolone	rhGH + Oxan.
At entry	4.57 (0.43)	4.80 (0.69)	4.72 (0.66)	5.31 (1.02)
3 months	NA	5.14 (0.97)	4.84 (1.37)	5.02 (1.23)
6 months	5.04 (0.98)	4.60 (0.49)	4.98 (0.81)	4.98 (0.57)
12 months	4.75 (0.47)	4.86 (0.54)	4.53 (0.33)	5.03 (1.08)
18 months	4.57 (1.29)	5.01 (0.69)	4.77 (0.55)	4.36 (0.69)
24 months	4.70 (0.68)	4.63 (0.43)	4.63 (0.69)	5.00 (2.11)

Mean (SD) Glycosylated Haemoglobin (HbA1) % of total Hb:

	No treatment	rhGH	Oxandrolone	rhGH + Oxan.
At entry	6.00 (0.97)	5.91 (0.67)	6.50 (0.69)	6.05 (0.74)
3 months	NA	6.33 (0.84)	5.83 (0.36)	6.44 (0.97)
6 months	6.61 (1.47)	5.85 (0.89)	6.02 (0.76)	6.52 (0.90)
12 months	5.95 (0.60)	6.38 (1.17)	5.60 (0.74)	6.37 (0.72)
18 months	6.05 (0.43)	6.13 (0.78)	6.10 (0.27)	5.98 (0.31)
24 months	6.06 (0.42)	6.14 (0.61)	6.45 (0.69)	6.51 (0.62)

Reference range = 4.7-7.9%

There were no significant changes in mean plasma glucose level or mean glycosylated haemoglobin in any of the groups.

However, one boy in the rhGH plus oxandrolone group did develop evidence of glucose intolerance. At three months into treatment random plasma glucose was 8.0 mmol/l with 1/2% glycosuria. Repeat urinalysis over the next few weeks revealed no further glycosuria, and random plasma glucose level at six months was unremarkable at 4.4 mmol/litre. At twelve months, random plasma glucose was 8.0 mmol/l, but with no glycosuria, and at 18 months plasma glucose was measured at 3.3 mmol/l. At 24 months random glucose was 9.4 mmol/l, with 2% glycosuria. Glycosylated haemoglobin during treatment ranged from 6.1-7.8 %, compared to a pre-treatment value of 5.5%. Islet cell antibodies were negative. Treatment was discontinued at 24 months, and a standard oral glucose tolerance test was performed.

Glucose Tolerance Test :

Time (mins)	Glucose (mmol/l)	Insulin mU/l
0	4.8	55.1
30	9.9	421.0
60	7.4	392.5
90	7.3	457.5
120	6.2	247.5

Although the glucose response is unremarkable, this boy had a very exaggerated insulin response to the oral glucose load, which is similar to that seen in girls with Turner syndrome who develop glucose intolerance during oxandrolone treatment (Wilson et al 1988). At no time was the boy clinically symptomatic. He has now been followed off treatment for two years. Random plasma glucose levels have been 4.8-6.1 mmol/l with HbA1 levels of 6.5-7.3%. Glycosuria has not been detected again.

Both oxandrolone and rhGH have been implicated in the development of glucose intolerance. It is impossible to know which drug if any was involved in this case. However it is a warning that one must be always vigilant to detect glucose intolerance in an apparently normal child being treated with either rhGH or an anabolic agent. No other child in this study developed either glycosuria or elevated random plasma glucose levels. It was not practical to bring our children to the clinic fasted and we had no opportunity to assess fasting insulin levels, which are more likely to detect more subtle problems with glucose tolerance.

In view of our experience with this boy, we would suggest that the oxandrolone effect on carbohydrate metabolism in normal young adolescents merits further investigation.

4. Effects on thyroid function

There has been little published about the effects of growth hormone on the thyroid function of normal children, though it has been shown to affect the thyroid function of GH deficient children (Pirazzoli et al 1992). Little is also known about the effects of oxandrolone on thyroid function in children, though the effects on athletes abusing anabolic agents are more widely documented (Alen et al 1987). Athletes using "large doses" of "illegal" anabolic steroids have been shown to have significant falls in TSH, T4, T3, Free T4, and thyroid binding globulin, but it is not known how these findings relate to pre-pubertal children receiving therapeutic doses of such agents.

We followed the thyroid function of our boys with measurements of total thyroxine, free thyroxine and thyroid binding globulin.

Mean (SD) Total T4 nmol/l:

	No treatment	rhGH	Oxandrolone	rhGH + Oxan.
At entry	114.3 (21.7)	112.4 (17.4)	107.8 (16.4)	105.5 (17.2)
3 months	NA	105.3 (15.9) *	90.1 (17.7) **	77.6 (22.0) **
6 months	115.8 (19.0)	125.0 (22.9)	91.7 (11.9) **	91.9 (16.8) **
12 months	112.4 (15.9)	120.2 (18.5)	99.0 (15.1)	97.1 (21.4)
18 months	106.0 (16.5)	115.2 (18.6)	94.5 (20.0)	87.9 (18.9)
24 months	101.5 (14.5)	107.3 (22.5)	107.5 (16.5)	95.0 (17.1)
		* p = 0.04	** p = <0.01	** p = 0.01

Reference range 70-180 nmol/l

Mean (SD) Free T4 pmol/l:

	No treatment	rhGH	Oxandrolone	rhGH + Oxan.
At entry	18.1 (2.8)	15.5 (2.0)	14.7 (2.1)	16.3 (2.2)
3 months	NA	15.7 (4.1)	12.0 (2.6)	13.2 (5.5)
6 months	13.5 (1.3)	14.6 (3.0)	14.2 (3.0)	13.3 (1.6)
12 months	14.0 (2.5)	14.0 (3.0)	14.1 (2.8)	13.3 (2.2)
18 months	14.8 (3.4)	13.9 (2.6)	13.0 (3.2)	12.7 (3.1)
24 months	12.9 (2.9)	15.5 (2.6)	12.3 (1.8)	12.5 (3.2)

Reference range 9-23 pmol/l

Mean (SD) Thyroid Binding Globulin mg/l :

	No treatment	rhGH	Oxandrolone	rhGH + Oxan.
At entry	23.0 (2.8)	23.2 (3.0)	22.2 (5.6)	23.4 (2.7)
3 months	NA	22.0 (4.3)	13.8 (3.1) *	17.5 (4.4)
6 months	23.0 (4.6)	23.7 (3.5)	17.8 (3.5)	17.0 (5.1)
12 months	22.7 (4.2)	23.2 (2.2)	17.6 (4.0)	18.0 (4.0)
18 months	21.6 (3.2)	24.8 (5.0)	14.0 (3.4)	19.6 (5.6)
24 months	20.9 (5.6)	22.0 (4.8)	17.5 (5.3)	18.2 (7.0)
			* p = 0.05	

Reference range 12-30 mg/l

There were no significant changes in total T4, free T4 or thyroid binding globulin during treatment with rhGH alone. In the boys treated with oxandrolone, either alone or in combination with rhGH, total thyroxine levels fell significantly by three months and remained low at six months, although there was some recovery at twelve months. Free T4 levels were maintained, and the fall in total thyroxine can be accounted for by the fall in thyroid binding globulin. If the results from all the boys taking oxandrolone (either alone or in combination with rhGH) are combined (n = 21) then the effect on thyroid binding globulin is dramatic. Total thyroxine levels fell at three months from a pre-treatment level of 103.0 to 80.5nmol/l (p = 0.001), with a concurrent fall in TBG form 22.7 to 16.1mg/l (p = 0.03). All but two boys treated with oxandrolone showed this trend. It is such a consistent feature that it has subsequently proved useful to us as a marker of compliance to oxandrolone therapy. The recovery of total thyroxine levels by 24 months in both oxandrolone treated groups is probably explained by the fact that most boys had completed their oxandrolone treatment by that time, the mean duration of oxandrolone treatment being 1.49 years.

5. Production of Growth Hormone Antibodies

The rhGH used in our study had an identical amino acid sequence to naturally occurring hGH, and is less antigenic (Rasmussen 1988) than methionyl GH. Antibodies to growth hormone were measured in all children at entry into the study and at six monthly intervals.

All samples were negative for rhGH antibodies.

6. Effects on liver function

Many studies have shown effects of anabolic steroids on the liver. 19 of 60 adult patients receiving long-term methyltestosterone had abnormal liver-function tests and 33 of 52 had abnormal liver scans, particularly those who had been treated for more than a year (Westaby et al 1977). The 17a-alkylated steroids seem to be particularly implicated in the development of cholestatic jaundice, peliosis hepatis, and liver tumours. These changes have been reported in athletes abusing large quantities of anabolic steroids including oxandrolone (Lamb 1984). Reversible rises in transaminases, bilirubin and alkaline phosphatase are also seen in such individuals. In patients taking smaller "therapeutic" doses of oxandrolone reversible increases in liver transaminases particularly serum glutamic-oxalacetic transaminase (SGOT) are seen both in adults (Sansoy et al 1971) and children (Geller 1968, Bettman et al 1971). In the children the SGOT normalised 1-3 months after stopping treatment.

We measured plasma alanine aminotransferase (ALT) and g-glutamyl transferase (GGT) at entry into the study and at 3 monthly intervals with the following results :

Mean (SD) ALT:

	No treatment	rhGH	Oxandrolone	rhGH + Oxan.
At entry	19.1 (4.1)	16.0 (3.0)	15.0 (3.7)	15.6 (4.6)
3 months	NA	18.5 (5.3)	15.1 (4.3)	17.3 (3.7)
6 months	17.4 (2.0)	17.9 (2.8)	15.7 (6.2)	18.3 (4.7)
12 months	17.6 (3.5)	16.5 (3.4)	16.4 (4.8)	19.6 (5.7)
18 months	19.3 (5.2)	21.2 (12.8)	28.8 (28.9)	28.9 (26.5)
24 months	19.5 (3.8)	16.8 (4.2)	22.5 (11.1)	20.9 (8.9)

Reference range 10-40 units/l

Mean (SD) GGT:

	No treatment	rhGH	Oxandrolone	rhGH + Oxan.
At entry	11.3 (1.3)	11.2 (3.1)	11.3 (2.6)	11.6 (1.2)
3 months	NA	11.3 (3.0)	10.4 (2.8)	11.4 (2.4)
6 months	12.3 (2.1)	11.8 (2.9)	11.9 (3.3)	11.7 (2.0)
12 months	12.7 (2.3)	11.4 (3.1)	12.3 (3.7)	11.7 (1.8)
18 months	12.3 (2.0)	11.6 (2.5)	11.5 (3.8)	12.9 (3.7)
24 months	13.3 (2.6)	11.5 (3.1)	14.8 (9.0)	12.6 (2.1)

Reference range <35 units /l

Although mean plasma ALT did not rise significantly during treatment, three boys had transient rises in ALT to levels above the laboratory reference range: one treated with rhGH alone - ALT 56 units/l after 18 months treatment, one treated with oxandrolone alone - ALT 87 units/l after 18 months treatment, and the third who received rhGH plus oxandrolone - ALT 98 units/l after 18 months treatment. When rechecked three months later the ALT had fallen to a normal level in all three boys. In none of the boys was there a concomitant change in GGT and all were clinically well.

7. Other side effects - testicular oedema

One of the boys in the group treated with rhGH from the outset developed a testicular abnormality. He had been treated with rhGH from the age of 10.8 years and was entirely pre-pubertal at the onset of therapy. He had undergone left orchidopexy at the age of 8 years. Puberty commenced at the age of 11.9 years and was uneventful with symmetrical testicular enlargement until the age of 13.3 years. At this time he had reached G2-3, PH1, AH1. The right testis was 6 mls in volume, but the left was 12 mls in volume and its consistency felt abnormal. Ultrasound scan suggested an abnormal area at one pole - possibly the sight of fixation at previous orchidopexy. Exploration of the scrotum and left orchidectomy was performed. At operation the testis was macroscopically very oedematous. Histology revealed a hydrocele, testicular oedema and chronic inflammation in the epididymis. rhGH was discontinued. The remaining testis continued to enlarge normally as puberty progressed.

When this boy developed the abnormal testicular enlargement, there was concern that he had developed a testicular neoplasm, particularly as he had undergone orchidopexy on this side, a recognised risk factor (United Kingdom Testicular Cancer Study Group 1994). The cause of the chronic inflammatory changes and the marked testicular oedema remains obscure. rhGH has been known to cause peripheral oedema in adults, but in this boy the changes were localised to the testis. There are no previous reports to implicate rhGH in this finding, but as it was a significant adverse event during treatment we notified the Committee of Safety of Medicines and the Kabi International Growth Study (KIGS) as it will be important to determine whether it is a recurring event in children treated with rhGH.

DISCUSSION

We aimed to determine whether treatment with rhGH or oxandrolone or rhGH plus oxandrolone improved height velocity in the short-term in peri-pubertal boys without growth delay. The boys who received "no treatment" for the first year showed a slight improvement in growth rate, which just reached statistical significance. The boys who received an active treatment did grow significantly better, with the best growth response occurring in those who received a combination of rhGH plus oxandrolone. There was no significant difference in the growth rates produced by either rhGH or oxandrolone singly - a similar result to that of Buyukgebiz et al (1990). The growth rate we produced with oxandrolone in our peri-pubertal boys was similar to that produced by Stanhope et al (1988) in their study in older boys with puberty delay, and was also comparable to that described by Papadimitriou et al (1991) in growth-delayed pre-pubertal boys.

We have reported two year data on our boys. In those treated with rhGH alone, and continued in a dose of 24iu/sq.m/week, we saw the typical waning of effect, although the second year growth rate was still better than that pre-treatment. There was no waning of effect over the second year in the boys who received oxandrolone, either singly or in combination with rhGH, but as we shall see later this is likely to be due to the effects on puberty in maintaining the growth response.

The study gave us the opportunity to compare two doses of rhGH (15iu and 24iu/sq.m/wk) in peri-pubertal boys. The growth rates produced over the first year of rhGH treatment were similar. Although the boys who received 15iu/sq.m/wk, after a year of no treatment, were older at the onset of active treatment than the boys who received 24iu, they were not clinically more advanced in puberty. As in the study reported in section 1, there appears to be little benefit in prescribing the larger dose of rhGH in this situation.

Skeletal maturation proceeded much more rapidly in those boys who received oxandrolone, either singly or in combination with rhGH. Over the two study years, bone age advanced by 3.25 years in the boys who received oxandrolone alone and 3.34 years in those who received oxandrolone in combination with growth hormone. This was significantly more than in the boys who received no treatment followed by 15iu rhGH (2.40 years) and those who received 24iu rhGH from the outset (2.24 years). It is of concern that bone maturation is more rapid in the boys who received the anabolic steroid. Like Sobel (1968), we observed rapid bone maturation in some of our boys, even after discontinuation of treatment. Our children received

oxandrolone for a relatively long period of time (compared to the studies in older boys with growth delay), and it may be that continued exposure to the agent leads to more marked effects on bone maturation. However there may be an alternative explanation, as there are significant differences in pubertal progress in the boys who received the oxandrolone.

The boys treated with oxandrolone, either singly or in combination with growth hormone were significantly more advanced in puberty after twelve months of the study than the boys who received rhGH alone or no treatment, with the differences being more marked by 24 months. The mean age at attainment of 4 ml testes - the traditional marker for the onset of puberty in boys - was between six to nine months earlier in the boys treated with oxandrolone either alone or in combination with rhGH compared to the boys who initially received either no treatment or rhGH alone. Our results suggest that oxandrolone treatment in these boys hastened the onset of puberty, and that puberty progresses more rapidly. The effects were seen even after discontinuation of the oxandrolone. These effects on puberty are likely to contribute to the more rapid advancement of bone age in the oxandrolone treated boys. The bone age continues to advance faster than chronological age even after discontinuing the oxandrolone and is presumably due to the increased amounts of endogenous androgens from the relatively more advanced puberty. We were not using an excessively large of oxandrolone, but were continuing it for a relatively longer period of time than is traditionally used in boys with growth delay. Perhaps using a smaller dose would have had less effect on skeletal maturation. It has suggested that 1.25 mg daily may be effective in boys with puberty delay. (Stanhope et al 1985).

Malhotra et al (1993) suggested that oxandrolone had androgenic effects which suppressed mean 24 hour serum LH and testosterone concentrations, with a rebound after treatment was stopped. We showed no significant differences in LH levels between the groups until 24 months, when median LH levels were higher in the groups treated with oxandrolone. As the average time of stopping oxandrolone was around eighteen months this may represent some rebound in LH after ceasing treatment. Alternatively as puberty had clinically progressed further in the boys treated with oxandrolone, one might have expected LH levels to be higher than in the rhGH and no treatment groups - oxandrolone may have been suppressing LH secretion during treatment. A similar pattern was observed in the random testosterone levels with the highest levels seen at 24 months in the two

groups treated with oxandrolone. Our results complement those of Malhotra et al (1993), but in a younger group of boys.

Oxandrolone has been used successfully to treat boys with puberty delay and is effective at bringing forward the timing of the pubertal growth spurt (Stanhope and Brook 1985, Stanhope et al 1988). We have now shown that it has marked effects on the timing of puberty and its rate of progression in boys without puberty delay. Although it promotes growth well in the short-term it appears to have significant effects on pubertal timing and may therefore limit the time available for growth.

We have concerns about the effects on final height in our oxandrolone treated boys. HSDS for bone age in the boys treated with oxandrolone alone gradually deteriorated over the two treatment years from -1.65 to -1.88. In the other three groups HSDS for bone age was better than pre-treatment at the end of the second year. Careful follow up to final height is required in this group of boys. Boys with familial short stature without growth delay already have a height prognosis at the lower end of the adult male range. Any intervention that might compromise this is not justified, however good the short-term gains.

There is still debate as to the mechanism of oxandrolone action in the peri-pubertal boy. All of our groups showed a rise in mean IGF1 at 6 months into the study, most marked in the groups receiving growth hormone either alone or in combination with oxandrolone. Although the oxandrolone group showed a rise in IGF1 at six months, this was not significantly different to the mean rise in the boys who received no treatment. There was no significant change in bALP in the boys who received no treatment. All the groups who received an active treatment showed an increase in bALP by three months, which was most marked in those who received both rhGH and oxandrolone in combination. It is of note that oxandrolone alone caused a rise in bALP not dissimilar to that in the rhGH groups, and that the rise in bALP in the combination group appeared cumulative. We suggest that this is further evidence that oxandrolone is exerting its effect directly at the growth plate rather than being mediated via IGF1.

Self esteem interestingly improved more in the oxandrolone treated group than in the boys treated with rhGH alone although the growth rates achieved were similar - this is possibly due to the more obvious virilising effects being more readily apparent to the boys than the growth-promoting effects - see Section 4 for more details.

The boys who were treated with rhGH either alone or in combination with oxandrolone became leaner as judged by skinfold thicknesses - a

similar effect to that which we saw in the pre-pubertal children. Body appearance of some of the boys in the combination group changed subjectively with the boys appearing "well muscled".

We did not observe any adverse effects of rhGH on cholesterol or triglyceride levels, but we did note that in both groups treated with oxandrolone mean plasma cholesterol levels fell. We were only measuring total cholesterol levels and are unable to comment whether or not this fall in total cholesterol is due to an alteration in the ratio of high density to low density lipoprotein cholesterol. This merits further investigation. One boy developed significant glucose intolerance with insulin resistance, which necessitated discontinuing his treatment. Both oxandrolone and rhGH have been implicated in the development of glucose intolerance. It is impossible to know which drug if any was involved in this case. One must be always vigilant to detect glucose intolerance in an apparently normal child being treated with either rhGH or an anabolic agent.

Anabolic agents, particularly methyltestosterone, have been implicated in liver dysfunction. We followed liver transaminases three monthly in our boys. Three of those treated with oxandrolone developed transient elevation of ALT, all of which recovered spontaneously. We did not note any similar changes in the boys treated with rhGH alone.

We found significant, but recoverable, changes in thyroid binding globulin in the boys treated with oxandrolone either alone or in combination with rhGH. A fall in total thyroxine was seen, although free T4 levels were maintained. These were characteristic findings, and have subsequently proved useful as a marker of compliance. Thyroid function tests in children receiving oxandrolone need to be interpreted with caution.

One boy treated with rhGH alone developed abnormal testicular enlargement. We were concerned at presentation that he had developed a neoplasm in the testis, particularly as he had predisposing factor. The histology was of oedema, though the aetiology remains obscure.

As in the younger children in section 1 we did not detect any parameters pre-treatment that are predictive of the response to treatment. There was no correlation between measured growth hormone levels (either physiological or stimulated), IGF1 or pre-treatment height velocity, and the height velocity at one year. This may be because we were dealing with a very clearly defined group of boys who were so similar in their pretreatment characteristics that we cannot find good markers to predict response. Again, as in the younger children, the increase in bALP at three months was a good marker of response to treatment at one year.

In summary, we have shown that we are able to promote the growth of peri-pubertal boys with familial short stature, at least in the short term. Although the growth responses to rhGH and oxandrolone are similar, oxandrolone appears to have considerable effects on the timing and tempo of puberty in these boys. This may lead to deleterious effects on final height. This anabolic agent must be used with caution, and the results of studies in boys with puberty delay must not be extrapolated to other groups of short boys without care. We would not at this stage recommend the use of oxandrolone in a boy without growth and maturational delay.

SECTION 3 : GROWTH PROMOTION IN BOYS WITH PUBERTY DELAY

3.1. Introduction

This study was designed to investigate the effects of recombinant human growth hormone (rhGH) in boys with puberty delay, and compare its efficacy to that of the orally active synthetic androgen testosterone undecanoate, either singly or in combination with rhGH. In contrast to the children in the previous studies (sections 1 and 2) the boys included in this study are older and their main problem is growth and puberty delay, rather than familial short stature.

Although the boys will be followed to adult height, I am presenting the short-term results ie. the first two years.

3.2. Definition of Constitutional Delay of Growth and Puberty

The first sign of male puberty is testiscular enlargement, most accurately determined with ultrasound, but more practically in comparison with standard ovoids - the "Prader" orchidometer (Zachmann et al 1974). Testicular volumes of 4 ml or more indicate that puberty is underway. According to Marshall and Tanner (1970) the average age at which Genitalia stage 2 is reached is 11.6 years, with 95% of boys reaching this between the ages of 9.5 and 13.5 years. The growth spurt starts at testicular volume 8 ml, with peak height velocity occuring at 12-15 ml, often two to three years after puberty has started. In a further study of normal adolescent growth and development patterns, Buckler (1990) documented the 50th centile for the attainment of genitalia 2+ to be 12.5 years. The first signs of puberty averaged 1.7 years before peak height velocity was reached which occured at genitalia stage 3-4.

In the U.K. the mean age of pubertal onset is such that only 3% of boys will have no signs of puberty by 13.8 years. Boys with no signs of puberty by 13.5 years may simply have delayed maturation, but could permanently lack the ability to develop in puberty, so it is reasonable to investigate those who present after this age. Distinguishing physiological delay from pathology may not always be possible clinically. Assessment must include physical examination and the appropriateness of the growth velocity determined in the context of the pubertal staging. Chronic systemic disease, hypothalamo-pituitary and other endocrine disorders must be excluded by careful history, examination and where appropriate investigation. Constitutional delay of growth and puberty (CDGP) is the most likely diagnosis in a healthy adolescent in whom stature is currently short for

the family but appropriate for the stage of puberty and skeletal maturation with normal height prognosis for the family.

Growth and puberty delay is a common cause of referral to growth clinics. Not only are the physical and sexual maturation of these boys delayed compared to their peers, but their growth spurt is consequently delayed and hence they are both short and physically underdeveloped compared to their contemporaries. Their more average peers are likely to have reached their growth spurt and hence the boys with puberty delay are falling further behind their contemporaries in terms of growth and maturation. Even if the boy is in early puberty when he presents it is likely that it will take another 18 months before peak height velocity is reached. Such boys with CDGP continue to grow for much longer than their peers, often not reaching their final adult height till after the age of 20 years. Their final height is usually acceptable but the delay in reaching it and the delay in attaining full physical maturation can cause significant distress to the boy.

Although many boys will be helped by an explanation of their problem and reassurance that full physical maturation will be reached in due course, many are so distressed by their problem that they seek intervention. It must always be remembered that CDGP is a normal variant and so any intervention must be safe and the benefits must outweigh any possible deleterious effects.

3.3. Effects of puberty delay

The effects of growth and puberty delay are both psychological and physical. There are reported effects on behaviour, self esteem, school attainment and success in adult life. For a full discussion of the psychological effects, please see section 4.2.3. When young adult men are asked about their experience of puberty delay many feel that their growth delay had affected their success at school, work or socially and many of these would have liked treatment to advance their growth spurt (Crowne et al 1990).

Although the psychological studies give a good picture of the feelings of boys who experience CDGP as a whole, there is likely to be a very wide spectrum. On an individual basis many boys cope without too many difficulties and may not come to medical attention. Those who come seeking help are likely to be at the extreme end of the spectrum in terms of height, lack of maturation and/or psychological distress.

Constitutional delay of growth and puberty may also lead to longer term physical effects. Studies of final height in boys with CDGP have suggested that those boys who experience late puberty reach an adult height in the lower range of normal (Bramswig et al 1990) but may not fully realise their expected potential for final height, in terms of what would be expected for their families (their target genetic height). Volta et al (1988) demonstrated that 40% of their patients with CDGP reached a final height which was in the 2 SD range below the genetic target height.
Crowne et al (1990) and La Franchi et al (1991) both showed a significant difference between final height and corrected mid-parental height with a mean difference of -6.5 cm and -5.1 cm respectively. Both these studies involved young men who had been referred to growth clinics because of short stature and may represent the most severe end of the spectrum of boys with puberty delay. Nevertheless these are exactly the sort of boys who present at growth clinics seeking help. The effects of intervention in boys with CDGP needs to be analysed in the context of these studies on untreated boys. Albanese and Stanhope (1993) also showed that boys with CDGP do not appear to attain ther genetic growth potential. The boys in their study had relatively poor spinal growth which did not improve at attainment of adult height.

Finkelstein et al (1992) demonstrated that radial and spinal bone mineral density was significantly lower in men aged 26 years with a history of delayed puberty compared to those who experienced puberty at an average time, suggesting that men in whom puberty was delayed may be at

increased risk for osteoporotic fratures when they are older. It may be that there is an optimal time for vertebral maturation in terms of both growth and mineralisation which is missed in boys with puberty delay.

3.4. Treatment of Puberty Delay : background literature review, with particular reference to testosterone undecanoate.

It has been recognised for many years that boys with puberty delay may suffer considerable psychological distress and merit treatment. Treatment could, in theory, be directed at the short stature or lack of physical maturity though as the two are so closely inter-related treatment directed to one aspect will affect the other.

One of the early forms of treatment for puberty delay in boys was with methyltestosterone. A large series published by Bayley et al (1957) reported 59 boys treated with methyltestosterone and a further 13 treated with methyltestosterone and "thyroid extract". The mean age of the boys was 13.7 years (range 10 to 18 years), and they were treated with a relatively low dose of 10 mg daily. Institution of methyltestosterone therapy was followed by a prompt growth spurt in the majority of the boys. There was no disruption of normal pubertal maturation. These boys did not appear to have marked skeletal maturation which is in contrast to the work reported by Sobel et al (1956) in a rather younger population and using larger doses of the drug. In the series of Bayley et al (1957) the most significant side effect was one of gynaecomastia requiring mastectomy in six boys. Kaplan et al (1973) treated 19 boys with a variety of androgens (methyltestosterone, fluoxymesterone, testosterone enanthate) and in a retrospective study compared them with 21 non-treated controls. Followed to reported final height, growth did not seem to have been compromised in the treated boys.

Concerns rose over the hepatic side effects of methyltestosterone, which include dose related cholestatic jaundice and hepatic neoplasia (Westaby et al 1977) and the drug is no longer available in the United Kingdom. Injectable forms of testosterone esters have since been widely used in the management of puberty delay, with testosterone enanthate being the most common form being reported.

Rosenfeld et al (1982) described a prospective randomised study in 16 boys aged 14-17 years. The boys received either testosterone enanthate (200mg IM, four times at 3 weekly intervals) or were observed with no treatment. This study is important as psychological studies (self image and social activity) were included, and psychological support and counselling were offered to both groups. The treated group showed an improvement in height velocity to 9.21 cm/year compared to a growth rate of 6.05 cm/year in the observation group. Both groups were said to have a disturbed self image prior to randomisation, and both showed improvement in self image over the course of twelve months with no significant differences between those who

were treated and those who were observed. A larger group of boys were similarly treated and reported by Wilson et al (1988) - some of the boys he reported were in Rosenfeld's group. 50 boys, mean age 15.0 years, were treated with testosterone enanthate. This study was not randomised and the decision to treat the individuals was based on "psychosocial difficulties". The psychological data is unfortunately poor, although in 18 of 19 treated boys treatment was said to be "helpful". Martin et al (1986) reported 58 boys treated with three varying doses of testosterone enanthate, with 14 controls. All treated boys showed an improvement in height velocity, but there was a trend that boys treated with the largest dose (200mg monthly for 9-12 months) had compromise of final height. Richman et al (1988) in a non-randomised uncontrolled study again described a beneficial effect of treatment with testosterone enanthate in a group of 15 adolescents mean age 14.1 years, who showed an improvement in height velocity within six months of starting treatment.

The common injectable form of testosterone in use in the United Kingdom is the intramuscuar depot preparation "Sustanon", a mixture of teststerone propionate, testosterone phenylpropionate, testosterone isocaproate, and testosterone decanoate. There are few detailed studies on its use in boys with puberty delay. de Lange et al (1979) described the treatment of 8 boys with monthly Sustanon for 6 months and demonstrated a good growth response without adverse effects on sexual maturation. A more recent study by Uruena et al (1992) of 44 boys treated with Sustanon 50 for a mean of 0.35 years showed a significant improvement in height velocity from 4.5 to 8.8 cm/year. Puberty appeared to progress more rapidly in the treated boys.

Intramuscular injections of testosterone esters have been the mainstay of treatment to promote puberty for many years. However there are problems with their usage. Even with low dosage, supraphysiological levels of testosterone are unavoidable in the first 48-72 hours after injection, followed by gradual waning of effect over the next 3-4 weeks. The pattern of testosterone levels is therefore very unphysiological compared to that found in early puberty in boys - normally there is a rise in testosterone in the morning (due to increasing nocturnal LH pulsatility), followed by a fall during the day. It is only when puberty is relatively far advanced that more or less constant testosterone levels are found. Administration of a depot testosterone preparation which is released over weeks in no way mimics the physiology of early to mid-puberty. More practically, depot testosterone

preparations need to be given by repeated IM injection, which are painful and disliked by many adolescents.

An alternative to injectable testosterone esters is the use of an oral preparation of testosterone. Pure unesterified testosterone is ineffective if administered orally, probably because it is rapidly metabolised in the intestinal wall and the liver. There have been two approaches to overcome this : i) administration by injection or implantation, or ii) modification of the testosterone molecule with the introduction of a methyl group at C17. The oral preparation of testosterone most often used in the past was methyltestosterone. However serious hepatic side-effects were reported and its use discontinued (Westaby et al 1977).

Testosterone undecanoate (17B-hydroxy-4-androsten-3-one 17B-undecanoate) is an oral testosterone preparation, in which the testosterone has been esterified with undecanoic acid. This enhances the lipophilic character of the steroid, and thus in the intestinal wall testosterone undecanoate can be incorporated into chylomicrons. It is then protected from metabolism by the intestinal wall enzymes and is transported via the lymphatics to the peripheral circulation before passing to the liver. It is most effective when administered as a solution in arachis oil (Nieschlag et al 1975) which itself stimulates the production of chylomicrons. In animal studies testosterone undecanoate had potent androgenic activity with no hepatic side effects. Hirschauser et al (1974) evaluated the effects on normal male volunteers and hypogonadal men. They suggested that oral testosterone undecanoate may be a more convenient form of testosterone replacement therapy.

Concerns were raised about high levels of plasma androgens after administration of the drug in a study in hypogonadal young men after a single dose of 40 mg testosterone undecanoate (Geere et al 1980). Total plasma androgens, but dihydrotestosterone levels in particular, were higher than one would expect in normal young men, attributed to the action of intestinal 5a-reductase. This work suggested that 40 mg daily may be too high an initial replacement dose. More positively the effect was short lived (peak usually by 2 hours, and return to baseline by 24 hours) which mimics more the episodic nature of testosterone secretion in adolescence.

More detailed pharmacokinetic studies (Butler et al 1992) in pre-pubertal boys have shown plasma total testosterone levels peak between 4-6 hours after administration of an oral dose of 40 mg, but there is wide variation even if the amount of dietary fat taken with the TU capsules is standardised. The peak levels initially appeared high (25-40 time basal

value), but total testosterone levels were significantly lower after three and six months treatment, with a parallel fall in sex hormone binding globulin. Levels of free testosterone remained constant during the treatment period, and were in an acceptable range (comparable to those found at stage 3-4 spontaneous puberty). High levels of dihydrotestosterone relative to testosterone were again found in this study. Thomas et al (1982) showed very similar results in a group of 8 boys with puberty delay. Longer term pharmacological studies have shown no significant hepatotoxic effects with testosterone undecanoate (Gooren 1986).

Butler et al (1992) showed that pre-pubertal boys treated with testosterone undecanoate showed an improvement in growth velocity to a level ususally seen at stage 3-4 puberty, which was also appropriate for the levels of circulating testosterone. Androgenisation progressed slightly more rapidly and this may be due to the high dihydrotestosterone : testosterone ratio as the external genitalia are more sensitive to dihydrotestosterone than testosterone during sexual differentiation. Spontaneous puberty progressed uneventfully in these boys, and treatment was stopped when testicular volumes of 6-8 ml were reached. It was concluded that oral testosterone undecanoate may be a suitable method of puberty induction in boys with puberty delay, and the dose of 40 mg daily was satisfactory. In a double blind study in boys with puberty delay, Gregory et al (1992) compared the growth promoting effects of a three month course of 40 mg testosterone undecanoate daily with placebo. The short course of oral testosterone undecanoate promoted growth during the 3 months of treatment and for the 3 months following treatment (with an increase in height velocity from 4.1 to 5.4 to 8.1 cm/yr). Fat free body mass also increased though there was no increase in muscle strength.

Boys who present with puberty delay have two main worries - their short stature and their lack of pubertal development. Treatment must ideally be directed to manage both of these problems ie. the boys want growth and development! However the two problems are intimately linked.

Evidence from the studies discussed above suggests that treatment with short courses of testosterone does speed up growth. The growth promoting effects are at least in part due to enhancement of endogenous GH release. During normal puberty there is a significant increase in spontaneous growth hormone secretion (Miller et al 1982, Mauras et al 1987, Rose et al 1991). This is predominantly due to augmentation of the size of GH pulses (Martha et al 1989). IGF1 levels also correlate with pubertal stage (Rosenfield et al 1983, Cara et al 1987). As a result of the

increased circulating GH concentrations fasting serum insulin concentrations rise (Hindmarsh et al 1988).

In boys with pubertal delay there are subtle "physiological " abnormalities of GH secretion. Eastman et al (1971) showed that insulin stimulated GH secretion was poor in boys with CDGP, and that after the onset of puberty there was a significantly increased GH response. Chalew et al (1988) showed that the 24 hour integrated concentration of growth hormone was lower in boys with pubertal delay compared to normal boys. Bala et al (1981) showed that IGF1 levels were lower in boys near pubertal age with CDGP than in age-matched controls. The poor GH secretory ability of boys with CDGP appears to be only occurring peri-pubertally, as GH secretion and IGF1 levels in pre-pubertal children with growth delay has been shown to be no different to controls (Lanes et al 1986).

Various groups (Link et al 1986, Chalew et al 1988) have shown that testosterone treatment in boys with CDGP increases both spontaneous GH secretion and IGF1 levels (Rosenfield and Furlanetto 1985). This appears to require chronic exposure to testosterone and does not occur after a single infusion of the agent (Foster et al 1989). The effects on GH and IGF1 are not seen if dihydrotestosterone is given rather than testosterone, suggesting that testosterone in vitro is acting via conversion to oestradiol rather than conversion to 5a-dihydrotestosterone. The growth response to testosterone is better than to dihydrotestosterone (Keenan et al 1993).

As it has been shown that spontaneous secretion of growth hormone is diminished in children with CDGP (see above), it has been suggested that treatment with rhGH might be a reasonable therapeutic manoeuvre. With the advent of the increased availability of rhGH it is important to determine whether it does have a role in the management of boys with pubertal delay. Fewer studies have explored this in detail to date. Beirich et al (1992) followed a group of 15 children (13 boys and 2 girls) with CDGP who were treated with GH (12-16iu/sq.m/wk) for an average of 3 years. The majority were pre-pubertal at the start of treatment. Short term results showed an initial increase in growth velocity, but long term follow up showed no improvement in final height. A comparison of the anabolic agent oxandrolone (rather than testosterone) with rhGH in the treatment of boys with constitutional delay of growth and puberty was made by Buyukgebiz et al (1990) in a group of 26 boys, mean age13.8 years. They compared 3 months treatment with oxandrolone (2.5 mg daily) with 12 months treatment with rhgH (20units/sq.m/week). Both groups showed a significant

improvement in height velocity, though it was better in the oxandrolone treated boys.

The boys with CDGP not only have concerns about their growth but also their degree of physical development. Any study of treatments must not only look at growth response but the effect on virilsation. Exogenous testosterone treatment in boys with CDGP produces increased virilisation. There is a suggestion that treatment with growth hormone increases the tempo of pubertal maturation (Darendeliler et al 1990), and hence rhGH treatment alone may enhance development in such boys. In hypopituitary boys testosterone only exerts its full growth-promoting action in the presence of normal endogenous growth hormone secretion or with sufficient hGH replacement (Aynsley-Green et al 1976). Because of the inter-related effects of GH and the gonadotrophin-sex steroid axis, treatment with a combination of growth hormone and testosterone may improve both growth and maturation better than either agent alone. Studies are required to determine whether this is the case, and not only compare growth rates but pubertal maturation.

The study we are presenting aims to answer some of these questions. In addition we wished to explore further the role of oral testosterone undecanoate in the management of boys with CDGP.

3.5. Study: Growth promotion in boys with puberty delay : growth hormone or testosterone undecanoate, singly or in combination.

The study reported was designed to determine whether there is an optimum method of promoting growth together with physical maturation in adolescents with puberty delay. It examines not only the physical effects of growth promotion but also the psychological effects.

3.5.1.The aims of the study were to determine in boys with puberty delay:

1) whether treatment with rhGH, testosterone undecanoate or a combination of rhGH plus testosterone undecanoate accelerates height velocity.
2) whether treatment with rhGH, testosterone undecanoate or rhGH plus testosterone undecanoate has effects on the rate of pubertal progression.
3) whether treatment with rhGH,testosterone undecanoate or rhGH plus testosterone undecanoate will improve final height.
4) the benefits (physical and psychological) of treatment.
5) the complications (if any) of treatment in boys.
6) whether there are any parameters that are predictive of the response to treatment.

3.5.2.Patient Recruitment

Boys were recruited from the growth and endocrine clinic at the Royal Hospital for Sick Children, Edinburgh, and attached peripheral clinics in Scotland. Children are referred to the growth clinic either by their general practitioners or the school medical service, or are tertiary referrals from other paediatricians.

The following inclusion criteria had to be met :

Age 14 years or more
Height at, or below, 3rd centile for chronological age (HSDS <-1.88)
Delayed puberty, defined as testicular volume 6 ml or less
No other cause found for the short stature and puberty delay

Ethical approval for the study was obtained from Lothian Health Board Committee on Medical Ethics. Parents and children were given detailed information about the study, and signed appropriate consent forms prior to entry.

3.5.3.Study protocol

Pre-treatment assessments:

1. Auxology

Prior to entry into the study the boys were assessed at the growth clinic for a minimum of 6 months, though the majority had measurements performed over at least one year.

Standing height was measured by a single observer (HS) using a fixed wall mounted Harpenden stadiometer. Sitting height was measured using a sitting stadiometer by a single observer (HS). Weight was measured using a standard balance. Measurements were compared to the standards of Tanner et al (1966, 1976). Triceps and subscapular skinfold thicknesses were measured using Holtain calipers by a single observer (HS) and compared to the standards of Tanner and Whitehouse (1975). Pubertal assessment was performed by a single observer (HS) using the standard ratings of Tanner (Tanner 1962, Marshall and Tanner 1969, Marshall and Tanner 1970). Testicular volume was estimated by comparison with the standard ovoids of a Prader orchidometer (Zachmann et al 1974).

Bone age was assessed by X-ray of left hand and wrist, and the Tanner Whitehouse II 20 bone method (Tanner et al 1983) of analysis performed by a single observer (HS).

Blood pressure was measured in the right upper limb by a single observer (HS) using an appropriate sized sphygmomanometer cuff for each boy (de Swiet et al 1992).

2. Endocrine assessment

a) Overnight profiles

Prior to entry into the study all the boys underwent overnight blood sampling form 20.00 hrs to 08.00 hrs, with samples collected at 20 minute intervals to measure plasma growth hormone. The boys were admitted to hospital in the early evening following a normal days activities and eating pattern. Topical anaesthetic cream (EMLA) was applied to an ante-cubital fossa and subsequently an indwelling intravenous cannula was inserted at least 45 minutes before blood sampling was commenced. The boys were allowed to be freely active, and eat and drink normally during the evening. They were strongly encouraged to be in bed by 10.00 pm with "lights out" at 10.30pm. In practice the majority slept soundly until 7.30-8.00 am the following morning. 2 ml samples of blood were taken every 20 minutes, collected into lithium heparin tubes, immediately centrifuged at -4C, then

separated and the plasma stored at -20C until assayed. All samples from a given boy were assayed in the same batch.

The samples were assayed in the Regional Hormone Laboratory, Edinburgh using an immunoradiometric assay (IRMA). The growth hormone profiles obtained were evaluated using the Munro modification of PULSAR program on an Apple Macintosh personal computer..

For an additional study, timed 12 hour urine collections (20.00 hrs to 08.00 hrs) were also obtained from the boys. Urinary growth hormone was measured in these samples at the Regional Hormone Laboratory, Edinburgh using an amplified enzyme immunoassay (Novo Nordisk).

Insulin like growth factor 1 (IGF1) levels were measured at 08.00 hrs in all boys. IGF1 was assayed by Novo Nordisk using a radio-immunassay.

Testosterone levels were measured in all boys at 08.00 hrs (Wu et al 1993).

b) Dynamic pituitary function tests

The following morning the boys underwent combined pituitary function tests, using either insulin-induced hypoglycaemia (0.15iu/kg) or clonidine (0.15mg per sq.m body surface area) together with TRH (7 micrograms/kg to a maximum of 200 micrograms) and LHRH (0.25 micrograms/kg). 29 of the boys underwent an insulin tolerance test, with the remainder having clonidine for the growth hormone provocation test. The tests were performed in a recognised growth centre, on a ward where the staff were well acquainted with the potential hazards of such tests and their management, and full facilities for resuscitation were available (Shah et al 1992). The tests were supervised by the same person (HS) on all occasions. There were no significant adverse events as a consequence of these tests.

The pituitary function tests were performed without the boy being "primed" with testosterone, as we were trying to fully evaluate the physiological status of the boy prior to treatment. The LHRH test used is a very low dose one and is likely to be more physiological than the supra-maximal stimulus of the conventional doses of 100 microgms or 2.5 microgms/kg (Hughes 1989). This study in combination with the studies in younger children allowed us to evaluate the low dose LHRH test as to whether it is a more useful way of assessing the imminence of puberty than the conventional dose.

3. Psychological assessment

The psychological studies were performed using a series of questionnaires involving parent, child and teacher reports.

For details please see Section 4.

Randomisation of treatment

The boys were randomised into one of three groups :

1) rhGH 24iu/sq./wk, given as a daily sub-cutaneous injection, increasing to 30iu/sq.m/wk after twelve months

or 2) testosterone undecanoate 40mg orally on alternate days, increasing to 40mg daily after twelve months

or 3) rhGH plus testosterone undecanoate in the above doses

The doses of rhGH and testosterone undecanoate increased after twelve months treatment to mimic the rise one would expect to see during spontaneous puberty.

Assessment of response

After entry into the study the boys were reviewed at three monthly intervals. Detailed auxological measurements (standing height, sitting height, weight, triceps and subscapular skinfold thicknesses, pubertal staging and blood pressure) were made every three months by a single observer (HS). Left hand and wrist X-ray was performed every six months, and bone age assessed by a single observer (HS) using the Tanner Whitehouse TW II method of analysis.

Haematological (full blood count wih differential white cell count, and in a subgroup T and B cell counts) and biochemical (liver function, renal function, glucose, HbA1, cholesterol, triglycerides, thyroid function) parameters were measured at entry and at three months, six months and at six monthly intervals thereafter. IGF1 was measured at entry and six monthly. Bone derived alkaline phosphatase was measured at entry, three months, six months and at six monthly intervals.

LH, FSH and testosterone levels were measured six monthly. Although these were random samples, all were taken between 09.00 and 12.00 in the morning.

3.5.4. Pre-treatment Growth status

Boys entered into the study

33 boys entered the study, all with the full written informed consent of their parents and themselves.

The mean age of the boys was 14.90 years (range 13.83 to 16.50)
The mean HSDS was -3.21 (range -4.93 to -1.77)
The mean height velocity was 4.07 cm/year (range 2.05 to 5.53)
The mean bone age was 12.57 years (range 9.5 to 14.7)
The mean bone age delay was 2.33 years (range 0.57 to 4.47)
All had testicular volume of 6 ml or less (range 02 to 06, median 04)

The mean height of the fathers was 175.3 cms (SD 7.09) = 50th centile
The mean height of the mothers was 158.2 cms (SD 6.64) = 25th centile

The mean target height of the boys was 173.3 cms (SD 5.45) = 25-50th centile
The mean predicted height at entry was 168.1 cms (SD 4.48) = 10-25th centile

The mean predicted height (calculated using the formula of Tanner 1983 based on height, bone age, growth rate and rate of bone maturation) at entry was significantly less than the target height (calculated as mid parental centile height) by 5.18 cms (SD 5.18, $p = <0.001$)

The mean birth weight of these boys was 3.00 kg (SD 0.54, range 1.87 - 3.88 kg). 5 children were born pre-term (at 35 or 36 weeks gestation) but none had major neonatal problems. Using a definition of small for gestational age (SGA) as < 10 th centile weight for gestational age, eight of the boys were SGA. Unfortunately accurate birth lengths were not available. The proportion of SGA infants in the cohort is less than in the previous two studies, probably a reflection of their mothers being taller.

3.5.5. Pre-treatment endocrine status

a) Growth hormone

Spontaneous growth hormone (GH) secretion is in a pulsatile fashion, and thus the GH profiles we obtained are a measure of the child's physiological GH status. The overnight growth hormone profiles we obtained from each child were analysed using the Munro modification of the PULSAR program. This program detects pulses of GH, and the overnight GH secretion can be described in terms of pulse amplitude (PA), sum of pulse amplitude, pulse interval, area under the curve and mean GH level. It has been found that there is an asymptotic relationship between HVSDS and pulse amplitude (Hindmarsh et al 1987), and that pulse amplitude is the best feature of the GH profile to relate to growth.

To summarise the results from the overnight GH profiles I have expressed the data as the means (SD) of mean PA, sum PA, and mean GH level, all expressed in mU/l, for the boys in the groups of the study. Also included in the table are the mean responses to the provocation test and mean IGF1 levels.

	All boys	rhGH	TU	rhGH+TU
Overnight GH				
Mean PA	17.9 (12.0)	16.3 (9.7)	19.1 (7.0)	18.3 (18.6)
Sum PA	74.2 (33.8)	76.0 (40.9)	79.1 (26.6)	66.3 (35.3)
Mean GH level	8.1 (3.9)	7.2 (3.2)	9.4 (3.7)	7.4 (4.9)
Peak stim. GH (mU/l)	23.8 (14.8)	23.7 (12.1)	19.8 (12.0)	28.9 (19.8)
IGF1 (U/ml)	0.83 (0.37)	0.83 (0.45)	0.93 (0.35)	0.72 (0.31)

There were no significant differences in the mean GH parameters measured overnight in the three groups, namely mean pulse amplitude (PA), sum of pulse amplitude or mean overnight GH level. There were no significant differences in the mean peak GH response to the GH provocation tests between the three groups. The wide range of peak stimulated GH responses (4.2 to 73.9 mU/l) reflects the fact that the boys were not "primed" with testosterone prior to the stimulation test and that 17 of the boys had peak GH responses less than the conventional " normal" lower limit of 20 mU/l.

The IGF1 results are expressed as Units/ml (1 Unit/ml = 37.4nmol/l) with the reference range quoted by the laboratory being 0.3 - 3.34 Units/ml

for children aged 11-16 yrs. The mean levels found in our boys are at the lower end of the expected range. There was no significant differences between the three treatment groups.

b) LHRH and testosterone levels

Measurement of overnight pulsatile LH secretion using highly sensitive assays has been shown to be the best way of detecting the hormonal onset of puberty (Wu et al 1990, 1991). However this was not practical to do in all our boys and so we used the LH response to a low dose of LHRH (0.25 microgms/kg) as a sensitive way to assess the pubertal activity of the hypothalamo-pituitary axis. Pre-pubertal children normally have baseline LH levels of <1.0 units/l, and will only show a small increment in the concentration of LH in response to this dose of LHRH. We have found that the pre-pubertal response of LH to LHRH is to a level of 3.8 units/l or less, whereas once puberty is underway levels of >4.5 units/l or more are acheived (see results for younger children in sections 1 and 2).

	All boys	rhGH	TU	rhGH+TU
Peak LH (units/l)	12.0 (6.48)	9.2 (4.25)	16.9 (7.20)*	8.8 (3.56)
08.00 Testost. (nmol/l)	3.5 (2.80)	3.0 (2.64)	4.3 (2.80)	2.9 (3.00)
			* p = 0.007	

All but two boys had an active response to low dose LHRH, defined as a peak LH response of 4.5 units/l or more. In our experience this suggests that the hypothalamo-pituitary axis is becoming pubertally active. The two boys who did not reach this level of response did show an increase in LH level over the basal value thus it is very unlikely that they have hypogonadotrophic hypogonadism.

The boys in the testosterone undecanoate treated group had a significantly greater mean LH response to LHRH than those in the other groups although clinically there was no significant difference in pubertal staging (see table above).

The mean LH response to low dose LHRH increases as puberty approaches - the mean peak LH response for these boys (12.0 units/l) in whom puberty was either imminent or in the early stages was higher than in the boys in section 2 who were less close to pubertal onset (5.18 units/l), and in turn their response was higher than the entirely prepubertal children in section 1 (3.30 units/l).

Early morning testosterone levels have been shown to be a useful marker of the imminence of puberty. Boys in whom early morning testosterone is <0.7 nmol/l are unlikely to enter puberty within the next 12 months (Wu et al 1993). All the boys had a detectable 08.00 testosterone (0.7 nmol/l or more). The mean 08.00 testosterone level was higher in the TU treated group although it did not reach statistical significance. There was no significant difference in median testicular volumes at entry into the study in the TU group compared to the other two groups (see table). This again reinforces the difficulty there is in estimating the hormonal activity of early pubertal boys who have the same clinical staging. The low dose LHRH test and/or an early morning testosterone level does identify which boys are more biochemically advanced in puberty.

3.5.6. Growth Results

Of the 33 boys who entered the study, three withdrew during the first year - two disliked the growth hormone injections, and one boy taking oral testosterone undecanoate refused further hospital follow-up.
The data on the 30 boys who completed at least one year is presented.

See table: summary of growth data

1 year growth results

30 boys entered the study and completed at least one year of follow-up

10 received rhGH

11 received testosterone undecanoate (TU)

9 received rhGH plus testosterone undecanoate.

There were no significant differences between the three groups at entry into the study in terms of: chronological age, height, height SDS, height velocity, pubertal staging, bone age, bone age delay, and predicted adult height.

rhGH group

10 boys completed at least one year of rhGH injections (24iu/sq.m/wk for the first year of treatment, increasing to 30iu/sq.m/wk for the second year). Over the first year of treatment they grew at a mean rate of 8.59 cm/yr compared to a mean rate of 4.36 cm/yr over the pre-study year. This improvement is highly significant ($p = <0.001$). Mean HSDS improved from -3.01 to -2.49 over the year which is significant at a level of $p = 0.01$. Mean bone age advance/chronological age advance was 1.22 years. Mean HSDS for BA improved from -0.57 to -0.44 though this is not significant. Mean predicted adult height did not change significantly (169.0 cms at one year compared to 169.8 cms at entry).

Testosterone Undecanoate group

The 11 boys who received and completed a year of testosterone undecanoate treatment also had a significant improvement in height velocity over the first year of treatment, from 4.13 cm /year to 8.48 cm/year ($p = <0.001$). This is not significantly different to the improvement in growth rate seen in the boys treated with growth hormone alone. Mean bone age advance /chronological age advance was 1.54 years, which was not significantly different to those treated with rhGH alone. Mean HSDS improved from -3.20 to -2.40 ($p = <0.001$), but mean HSDS for BA did not

	rhGH	TU	rhGH + TU
At entry:			
No. boys	10	11	9
Age (yrs)	14.72 (0.66)	15.13 (0.30)	14.79 (0.88)
HSDS	-3.01 (0.61)	-3.20 (0.81)	-3.46 (0.57)
HV (cm/yr)	4.36 (0.87)	4.13 (0.72)	3.81 (0.62)
Bone age (yrs)	12.39 (1.69)	12.68 (0.68)	12.51 (0.65)
BA delay (yrs)	2.31 (1.30)	2.45 (0.67)	2.28 (0.76)
HSDS for BA	-0.57 (-0.65)	- 0.45 (0.98)	-0.99 (0.47)
PAH (cms)	169.8 (3.43)	169.0 (5.19)	166.0 (3.82)
At one year:			
No. boys	10	11	9
HSDS	-2.49 (1.01)	-2.40 (0.87)	-2.74 (0.54)
HV (cm/yr)	8.59 (1.94)	8.48 (0.79)	9.91 (1.91)
dBA/dCA	1.22 (0.48)	1.54 (0.76)	1.28 (0.68)
HSDS for BA	-0.44 (0.67)	-0.69 (0.78)	-0.76 (0.68)
PAH (cms)	169.0 (3.13)	167.9 (4.30)	166.3 (2.60)
At two years:			
No. boys	9	9	7
HSDS	-1.88 (1.16)	-1.34 (0.97)	-1.56 (0.48)
HV (cm/yr)	8.13 (2.11)	7.26 (1.92)	9.36 (2.48)
dBA/dCA	1.42 (0.23)	1.08 (0.44)	1.17 (0.71)
HSDS for BA	-0.50 (0.61)	-0.71 (0.88)	-0.72 (1.06)
PAH (cms)	170.7 (5.18)	172.1 (7.01)	170.0 (3.51)

Study 3 : Summary of Growth Data

change significantly (-0.69 compared to -0.45 at entry) nor did mean predicted adult height (167.9 cms compared to 169.0 at entry).

rhGH plus Testosterone Undecanoate group

The 9 boys who completed a year of combination of rhGH (24iu/sq.m/week) and testosterone undecanoate (40mg on alternate days) grew fastest over the first year of treatment, with improvement in height velocity from 3.81 cm/year to 9.91 cm/year ($p = <0.001$). This was significantly better than the TU group ($p = 0.04$), but not significantly different to rhGH alone. Mean bone age advance/chronological age advance was 1.28 years, not significantly different to other two groups. Mean HSDS improved from -3.46 to -2.74 ($p = <0.001$), but HSDS for BA did not change significantly (-0.76 compared to - 0.99 at entry) nor did mean predicted adult height (166.3 cms compared to 166.0 at entry).

2 Year Growth Results

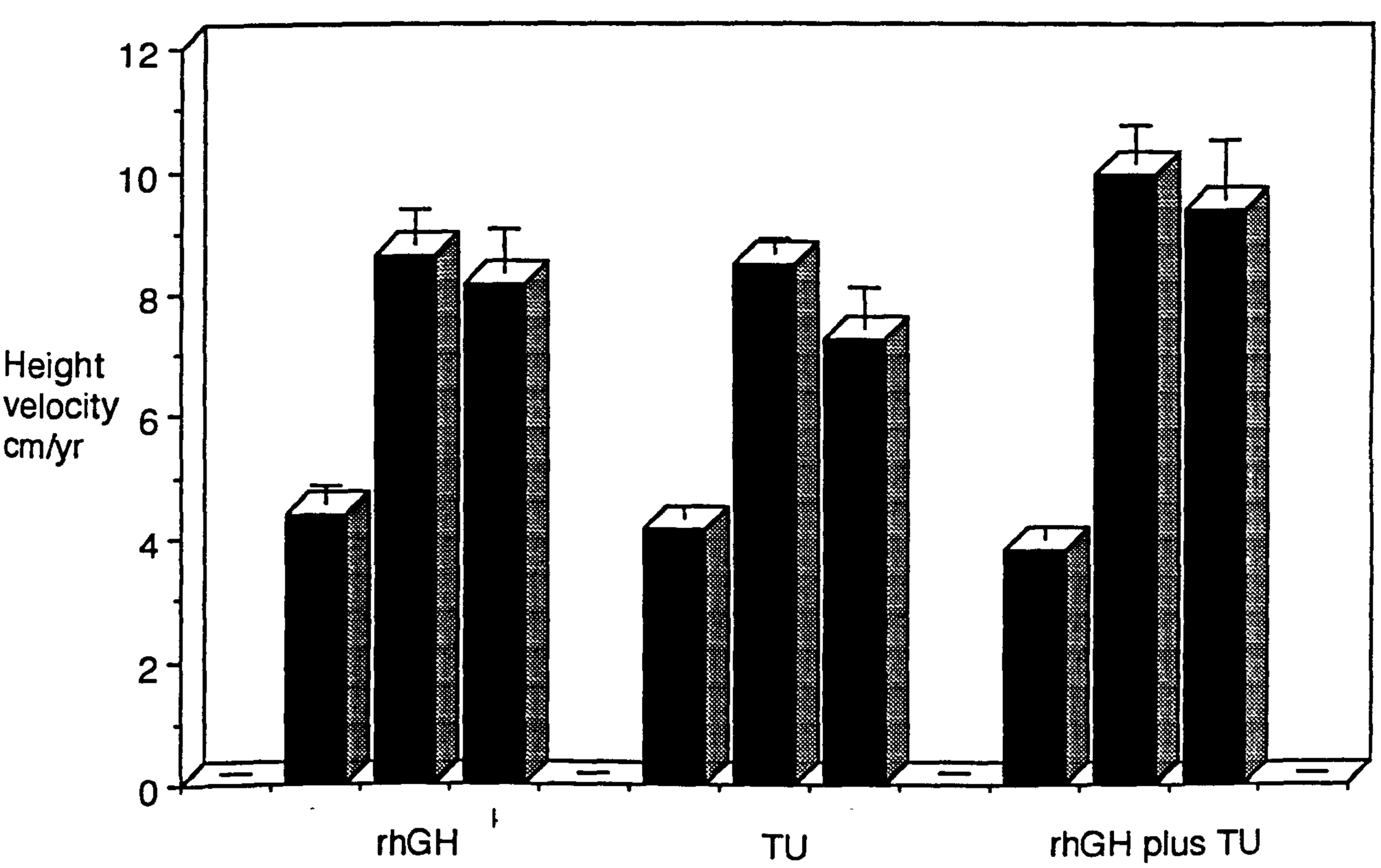
rhGH group

The boys who received 24iu/sq.m/wk of rhGH from the start of the study continued to grow well over the second year of treatment. The dose of rhGH was increased to 30iu/sq.m/wk over the second year to mimic the increase in endogenous GH secretion which is seen as puberty progresses. Mean height velocity during the second treatment year was 8.13 cm/year. This growth rate is slightly slower than that over the first year, but not significantly different. Mean HSDS continued to improve and was -1.88 at the end of the second study year - a significant improvement over mean HSDS at one year ($p = <0.001$). Mean HSDS for BA did not change significantly over the second treatment year (-0.50 compared to -0.44 at one year), nor did mean predicted adult height (170.7 cms at two years compared to 169.0 at one year). Mean BA advance/chronological age advance was 1.42 years over the second study year, giving a total mean bone age advance over the two years of 2.64 years.

Testosterone Undecanoate group

The boys who received testosterone alone from the onset of the study continued to grow well during the second study year. The dose of testosterone was increased to 40mg daily during the second treatment year. The growth rate during this year was 7.26 cm/year which was not significantly different to that acheived during the first year. Mean HSDS

Height velocity (SE) at entry, 12 months and 24 months



continued to improve and was -1.34 at the end of the second study year - a significant improvement over mean HSDS at one year (p = <0.001). Mean HSDS for BA did not change significantly over the second treatment year (-0.71 compared to -0.69 at one year), although mean predicted adult height improved (172.1 cms at two years compared to 167.9 at one year, p = 0.005). Mean bone age advance/chronological age advance was 1.08 years, giving a total mean bone age advance of 2.62 years over the two study years.

rhGH plus Testosterone Undecanoate group

The boys who received a combination of rhGH and testosterone from the start of the study continued both drugs during the second year at the increased doses (30iu/sq.m/wk of rhGH and 40mg daily for testosterone undecanoate).The mean height velocity of these boys during this year was 9.36 cm/year (not significantly different to that during the first year). Mean HSDS for BA did not change significantly over the second treatment year (-0.72 compared to -0.76 at one year), although mean predicted adult height improved (170.0 cms at two years compared to 166.3 at one year, p = 0.005). Mean bone age advance/chronological age advance over the second year was 1.17 years, giving a total mean bone age advance of 2.45 years over the two study years.

3.5.7.Effects on puberty

See table of pubertal status at 0, 12 and 24 months.

The three groups were similar at entry into the study in terms of their clinical pubertal staging, and 08.00 testosterone levels, although the boys in the TU group had a rather more active LH response to low dose LHRH (see comments section 3.5.5)

There were no significant differences between the three groups with regard to the rate of pubertal progression - median testicular volume and clinical pubertal staging at six months, one year and two years were very similar in all groups, as was the mean age at which 12 ml testes were reached.

It is important to note that increase in testicular volume proceeded normally in the two groups who received oral testosterone undecanoate - this low dose did not appear to inhibit testicular growth.This is an important

	rhGH	Testosterone undecanoate	Test. undecanoate + rhGH
At entry : No. boys Age (yrs) Median test. vol (mls) Median G stage Median PH stage Median AH stage	 10 14.72 05 (range 02-06) G2 (range G1-G2) PH1+ (range PH1-PH2) AH1 (all AH1)	 11 15.13 04 (range 03-06) G2 (all) PH1+ (range PH1 - PH2) AH1 (all AH1)	 9 14.79 04 (range 03-06) G2 (range G1-G2) PH1+ (range PH1-PH2) AH1 (range AH1 - AH2)
At six months : Median test. vol (mls)	 06 (range 02 -15)	 06 (range 04-10)	 06 (range 04-10)
At one year : No. boys Median test. vol (mls) Median G stage Median PH stage Median AH stage	 10 10 (range 03-15) G3 (range G2-G4) PH2 (range (PH1-PH3) AH1 (range AH1-AH2)	 11 10 (range 06-12) G3 (range G2-G4) PH2+ (range PH2-PH3) AH1 (range AH1 - AH2)	 9 10 (range 06-12) G3 (range G2-G4) PH2+ (rangePH2-PH3) AH1 (range AH1-AH2)
At two years : No. boys Median test. vol (mls) Median G stage Median PH stage Median AH stage	 9 15 (range 06-25) G4 (range G2-G5) PH3 (range PH2-PH4) AH2 (range AH1-AH3)	 9 15 (range 10-20) G4 (range G4 - G5) PH4 (range PH3-PH4) AH2 (range AH2-AH3)	 7 15 (range 12-25) G4 (range G4-G5) PH4 (range PH3-PH4) AH2 (range AH1-AH3)
Mean age at 12 ml testes (yrs)	16.10 (n=8)	16.59 (n=10)	16.05 (n=9)

Study 3: Summary of Puberty Data

consideration as there have been concerns about the effects of other anabolic agents on testicular growth in animal studies (Grokett et al 1992).

It has been suggested that when other anabolic agents, particularly oxandrolone, are used to treat boys with CDGP there is suppression of mean 24 hour serum LH and testosterone concentrations, with a rebound after treatment is stopped (Malhotra et al 1993, and see section 2.5.7). It is important to determine whether testosterone undecanoate produces a similar effect. Our boys were not studied in as much detail, but we do have serial random LH and testosterone levels, measured three monthly during treatment.

Mean (SD) LH levels (units/l) during treatment :

	rhGH	TU	rhGH+TU
At entry	1.86 (1.34)	2.10 (1.28)	1.24 (0.31)
6 months	1.80 (1.34)	1.97 (0.79)	1.69 (0.86)
12 months	2.25 (1.23)	2.96 (2.04)	2.21 (0.88)
18 months	2.97 (1.88)	3.30 (1.24)	2.70 (1.30)
24 months	3.80 (2.14)	3.90 (0.71)	3.80 (1.30)

Random LH levels are not a particularly good marker of hypothalamo-pituitary function as LH is secreted in a pulsatile manner and it is impossible to know where in a pulse a random level has been taken. However, as puberty progresses one would expect mean random levels in a group of boys to gradually increase as the chances of sampling from a pulse of LH increases. Mean levels across groups are a crude way of looking at the progresion of LH secretion. There were no significant differences between the three groups in mean random LH levels measured at entry or six monthly during the treatment period. In particular treatment with exogenous testosterone did not suppress LH secretion as there was a steady trend for LH to increase with time, implying that normal maturation of the hypothalamo-pituitary axis is proceeding.

Mean (SD) Testosterone levels (nmol/l) during treatment :

	rhGH	TU	rhGH+TU
At entry	2.43 (2.04)	2.06 (1.47)	2.20 (2.53)
6 months	4.54 (4.18)	8.86 (12.23)	3.07 (1.84)
12 months	8.79 (7.48)	9.81 (6.84)	11.60 (8.56)
18 months	12.68 (6.82)	14.93 (5.11)	17.55 (4.87)
24 months	12.38 (4.99)	18.04 (2.01)	15.08 (5.42)

Mean testosterone levels were generally higher in the boys receiving oral testosterone undecanoate either singly or in combination with rhGH than in the boys who received rhGH alone, which is not suprising as they were receiving exogenous testosterone in addition to their own endogenous production. It is perhaps more surprising that the measured levels were not higher. During the first year of the study the boys received the oral testosterone on alternate days, but during the second year it was given on a daily basis. Thus measured testosterone levels at six and twelve months may reflect testosterone taken on that day or at least 24 hours previously. There was a very wide variation in the testosterone levels measured. A maximum level of 44 nmol/l was measured in one boy. 12 boys had levels of 10 nmol/l (lower limit of adult reference range) or greater at 6 or 12 months, and at 18-24 months all but three boys had random levels above10 nmol/l. The levels that we found in the boys who received testosterone undecanoate (either singly or in combination) at six months amd twelve months were similar to those found by Butler et al (1992) in their group of peripubertal boys.

3.5.8. Effects on biochemical markers of growth

Two biochemical markers of growth have been measured in these boys. Insulin-like growth factor 1 (IGF1) is predominately produced by liver, and is known to rise in GH-deficient children treated with growth hormone, an also in normal boys as puberty progresses. Similarly bone-derived alkaline phosphatase (bALP), a more specific marker of bone turnover, increase during the pubertal growth spurt and also in response to growth promoting agents (see sections 1.5.8. and 2.5.8).

1. IGF1

IGF1 was measured at entry into the study and at six months. The results are expressed as units/ml (1 Unit/ml = 37.4 nmol/l). The reference range quoted by the laboratory is 0.28 -1.36 Units/ml, or more usefully divided into age ranges, with the reference range for 11-16 year old adolescents being 0.3 - 3.34 Units/ml. The mean levels at entry in our boys are at the lower end of the reference range.There were no significant differences between the three treatment groups at entry into the study.

	rhGH	TU	rhGH+TU
At entry	0.87 (0.46)	0.94 (0.36)	0.73 (0.33)
6 months	1.45 (0.70) *	1.24 (0.43) *	1.74 (0.61) *
12 months	1.61 (0.55)	1.57 (0.44)	1.70 (0.71)
18 months	2.00 (0.71)	1.80 (0.60)	2.05 (0.90)
24 months	2.00 (0.75)	2.12 (0.44)	2.65 (0.75)
	* p = 0.002	* p = 0.007	* p = <0.001

There were no significant differences in mean IGF1 levels at entry into the study between the three groups. All groups showed a significant rise in IGF1 at 6 months of treatment. This was most marked in the group receiving combination therapy, which was the group who grew the fastest. The steady increase in plasma IGF1 seen over the course of the two years is similar to the pattern seen in spontaneous puberty (Cara et al 1987).

2. Bone-derived alkaline phosphatase (bALP)

This was measured at 3 monthly intervals in all of the boys. bALP was measured by an in-house lectin affinity electrophoresis method at the Royal Hospital for Sick Children (Crofton 1992).

The results are expressed as:

Mean (SD) increase in bALP (units/l) at three months and six months above the baseline measurement :

	Increase in bALP at three months	Increase in bALP at six months
rhGH	+163.0 (20.1)	+172.5 (160.6)
TU	+38.0 (67.5)	+123.1 (113.9)
rhGH + TU	+262.7 (100.4)	+219.8 (144.3)

bALP increased in all groups by three months of treatment, but it was significantly better in the two groups treated with rhGH than in those boys who received TU alone ($p = 0.008$). By six months the increase in bALP in the TU treated group was of the same order as the rhGH group.The increase in bALP in the boys who received combination treatment appeared to be cumulative, compared to the single treatment groups, but this did not reach statistical significance.

3.5.9. Side effects of treatment

1. Effects on body fat

Growth hormone has well known lipolytic and anabolic effects. It has long been recognised that growth hormone deficient children have increased body fat compared to normal children, and that when they are treated with replacement growth hormone they become leaner (Tanner et al 1977). The greatest effect appeared to be within the first three months of treatment.

Skinfold thicknesses are a practical way to estimate body fat (Brook 1971, Durnin and Womersley 1974). In order to determine the effects of rhGH on the body fat of short normal children, we measured triceps and subscapular skinfold thicknesses at entry into the study and at three monthly intervals. The measurements were made by a single observer (HS) using Holtain calipers. The individual measurements were log transformed and then expressed as the mean sum (SD) of log transformed triceps and subscapular skinfold measurements.

	rhGH	TU	rhGH+TU
At entry	343 (49.2)	345 (43.5)	347 (36.7)
3 months	332 (46.7)	345 (41.2)	340 (37.4)
6 months	316 (52.9)*	343 (36.1)	335 (24.4)*
12 months	338 (71.8)	336 (35.8)	330 (20.1)*
18 months	349 (70.4)	331 (27.6)	330 (26.0)
24 months	364 (75.6)	334 (33.5)	336 (26.1)
	* p = 0.02		* p = 0.03

The boys who received rhGH alone during the first year of the study had a significant decline in mean sum of log skinfold thickness, and thus a decrease in body fat. This reached significance by six months and then showed recovery by twelve months, and was similar to the effect seen in the prepubertal children and peri-pubertal boys (Sections 1.5.9. and 2.5.9). The boys who received TU as a single agent did not show this same decrease in skinfold thicknesses. The boys who were treated with TU alone did not show any significant changes in skinfold thickness, though there is a steady downward trend over the two years probably reflecting the change in body fat distribution as puberty is progressing (Buckler 1989). The boys treated with a combination of rhGH and TU showed similar changes in skinfold thicknesses to the boys treated with rhGH alone.

rhGH has effects on the body fat of pubertal boys, making them transiently leaner.

2. Effects on cholesterol and triglyceride levels:

There have been concerns about the effects of anabolic agents on plasma lipid levels (see section 2.5.9). Changes in cholesterol distribution (a decrease in high density lipoprotein cholesterol and apoprotein levels and rise in low density lipoprotein cholesterol) have been reported in athletes who abuse anabolic steroids (Lamb 1984, Webb et al 1984). McCaughey et al (1993) have suggested that growth hormone treatment in normal short children alters plasma cholesterol and triglyceride levels.

In our study, plasma cholesterol and triglyceride levels were measured at entry into the study and at three months, six months and then at six monthly intervals. The baseline values were often fasting (taken at the time of the child's GH provocation test) and the subsequent levels were non-fasting, as it was not practical to bring the children to clinic fasted. This will not affect the cholesterol level but may affect the triglyceride levels, making them subject to wider variation.

Mean (SD) cholesterol mmol/l:

	rhGH	TU	rhGH+TU
At entry	4.33 (0.72)	4.55 (0.55)	4.81 (0.92)
3 months	4.35 (0.77)	4.54 (0.78)	4.79 (0.64)
6 months	4.49 (0.73)	4.13 (0.66)	4.70 (0.80)
12 months	4.10 (0.62)	4.08 (0.63)	4.34 (0.82)
18 months	4.15 (0.44)	4.16 (0.70)	4.08 (0.44)
24 months	3.95 (0.39)	4.22 (0.34)	4.16 (0.35)

Normal range = 2.5-6.3 mmol/l

There was a downward trend in mean plasma cholesterol over the two years in all groups, but did not reach significance. There was no evidence that rhGH increases mean plasma cholesterol in the boys we treated.

Mean (SD) triglyceride mmol/l:

	rhGH	TU	rhGH+TU
At entry	0.73 (0.29)	1.00 (0.40)	0.72 (0.15)
3 months	0.89 (0.30)	0.92 (0.30)	1.01 (0.44)
6 months	0.92 (0.48)	0.97 (0.42)	0.81 (0.30)
12 months	1.01 (0.60)	0.87 (0.32)	0.72 (0.19)
18 months	0.84 (0.39)	0.94 (0.59)	0.87 (0.34)
24 months	1.00 (0.54)	1.08 (0.54)	0.84 (0.32)

Normal range = 0.1-1.5 mmol/l

There were no significant changes in triglyceride levels in the boys treated with either rhGH, TU or a combination of the two.

3. Carbohydrate metabolism
- effects on blood glucose and glycosylated haemoglobin

There are theoretical concerns that the growth hormone treatment of normal short children may lead to impaired glucose tolerance (see section 1.6.) Similarly there are concerns about the effects of anabolic steroids on glucose metabolism (see section 2.5.9.)

In our study randon plasma glucose levels and HbA1 were measured at entry into the study and at 3 months, 6 months and then at 6 monthly intervals. We did not have the opportunity to measure serial fasting plasma insulin levels. Urine was checked for glycosuria at 3 monthly intervals.

Mean (SD) plasma glucose mmol/l:

	rhGH	TU	rhGH+TU
At entry	5.04 (0.45)	5.25 (0.94)	5.13 (0.64)
3 months	5.17 (0.68)	4.60 (0.64)	4.50 (0.68)
6 months	4.96 (0.19)	5.07 (0.64)	5.09 (0.56)
12 months	5.10 (0.38)	4.87 (0.70)	4.96 (0.79)
18 months	5.20 (0.98)	4.39 (0.97)	5.05 (1.01)
24 months	4.55 (0.79)	5.08 (0.47)	4.58 (1.00)

There were no significant changes in random glucose levels in any of the treatment groups.

Mean (SD) Glycosylated Haemoglobin (HbA1) % of total Hb:

	rhGH	TU	rhGH+TU
At entry	6.33 (0.67)	5.87 (0.76)	6.60 (0.73)
3 months	6.27 (0.74)	6.68 (1.13) *	5.79 (0.97)
6 months	5.81 (0.53)	6.09 (0.57)	6.54 (0.77)
12 months	5.87 (0.84)	6.30 (0.84)	6.47 (0.62)
18 months	6.00 (0.84)	6.46 (0.78)	6.40 (0.37)
24 months	5.48 (0.26)	6.80 (0.71) **	6.40 (0.98)
		* p = 0.05 ** p = 0.03	

Reference range 4.7-7.9%

Mean HbA1 in the boys treated with TU either alone or in combination with rhGH tended to be higher than in the rhGH group. In the TU group mean HbA1 levels were significantly higher than baseline at three months and twelve months. However all values were within the reference range.

None of the boys developed glycosuria, and we did not demonstrate any convincing evidence of glucose intolerance. However this does not

mean that there was no development of insulin resistance which could have occurred. It was not practical to bring our children to the clinic fasted and therefore had no opportunity to assess fasting insulin levels.
The glucose metabolism of boys with puberty delay being treated with anabolic agents, including testosterone, merits further investigation.

4. Effects on thyroid function

There has been little published about the effects of growth hormone on the thyroid function of normal children, though it has been shown to affect the thyroid function of GH deficient children (Pirazzoli et al 1992). Little is also known about the effects of testosterone treatment on thyroid function in boys, though the effects on athletes abusing anabolic agents are more widely documented (Alen et al 1987). We have shown significant effects on total thyroxine and thyroid binding globulin in peripubertal boys taking "therapeutic" doses of oxandrolone (see section 2.5.9.)

We followed the thyroid function of our boys with measurements of total thyroxine, free thyroxine and thyroid binding globulin.

Mean (SD) Total T4 nmol/l:

	rhGH	TU	rhGH+TU
At entry	103 (29.4)	107 (16.8)	101 (18.9)
3 months	99 (19.5)	97 (10.8) *	95 (15.1)
6 months	106 (23.2)	98 (14.5)	96 (10.1)
12 months	97 (18.9)	103 (16.0)	92 (16.7)
18 months	110 (26.2)	104 (26.4)	86 (16.5)
24 months	117 (17.2)	110 (15.9)	105 (26.3)
		* p = 0.04	

Reference range 70-180 nmol/l

Mean (SD) Free T4 pmol/l:

	rhGH	TU	rhGH+TU
At entry	12.9 (0.60)	15.8 (1.92)	16.5 (3.54)
3 months	13.4 (1.61)	13.5 (2.96)	15.9 (0.92)
6 months	14.5 (2.61)	14.1 (2.00)	16.1 (1.41)
12 months	14.1 (2.13)	11.3 (1.54) *	13.7 (2.44)
18 months	15.7 (1.23)	13.8 (0.69)	NA
24 months	NA	13.1 (1.84)	NA
		* p = 0.02	

Reference range 9-23 pmol/l

Mean (SD) Thyroid binding globulin mg/l:

	rhGH	TU	rhGH+TU
At entry	20.8 (3.59)	18.4 (6.50)	16.5 (3.54)
3 months	19.0 (4.16)	20.0 (8.46)	16.0 (1.41)
6 months	19.8 (4.35)	19.6 (10.85)	20.0 (0.00)
12 months	20.0 (5.60)	16.2 (7.41)	17.0 (2.00)
18 months	20.3 (1.16)	19.0 (7.35)	12.0 (NA)
24 months	NA	20.3 (3.06)	11.0 (NA)

Reference range 12-30 mg/l

Although there were trends for total thyroxine to fall in the boys treated with TU alone or a combination of TU these were not as striking as in the younger boys treated with oxandrolone. We did not detect any effect of TU on thyroid binding globulin - in contrast to the fall in thyroid binding globulin we saw with oxandrolone.

Testosterone undecanoate has less effect on thyroid function than oxandrolone.

5. Production of Growth Hormone Antibodies

The rhGH used in our study had an identical amino acid sequence to naturally occurring hGH, and is less antigenic (Rasmussen 1988) than methionyl GH. Antibodies to growth hormone were measured in all children at entry into the study and at six monthly intervals.

All samples were negative for rhGH antibodies.

6. Effects on liver function

There have been concerns in tha past about the effects of testosterone derivatives on the liver, particularly methyltestosterone (Westaby et al 1977) leading to its withdrawal. Therefore it was important that we closely follwed the liver function of the boys in this study.

We measured plasma alanine aminotransferase (ALT) and g-glutamyl transferase (GGT) at entry into the study and at 3 monthly intervals with the following results :

Mean (SD) ALT:

	rhGH	TU	rhGH+TU
At entry	16.7 (4.7)	16.7 (4.5)	14.6 (4.0)
3 months	19.2 (8.3)	15.9 (5.0)	15.6 (5.2)
6 months	19.5 (6.4)	16.1 (3.0)	14.2 (1.3)
12 months	15.4 (3.4)	16.1 (2.1)	12.1 (1.8)
18 months	15.3 (3.0)	15.1 (3.0)	13.0 (2.7)
24 months	14.7 (3.7)	15.0 (3.7)	16.2 (5.6)

Reference range 10-40 units/l

Mean (SD) GGT:

	rhGH	TU	rhGH+TU
At entry	10.8 (3.0)	12.1 (2.1)	11.1 (2.6)
3 months	11.8 (1.7)	13.4 (1.7)	11.8 (2.1)
6 months	11.7 (1.6)	12.7 (2.0)	12.3 (2.7)
12 months	11.8 (1.7)	13.5 (1.7)	12.6 (3.5)
18 months	11.4 (1.5)	13.6 (0.9)	11.5 (2.6)
24 months	10.2 (2.2)	13.8 (2.0)	14.2 (2.8)

Reference range <35 units /l

There were no significant changes in mean plasma ALT or GGT in any of the groups. No boy had a measured ALT or GGT above the laboratory reference range at any time. We did not detect any worrying effects on liver function from either testosterone undecanoate or rhGH treatment.

Discussion

Rosenfeld (1982) has recommended various criteria for the trearment of boys with puberty delay: minimum age 14 years, height <5th centile, puberty stage G1 or G2 with serum testosterone <100ng/100ml, and impaired self image and social withdrawal not responding to reassurance. We aimed to determine whether we could promote the growth of such boys with rhGH, oral testosterone undecanoate, or a combination of both treatments. We have shown that it is possible to do this. The boys who received combination treatment grew fastest over the two years of treatment. Over the first year their growth rate was just significantly better than those who received testosterone undecanoate alone, but not from those who received rhGH alone. The growth response of those boys treated with oral testosterone undecanoate was as good as that acheived by rhGH alone - without the need for daily injections and at a cost of £100 per year compared to £10,000 per year for rhGH.

We explored whether treatment with rhGH or testosterone undecanoate or rhGH plus testosterone undecanoate have effects on the rate of pubertal progression. At entry into the study the majority of the boys were in early puberty - to fulfil the entry criteria they had to be no further in puberty than 6 ml testicular volumes. The three groups were similar at entry into the study in terms of their clinical pubertal staging, and 08.00 testosterone levels, although the boys in the TU group had a rather more active LH response to low dose LHRH. Puberty appeared to progress at very similar rates in all three groups. There were no significant differences between the three groups with regard to the rate of pubertal progression - median testicular volume and clinical pubertal staging at six months, one year and two years were very similar in all groups, as was the mean age at which 12 ml testes were reached, as was the time that they took to progress from 6 to 12 ml testes (mean of 1.38 years in rhGH group, 1.46 years in TU group, and 1.26 years in the combination group). The median testicular volume in all groups was 10 ml at one year, and 15 ml at two years. It is really only at a testicular volume of 10 ml that one would be expecting the growth rate to speed up as peak height velocity is reached. The boys reached median testicular volume of 10 ml at one year, and, as they had been growing quickly over the preceeding twelve months, this is likely to be due to the treatment given. The growth rates we produced are similar to those acheived by other workers using different testosterone analogues (Rosenfeld et al 1982), oxandrolone or rhGH (Buyukgebiz et al 1990)

The increase in testicular volume proceeded normally in the two groups who received oral testosterone undecanoate - this low dose did not appear to inhibit testicular growth. It has been suggested that when other anabolic agents, particularly oxandrolone, are used to treat boys with CDGP there is suppression of mean 24 hour serum LH and testosterone concentrations, with a rebound after treatment is stopped (Malhotra et al 1993). There were no significant differences between the three groups in mean random LH levels measured at entry or six monthly during the treatment period. In particular treatment with exogenous testosterone did not suppress LH secretion as there was a steady trend for LH to increase with time, implying that normal maturation of the hypothalamo-pituitary axis is proceeding.

As puberty progressed at similar rates in the three groups we are not particularly concerned about differing rates of puberty affecting adult height in these groups (cf. section 2 - the effects of oxandrolone on peri-pubertal boys). HSDS for bone age initially improved, then deteriorated in the boys treated with rhGH alone, and predicted adult height after two years was not significantly different to prior to treatment. In the boys treated with testosterone undecanoate alone HSDS for bone age tended to deteriorate over the two years, but again predicted adult height after two years was not significantly different to pre-treatment. In the combination group HSDS for bone age improved steadily over the two years, with a final improvement in predicted adult height. It is unlikely that final height in these boys will improve significantly as a result of the treatment, and they will eventually fall at the lower end of the range of their target height. Thus treatment with growth promoting agents is unlikely to improve the final height prognosis of boys with puberty delay. We will follow these boys to final height to determine how close to their target heights they have reached, and compare them to the published studies of untreated boys with CDGP (Crowne et al 1990). The final height acheivement in Albanese and Stanhope's group of boys with constitutional delay of growth and puberty (1993) was significantly lower than their target height and appeared unaltered irrespective of whether treatment was administered. They attributed the failure to acheive the target height on relatively short spinal length in the boys with CDGP.

In the children followed by Bierich to final height (1992), the mean final height did not differ from predicted height. Adult height did however fall below target height as in previous studies of untreated boys with CDGP. Disappointingly growth hormone treatment did not appear to increase final height. Our results at this stage would appear to concur with these findings.

Our studies at this stage do not enable us to answer any question on bone density. Assuming there is a critical time for laying down bone, it would be interesting to perform bone density studies on our boys once they have completed their growth, and compare their bone density to other untreated boys with CDGP. If the bone density in the treated boys was better than in untreated boys then it would prove to be an additional reason to bring forward the timing of the pubertal growth spurt in boys with CDGP.

The boys we treated with rhGH did become transiently leaner - a similar effect to that which we saw in the younger children. We did not encounter any problems in carbohydrate or fat metabolism in these boys. In contrast to the younger boys treated with oxandrolone (section 2) we did not detect any abnormalities in thyroid function in the boys who received testosterone undecanoate, nor were there any problems with liver function.

We were unable to find any specific markers prior to treatment that predicted the boys response to therapy. None of the measured growth hormone parameters, nor pre-treatment IGF1 levels predicted their response to treatment.

Although the boys were clinically all at a similar stage of puberty prior to entry, there were obviously some who were biochemically further advanced. The low dose LHRH test and/or an early morning testosterone level does identify these boys. One may be less keen to intervene in a boy who is biochemically more pubertally active than one in whom the gonadal axis hormones are relatively quiescent. It is difficult to justify putting boys with CDGP through growth hormone provocation tests unless there is something unusual about the boy to make one more concerned about abnormal GH secretion.

There appears to be no advantage in prescribing rhGH either alone, or in combination with testosterone undecanoate for boys with CDGP. Similar results are acheived with oral testosterone undecanoate alone, without the need for daily injections and at a reasonable cost (£48 per year for testosterone undecanoate 40 mg on alternate days, compared to £11,500 per year for 4iu per day of growth hormone). There were no significant adverse effects from the oral testosterone preparation we used. 40 mg of testosterone undecanoate on alternate days is a practical way of starting treatment, but with increased availability of 20 mg capsules it may be better to use a lower daily dose.

SECTION 4 : THE PSYCHOLOGICAL EFFECTS OF SHORT STATURE AND ITS TREATMENT IN THE SHORT NORMAL CHILD

4.1. Introduction

It is traditionally assumed that children with short stature have social, academic and psychological difficulties, with low self-esteem, social isolation, withdrawal and immaturity. It is important to understand the background literature as to why this assumption has built up, and to understand the relevance to the children we included in our growth studies.

In mythology there are numerous references to "elves" and "dwarves" who are almost universally seen as small, evil, powerful people with unusual mystic properties. Short stature has been entangled with concepts of ugliness and deformity, but also with child like innocence and mischief. Short people have been regarded as both inferior and sinister, yet sometimes possessing special skills. It is no wonder that peoples' perceptions of a small person may be clouded by the background of these stereotypes, and colour the attitudes to both adults and children who are small. Society does not always view short people kindly, whereas conversley tall people appear to have advantage in our society - taller people are more likely to be better educated, in employment, employed in higher status occupations, and to have lower age -standardised death rates than their shorter counterparts (Macintyre 1988).

Growth promotion in a child with a pathological cause of short stature eg growth hormone deficiency leads to an improvement in short term height velocity, final height, and possibly psychological benefit (see literature review below). There is no dispute that these children should be given treatment.

The benefits of growth promotion in the short normal child are much more difficult to assess. Growth promotion in a short but otherwise normal child may not significantly improve final height, but as we have shown in the previous chapters, does lead to a significant short term improvement in height velocity. This means that the child will be growing much more quickly than his peers and will begin to "catch them up". This may be more important psychologically to the child than the possibility of a slight improvement in final height in many years time. Indeed children live for the "here and now" and have poor concept of future benefits or side-effects. As we have seen this period of more rapid growth usually only lasts 18-24 months before the growth velocity begins to plateau.

It is important to try and ascertain whether or not this growth promotion does lead to a psychological improvement, as this is one of the main arguments for justifiying treatment in the short normal child. The dilemma remains of what is the most cost effective, least invasive, safe

treatment for short normal children with or without pubertal delay, and what level of psychological distress needs to be present to justify treatment. One must elucidate whether growth promotion itself does lead to improved self esteem, altered behaviour and academic attainments .

One must also try and ascertain whether or not the child has unrealistic hopes of the treatment, as perceived treatment failure may lead to deleterious psychological effects. In the context of our studies we included a placebo group in one of the studies. This gives an opportunity to investigate the psychological effects of a year of placebo injections, in addition to the comparison groups who received no treatment.

4.2. Psychological effects of short stature - background literature review

When reviewing the background literature on the psychological effects of short stature it is important to look separately at the groups that have been studied and not mix all short children together for psychological analysis - the cause of the short stature may affect the response to it. Different diagnostic groups of short children exhibit distinct characteristics, and there will be differing psychological dilemmas at varying ages and stages of puberty. The methodology in the studies varies considerably and again must be considered when comparing results to our studies.

4.2.2. Pathological causes of short stature

The behaviour and self esteem of children with pathological causes of their short stature has been explored by various groups. Steinhausen and Stanhnke (1976) described psychoendocrinological studies in 32 "dwarfed children and adolescents" - 16 with growth hormone deficiency, 16 of uncertain cause and control children. They used intelligence scales and personality scores. The "dwarfed" children were less agressive, less excitable, less dominant, more conscientious, more tender minded, less shrewd, more controlled and less tense than normal stature controls. "Inadequate coping" was present in almost three quarters of the group, with taunting by adults appearing as a problem.

Stace and Danks (1981) reviewed the experience of 75 children with bone dysplasias, and of their parents utilising a parent interview. Affected children were usually popular and extroverted in their early school years, but became socially isolated and depressed in their teenage years. Unsettled behaviour was frequent at school, reflected in repetition of grades and changes of school. There was a high incidence of mental disturbance and antisocial behaviour in many of the families.

Holmes et al (1982) studied parents' and teachers' differing views of short children's behaviour, but in a very heterogeneous goup of 56 children (including growth hormone deficiency, Turner syndrome and constitutional delay). All groups except young constitutional delay patients were rated by their parents to have significant problems in school functioning. Grade retention (a more common occurence in US schools than in the UK) occurred relatively more often, with immaturity and small size being perceived by parents as the reason to retain the child. Pollitt and Money (1964) suggested that short children do not do as well at school as their IQ

should predict ie. they underacheive, have poor concentration and a short attention span. However this study was small (15 patients) with very mixed causes of short stature, some of which may have had associated specific learning disorders. Holmes et al (1985) explored the social and school competencies in 47 children with short stature over 3 years (17 with growth hormone deficiency, 9 with Turner syndrome and 21 with constitutional growth delay), all of whom had at least average intelligence. They suggested that the children appear to undergo an age related decline in adjustment during early adolescence which was related to greater social isolation.

It has to be remembered that pathological causes of short stature may have associated specific learning problems eg. girls with Turner syndrome are recognised to have a higher incidence of specific visuo-spatial problems. Treatment with GH in growth hormone deficiency has not been shown to influence intelligence (Meyer-Bahlburg et al 1978).

Studies of short stature adults suggest they are disadvantaged, especially in their long-term social relationships and employment prospects (Underwood 1991). They are seen less positively by their peers, and perceive themselves less favourably.

Although many of these studies include some children with normal variant short stature they are mixed with the children with pathological causes of short stature. This makes it very difficult to tease out the effects of normal variant short stature.

4.2.2. Normal variant short stature

Most of the literature concentrates on children who have organic causes for their short stature and may include some short normal children mixed in the the studies (see above). Less has been specifically written about the short normal child, espsecially pre-pubertally.

The psychosocial effects of normal variant short stature have been studied specifically by Gordon et al (1982). 20 boys and 4 girls aged 6 -12 years, with height < 5th centile and constitutional delay were matched with 23 children of normal height, and comparable age, intelligence, sex and socioeconomic status. They were studied with various tools for both parents (Child Behaviour Checklist, Maryland Parent Attitude Survey, Family Functioning Index) and child (Piers-Harris Self Concept Scale). They reported that "children with constitutional short stature have significantly more behaviour problems and less self esteem than a matched control group with normal height. A general picture emerges of socially withdrawn

and aloof children who express emotional concerns internally and tend to view themselves less favourably than do their taller peers". Parents reported significantly higher somatic complaints, schizoidal tendencies and social withdrawal in the children with CDGP. Indices of self esteem tended to be lower in the short children, who more often saw themselves as unhappy and unpopular, though there was little evidence of hyperactive or aggressive behaviour.

The school progress and academic acheivements of the children with constitutional delay of growth was explored by Gordon et al (1984). Their group of children with constitutional growth delay (20 boys, 4 girls aged 6 -12 years, height < 5th centile, matched with 23 children of normal height, and comparable age and socioeconomic status) were not any more subject to school-related difficulties than their taller peers as assessed by their intellectual acheivements, visuo-motor functioning and behaviour ratings from teachers. However Gold et al (1978) had suggested a different picture in their study of children with CDGP and familial short stature (FSS). They compared case record analysis of 591 children with CDGP and 435 with FSS with detailed evaluation of 37 CDGP and 8 FSS. A higher incidence of learning problems in CDGP than in FSS was identified, but based on parental descriptions rather than standardised tests. In the smaller numbers with detailed follow up (school records, follow-up questionnaire, teacher evaluation) there seemed to be an increased frequency of minimal brain dysfunction and hyperactivity in CDGP, with growth delayed children more likely to be described as immature.

Not all short children appear to have such significant problems. 140 pre-pubertal normal short children in the community who were relatively unselected (ie had not been referred to a specialist growth centre) were studied by Voss et al (1991) in the Wessex growth study. Compared to controls they had unimpaired self esteem and normal patterns of behaviour, but a tendency towards hyperactivity and poor concentration. Apart from poorer reading attainments which may relate to superadded social deprivation these children appeared to be functioning well psychologically.

It is likely that there will be differences in the self esteem of children who present to a paediatrician because of concerns (either their own or parental) about their short stature than in those who are unselected. Much of the literature attributes behaviour problems in the short child (either from a pathological cause or normal variant) to differences in parenting, particularly the tendency to treat the short child younger than his chronological age.

4.3.3. Puberty delay

More has been written specifically about the psychological effects of pubertal delay, and it has been recognised for many years that pubertal delay can lead to psychological difficulties.

There are reported effects on behaviour and self esteem in boys who experience late puberty. Jones and Bayley (1950) studied physical maturation among boys as related to their behaviour and compared 16 growth accelerated boys, and 16 growth retarded boys aged 14 yrs or above, the diagnosis being based on skeletal maturation. Although the tools used were imperfect (Institute of Child Welfare ratings, and observational studies), they recognised that physically growth retarded boys exhibited many forms of relatively immature behaviour. Some were overactive and strove for attention, whilst others were withdrawn. Mussen and Jones (1957) in a study exploring the self-conceptions, motivations, and interpersonal attitudes of late and early maturing boys compared 17 boys who were physically delayed to 16 growth accelerated boys at the age of 17 years. More of the late maturing boys revealed strong feelings of inadequacy, dependent needs and negative self-concepts. More of the late maturers regarded their parents as highly dominating and rejecting. Aggression was more common in the early maturers who were also more self-confident.

Various studies have examined the effects that late attainment of physical maturity may have on school and academic acheivements. Douglas and Ross (1964) related the age of puberty to educational ability, attainment and school leaving age in the National Survey of Health and Development - a longitudinal study of 5000 children born in 1946. Those who reached puberty early had fared better in tests of ability and attainment at ages 8, 11 and 15 years, went on to achieve more passes at GCE O-level and stayed on longer at school.

Duke et al (1982) assessed the educational correlates of early and late sexual maturation in adolescence by reviewing 5735 Caucasian males and females, aged 12-17 years in the National Health Examination Survey. Late maturing boys were at a disadvantage compared to mid-maturers with lower aspirations and lower mean scores on WISC and WRAT (Wide Range Achievement test) even when controlled for IQ, althought there were no differences in the teachers' reports of their behaviour. The association was strongest in the older boys who were most out of physical harmony with their peers.

Jones (1957) examined the later careers of boys who were early or late maturing. The boys, who were reviewed at the age of thirty years or

more, had been originally classified as physically accelerated or retarded in terms of skeletal age during adolescence (the same boys as in Jones and Bayley 1950). There were no significant differences in adult height or masculinity between the two groups but there were some personality differences with higher scores for the early maturers on "good impression" and "socialisation". There were no differences in marital status, family size or educational level.

Sartorio et al (1990), however, reported that the social outcome of adults with constitutional growth delay was not as good as age, sex and social matched controls and that individuals with CDGP suffer from social problems in a similar way to those with growth hormone deficiency (GHD). In a structured questionnaire 45 adults (32 male, 13 female) who had experienced CDGP, and had reached final height (mean 161.9 cms in the men and 155.1 cms in the women) were interviewed aged 19-35 yrs. 8.8% were married (cf 39.8% controls cf 1.8% GHD), 84.4% were still living with their parents (cf 35.2% controls, 62.6% GHD) and 32.8% were unemployed or in part time work (cf 18.5% controls, 41% GHD). The educational achievements in the CDGP group were similar to control group. Socially they were more isolated, and preferred single sport activities rather than team sports (Sartorio et al 1990). When young adult men were asked about their experience of puberty delay over half felt their growth delay had affected their success at school, work or socially and many of these would have liked treatment to advance their growth spurt (Crowne et al 1990).

Although these studies give a good picture of the feelings of boys who experience CDGP as a whole, there is likely to be a very wide spectrum. On an individual basis many boys cope without too many difficulties and may not come to medical attention. Those who come seeking help are likely to be at the extreme end of the spectrum in terms of height, lack of maturation and/or psychological distress.

4.3.4. Effects of growth promotion

Much of the work on the psychological effects of growth promoting treatments has been with growth hormone deficient patients treated with GH Money and Pollitt (1966) investigated the personality maturation and response to GH treatment in hypopituitary children using a structured interview with patients and parents, and assessed school reports. The psychological maturation achieved depended on the success of the parents to treat the child to his chronological age rather than size. Almost all felt that GH treatment necessitated a major readjustment.

Mitchell et al (1986) in a retrospective evaluation of psychosocial impact of long term growth hormone therapy investigated 58 (44 men, 14 women) GH deficient adults who had received growth hormone treatment, reported lack of adequate relationships with peers of same sex and age, and difficulties with heterosexual relationships. 60% had been treated as younger than their chronological age, and 12% believed their school performance to be less than average although there was no difference in attainments compared to their siblings. They appeared to be less positive about themselves physically, their rate of unemployment was double that of the country and 35 remained single. GH treatment was regarded positively by most of patients, but the expectations of treatment had been met in only 64% of patients.

Dean et al (1985) in a study of 116 adults (86 men, 30 women, aged 18-38 years) with growth hormone deficiency, examined the educational, vocational, and marital status of GHD adults treated with GH during childhood by means of a structured intervew. Although educational acheivements were similar to their parents and siblings, their employment rate was less than parents or siblings (35.4% unemployed) and marital rate was <30% of the expected age adjusted rate. The poor outcome was unrelated to the response to GH therapy and these workers suggested that "increased height does not necessarily lead to normal social integration into adult life".

There are few placebo controlled studies looking at the psychological effects of growth promotion. Some work in adults is now beginning to be published. McGauley et al (1989) investigated the psychological effects of rhGH in 24 GHD adults in a double blind placebo controlled 6 month trial of rhGH. At entry the GHD adults had lower scores than controls suggesting they may be psychologically compromised. Treatment with rhGH produced improvement in some psychological areas compared to the placebo-treated group.

It is very difficult to know how to relate the results of these studies in GH deficient children and adults to our group of short normal children. One cannot assume they have either the same psychological characteristics at entry to the studies, or that their responses to treatment will be similar. To date, little has been published on the psychological effects of treating the short normal child. Boulton et al (1991) have described their experience in 66 short children without GH deficiency aged 5-15 years who were treated with rhGH for two years. They used the "Attitude to Growth " scale and the Piers Harris Self Concept Scale. They felt that the emotional attitude and

perceptions of the short, slowly growing children improved over the two years of growth hormone treatment.

4.2.5. Expecations of treatment and effects of "treatment failure"

Kusalic et al (1972) investigated the psychodynamic aspects of short stature and the response to GH treatment in 11 children with hypopituitarism. Prior to treatment they were described as having immature dependent behaviour with low self esteem, and absence of aggressive impulses. After six months of treatment there was increased depression and increased anger, coupled with an unrealistic hope in the "magical effect" of the hormone. Rotnem et al (1979) described the psychological sequelae of relative "treatment failure" for 11 children who were receiving human GH replacement for GHD during one year of GH therapy. During treatment the majority of children showed increased growth but failed to grow to the degree they and their families anticipated. Despite the fact that their clinicians considered treatment a success, the children perceived their treatment to be a failure because of overestimation of the final result. Their expectations of growth were heightened by the elaborate endocrine evaluations and hospital admissions. There was an increased emergence of depressive themes when the psychosocial response to therapy were . examined. It is important that health professionals are aware of the disparity between wish and realization of therapeutic success.

The worries of parent's due to the child's short stature are somewhat different to the child's own concerns. The parent will worry about the diagnosis, curability, expected adult height, intelligence, life expectancy and health, sexual development and the quality of life, all of which are relatively long term outcomes. The child on the other hand is much more concerned with the "here and now", his short stature compared to his peer group, obvious disproportion or deformity and lack of appropriate sexual development being uppermost in his worries. He is less likely than his parents to be concerned about potential long term side effects of any treatment, but will worry more about the discomfort of a therapy. These priorities in thought may influence the perceived benefits of any therapeutic manoeuvures and need to be taken into account when assessing expectations of treatment and response. Boulton et al (1991) emphasised the need for parents to undergo careful evaluation before their children are offered GH treatment, so that their understanding of their child's perceptions are clarified, and their own expectations of treatment are realistic.

4.3. Study : The effects of growth promotion on the psychological status of short normal children

4.3.1. Aims of the study
4.3.2. Patient recruitment
4.3.3. Study protocol
4.3.4. Results

4.3.1. The aims of the study were

1) to determine whether the short normal children we enrolled into the trials of growth promoting agents were psychologically impaired.
2) to determine whether treatment with growth promoting agents affects psychological well being.
3) to determine whether specific treatments at differing ages and stages of maturation have differing psychological effects

4.3.2. Patient recruitment

All children who were enrolled in the studies of growth promoting agents (see Sections 1, 2 and 3) together with their families were asked to participate in the psychological studies. Children under 8 years of age were excluded, as the methods used are not appropriate and have not been validated for this age group. Participation in the psychological studies was not obligatory to enter the clinical trials but we encouraged all the families eligible to participate and only a minority declined. The few families who declined were typical of the group as a whole.

4.3.3. Study protocol

The psychological studies were performed using a series of questionnaires involving parental, child and teacher reports. The questionnaires used were chosen to yield maximal information with minimal intrusion into the lives of the children and their families.

Parents : Conners' parent Questionnaire
General Health Questionnaire
Family Assessment Measure
Importance Questionnaire

Child : Harter Self Perception Profile
Importance Questionnaire
Child Manifest Anxiety Scale
Children's Depression inventory
Locus of Control

Teacher : Rutter Teacher Questionnaire

The Conners' 93 item Parents Questionnaire was used to cover the child's reported behaviour. In order to focus on the child's view of self we used the Harter Self-Perception Profile, the Children's Depression Inventory, and the Revised Child Manifest Anxiety Scale. We also included the Nowicki-Strickland Locus of Control Scale. A measure of family functioning - the General Adaptation scale of the Family Assessment Measure - was completed by both parents individually and by children over the age of 12 years. Both parents completed the General Health Questionnaire (of Goldberg) to asses their current level of psychological distress. The Rutter Teacher Questionnaire was used to assess school behaviour.

I am presenting details of two of the questionnaires which appear to yield the most relevant information for this group of short normal children - the Conners' Parent Questionnaire and the children's Harter Self Perception Questionnaire.

Conners' Parent Rated Child Behaviour Questionnaire

This assesses nine areas of behaviour

A conduct disorder
B anxious - shy
C restless - disorganised
D learning problem
E psychosomatic
F obsessive compulsive
G antisocial
H hyperactive - immature
I 10-item hyperactivity index

using 93 questions / statements which cover many aspects of life eg. problems of eating, problems of sleep, fears and worries, physical symptoms, immaturity, trouble with feelings, restlessness, temper, perfectionism. The parent is asked to read each statement and decide how much they think the child has been bothered by the problem during the past month, scoring from 1 to 4 (1 = not at all, 2 = just a little, 3 = pretty much, 4 = very much). The respondents answers are then transferred to the scoring key, and then the raw data converted to T-scores (transformed scores) using a profile grid. Thus the results of the Conners' questionnaire are expressed as T-scores for each of the ares of behaviour. These T-scores are compared to established norms (based on US children) and can identify how the child compares to children not specifically identifed as having a diagnosable behaviour problem. T-scores have a mean of 50 and a standard deviation of 10, hence a T-score of 45-55 is "average". High scale scores are indicative of having a problem while low scale scores indicate the absence of the problem. Hyperactivity is scored slightly differently, and a score of 15 or more is a criterion for identifying hyperactive children.

The Conners' parent rating scale we used was developed on a sample of 683 children between the ages of 6 and 14 years. The Conners' rating scales are well established and appear to have strong explanatory and predictive utility in child behaviour problems, and have been shown to be valid in many studies (Conners).

The Harter Self perception Profile for children

This measure was devised to evaluate children's domain-specific judgements of their competence, as well as a global perception of their worth or esteem as a person. It contains six seperate subscales tapping five specific domains, as well as global self-worth :

Specific domains
1. Scholastic competence (SC)
2. Social acceptance (SA)
3. Athletic competetnce (AC)
4. Physical appearance (PA)
5. Behavioural conduct (BC)

plus 6. Global self-worth (G)

The child is presented with a questionnaire entitled "What I am like" consisting of 36 statements (six for each of the subscales). The child is then presented with the series of statements and asked to decide which kind of child is most like him or her, and then asked whether this is only "sort of true" or "really true" for him or her.

eg.	Some kids often forget what they learn	BUT	Other kids can remember things easily

Each item is scored on a scale from 1 to 4, where a score of 1 indicates low perceived competence and a score of 4 reflects high perceived competence. Harter originally standardised this questionnaire on school children living in Colorado. Normative data for Scottish schoolchldren has been provided by Hoare et al (1993), who validated the use of a modified Harter Profile in over 4000 Lothian school children from 8-15 years of age.

The questionnaires were completed before treatment and at yearly intervals during treatment. Baseline measures were recorded when the children came for overnight blood sampling prior to entry into the study.

4.3.4. Results

I am presenting details of two of the questionnaires which appear to yield the most relevant information for this group of short normal children - the Connors Parent Questionnaire and the children's Harter Self Perception Questionnaire. I will refer to the results of the other studies where they are relevent and informative.

Children included in the psychological studies

96 children were included in the psychological part of the studies (19 from study 1, 38 from study 2, 29 from study 3).
The social class distribution of the children in the studies (13% social class I and II, 55% social class III, and 32% social classes IV and V) differed from our base population. This is also reflected in the intellectual and attainment level of our children who scored above, though not significantly, the population mean for IQ on the Scottish revision of WISC, and had higher attainments on the vocabulary and arithmetic subscales of the the British Ability Scales.

Results of Conners' Parent Questionnaire

It must be remembered that the Conners' questionnaire is an assessment of the child's reported behaviour by the parents. The results of the Conners' questionnaire are expressed as T-scores (see methods). T-scores have a mean of 50 and a standard deviation of 10, hence a T-score of 45-55 is "average". High scale scores are indicative of having a problem while low scale scores indicate the absence of the problem.
Interpretive guidelines for T-scores

>70	Very much above average
66-70	Much above average
56-60	Above average
45-55	Average
40-44	Slightly below average
35-39	Below average
30-34	Much below average
<30	Very much below average

T-scores of 70 or more are generally regarded as clinically significant. Areas of relative strength and weakness in the profiles may become apparent by analysing the scores for the nine areas of behaviour. Hyperactivity is scored

differently, and a score of 15 or more is a criterion for identifying hyperactive children.

At entry: baseline T-scores Mean and 95% confidence limits

We have expressd the results as per each trial group which will divide the children by age and state of pubertal maturation. This may be important as differing psychological effects could be seen at differing ages and stages of maturation.

	Study 1	Study 2	Study 3
A (conduct disorder)	45.9 (43.2-48.6)	46.1 (43.6-48.6)	46.7 (43.4-49.0)
B (anxiety)	48.4 (44.8-51.9)	50.8 (47.9-53.7)	48.0 (45.4-50.6)
C (restlessness)	54.7 (49.1-60.2)	55.8 (52.5-59.0)	51.9 (48.8-54.9)
D (learning problems)	46.9 (43.4-50.2)	49.0 (46.3-51.7)	49.2 (46.5-51.8)
E (psychosomatic)	49.9 (46.4-53.4)	53.6 (50.2-57.0)	50.0 (47.3-52.8)
F (obsessional)	50.5 (45.9-55.0)	50.9 (47.6-54.2)	52.0 (48.6-55.4)
G (antisocial)	42.4 (40.1-44.7)	44.7 (42.3-47.0)	44.1 (41.2-46.6)
H (immaturity)	48.9 (49.4-52.1)	49.4 (46.5-52.2)	46.4 (44.0-48.8)
I (hyperactivity)	18.7 (15.8-21.5)	18.9 (16.9-20.8)	17.4 (15.4-19.3)

In all three study groups, all areas of reported behaviour at entry into the study were within the normal range, apart from hyperactivity. All three groups show elevated scores on the hyperactivity index, with 71% of the children having a score of more than 15, the traditional cut-off point for clinical disorder. A similar trend was seen on the Rutter Teacher's Questionnaire.

There were no obvious between the groups reflecting changes in perceived behaviour with increasing age or stage of maturation.

One year Conners' results:

Analysis of the "no treament" groups in the two studies involving the younger children enables us to determine whether there are any psychological benefits of attending specialised growth clinic on a regular basis.

"No treatment" children (n=17)

Mean Conners' T-scores (95% confidence limits)

		Wilcoxon signed ranks (p)
A (conduct disorder)	45.8 (41.8-49.7)	0.31
B (anxiety)	50.9 (46.0-55.7)	0.60
C (restlessness)	51.2 (47.7-54.7)	0.06
D (learning problems)	47.8 (43.8-51.8)	0.81
E (psychosomatic)	51.1 (46.0-56.0)	0.31
F (obsessional)	51.7 (45.6-57.7)	0.94
G (antisocial)	42.0 (39.2-44.7)	0.06
H (immaturity)	45.6 (42.5-48.6)	0.10
I (hyperactivity)	16.5 (14.6-18.4)	chi square 1

There were no perceived significant psychological changes in the children receiving no treatment.

The relatively small number of children receiving placebo injections yet who were old enough to be included in the psychological studies enabled us to begin to determine whether placebo injections changed perceived behaviour. Although there were no statistically significant changes (perhaps due to the small sample size) there was a trend for most problem behaviours to increase.

Children treated with rhGH alone (n=32)

Mean Conners' T-scores (95% confidence limits)

		Wilcoxon signed ranks (p)
A (conduct disorder)	42.1 (39.3-45.7)	0.02
B (anxiety)	46.4 (42.6-50.3)	0.007
C (restlessness)	50.2 (46.5-53.8)	0.02
D (learning problems)	45.5 (43.1-47.9)	0.44
E (psychosomatic)	47.3 (43.9-50.6)	0.04
F (obsessional)	48.2 (44.9-51.5)	0.09
G (antisocial)	42.2 (39.2-45.2)	0.37
H (immaturity)	43.4 (40.1-46.6)	0.005
I (hyperactivity)	15.7 (13.8-18.1)	chi square 0.996

In the children treated with rhGH alone in all three studies there were some clear changes in reported behaviour especially in the areas of anxiety and Immature behaviour and to a lesser effect in conduct disorder, restlessness, and psychosomatic complaints. Little change occurred in the perceived parameters of learning problems, obsessional or publically antisocial behaviour and hyperactivity. In the younger children the changes appeared to be more prominent in the more "internalised" problems such as anxiety, psychosomatic complaints and immature behaviour.

It is important to try and establish whether this is due to the improved growth rate produced by rhGH, or a more central effect of rhGH. In order to do this we compared the effects on the Conners' scores of other growth-promoting agents. There were weak effects on immature behaviour and publically antisocial behaviour in the boys treated with oxandrolone alone or in combination with rhGH, but no other behaviour effects of significance occurred. In the older boys with puberty delay, neither testosterone undecanoate alone or in combination with rhGH, produced statistically significant changes in behaviour.

rhGH treatment does appear to shift the reported behaviour of children in a positive direction, though both before and after one year of treatment the parameters measured are within the normal range. Therefore one is only looking at trends within a normal range. Perceived hyperactive behaviour was not altered by any of the treatments.

Results of Harter Self Perception Profile

The Conners' questionnaire reflect the parents views of their child's behaviour. It is important to balance this with an understanding of how the child is seeing himself. The Harter Self-perception profiles enable us to do this.

In contrst to the soring of the Conners' scale, the higher the score on the Harter scale, the <u>better</u> the childs self esteem

At entry: expressed as mean scores (95% confidence limits)

	Study 1	Study 2	Study 3	Local population (SD)
SC social competence	2.67 (2.40-2.95)	2.75 (2.60-2.90)	2.70 (2.48-2.91)	2.74 (0.63)
SA social acceptance	2.88 (2.59-3.16)	3.01 (2.80-3.22)	3.38 (3.20-3.55)	3.04 (0.65)
AC athletic competance	2.74 (2.48-3.00)	3.12 (2.93-3.31)	3.03 (2.82-3.24)	2.98 (0.66)
PA physical appearance	2.65 (2.40-2.89)	2.77 (2.56-2.94)	2.63 (2.44-2.82)	2.89 (0.67)
BC behavioural conduct	2.66 (2.41-2.94)	2.70 (2.53-2.88)	2.63 (2.45-2.81)	2.61 (0.58)
G global self-esteem	3.22 (2.93-3.50)	3.10 (2.92-3.29)	2.96 (2.76-3.15)	3.05 (0.58)

Before entry into the studies the children appeared to be normal and comparable to the local population. There were no obvious trends with age or increasing maturation.

In the Importance Questionnaires, parents and children differed in their view of small-sized children, with the children considering social acceptance, athletic competence and physical appearance more important than their parents.

One year Harter results

There were "no treatment" groups in two of the studies, and we can use these children's data to determine whether or not attendance at a specialised growth clinic alters the child's perception of themselves.

"No treatment" children:

	Mean (95% conf. limits)	Wilcoxon Signed Rank (p)
SC	2.78 (2..49-3.07)	0.96
SA	3.31 (3.00-3.62)	0.17
AC	3.23 (2.95-3.52)	0.06
PA	2.70 (2.41-2.99)	0.53
BC	2.83 (2.59-3.06)	0.11
G	3.20 (2.90-3.49)	0.55

There was a trend for change in perceived athletic competence, but this did not reach significance, and may be a reflection of age and practice. Apart from this, there were no changes in the child's self esteem during a year of "no-treatment". This is similar to the results of the Conners' questionnaires for parental perception of the child's behaviour.

Placebo treatment:

The relatively small number of children receiving placebo injections yet who were old enough to be included in the psychological studies enabled us to begin to determine whether placebo injections changed the child's self esteem.

	Mean (95% conf. limits)	Wilcoxon Signed Rank (p)
SC	2.38 (1.46-3.31)	0.04
SA	3.23 (2.83-3.63)	0.58
AC	2.90 (2.26-3.54)	0.91
PA	2.67 (1.74-3.59)	0.46
BC	2.05 (1.08-3.02)	0.46
G	2.88 (2.19-3.58)	0.07

There is a large fall in perceived scholastic competence, and downward trends in satisfaction with physical appearance, behavioural conduct, and global self esteem. Although the numbers in this group are small, these trends are worrying and should alert us to the dangers of placebo treatments in children, particularly in the contexts where they may be more aware that treatment may not be working eg. the child inspecting his growth chart at the clinic will be looking for an upward trend in the spots on the chart and may become disillusioned if this is not happening as he expects.

In order to determine whether or not active treaments improve self esteem we must analyse the Harter responses to them

rhGH : one year results

We have included all children in the three studies treated with rhGH alone

	Mean (95% conf. limits)	Wilcoxon Signed Rank (p)
SC	2.76 (2.51-3.03)	0.02
SA	3.23 (2.97-3.49)	0.15
AC	3.08 (2.74-3.42)	0.88
PA	2.68 (2.37-2.99)	0.46
BC	2.70 (2.41-2.98)	0.64
G	2.97 (2.65-3.29)	0.32

Only perceived scholastic competence is seen as significantly better with only weak trends to improvement in other domains of self-evaluation.

Oxandrolone : one year results

It is of interest to compare the child's self esteem after a year of oxandrolone - there was no significant difference in growth rate between the children treated with hGH after one year compared to those treated with the anabolic agent oxandrolone, but pubertal maturation was more rapid (see section 2.5). This treatment is occurring at a time when the boy is sensitive not only about his height, but about his pubertal maturation compared to his peers.

	Mean (95% conf. limits)	Wilcoxon Signed Rank (p)
SC	3.16 (2.74-3.57)	0.21
SA	3.60 (3.40-3.80)	0.01
AC	3.28 (2.81-3.75)	0.17
PA	3.07 (2.65-3.48)	0.48
BC	3.03 (2.68-3.39)	0.03
G	3.58 (3.39-3.76)	0.03

Oxandrolone appears to have more effect on self esteem than rhGH alone, particularly in the domains of social acceptance, behavioural conduct and global self worth. It is unlikely that it is just the effect on growth that produces this effect as these children are growing at a very similar rate to those treated with rhGH alone. It is interesting to speculate as to whether it is the effect of the anabolic agent on the timing of puberty, or whether there is a central nervous system effect from the anabolic steroid. Combining rhGH with oxandrolone appears to blunt the effects, except on behavioural conduct, even though this combination is producing the most rapid growth rates.

In the older boys with puberty delay, testosterone undecanoate produces an improvement in athletic competence. This is not maintained in the combination treatment with rhGH, which produces only improved perceived behavioural conduct. Again there are puzzling disassociations between the increase in growth rate and the changes in self esteem.

4.4. Discussion

The psychological studies were performed using a series of questionnaires involving parental child and teacher reports, though the best method of psychological assessment is arguable (Wiklund et al 1991). We chose a questionnaire study to yield maximal information with minimal intrusion into the lives of the children and their families.

The children included in our studies are a relatively socially and intellectually advantaged section of our community. This may be due to a bias in referral patterns to a tertiary growth centre compared to a non-selected group of short children. Referred children with growth problems probably do differ substantially from those short normal children who do not reach hospital clinics. There may well be differences in the self esteem of children who present to a paediatrician because of concerns (either their own or parental) about their short stature than in those who are unselected. However the pre-treatment status of our children is similar to that found by Voss et al (1991) in a non-selected group of short British children in the Wessex growth study. Our children were remarkably "normal" prior to entry into the studies compared to those children studied by other workers eg Gordon et al (1982). They did not depart from the normal population in behaviour or self-esteem. As a group they functioned well cognitively and emotionally. There were obvious clinical exceptions to this general rule, but other stressful processes may have been implicated. It is possible that cultural differences between the USA and the UK may partly explain the differing results.

When one is dealing with relatively "normal" subjects to start with, it is much more difficult to detect significant changes compared to when one is dealing with a psychologically disturbed population. We are looking for trends within a normal range, rather than changes from abnormality to normality. These are bound to be much more subtle, and their clinical significance harder to assess. In addition the numbers of children in our treatment groups are relatively small in number, making it difficult to interpret differences between groups.

Children in all "active" treatment groups showed an increase in their rate of growth. There does not appear to be a close correlation between the rate of growth induced and psychological change produced. The children who grew the fastest with a combination of treatments (rhGH plus oxandrolone) did not exhibit the most marked psychological changes. This means that when considering a growth promoting treatment, the end point is not necessarily just the rate of growth that can be achieved.

Self esteem appeared to be most responsive to the anabolic steroid oxandrolone - perhaps this is due to the effects we saw on maturation or possibly a central effect of the agent. In boys with puberty delay, testosterone has weak effects on behaviour and improved self esteem. Adding rhGH did not improve outcomes, either in terms of growth rate or psychological changes.

rhGH alone did produce positive changes in perceived behaviour and self esteem though these effects are subtle, and are trends within a normal range. One has to ask whether it was worth the daily injection, together with the risk of potential side effects.

We need to be aware of the children who may have perceived their treatment to be a failure. In our studies we were concerned to detect a decline in self-esteem in the placebo group, consistent with the weaker effect on perceived behaviour. Great caution needs to be taken when considering a placebo limb of a clinical study in children. However we recognise that such a placebo control group is a necessary part of any new unproven treatment study, and is appropriate when considering psychological effects.

Many short normal children present to growth clinics and they and their parents do seem concerned by their slow growth. They seem at risk of being socially stressed adults. It is possible that psychological interventions may be as effective as medical treatment in the future, but it is extremely difficult to shift attitudes and social reactions. If the intervention should be psychological rather then medical it needs to be directed to specific areas of stress with judicious medical intervention in certain cases. Careful studies must be done as any psychotherapy needs to be very carefully evaluated - just as with any other medical therapeutic intervention.

The dilemma still remains of what is the most cost effective, least invasive safe treatment for short normal children with or without pubertal delay, and what level of psychological distress suggests treatment may be helpful. Any psychological changes produced are subtle and not easy to

measure. Although it is relatively easy to make children grow better, it is not as easy to improve them psychologically.

SUMMARY AND CONCLUSIONS

We have shown unequivocally that it is possible to promote the growth of the short normal child.

In the pre-pubertal child with familial short stature we have shown that rhGH injections will improve growth for a period of 12-24 months following which the child will grow along his or her new centile. Using a dose of 15iu/sq.m/wk of rhGH produced the same effects on both growth and the biochemical markers of growth as 24iu/sq.m/wk. We have shown that regular attendance at a specialised growth clinic does not itself improve either growth or psychological well being. There was no placebo effect from daily injections, but there were worrying trends in psychological status following a year of placebo injections. We do not feel there is any place for further placebo controlled studies in the pre-pubertal short normal child.

Although growth is improved over the short-term, it is unlikely that final height will improve significantly. This is predominantly due to the effects that rhGH treatment has on puberty - the onset of puberty tends to be earlier in girls, whereas the tempo of puberty is faster in boys. This means that any gain in height achieved pre-pubertally is likely to be lost as final height will be reached at an earlier than average age.

In the peri-pubertal boys with familial short stature we again showed it is possible to improve growth in the short term with either rhGH, oxandrolone, or a combination or the two agents. 15iu/sq.m/wk of rhGH produced similar effects to 24iu/sq.m/wk in terms of growth and the biochemical markers of growth. Oxandrolone produced a similar growth response to rhGH. The fastest growth rate was achieved with a combination of rhGH and oxandrolone.

Oxandrolone, either alone or in combination with rhGH, had significant effects on the timing and tempo of puberty. Boys treated with the anabolic steroid entered puberty earlier than those who received rhGH or no treatment. Skeletal maturation advanced much more rapidly in the boys who received oxandrolone, either alone or with rhGH. Self esteem improved most in the boys treated with oxandrolone. However as final height will be reached more quickly, the time available for growth will be limited and final height possibly compromised. The boys in our study had familial short stature, with a target height at the lower end of the adult range. Any treatment that may compromise this is not justified and we would not recommend oxandrolone for boys without growth delay.

In older boys with puberty delay, treatment with rhGH, oral testosterone undecanoate, or a combination of the two was equally effective. Puberty progressed normally in all three groups, and skeletal maturation was not different between the groups. Oral testosterone undecanoate is a safe, effective treatment for boys with puberty delay, and there is no indication to prescribe rhGH in this situation.

We must remember that the children we treated were normal healthy individuals, and that any treatment we propose must be as safe as possible.Significant adverse events occurred in three of the children included in our studies, necessitating discontinuation of treatment. One boy receiving rhGH developed hepatic failure and required liver transplantation. One boy receiving rhGH developed unilateral testicular swelling and underwent orchidectomy. It is not likely that these adverse events were attributable to the rhGH. One boy receiving rhGH and oxandrolone developed significant glucose intolerance with insulin resistance. Three other boys receiving oxandrolone developed transient abnormalities in ALT. Almost all boys receiving oxandrolone developed reversible depression of thyroid binding globulin. All children receiving rhGH became transiently leaner. Treatment with any growth promoting agent must not be undertaken lightly, particularly in the short normal child, and regular detailed checks must be performed to detect any side effects.

The psychological effects of growth promotion are both subtle and complex. Our study population was psychologically normal at the outset and we were observing changes within the normal range. Although final height is unlikely to improve there are subtle improvements in the psychological status of the children treated with rhGH. However it is not just a matter of producing fast growth - as boys treated in the combination therapy groups, in whom growth was the fastest, did not show the best psychological changes.

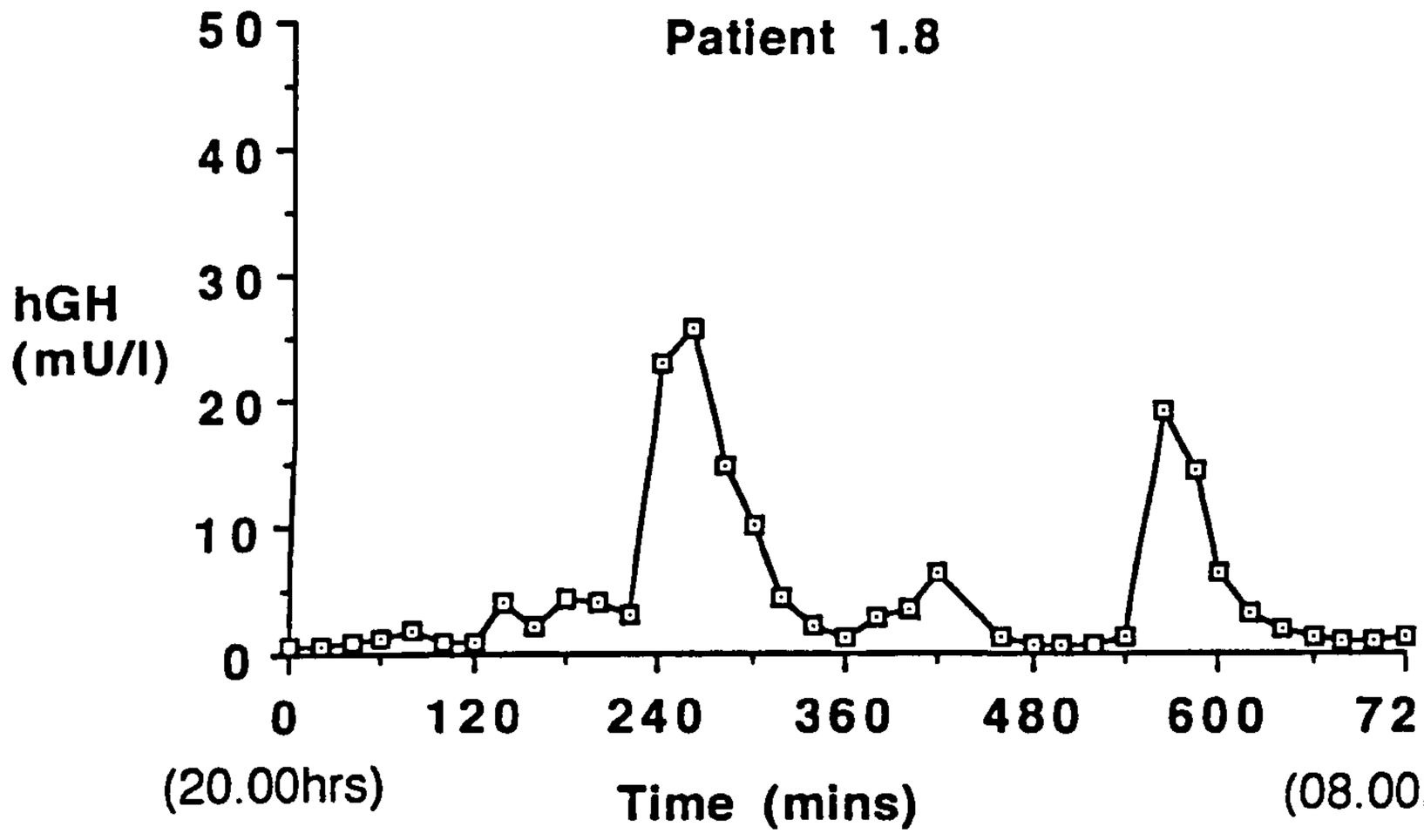
It has to be debated whether these subtle psychological changes justify the "costs" of growth promoting treatments - not only in financial terms, but the risk of potential side effects. Psychological intervention may be as effective, and merits further study.

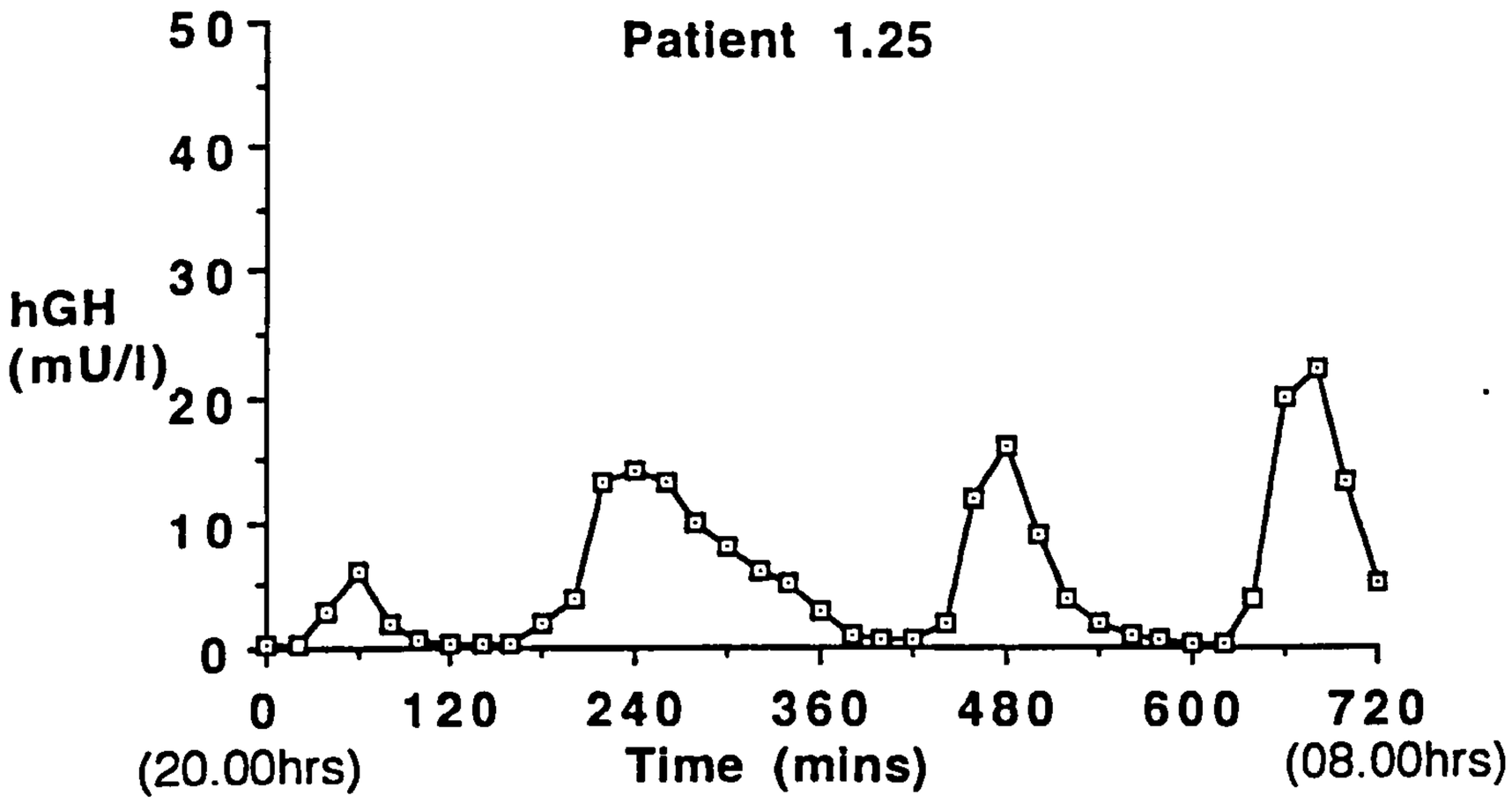
It is difficult to justify the use of rhGH in young children with familial short stature, or in boys with puberty delay. Growth hormone must not be used indiscriminately in the short normal child.

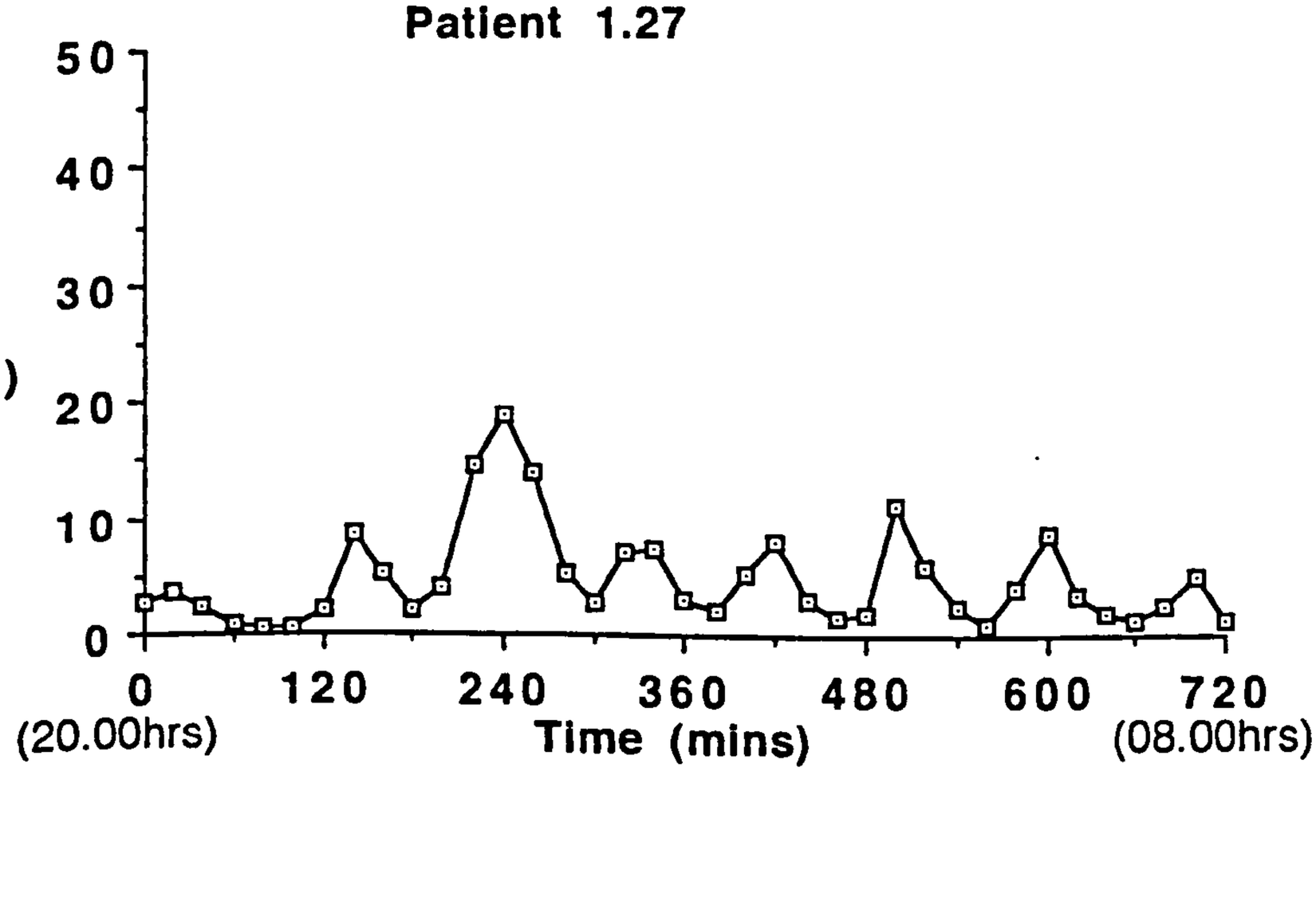
APPENDIX

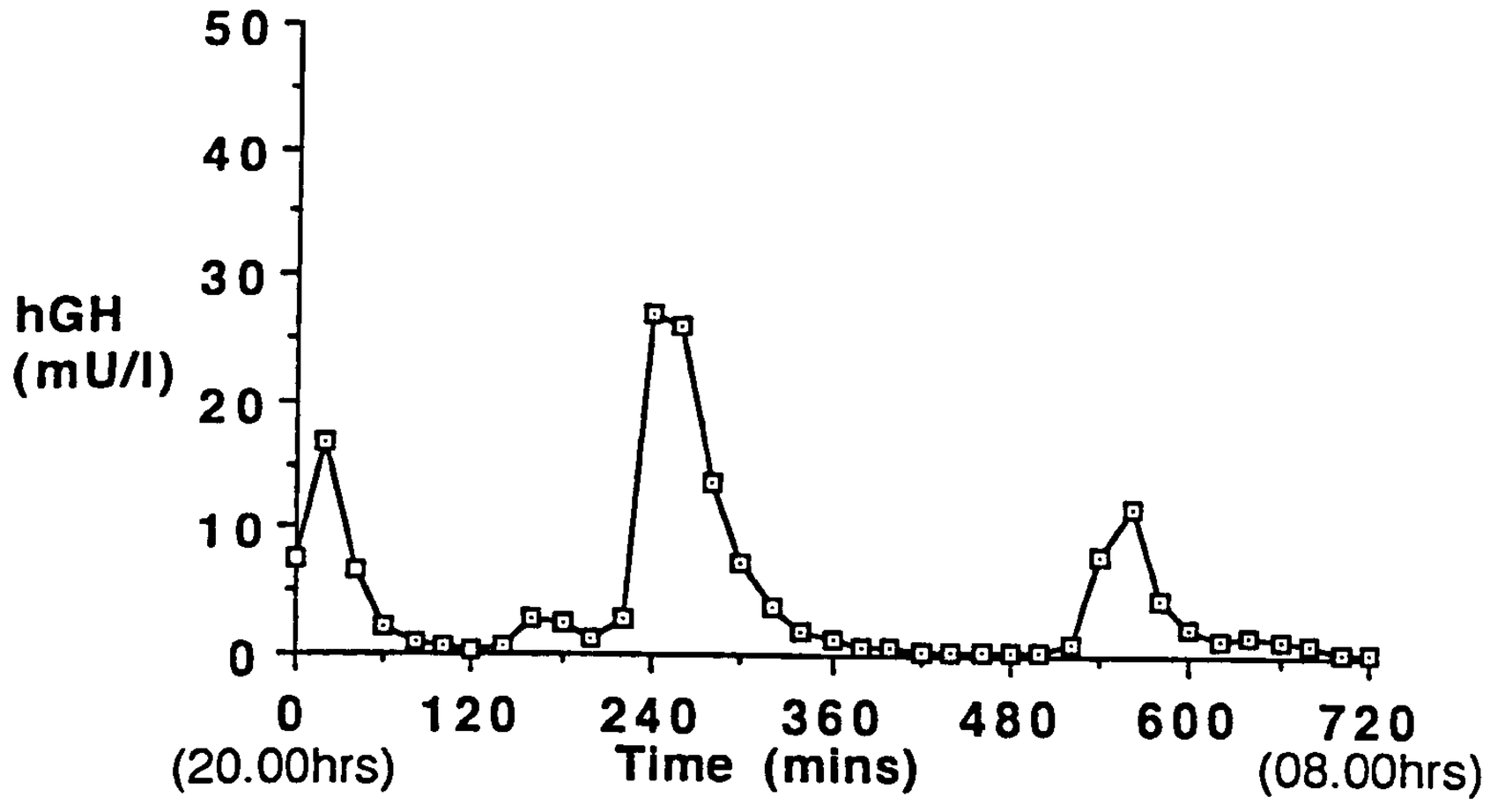
1. Examples of overnight growth hormone profiles

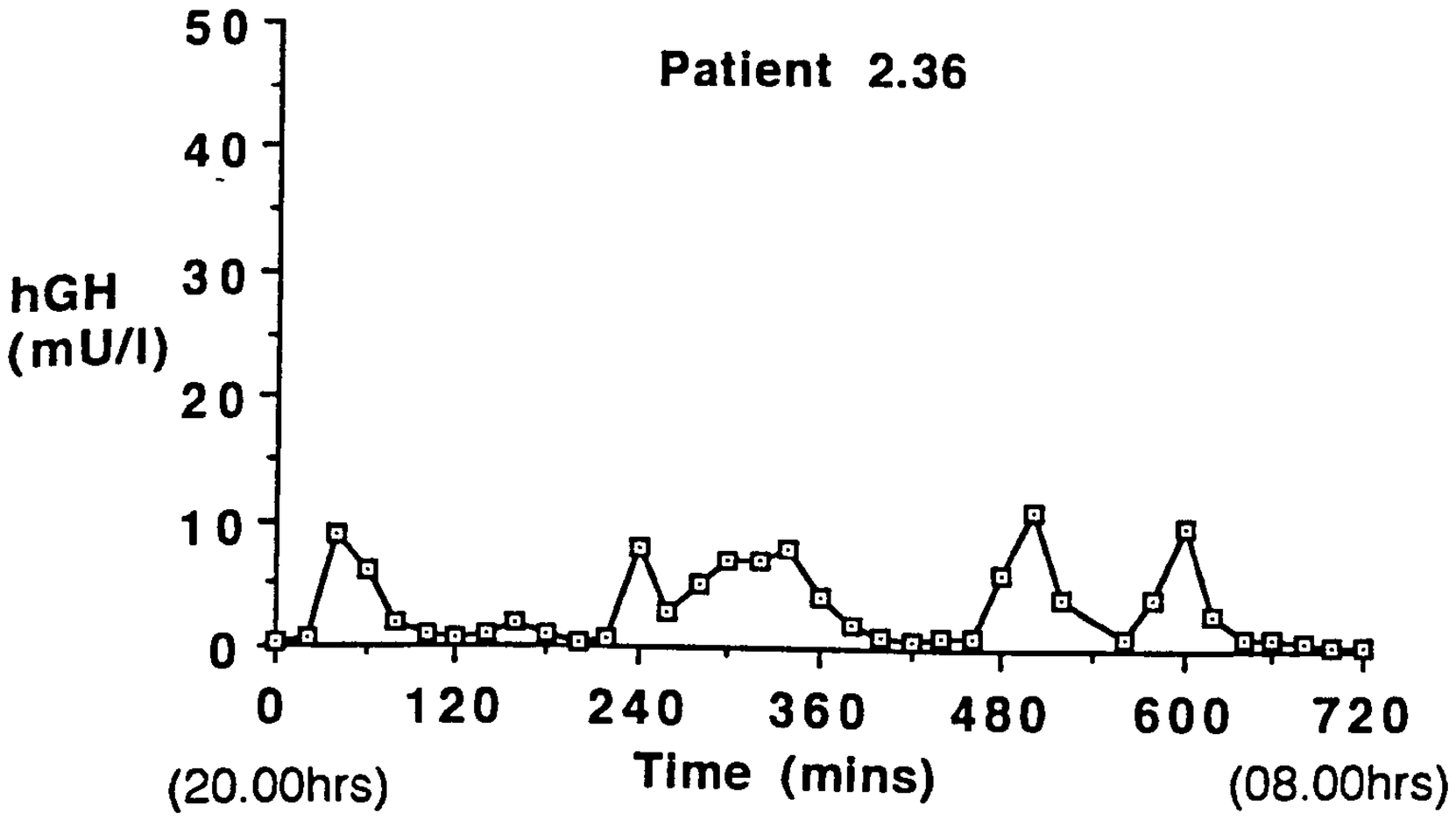
2. Examples of growth charts from children included in the studies

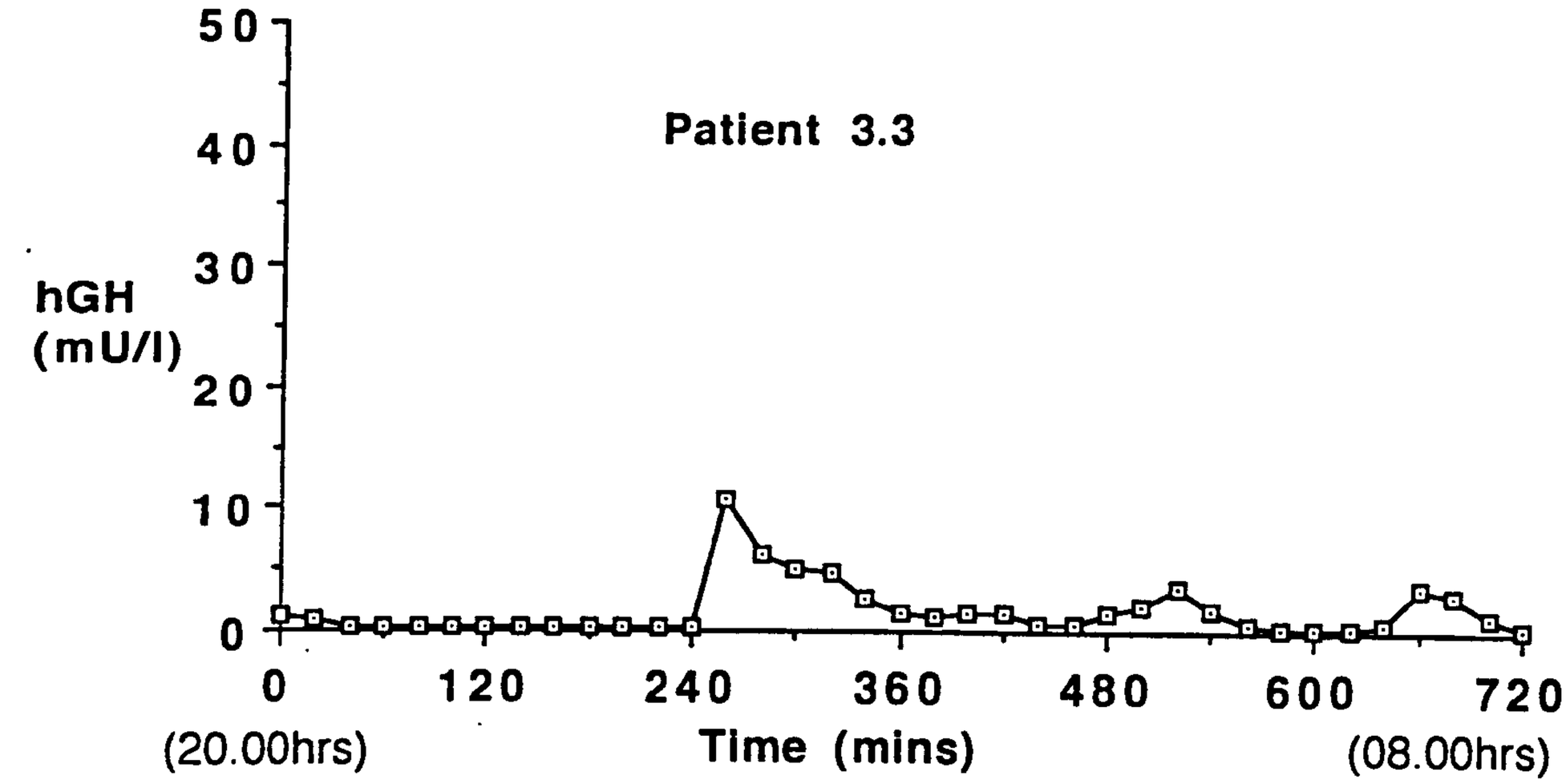

Patient 1.8
hGH
(mU/l)
50
40
30
20
10
0
0
(20.00hrs)
120
240
360
480
600
720
(08.00hrs)
Time (mins)

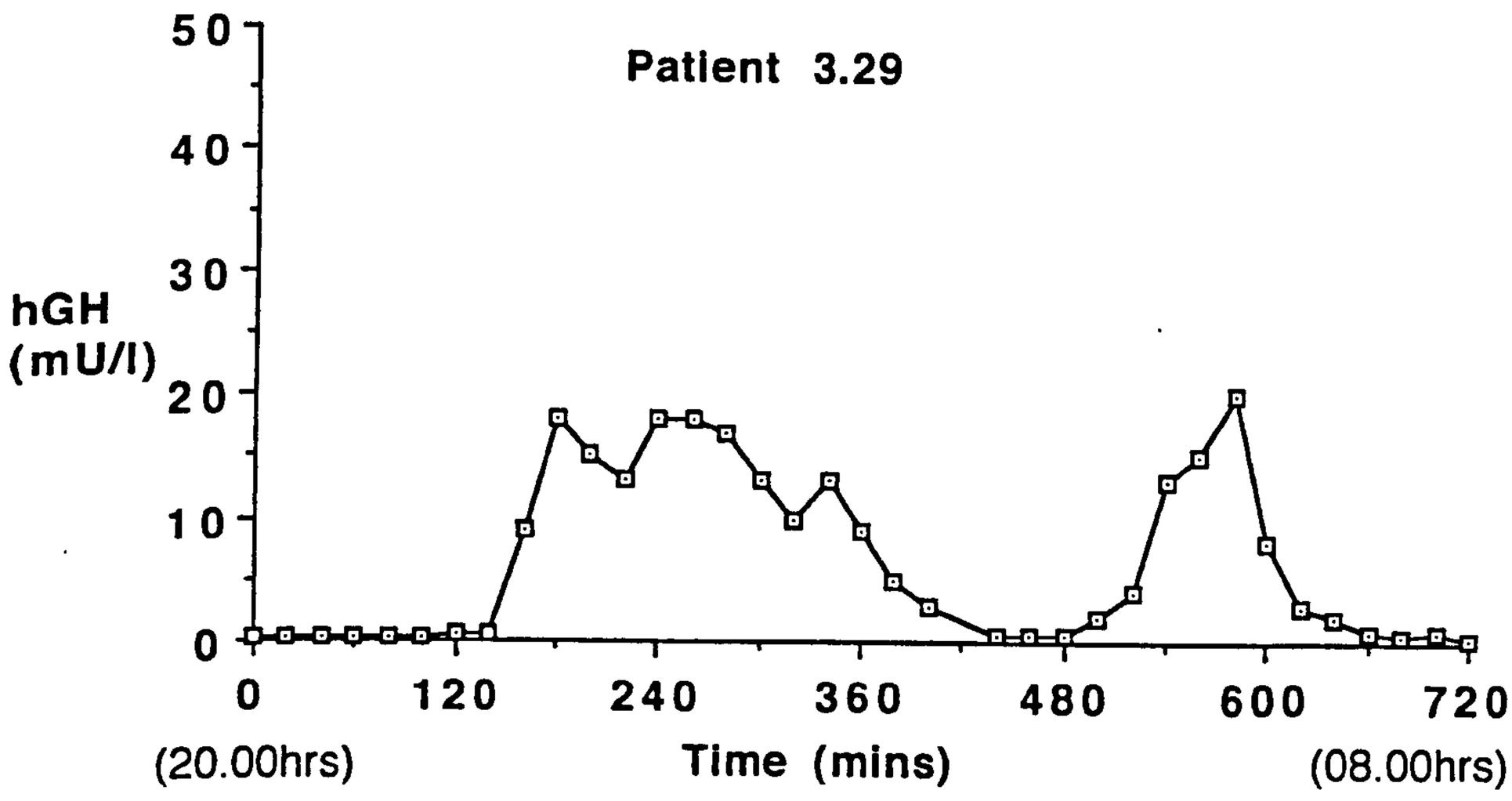

Patient 1.25
hGH
(mU/l)
50
40
30
20
10
0
0
(20.00hrs)
120
240
360
480
600
720
(08.00hrs)
Time (mins)

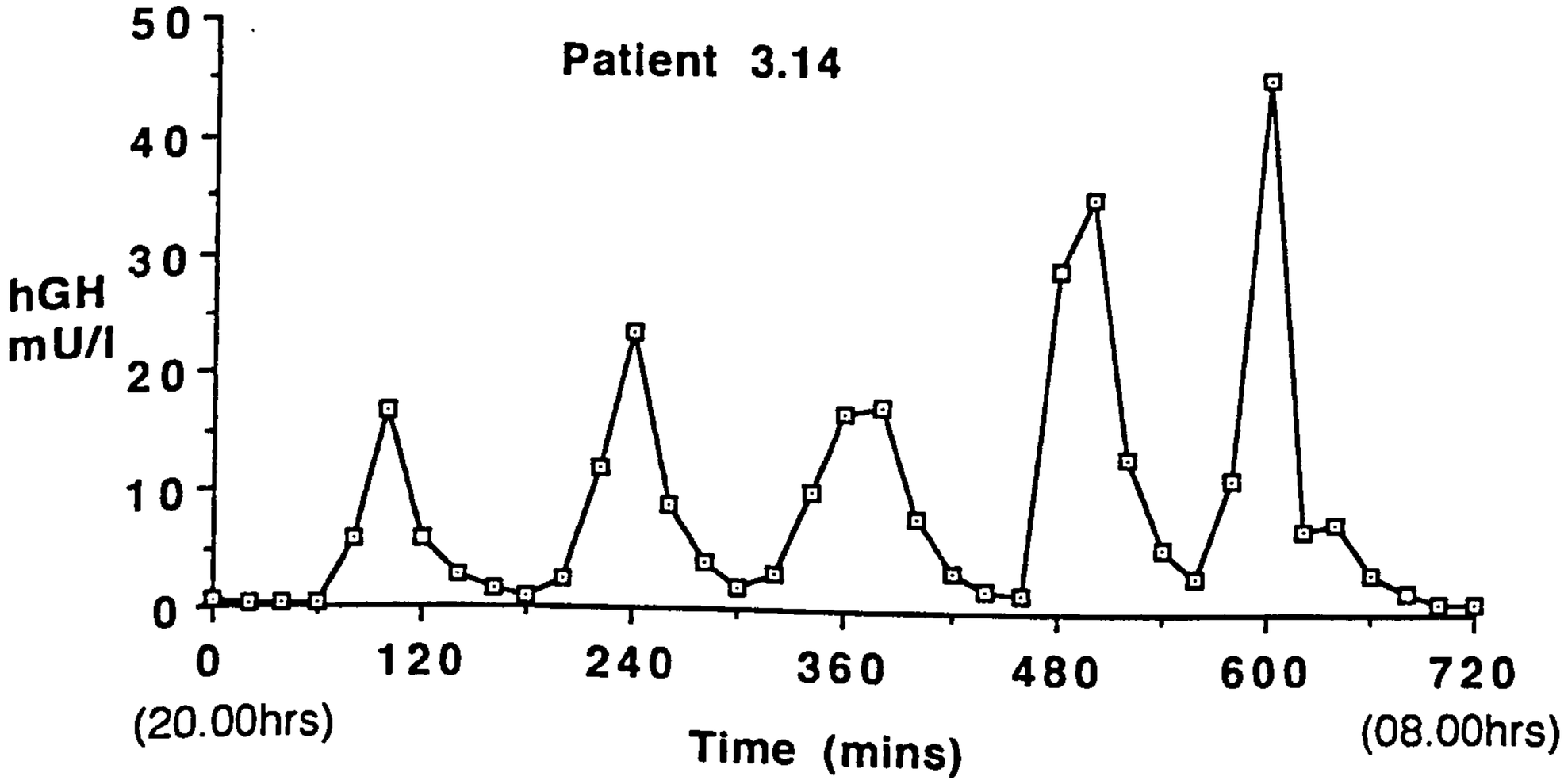

Patient 1.27
hGH
(mU/l)
50
40
30
20
10
0
0
(20.00hrs)
120
240
360
480
600
720
(08.00hrs)
Time (mins)


Patient 2.41
50
40
30
20
10
0
hGH
(mU/l)
0
120
240
360
480
600
720
(20.00hrs)
Time (mins)
(08.00hrs)


Patient 2.36
50
40
30
20
10
0
hGH
(mU/l)
0
120
240
360
480
600
720
(20.00hrs)
Time (mins)
(08.00hrs)


Patient 3.3
hGH
(mU/l)
50
40
30
20
10
0
0
120
240
360
480
600
720
(20.00hrs)
Time (mins)
(08.00hrs)


Patient 3.29
hGH
(mU/l)
50
40
30
20
10
0
0
120
240
360
480
600
720
(20.00hrs)
Time (mins)
(08.00hrs)


Patient 3.14
hGH
mU/l
50
40
30
20
10
0
0
120
240
360
480
600
720
(20.00hrs)
Time (mins)
(08.00hrs)

PATIENT 1.16
rhGH 24 in/sq.m/wk

GIRLS Height

Longitudinal standards — 97, 50, 3

Limits for single occasions (cross-sectional) — 97, 3

B2

GH

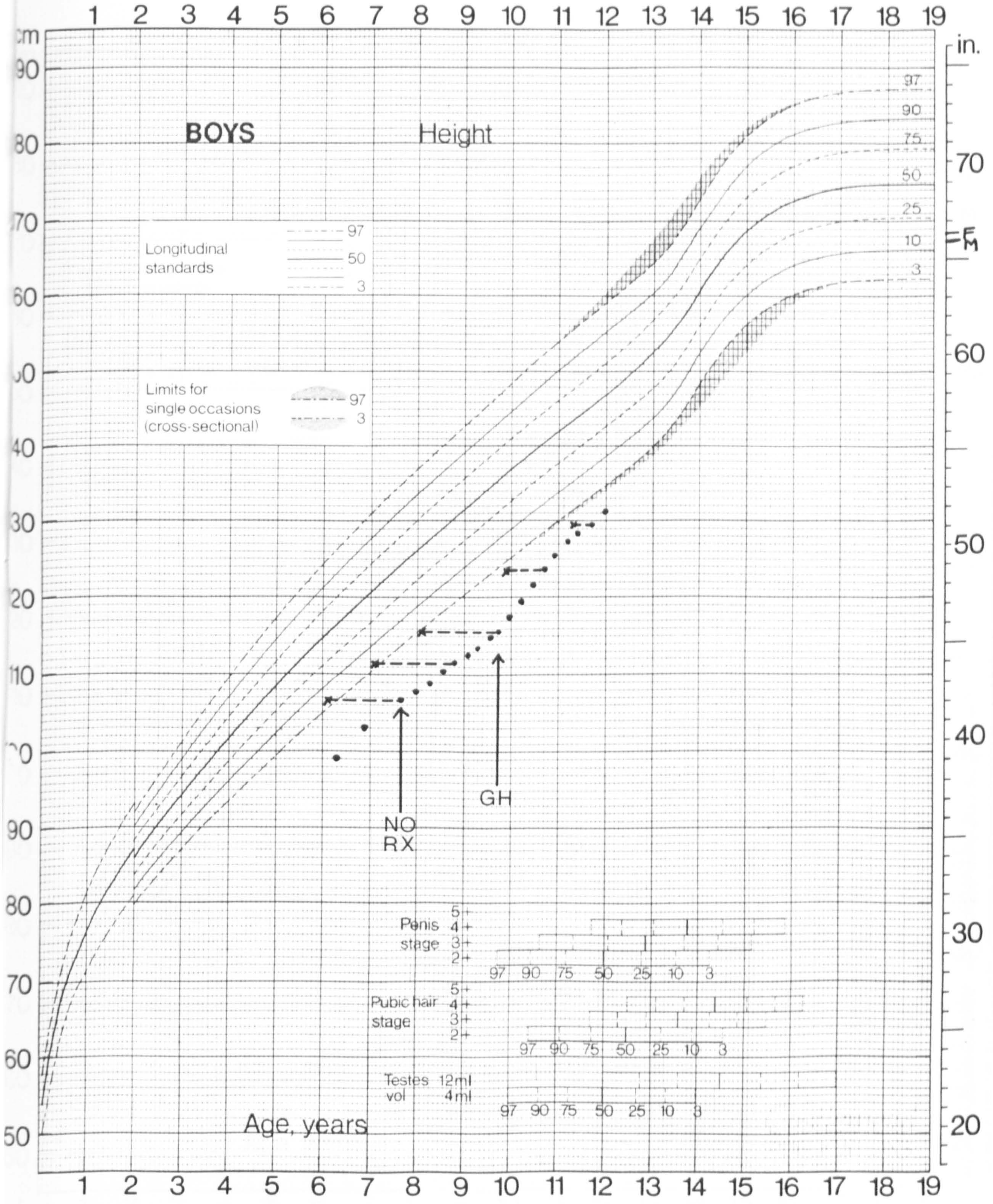
F

Age, years

in

cm

PATIENT 1.18
No treatment, then rhGH 15iu/m²/wk



PATIENT 1.23
PLACEBO, then rhGH 15 in/sq m/wk

BOYS Height

Longitudinal standards — 97, 50, 3

Limits for single occasions (cross-sectional) — 97, 3

PL

GH

Penis stage — 5+, 4+, 3+, 2+ — 97 90 75 50 25 10 3

Pubic hair stage — 5+, 4+, 3+, 2+ — 97 90 75 50 25 10 3

Testes vol — 12ml, 4ml — 97 90 75 50 25 10 3

Age, years

cm: 90 80 70 60 50 40 30 20 10 90 80 70 60 50

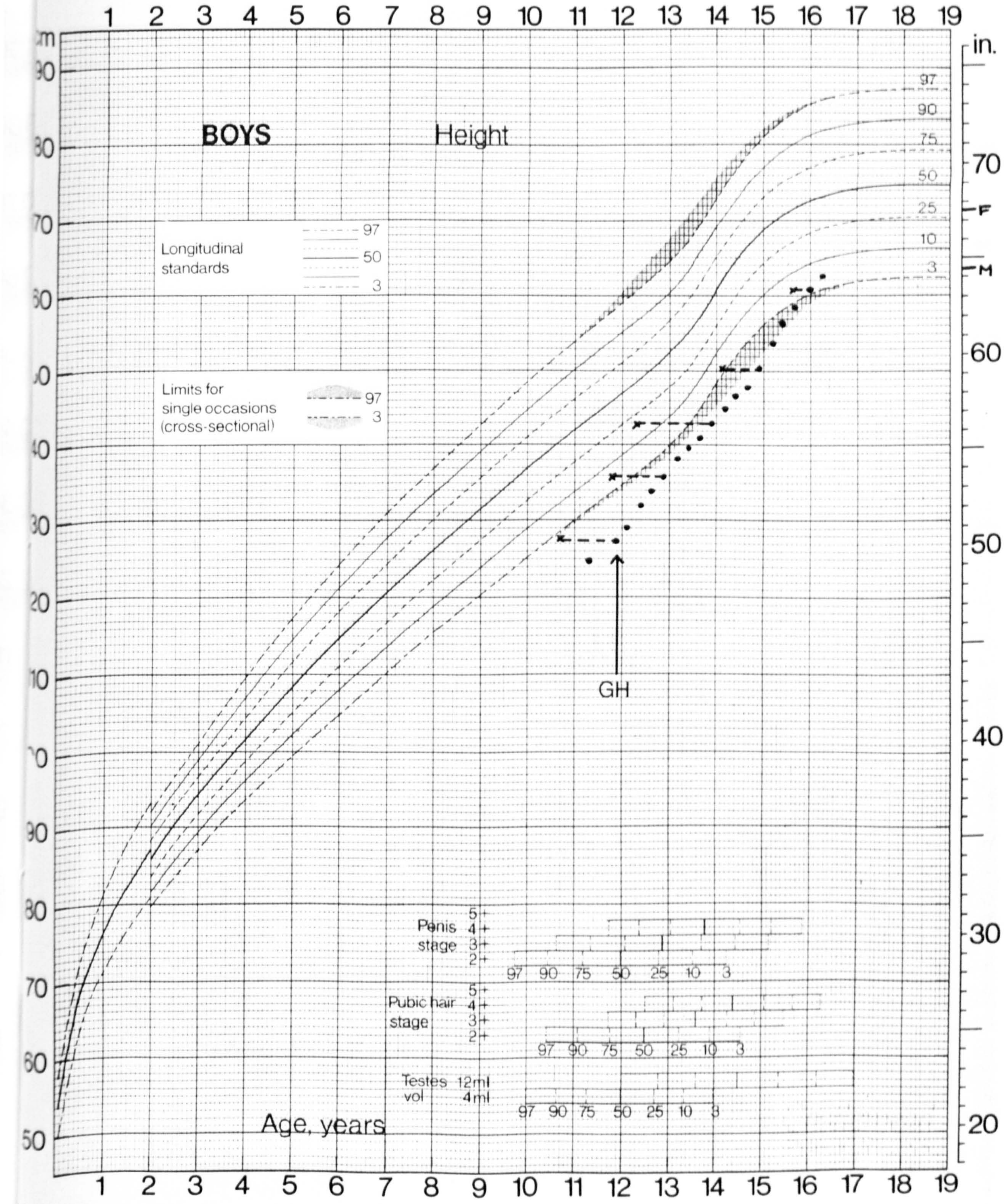
in.: 70 60 50 40 30 20

M F

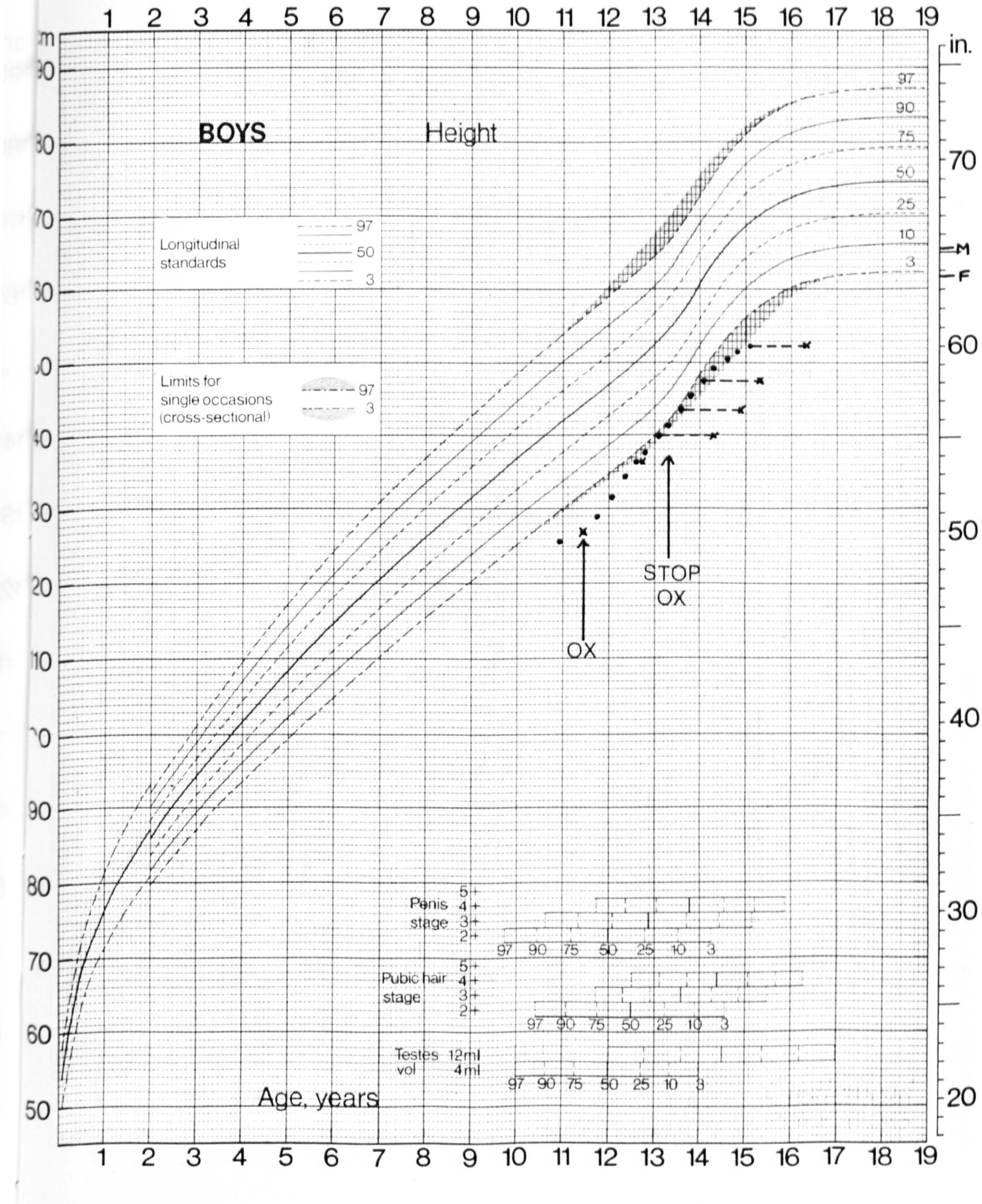
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

PATIENT 2.9.
rhGH plus oxandrolone

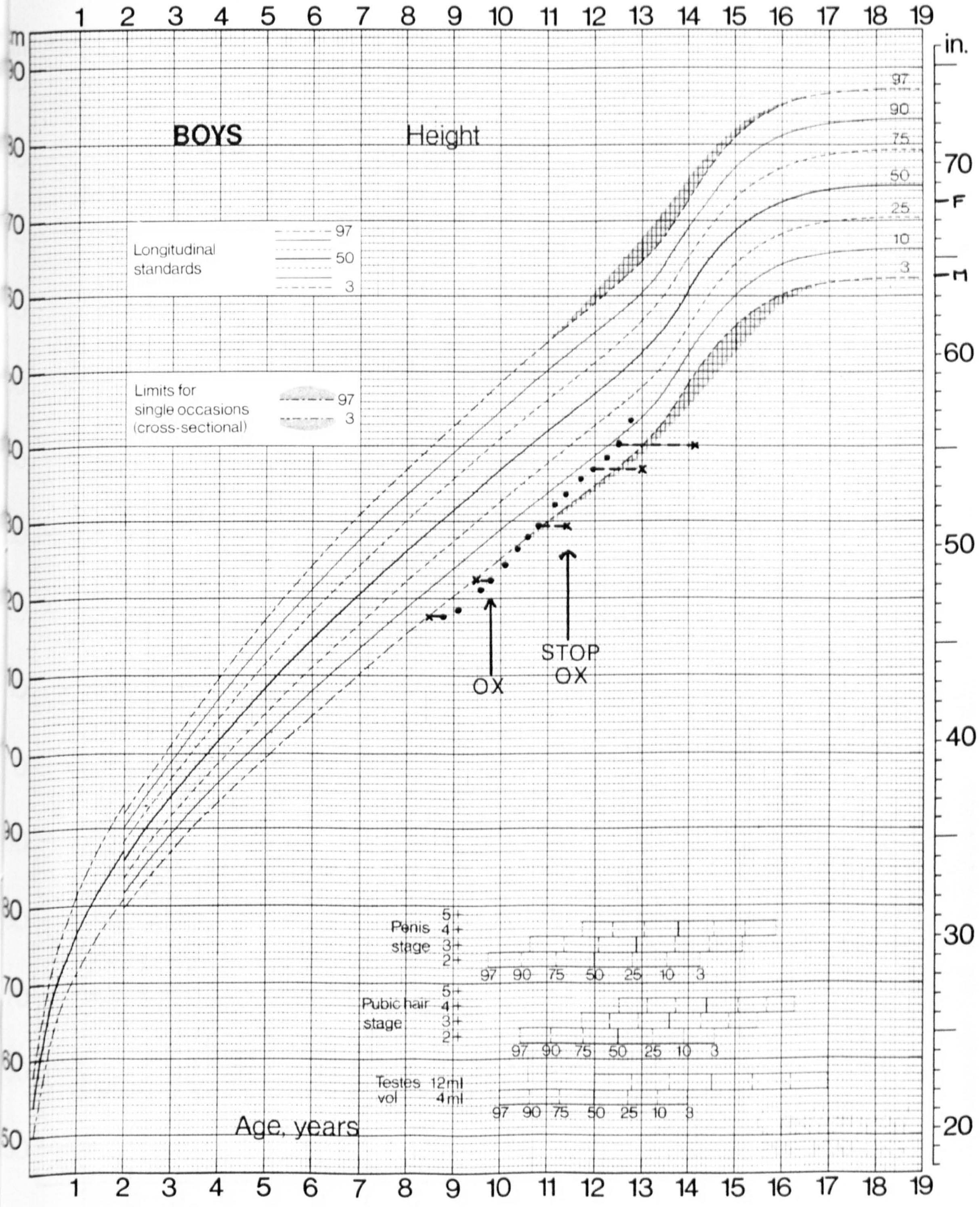
PATIENT 2.16
rhGH 24 in/sq.m/wk



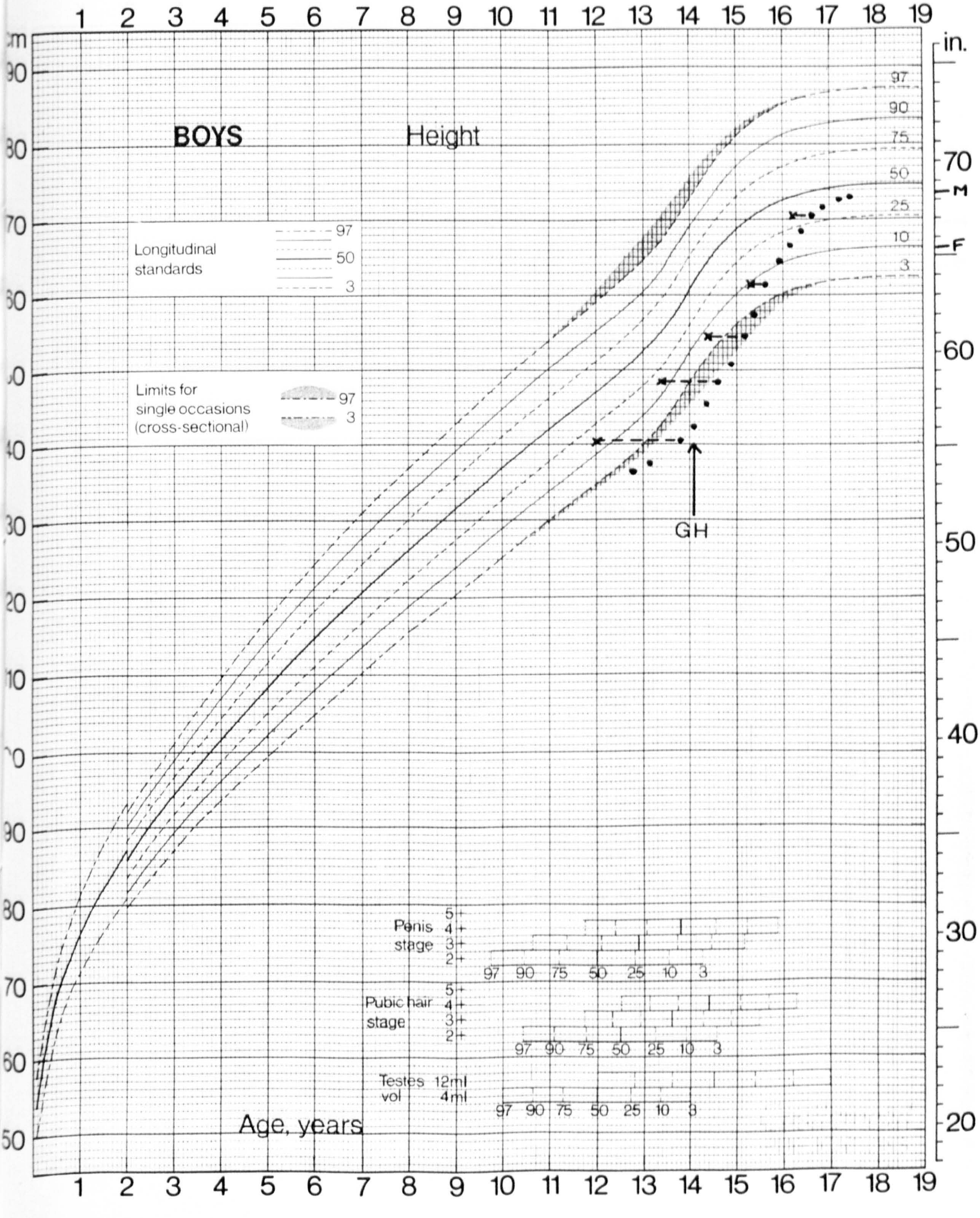
PATIENT 2.22
Oxandrolone



PATIENT 2.34
Oxandrolone

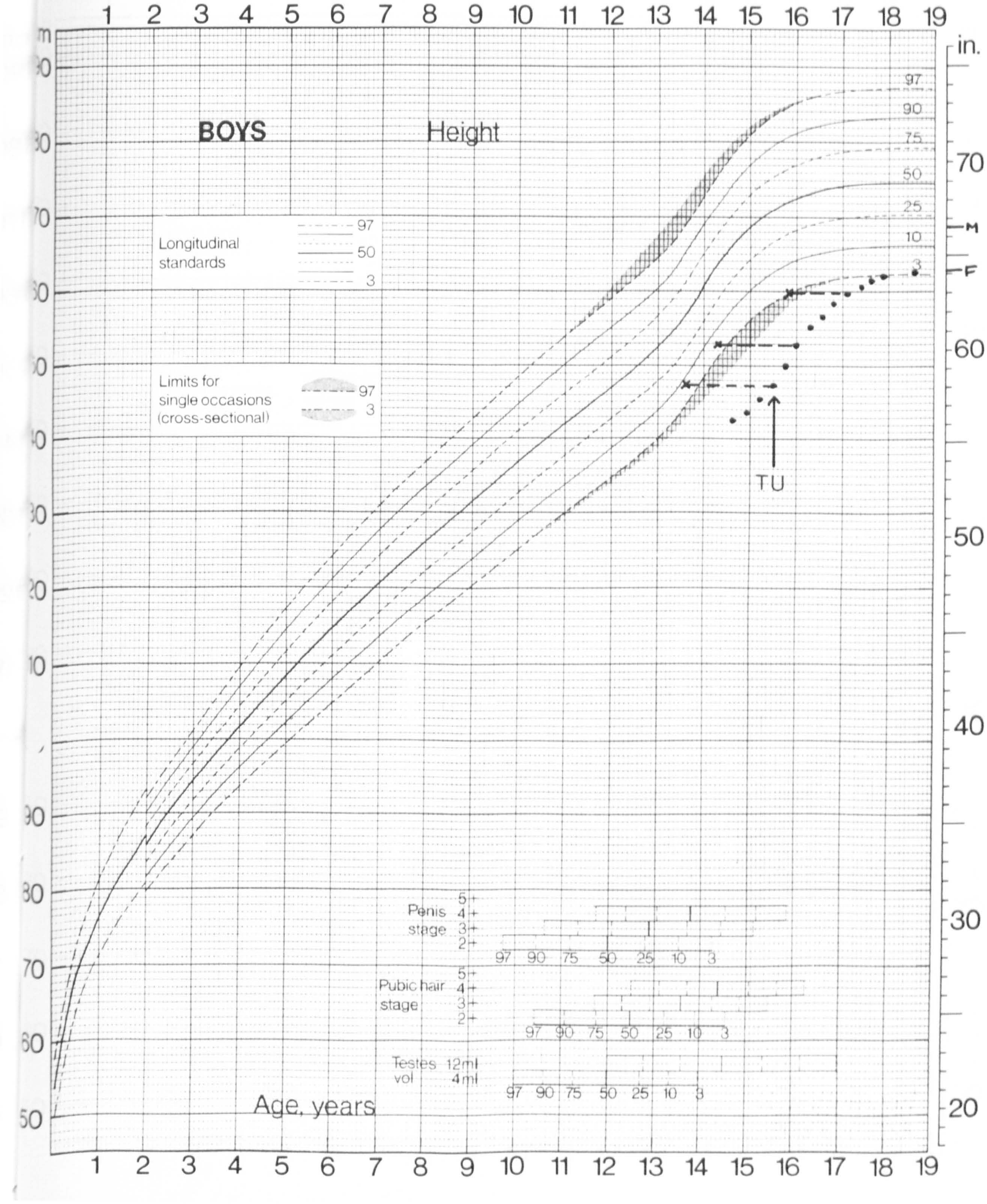


PATIENT 3.1
rhGH



PATIENT 3.2.

Testosterone undecanoate



PATIENT 3.15
rhGH plus Testosterone undecanoate

BOYS Height

Longitudinal standards — 97, 50, 3

Limits for single occasions (cross-sectional) — 97, 3

GH TU

Penis stage — 5, 4, 3, 2 — 97 90 75 50 25 10 3

Pubic hair stage — 5, 4, 3, 2 — 97 90 75 50 25 10 3

Testes vol — 12ml, 4ml — 97 90 75 50 25 10 3

Age, years

cm: 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50

in.: 70 60 50 40 30 20

F

M

REFERENCES

Ackland FM, Jones J, Buckler JMH, Dunger DB, Rayner PHW, Preece MA. 1990.
Growth hormone treatment in non-growth hormone deficient children: effects of stopping treatment.
Acta Paediatr Scand (suppl) 366: 32-7.

Albanese A, Stanhope R. 1993.
Does constitutional delayed puberty cause segmental disproportion and short stature?
Eur J Pediatr 152: 293-6.

Albertsson-Wikland K. 1986.
Growth hormone treatment in short children.
Acta Paediatr Scand (Suppl) 325: 64-70.

Albertsson-Wikland K, Rosberg S. 1988.
Analyses of 24-hour growth hormone profiles in children: relation to growth.
J Clin Endocrinol Metab 67: 493-500.

Alen M, Rahkila P, Reinila M, Vihko R. 1987.
Androgenic-anabolic steroid effects on serum thyroid, pituitary and steroid hormones in athletes.
Am J Sports Med 15: 357-61.

Aynsley-Green A, Zachmann M, Prader A. 1976.
Interrelation of the therapeutic effects of growth hormone and testosterone on growth in hypopituitarism.
J Pediatr 89: 992-9.

Bala RM, Lopatka J, Leung A, McCoy E, McArthur RG. 1981.
Serum immunoreactive somatomedin levels in normal adults, pregnant women at term, children at various ages, and children with constitutionally delayed growth.
J Clin Endocrinol Metab 52: 508-12.

Bayley N, Gordan GS, Lisser H. 1957.
Long-term experiences with methyltestosterone in short immature boys.
Pediatr Clin North Am 4: 819-25

Bettmann HK, Goldman HS, Abramowicz M, Sobel EH. 1971.
Oxandrolone treatment of short stature: effect on predicted mature height.
J Pediatr 79: 1018-23.

Bierich JR, Nolte K, Drews K, Brugmann G. 1992.
Constitutional delay of growth and adolescence. Results of short-term and long-term treatment with GH.
Acta Endocrinologica 127: 392-6.

Boulton TJC, Dunn SM, Quigley CA, Taylor JJ, Thompson L et al. 1991.
Perceptions of self and short stature: effects of two years of growth hormone treatment.
Acta Paediatr Scand (suppl) 377: 20-7.

Boulton TJC, Smith R, Single T. 1992.
Psychosocial growth failure: a positive response to growth hormone and placebo.
Acta Paediatr 81: 322-5.

Bowman S. 1990.
Anabolic steroids and infarction.
BMJ 300: 750.

Bramswig JH, Fasse M, Holthoff M-L, von Lengerke HJ, von Petrykowski W, Schellong G. 1990.
Adult height in boys and girls with untreated short stature and constitutional delay of growth and puberty: accuracy of five different methods of height prediction.
J Pediatr 117: 886-91.

Brook CGD. 1971
Determination of body composition of children from skinfold measurements.
Arch Dis Child 46: 182-4.

Brown P, Preece MA, Will RG. 1992.
"Friendly fire" in medicine : hormones, homografts, and Creutzfeldt-Jakob disease
Lancet 340: 24-7

Buchanan CR, Law CM, Milner RDG. 1987.
Growth hormone in short, slowly growing children and those with Turner's syndrome.
Arch Dis Child 62: 912-6

Buckler J. 1990.
A longitudinal study of adolescent growth.
Springer-Verlag London Limited.

Butler GE, Sellar RE, Walker RF, Hendry M, Kelnar CJH, Wu FCW. 1992.
Oral testosterone undecanoate in the management of delayed puberty in boys: pharmacokinetics and effects on sexual maturation and growth.
J Clin Endocrinol Metab 75: 37-44.

Buyukgebiz A, Hindmarsh PC, Brook CGD. 1990.
Treatment of constitutional delay of growth and puberty with oxandrolone compared with growth hormone.
Arch Dis Child 65: 448-52.

Cara JF, Rosenfeld RL, Furlanetto RW. 1987.
A longitudinal study of the relationship of plasma somatomedin-C concentration to the pubertal growth spurt.
Am J Dis Child 141: 562-4.

Chalew SA, Udoff LC, Hanukoglu A, Bistritzer T, Armour KM, Kowarski AA. 1988.
The effect of testosterone therapy on spontaneous growth hormone secretion in boys with constitutional delay.
Am J Dis Child 142: 1345-8.

Clayton PE, Shalet SM, Price DA, Addison GM. 1988.
Growth and growth hormone responses to oxandrolone in boys with constitutional delay of growth and puberty (CDGP).
Clin Endocrinol 29: 123-30.

Conners CK.
Conners' Rating Scales Manual.
MHS.

Corvol M-T, Carrascosa A, Tsagris L, Blanchard O, Rappaport R. 1987.
Evidence for a direct in vitro action of sex steroids on rabbit cartilage cells during skeletal growth: influence of age and sex.
Endocrinology 120: 1422-9.

Cowell CT. 1990.
Effects of growth hormone in short, slowly growing children without growth hormone deficiency.
Acta Paed Scand (suppl)366: 29-30.

Crofton PM. 1992.
Wheat-germ lectin affinity electrophoresis for alkaline phosphatase isoforms in children: age-dependent reference ranges and changes in liver and bone disease.
Clin Chem 38: 663-700.

Crowne EC, Shalet SM, Wallace WHB, Eminson DM, Price DA. 1990.
Final height in boys with untreated constitutional delay in growth and puberty.
Arch Dis Child 65: 1109-1112.

Dalboge H, Dahl H-HM et al. 1987.
A novel enzymatic method for production of authentic hGH from an E.Coli produced hGH-precursor.
Bio-Technology 5: 161-4

Danowski TS, Lee FA, Cohn RE, D'Ambrosia RD, Limaye NR. 1965.
Oxandrolone therapy of growth retardation.
Amer J Dis Child 109: 526-532.

Darendeliler F, Hindmarsh PC, Preece MA, Cox L, Brook CGD. 1990.
Growth hormone increases rate of pubertal maturation
Acta Endocrinologica (Copenh) 122, 3: 414-6.

Darendeliler F, Hindmarsh PC, Brook CGD. 1990.
Dose-response curves for treatment with biosynthetic human growth hormone.
J Endocrinol 125: 311-6.

Dean HJ, Kellett JG, Bala RM, Guyda HJ, Bhaumick B, Posner BI et al. 1982.
The effect of growth hormone treatment on somatomedin levels in growth hormone deficient children.
J Clin Endocrinol Metab. 55: 11267-73.

Dean HJ, McTaggart TL, Fish DG, Friesen HG, 1985.
The educational, vocational and marital status of growth hormone deficient adults treated with growth hormone during childhood.
Am J Dis Child 139: 1105-10.

de Lange WE, Snoep MC, Doorenbos H. 1979.
The effect of short-term testosterone treatment in boys with delayed puberty.
Acta Endocrinologica 91: 177-83.

de Swiet M, Fayers P, Shinebourne EA. 1992.
Blood pressure in the first 10 years of life : the Brompton study
BMJ 304: 23-6.

Diekama DS. 1990.
Is taller really better? Growth hormone treatment in short children.
Perspectives in Biology and Medicine 34: 109-23.

Douglas JWB, Ross JM. 1964.
Age of puberty related to educational ability, attainment and school leaving age.
J Child Psychol Psychiatr 5: 185-96.

Duke PM, Carlsmith JM, Jennings D, Martin JA, Dornbusch SM, Gross RT et al 1982.
Educational correlates of early and late sexual maturation in adolescence.
J Pediatr 100: 633-7.

Durnin JVGA, Womersley J. 1974.
Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years.
Br J Nutr 32: 77-97

Eastman CJ, Lazurus L, Stuart MC, Casey JH. 1971.
The effect of puberty on growth hormone secretion in boys with short stature and delayed adolescence.
Aust NZ J Med 2: 154-9.

Editorial. 1992.
Call to halt US growth hormone trials in children.
Lancet 340: 110.

Ferenchick GS. 1991.
Are androgenic steroids thrombogenic?
NEJM 322: 476.

Finkelstein JS, Neer RM, Biller BMK, Crawford JD, Klibanski A. 1992.
Osteopenia in men with a history of delayed puberty.
NEJM 326: 600-4.

Foster CM, Hopwood NJ, Hassing JM, Hale PM, Mendes T, Kelch RP et al. 1989.
Nocturnal serum growth hormone concentration is not augmented by short-term testosterone infusion in pubertal boys,
Ped Res 26: 320-4.

Fox M, Minit AS, Liddle GW. 1962.
Oxandrolone: a potent anabolic steroid of novel chemical configuration.
J Clin Endocrinol Metab 22: 921-4.

Geere G, Jones J, Atherden SM, Grant DB. 1980.
Plasma androgens after a single oral dose of testosterone undecanoate.
Arch Dis Child 55: 218-20.

Geller J. 1968.
Oxandrolone effect on growth and bone age in idiopathic growth failure.
Acta Endocrinologica 59: 307-16.

Gertner JM, Genel M, Gianfredi SP, Hintz RL, Rosenfeld RG,
Tamborlane WV, Wilson DM. 1984.
Prospective clinical trial of human growth hormone in short children without growth hormone deficiency.
J Pediatr 104: 172-6.

Gold R. 1978.
Constitutional growth delay and learning problems.
J Learn Dis 11: 36-8.

Gooren LJG. 1986.
Long-term safety of oral androgen testosterone undecanoate.
Int J Androl 9: 21-6.

Gordon M, Crouthamel C, Post EM, Richman RA. 1982.
Psychosocial aspects of constitutional short stature: Social competence, behaviour problems, self esteem and family functioning.
J Pediatr 101: 477-80.

Gordon M, Post EM, Crouthamel C, Richman RA. 1984.
Do children with constitutional delay really have more learning problems?
J Learn Dis 17: 291-3

Gregory JW, Greene SA, Jung RT et al.??
Metabolic effects of growth hormone treatment: an early predictor of growth response?
Arch Dis Child 68: 205-9.

Gregory JW, Greene SA, Thompson J, Scrimgeour CM, Rennie MJ. 1992.
Effects of oral testosterone undecanoate on growth, body composition, strength and energy expenditure of adolescent boys.
Clin Endocrinol 37: 207-13.

Grokett BH, Ahmad N, Warren DW. 1992.
The effects of an anabolic steroid (oxandrolone) on reproductive development in the male rat.
Acta Endocrinologica 126: 173-8.

Harter S. 1985.
Manual for the self-perception profile for children
University of Denver 1985.

Hindmarsh P, Brook CGD. 1987.
Effect of growth hormone on normal short children.
BMJ 295: 573-7.

Hindmarsh P, Smith PJ, Brook CGD, Matthews DR. 1987.
The relationship between height velocity and growth hormone secretion in short prepubertal children.
Clin Endocrinol 27: 581-91.

Hindmarsh P, Di Silvio L, Pringle PJ, Kurtz AB, Brook CGD. 1988.
Changes in serum insulin concentration during puberty and their relationship to growth hormone.
Clin Endocrinol 28: 381-8.

Hindmarsh PC, Smith PJ, Pringle PJ, Brook CGD. 1988.
The relationship between the response to growth hormone therapy and pretreatment growth hormone secretory status.
Clin Endocrinology 28: 1-5.

Hindmarsh PC, Pringle PJ, DeSilvo L, Brook CGD. 1990.
Effect of 3 years of growth hormone therapy in short normal children,
Acta Paediatr Scan (Suppl) 366: 6-12.

Hindmarsh PC, Brook CGD. 1992.
Long term effects of growth hormone (GH) treatment in short normal children.
Hormone Research 37.S4: 2

Hintz RL, Rosenfeld RG, Wilson DM et al. 1982.
Biosynthetic methionyl human growth hormone is biologically active in adult man.
Lancet: 1276-9.

Hirschhauser C, Hopkinson CRN, Sturm G, Coert A. 1974.
Testosterone undecanoate: a new orally active androgen.
Acta Endocrinologica 80: 179-87.

Hoare P, Elton R, Greer A, Kerley S. 1993.
The modification and standardisation of the Harter self-esteem questionnaire with Scottish school children.
Eur Child and Adol Psychiat 2: 19-33.

Holmes CS, Hayford JT, Thompson RG. 1982.
Parents and teachers differing views of short children's behaviour.
Child: Care, health and development 8; 327-36.

Holmes CS, Karksson JA, Thompson RG. 1985.
Social and school competencies in children with short stature : longitudinal patterns
Developmental and Behavioural Pediatrics 6: 263-7

Holmes CS, Karksson JA, Thompson RG. 1986.
Longitudinal evaluation of behaviour patterns in children with short stature in Stabler B, Underwood LE (ed). 1986. Slow grows the child. Psychosocial aspects of growth delay. Hillsdale, LawrenceErlbaum Associates : 1-13

Hopwood NJ, Kelch RP, Zipf WB, Hernandez RJ. 1979.
The effect of synthetic androgens on the hypothalamo-pituitary-gonadal axis in boys with constitutionally delayed growth.
J Pediatr 94: 657-662.

Hopwood NJ, Hintz RL, Gertner JM, Attie KM, Johanson AJ, Baptista J et al. 1993.
Growth response of children with non-growth-hormone deficiency during three years of growth hormone therapy.
J Pediatr 123: 215-22.

Hughes IA 1989.
Handbook of endocrine investigations in children
Butterworth and Co.

Jackson ST, Rallison ML, Buntin WH, Johnson SB, Flynn RR. 1973.
Use of oxandrolone for growth stimulation in children.
Am J Dis Child 126: 481-4.

Jones MC. 1957.
The later careers of boys who were early or late-maturing.
Child Devel 28: 113-28.

Jones MC, Bayley N. 1950.
Physical maturing among boys as related to behaviour.
J Educat Psychol 41: 129-47.

Joss EE, Schmidt HA, Zuppinger KA. 1989.
Oxandrolone in constitutionally delayed growth, a longitudinal study up to final height.
J Clin Endocrinol Metab 69: 1109-15.

Kaplan JG, Moshang Jr T, Bernstein R, Parks JS, Bongiovanni AM. 1973.
Constitutional delay of growth and development: effects of treatment with androgens.
J Pediatr 82: 38-44.

Kaplan SL, Underwood LE, August GP et al. 1986.
Clinical studies with recombinant-DNA-derived methionyl human growth hormone in growth hormone deficient children.
Lancet 1: 697-700.

Keenan BS, Richards GE, Ponder SW, Dallas JS, Nagamani M, Smith ER. 1993.
Androgen-stimulated pubertal growth: the effects of testosterone and dihydrotestosterone on growth hormone and insulin-like growth factor-1 in the treatment of short stature and delayed puberty.
J Clin Endocrinol Metab 76: 996-1001.

Kelnar CJH, Tanaka T. 1994.
Is there a place for short bursts of growth hormone treatment in short children without growth hormone deficiency?
Acta Paediatr Suppl : in press.

Koch TK, Berg BO, De Armond SJ, Gravina RF. 1985.
Creutzfeldt-Jakob disease in a young adult with idiopathic hypopituitarism.
NEJM 313: 731-3

Kowarski AA, Schneider J, Ben-Galim E, Weldon VV, Daughaday WH. 1978.
Growth failure with normal serum RIA-GH and low somatomedin activity: somatomedin restoration and growth acceleration after exogenous GH.
J Clin Endocrinol Metab 47: 461-4.

Knuth UA, Maniera H, Nieschlag E. 1989.
Anabolic steroids and semen parameters in bodybuilders.
Fertil Steril 52: 1041-7.

Kusalic M, Fortin C, Gauthier Y. 1972.
Psychodynamic aspects of dwarfism: response to growth hormone treatment.
Canad Psychiat Ass J 17: 29-34.

LaFranchi S, Hanna CE, Mandel SH. 1991.
Constitutional delay of growth: expected versus final adult height.
Pediatrics 87: 82-7.

Lamb DR. 1984.
Anabolic steroids in athletics: how well do they work and how dangerous are they?
Am J Sports Medicine 12: 31-8.

Landon J, Wynn V, Houghton BJ, Cooke JNC. 1962.
Effects of anabolic steroid, methandienone, on carbohydrate metabolism in man II. Effect of methandienone on response to glucagon, adrenalin, and insulin in the fasted subject.
Metabolism 11: 513-23.

Landon J, Wynn V, Samols E. 1963.
The effect of anabolic steroids on blood sugar and plasma insulin levels in man.
Metabolism 12: 924-35.

Lanes R, Bohorquez L, leal V, hernandez G, Borges M, Hurtado E, Moncada G. 1986.
Growth hormone secretion in patients with consitutional delay of growth and pubertal development.
J Pediatr 109: 781-3.

Lantos J, Siegler M, Cuttler L. 1989.
Ethical issues in growth hormone therapy.
JAMA 261: 1020-4.

Larsson B, Vaara I. 1992.
Cholesterol screening of seven-year-old children. How to identify children at risk.
Acta Paediatr 81: 315-8.

Lennon D, Saunders FJ. 1964.
Anabolic activity of 2-oxa-17 alpha methyl dihydrotestosterone (oxandrolone) in castrated rats.
Steroids 4: 689.

Limbeck GA, Ruvalcaba RHA, Mahoney CP, Kelley VC. 1971.
Studies on anabolic steroids. IV. The effects of oxandrolone on height and skeletal maturation in uncomplicated growth retardation.
Clin Pharm and Therapeutics 12: 798-805.

Lin T-H, Kirkland RT, Sherman BM, Kirkland JL. 1989.
Growth hormone testing in short children and their response to growth hormone therapy.
J Pediatr 115: 57-63.

Link K, Blizzard RM, Evans WS, Kaiser DL, Parker MW, Rogol AD. 1986.
The effect of androgens on the pulsatile release and the twenty-four-hour mean concentration of growth hormone in peripubertal males.
J Clin Endocrinol Metab 62: 159-64.

Loche S, Corda R, Lampis A, Puggioni R, Cella SG, Muller EE et al. 1986.
The effect of oxandrolone on the growth hormone response to growth hormone releasing hormone in children with constitutional growth delay.
Clin Endocrinol 25: 195-200.

Macintyre S. 1988.
Social correlates of human height.
Sci Prog, Oxford 72: 493-510.

McCaughey ES, Voss LD, Mulligan J, Betts PR. 1993.
Metabolic consequences of high dose GH treatment.
British Paediatric Association : 47.

Malhotra A, Poon E, Tse W-Y,Pringle PJ, Hindmarsh PC, Brook CGD. 1993.
The effects of oxandrolone on the growth hormone and gonadal axes in boys with constitutional delay of growth and puberty.
Clin Endocrinology 38: 393-8.

Marshall WA, Tanner JM. 1969.
Variations in the patterns of pubertal changes in girls.
Arch Dis Child 44: 291-303.

Marshall WA, Tanner JM. 1970.
Variations in the patterns of pubertal changes in boys.
Arch Dis Child 45: 13-23.

Martha Jr PM, Rogol AD, Veldhus JD, Kerrigan JR, Goodman DW, Blizzard RM. 1989.
Alterations in the pulsatile properties of circulating growth hormone concentrations during puberty in boys.
J Clin Endocrinol Metab 69: 563-70.

Marti-Henneberg C, Niirianen AK, Rappaport R. 1975.
Oxandrolone treatment of constitutional short stature in boys during adolescence: effect on linear growth, bone age, pubic hair, and testicular development.
J Pediatr 86: 783-8.

Martin MM, Martin ALA, Mossman KL. 1986.
Testosterone treatment of constitutional delay in growth and development: effect of dose on predicted versus definitive height.
Acta Endocrinologica suppl 279: 147-52.

Mauras N, Blizzard RM, Link K, Johnson ML, Rogol AD, Veldhus JD. 1987.
Augmantation of growth hormone secretion during puberty: evidence for a pulse amplitude-modulated phenomenon.
J Clin Endocrinol Metab 64: 596-601.

Melvin KEW, Wright AD, Hartog M, Antcliff AC, Copestake AM, Fraser TR. 1967.
Acute metabolic response to human growth hormone in different types of dwarfism.
BMJ. 196-9.

Meyer-Bahlburg HFL, Feinman JA, MacGillivray MH, Aceto Jr T. 1978.
Growth hormone deficiency, brain development, and intelligence.
Am J Dis Child 132: 565-72.

Miller JD, Tannenbaum GS, Colle E, Guyda HJ. 1982.
Daytime pulsatile growth hormone secretion during childhood and adolescence.
J Clin Endocrinol Metab 55: 989-94.

Milner RDG, Russell-Fraser T, Brook CGD et al. 1979.
Experience with human growth hormone in Great Britain : the report of the MRC working party.
Clinical Endocrinology 11: 15-38.

Mitchell CM, Joyce S, Johanson AJ, Libber S, Plotnick L, Migeon CJ et al. 1986.
A retrospective evaluation of psychosocial impact of long-term growth hormone therapy.
Clin Ped 25: 17-23.

Moore WV, Moore KC, Gifford R, Hollowell JG, Donaldson DL. 1992.
Long-term treatment with growth hormone of children with short stature and normal growth hormone secretion.
J Pediatr 120: 720-8.

Money J, Pollitt E. 1966.
Studies in the psychology of dwarfism II. Personality maturation and the response to growth hormone in hypopituitary dwarfs.
J Paediatr 68: 381-90.

Mussen PH, Jones MC. 1957.
Self-conceptions, motivations, and interpersonal attitudes of late and early maturing boys.
Child Development 28: 243-56.

Nieschlag E, Mauss J, Coert A, Kicovic P. 1975.
Plasma androgen levels in men after oral administration of testosterone or testosterone undecanoate.
Acta Endocrinologica 79: 366-74.

Papadimitriou A, Wacharasindhu S, Pearl K, Preece MA, Stanhope R. 1991.
Treatment of constitutional growth delay in prepubertal boys with a prolonged course of low dose oxandrolone
Arch Dis Child 66: 841-3.

Pirazzoli P, Cacciari E, Mandini M, Sganga T, Capelli M, Cicognani A, Gualandi S. 1992.
Growth and thyroid function in children treated with growth hormone.
J Pediatr 121: 210-3.

Plotnick LP, Van Meter CDRQ, Kowarski AA. 1983.
Human growth hormone treatment of children with growth failure and normal growth hormone levels by immunoassay: lack of correlation with somatomedin generation.
Pediatrics 71: 324-7.

Pollitt E, Money J. 1964.
Studies in the psychology of dwarfism I. Intelligence quotient and school achievement.
J Pediatr 64: 415-21.

Powell-Jackson J, Weller RO, Kennedy P, Preece MA, Whitcombe EM, Newsom-Davis J. 1985
Creutzfeldt-Jakob disease after administration of human growth hormone.
Lancet : 244-6

Preece MA, Tanner JM, Whitehouse RH, Cameron N. 1976.
Dose dependence of growth response to human growth hormone in growth hormone deficiency.
J Clin Endocrinol Metab 42: 477-83.

Raben MS. 1958.
Treatment of a pituitary dwarf with human growth hormone
J Clin Endocrinol Metab 18: 901-3.

Raben MS. 1959.
Human growth hormone
Recent Progress in Hormone Research 15: 71-114.

Raiti S, Trias E, Levitsky L, Grossman MS. 1973.
Oxandrolone and human growth hormone.
Am J Dis Child 126: 597-600.

Rasmussen LH, Zachmann M, Nilsson P. 1988.
Authentic recombinant human growth hormone. Results of a multicentre clinical trial in patients with growth hormone deficiency.
Helv Paediat Acta 43: 443-8.

Report of the Committee on Growth Hormone Use of the Lawson Wilkins Pediatric Endocrine Society, May 1985
The Journal of Pediatrics 107: 10-12.

Rose SR, Municchi G, Barnes KM, Kamp GA, Uriarte M et al. 1991.
Spontaneous growth hormone secretion increases during puberty in normal boys and girls.
J Clin Endocrinol Metab 73: 428-35.

Rosenfeld RG, Northcraft GB, Hintz RL. 1982.
A prospective, randomized study of testosterone treatment of constitutional delay of growth and development in male adolescents.
Pediatrics 69: 681-7.

Rosenfield RL, Furlanetto R, Brock D. 1983.
Relationship of somatomedin-C concentrations to pubertal changes.
J Pediatr 103: 723-8.

Rosenfield RL, Furlanetto R. 1985.
Physiologic testosterone or estradiol induction of puberty increase plasma somatomedin-C.
J Pediatr 107: 415-7.

Rotnem D, Cohen DJ, Hintz RL. et al 1979.
Psychological sequelae of relative "treatment failure" for children receiving growth hormone replacement.
J Am Acad Child Psychiat 18: 505-20.

Rudman D, Kutner MH, Blackston RD, Jansen RD, Patterson JH. 1979.
Normal variant short stature: subclassification based on responses to exogenous human growth hormone
J Clin Endocrinol Metab 49: 92-9.

Rudman D, Kutner MH, Goldsmith MA, Kenny J, Jennings H, Bain RP. 1980.
Further observations on four subgroups of normal variant short stature.
J Clin Endocrinol Metab 51: 1378-84.

Rudman D, Kutner MH, Blackston RD, Cushman RA, Bain RP, Patterson JH. 1981.
Children with normal-variant short stature : treatment with human growth hormone for six months.
NEJM 305: 123-31.

Sansoy OM, Roy AN, Shields LM. 1971.
Anabolic action and side effects of oxandrolone in 34 mental patients.
Geriatrics 26: 139-43.

Sartorio A, Morabito F, peri G, Conti A, Faglia G. 1990.
The social outcome of adults with constitutional growth delay
J Endocrinol Invest 13: 593-5.

Shah A, Stanhope R, Matthew D. 1992.
Hazards of pharmacological tests of growth hormone secretion in childhood.
BMJ 304: 173-4.

Smith PJ, Hindmarsh PC, Brook CGD. 1988.
Contribution of dose and frequency of administration to the therapeutic effect of growth hormone.
Arch Dis Child 63: 491-4.

Sobel EH, Raymond CS, Quinn KV, Talbot NB. 1956.
The use of methyltestosterone to stimulate growth: relative influence on skeletal maturatiuon and linear growth.
J Clin Endocrinol Metab 16: 241-8.

Sobel EH. 1968.
Anabolic steroids.
in Clinical Endocrinology vol.2 789-87. eds. Ashwood AB, Cassidy CE

Spilliotis BE, August GP, Hung W, Sonis W, Mendelson W, Bercu BB. 1984.
Growth hormone neurosecretory dysfunction: a treatable cause of short stature.
JAMA 251: 2223-30.

Stace L, Danks DM. 1981.
A social study of dwarfing conditions II. The experience of children with bone duysplasias, and of their parents.
Aust Paediatr J 17: 172-6.

Stanhope R, Brook CGD. 1985.
Oxandrolone in low dose for constitutional delay of growth and puberty in boys.
Arch Dis Child 60: 379-81.

Stanhope R, Noone C, Brook CGD. 1985.
A search for the lowest dose of oxandrolone which is effective for the treatment of constitutional delay of growth and puberty in boys.
Ped Res 19: 634.

Stanhope R, Buchanan CR, Fenn GC, Preece MA. 1988.
Double blind placebo controlled trial of low dose oxandrolone in the treatment of boys with constitutional delay of growth and puberty.
Arch Dis Child 63: 501-5.

Steinhausen H-C, Stahnke N. 1976.
Psychoendocrinological studies in dwarfed children and adolescents.
Arch Dis Child 51: 778-83.

Tanner JM. 1962.
Growth at adolescence 2nd edition.
Oxford, Blackwell Sci. Pupl.

Tanner JM, Whitehouse RH, Talaishi M. 1966.
Standards from birth to maturity for height, weight, height velocity and weight velocity: British children 1965 Part I.
Arch Dis Child 41: 454-71.

Tanner JM, Whitehouse RH, Talaishi M. 1966.
Standards from birth to maturity for height, weight, height velocity and weight velocity: British children 1965 Part II.
Arch Dis Child 41: 613-35.

Tanner JM, Whitehouse RH, Hughes PCR, Vince FP. 1971.
Effect of human growth hormone treatment for 1-7 years on growth of 100 children with growth hormone deficiency, low birth weight, inherited smallness, Turner's syndrome, and other complaints.
Arch Dis Child 46: 745-82.

Tanner JM, Whitehouse RH. 1975.
Revised standards for triceps and subscapular skinfolds in British children.
Arch Dis Child 50: 142-5.

Tanner JM, Whitehouse RH. 1976.
Clinical longitudinal standards for height, weight, height velocity and weight velocity and the stages of puberty.
Arch Dis Child 51: 170-9.

Tanner JM, Hughes PCR, Whitehouse RH. 1977.
Comparative rapidity of response of height, limb muscle and limb fat to treatment with human growth hormone in patients with and without growth hormone deficiency.
Acta Endocrinologica 84: 681-96

Tanner JM, Landt KW, Cameron N, Carter BS, Patel J. 1983.
Prediction of adult height from height and bone age in childhood.
Arch Dis Child 58: 767-76.

Thomas M, de Muinck Keizer S, Drop S, Degenhart HJ. 1982.
Effects of oral testosterone undecanoate treatment in boys with delayed puberty.
Ped Res 16: 907.

Tse W-Y, Buyukgebiz A, Hindmarsh PC, Stanhope R, Preece MA, Brook CGD. 1990.
Long-term outcome of oxandrolone treatment in boys with constitutional delay of growth and puberty.
J Pediatr 117: 588-91.

Underwood LE. 1991.
The social cost of being short: societal perceptions and biases.
Acta Paediatr Scand (suppl) 377: 3-8.

United Kingdom Testicular Cancer Study Group. 1994.
Aetiology of testicular cancer: association with congenital abnormalities, age at puberty, infertility, and exercise.
BMJ 308: 1393-9.

Uruena M, Pantsiotou S, Preece MA, Stanhope R. 1992.
Is testosterone therapy for boys with constitutional delay of growth and puberty associated with impaired final height and suppression of the hypothalamo-pituitary-gonadal axis.
Eur J Pediatr 151: 15-8.

Van Vliet G, Styne DM, Kaplan SL, Grumbach MM. 1983
Growth hormone treatment for short stature
NEJM 309: 1016-22.

Volta C, Ghizzoni L, Buono T, Ferrari F, Virdis R, Bernasconi S. 1988.
Final height in a group of untreated children with constitutional growth delay.
Helv Paediat Acta 43: 171-6.

Voss LD, Bailey BJR, Mulligan J, Wilkin TJ, Betts PR. 1991.
Short stature and school performance - the Wessex growth study.
Acta Paed Scand (suppl) 377: 29-31.

Wales JKH, Milner RGD. 1989.
Changes in leg length and height during treatment with somatropin.
Arch Dis Child 64: 1708-12.

Walker J, Chaussain JL, Bougneres PF. 1989.
Growth hormone treatment of children with short stature increases insulin secretion but does not impair glucose disposal.
J Clin Endocrinol Metab 69: 253-8.

Walker JM, Bond SA, Voss LD, Betts PR, Wootton SA, Jackson AA. 1990.
Treatment of short normal children with growth hormone - a cautionary tale?
Lancet 336: 1331-4.

Webb OL, Laskarzewski PM, Glueck CJ. 1984.
Severe depression of high-density lipoprotein cholesterol levels in weight lifters and body builders by self-administered exogenous testosterone and anabolic-androgenic steroids.
Metabolism 33: 971-5.

Westaby D, Ogle SJ, Paradinas FJ, Randall JB, Murray-Lion IM. 1977.
Liver damage from long term methyltestosterone.
Lancet ii: 261-3.

Wiklund I, Albertsson-Wikland K, Wiren L. 1991.
How should the psychosocial impact of short stature and response to growth hormone treatment be assessed in children.
Acta Paediatr Scand (suppl) 377: 174-5.

Wilson DM, Frane JW, Sherman B, Johanson AJ, Hintz RL, Rosenfeld RG et al. 1988.
Carbohydrate and lipid metabolism in Turner syndrome: effect of therapy with growth hormone, oxandrolone, and a combination of both.
J Pediatr 112: 210-7.

Wilson DM, Kei J, Hintz RL, Rosenfeld RG. 1988.
Effects of testosterone therapy for pubertal delay.
Am J Dis Child 142: 96-9.

Wit JM, Fokker MH, de Muinck Keizer-Schrama SMPF, OOstdijk W, Gons M, Otten BJ et al (Dutch Growth hormone Working Group). 1989.
Effects of two years of methionyl growth hormone therapy in two dosage regimens in pre-pubertal children with short stature, subnormal growth rate, and normal growth hormone response to secretagogues.
J Pediatr 115: 720-5.

Wu FCW, Butler GE, Kelnar CJH, Sellar (1990)
Patterns of pulsatile luteinising hormone secretion before and during the onset of puberty in boys: a study using an immunoradiometric assay.
J Clin Endocrinol Metab 70: 629-37.

Wu FCW, Butler GE, Kelnar CJH, Stirling HF, Huhtaniemie I (1991)
Patterns of luteinising hormone and follicle stimulating hormone secretion in prepubertal (midchildhood) boys and girls and patients with idiopathic hypogonadotrophic hypogonadism (Kallmann's syndrome) : a study using an ultrasensitive time-resolved immunofluorometric assay
J Clin Endocrinol Metabol 72 1229-1237

Wu FCW, Brown DC, Butler GE, Stirling HF, Kelnar CJH. 1993.
Early morning plasma testosterone is an accurate predictor of imminent pubertal development in prepubertal boys.
J Clin Endocrinol Metab 76: 26-31.

Zachmann M, Prader A, Kind HP, Hafliger H, Budliger H. 1974.
Testicular volume during adolescence: cross sectional and longitudinal studies.
Helv Paediat Acta 29: 61-72.

Zadik Z, Landau H, Limoni Y, Lieberman E. 1992.
Predictors of response to growth hormone in otherwise normal short children.
J Pediatr 121: 44-8.

Zanganeh F, Steiner MM. 1967.
Oxandrolone therapy in growth retardation of children.
Amer J Dis Child 113: 234-241.

ACKNOWLEDGEMENTS

I would like to acknowledge the help, advice and encouragement of Dr. CJH Kelnar, Royal Hospital for Sick Children, without whose support this work would not have been achieved.

I would like to thank the following consultant paediatricians in Scotland for allowing me to include their patients in this study:
Dr R Simpson, Dr P Clow, Dr R Thomson, Dumfries and Galloway Royal Infirmary
Dr C Galloway, Raigmore Hospital Trust, Inverness
Dr M Donaldson, Royal Hospital for Sick Children, Glasgow
Dr A Blair, Dr M Macmillan, Dr C Steer, Victoria Hospital, Kirkcaldy

I acknowledge Novo Nordisk for supporting these studies and providing the rhGH.