Growth and Segmental Proportion in Children with Prepubertal Growth Delay and Constitutional Delay of Growth and Puberty: Natural History and the Effect of Therapeutic Intervention.

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SECTION 1:
ABSTRACT
Despite being considered a variant of normal, constitutional delay of growth and puberty (CDGP) is a condition which should be regarded as a disorder. Final height and body proportion attainment in boys and girls who had a delayed onset of pubertal development were retrospectively analysed. Final height was below both genetic target and the expected normal range for the adult population. A relatively short sitting height was a characteristic feature of delayed puberty at presentation and did not correct at final height attainment. Predictive factors of final height outcome at presentation were identified. Attempts have been made to prevent body segmental disproportion and hopefully improve final height attainment by treating growth delay before delayed puberty was manifest. Oxandrolone was used in short boys in late prepuberty and growth hormone (GH) in short children during childhood. Preliminary data suggest height and growth deceleration in sitting height may be preventable with early anabolic steroid treatment. High dose GH treatment improved short-term growth in these children, but this was followed by a significant growth deceleration when GH treatment ceased.

Doubts have been raised on the efficacy of oral testosterone to induce a growth acceleration in boys with CDGP. It has been demonstrated, using a randomised study, that a similar anthropometric response is obtained using either oral oxandrolone or oral testosterone. The interaction between sex/anabolic steroids and GH has been studied in clinical models to understand the mechanisms involved in the pubertal growth spurt. Finally the modulation of the somatotrophic - IGF-1 - IGF BP-3 axis and of gonadotrophin secretion following administration of GH to prepubertal short children with growth delay has been studied.
In conclusion these data suggest a new approach to therapy in CDGP may be necessary. Treatment may be appropriate not only for psychological reasons but also for optimising both final height and body proportion.
SECTION 2:
AIMS OF THE THESIS
The aims of this thesis were:

1. To determine whether children with CDGP reach a final height appropriate for their genetic potential.

2. To study the effect of delayed onset of pubertal maturation on segmental body proportion.

3. To identify predictive factors of final height outcome at presentation in boys with CDGP.

4. To attempt to prevent segmental body disproportion and improve final height by treating growth delay before delayed puberty becomes manifest.

5. To test the efficacy of oral testosterone versus oral oxandrolone to induce growth acceleration and secondary sexual characteristics.

6. To understand the mechanisms by which the synergistic action between growth hormone and sex steroids takes place during the pubertal growth acceleration.

7. To study the effect of pharmacological doses of growth hormone treatment on spontaneous growth hormone profile and gonadotrophin secretion in childhood.

Research in this field may increase our knowledge of normal and delayed sexual maturation and contribute to a different therapeutic approach aimed to optimise growth, bone accretion and psychological well being in the adolescent with constitutional delay of growth and puberty.
SECTION 3 :

HISTORICAL INTRODUCTION
3.1- Normal Pubertal Development and the Pubertal Growth Spurt

During puberty there is a progressive series of physical changes with the acquisition of secondary sexual characteristics due to the maturation of gonadal function, associated with growth acceleration and resulting in the attainment of fertility and final height. The average age for the onset of the first signs of pubertal maturation is 11.4 years in girls (Marshall et al, 1969) and 12.0 in boys (Marshall et al, 1970), with a mean difference in onset of 6 months, and are indicated by the onset of breast development and increase in testicular volume respectively. There is a close relationship between the appearance of secondary sexual characteristics and the onset of pubertal growth spurt and the sequence of this relationship differs between the sexes. In girls (Marshall et al, 1969) the onset of breast development is coincident with the onset of the growth acceleration, with a peak at Tanner stage 2 to 3 of breast maturation. Boys (Marshall et al, 1970) commence to grow at a faster rate 2 years later than girls as they commence to exhibit pubertal growth acceleration relatively late in puberty, when genitalia stage 3-4 (10 ml testicular volume) is attained. The overall height gain achieved during puberty is 25 cm in girls and 28 in boys (Tanner et al, 1976a). The mean height difference between adult men and women is 13 cm (Preece et al, 1992) and this results partly from +3 cm gained in boys from their more intense growth spurt, and partly from the 2 extra years of growth at around 5 cm/year in boys before the onset of the pubertal growth spurt. During the pubertal growth spurt, spinal growth is relatively delayed compared to leg length (Nielsen et al, 1986). Therefore, there is a stage of development when boys and girls have relatively long legs before attaining normal adult
proportion. Progress through puberty can take a considerable length of time. There is a variation in the time spent at any given stage of puberty but each stage of puberty (classified according to Tanner's standards) should last no more than 9 months and if longer, arrested puberty should be suspected (Tanner JM, 1962). Loss of consonance in the sequence of sexual maturation or in the relationship with the growth acceleration should always be investigated as it points to the presence of an endocrinopathy. Historical evidence suggests that puberty occurs at an earlier age today than in the past. During the last century in industrialised countries a "secular trend" in growth and puberty has occurred (Tanner JM, 1981) and children have grown at a faster rate and had an earlier onset of sexual maturation and menarche. This is mainly due to improved psychosocial-nutritional aspects as well as improved management of medical conditions and in many areas this has now slowed down or stopped.

3.2- Endocrinology of Puberty

In vertebrates the reproductive activity is closely regulated by the interaction between neuronal and endocrine systems: gonadal activity is regulated by the pituitary gland which is under the hypothalamic control. The timing of onset of pubertal development is also under neurologic control and is influenced by genetic and environmental factors. Puberty does not begin until the appearance of pulsatile GnRH secretion released by the hypothalamus. Pulsatile GnRH secretion induces the pulsatile release of gonadotrophin (LH) by the pituitary gonadotroph cells, which results in gonadal activation. The relation of pulsatile GnRH with pulsatile LH secretion has been described in monkeys (Knobil et al, 1978 and 1980) and in humans (Reiter EO, 1987a) and the induction of puberty has been
reproduced in immature monkeys (Wildt et al, 1980) and humans (Delemarre et al, 1983; Stanhope et al, 1987a) by exogenous administration of pulsatile GnRH.

During foetal life, GnRH-producing cells migrate from the medial olfactory placode of the nose to the preoptic area and anterior hypothalamus where they will be located for the remainder of life (Schwanzel-Fukuda et al, 1992). Following this anatomical step the hypothalamic-pituitary-gonadal axis becomes fully functional during foetal life (Siler-Khodr et al, 1974). Plasma gonadotrophin and pituitary contents gradually increase, with a maximum at 20 weeks of gestational age. At birth, a transient rise in gonadotrophin and gonadal sex steroids is seen and this is probably caused by the withdrawal of placental steroids (Winter et al, 1975 and 1976). During the first months of life plasma levels of gonadotrophins rise intermittently to adult values (Waldhauser et al, 1981). FSH pulsatility is greater in female infants while LH pulsatility is greater in boys. The high gonadotrophin levels are associated with increased serum sex hormones (testosterone in boys and oestrogen in girls). By 6 months of age in boys and 1-2 years in girls gonadotrophin levels decline to prepubertal values (Winter et al, 1975; Forest MG, 1990). With sensitive radio-immunoassays infrequent LH pulses have been detected in prepubertal children (Dunkel et al, 1990; Jakacki et al, 1982; Yen et al, 1993). The pulses have lower amplitude and frequency than those described in pubertal children or adults. The neuroendocrine hallmark of the onset of puberty can be identified by measuring LH during sleep: an increase of the amplitude of LH secretion during the night in response to an increase in pulsatile GnRH secretion is the first sign of puberty. Boyar (Boyar et al, 1974 and 1976) described the mainly sleep-associated pulsatile release of LH in early and midpuberty. An
enhanced release of LH can also be shown in response to intravenous exogenous GnRH (Dickerman et al, 1976). In boys the nocturnal increase of LH is associated with testosterone secretion (Boyar et al, 1974); in girls the rise in oestradiol levels occurs the next morning (Boyar et al, 1976). A day-night rhythm in FSH secretion has not been described. Later in puberty the LH secretion continues to increase both in frequency and amplitude until Tanner stage 4 in boys (Wennink et al, 1989) and Tanner stage 3 in girls (Wennink et al, 1990) are attained. The LH secretion then remains at a plateau level. In Fig. 1 the changes in the pattern of FSH and LH secretion in early infancy, childhood and puberty is illustrated in both sexes. In late puberty and in adulthood the LH day-night difference disappears and high amplitude pulses occur during the day as well as the night. Pulse frequency of gonadotrophin secretion has approximately 2 hour cycle in adult men (Spratt et al, 1988) and about 1 hour cycle during the mid-follicular phase in women (Crowley et al, 1985). It has been hypothesised that the regulation of the qualitative changes in LH bioactivity may be an important regulation factor of the pubertal development (Reiter et al, 1987b).

Different neuroendocrine mechanisms regulate the pituitary-gonadal function (Fig. 2 and 3). Depending on doses, time and previous hormonal status, gonadal hormones may inhibit or stimulate gonadotrophin secretion acting at the pituitary and/or hypothalamic levels (long-loop feedback); gonadotrophins may directly modulate their concentration, acting on hypothalamus (short-loop feedback) and finally GnRH may influence its own secretion (ultrashort-loop feedback). Other hormones are involved in the pituitary-gonadal axis regulation, such as inhibins, which are polypeptide hormones produced the gonads (Ying SY, 1988). Neuronal influences on pituitary-gonadal axis also derive from several parts of the
brain and are capable of either stimulation or inhibition. The mechanism by
which one releasing factor can control the secretion of both gonadotrophins
is still not completely understood. Changes in the frequency and probably in
the amplitude of GnRH secretion may modulate the pattern of FSH and LH
secretion (Grumbach et al, 1990). High frequency of GnRH secretion is
associated with relatively high levels of FSH secretion, as occur during the
follicular phase of the menstrual cycle (Reame et al, 1984), and lower
frequency is mainly associated with LH secretion, as during the luteal phase
(Reame et al, 1984).

Pulsatile nocturnal gonadotrophin secretion results in a multicystic ovarian
morphology (Stanhope et al, 1985), increase in testicular size, and gonadal
secretion of sex steroids, which, induce secondary sexual characteristics
and as a secondary action modulate hypothalamic function. Testosterone
is the main hormone produced by the Leydig cells of the testes in response
to LH secretion. It induces the development of a male body habitus and
change in the voice while dihydrotestosterone, derived by peripheral tissue
conversion of testosterone, induces the development of penis, prostate and
beard growth. Sperm in the urine (spermarche) occurs at an early stage of
pubertal maturation (from 6 ml testicular volume) and the first conscious
ejaculation at mean chronological age of 13.5 years (Nielsen et al, 1986;
Richardson et al, 1978). By contrast to the early acquisition of potential
for fertility in boys, in girls the menstrual cycle becomes ovulatory only
after 1-2 years following menarche (Apter et al, 1977).
Fig. 1 - Change in the pattern of pulsatile FSH and LH in early infancy, childhood and puberty (From Grumbach et al, 1992).
Fig. 2 - Schematic diagram of regulation of gonadotrophin secretion in females.
Fig. 3 - Diagram of gonadotrophin control system in males.
3.3- Sex Steroid and Growth Hormone Secretion during Puberty

The hormonal mechanisms involved in growth during puberty are not fully understood but it is recognised that both sex steroids and GH are essential for the pubertal growth spurt as the absence of either factor, such as in isolated GH deficiency or hypogonadism, leads to failure of the pubertal growth spurt.

GH is secreted by the pituitary gland in a pulsatile fashion with peaks at approximately 3-hourly intervals under the regulation of GHRH and somatostatin (Frohman et al, 1986), which are in turn regulated by brain neurotransmitters. It is postulated that somatostatin controls the timing of GH pulse, whereas GHRH determine the amplitude of GH peak (Clark et al, 1988; Hindmarsh et al, 1991). GH action on peripheral tissue is mediated by insulin-like growth factor I (IGF-I), synthesis of which is GH-dependent. During puberty spontaneous GH secretion (Mauras et al, 1987; Delamarre-van de Wall et al, 1991; Albertsson-Wikland et al, 1994) and GH response to provocative stimuli (Frasier et al, 1970) are markedly increased. This occurs as a result of an increase of GH amplitude pulse, while pulse frequency remains unaltered. IGF-I levels also increase during puberty, reflecting increased GH concentration. Gonadal steroids are considered to have a central role in mediating the changes in GH pulse amplitude (Liu et al, 1987; Ulloa-Aguirre et al, 1990). The synergism between GH and sex steroid was initially shown (Aynsley-Green et al, 1976) by using testosterone in patients with isolated gonadotrophin deficiency and anorchia and in patients with GH deficiency (alone or in simultaneous combination with GH). Testosterone alone increases growth
rate but at a lesser extent than when it is associated with GH. By contrast, GH alone is not able to induce a pubertal growth spurt of normal magnitude, as shown by the lack of growth acceleration in patients with Kallmann syndrome without substitutive sex steroid treatment. The mechanism whereby testosterone interacts with GH is unclear. So far, two main principal mechanisms can be hypothesised: an activation of the somatotropic axis by testosterone via its aromatization to oestradiol or a direct action of the untransformed steroid on androgen receptors located in peripheral tissues. The first hypothesis is supported by the positive correlation between plasma oestradiol and integrated growth hormone concentrations and by the absence of a correlation with testosterone values (Ho et al, 1987). It is also well known that testosterone and oestrogen are able to increase the response of GH to pharmacological stimuli. Indeed, sex steroid "priming" is used to test pituitary function before the onset of the growth spurt, when, without priming, pharmacological and physiological tests may reveal GH insufficiency (Eastman et al, 1971; Bierich JR, 1987). The second hypothesis is suggested by the induction of the growth spurt by non-aromatizable androgens (such as dihydrotestosterone or oxandrolone), without an activation of the somatotropic axis in boys with delayed puberty (Keenan et al, 1993). By contrast, doubling the dosage of GH during puberty in patients with isolated GH deficiency (Stanhope et al, 1992) does not appear to improve either growth rate or final height prognosis. Such an increased dose during puberty is associated with a faster rate of progress through puberty and it may be due to an augmentation of gonadotrophin action on the gonad (Adashi et al, 1985).
3.4- Constitutional Delay of Growth and Puberty (CDGP): Definition and Auxology

The range of age of onset of puberty in normal children is wide. A delayed onset of more than two standard deviations from the mean (13.4 in girls and 13.8 in boys) is defined as delayed puberty. The most frequent disorder in which puberty and its associated growth spurt are delayed is "Constitutional Delay of Growth and Puberty" (CDGP). According to Wilkins (Wilkins L, 1957), who coined the name "Constitutional Delay of Growth and Adolescence", the most significant features of this condition are 1.- Retarded linear growth which leads to short stature; 2.- Delayed bone age maturation; 3.-In older patients, associated delayed puberty; 4.- Familial pattern in many cases. As growth potential is related to the degree of bone maturation, it is the delay in bone maturation which should permit a final height within the normal range. Presentation of CDGP is more common among boys who are more socially affected than girls making them more likely to present for medical attention. On the other hand the incidence of precocious sexual maturation is higher in girls. This may be due to a sex difference in the pituitary response to GnRH with the girls having an increased sensitivity (Stanhope et al, 1987a).

Characteristically, the majority of these patients have normal birth weight and body length. Growth failure commences to manifest usually around the second or third year. On school entry they are among the smallest of their peer group as they usually grow at a slower rate in comparison to them. A progressive retardation in bone maturation runs parallel with slow growth. Their growth curve continues to lie at or below the tenth centile on the distance chart and at the time of normal puberty they drop far below the
normal centiles. While their peer group experience pubertal growth acceleration their growth velocity is extremely reduced with a nadir which may be below 3 cm per year. The pubertal growth spurt is usually delayed by 2 to 4 years and the total period of growth may come to its ends only at the age of twenty or more years. Fig. 4 illustrates the pattern of growth in a boy with CDGP. The onset of puberty occurred at the age of 13.8 years but the pubertal growth spurt was delayed until the age of 16 yrs. Skeletal maturation is usually delayed by 2 to 4 years and this delay precedes the delayed puberty in CDGP. Sometimes growth delay becomes manifest only during late prepuberty and delayed bone maturation is a sequela of the retarded puberty, the so called condition "Constitutional Delay of Puberty and Growth". When the pubertal growth spurt occurs in patients with CDGP, its duration, peak height velocity and consequently the total pubertal height gain are reduced. However, the increased prepubertal height gain (Bourguignon JP, 1988), should counterbalance the reduced pubertal growth spurt so that final height should not be compromised. Nevertheless, data on final height from non-treated boys with CDGP have shown that final height impairment may occur. In Table 1 published data on final height, predicted height and adult height from boys with untreated CDGP are summarised (Preece et al, 1980; Ranke et al, 1982; Volta et al, 1988; Crowne et al, 1990; Holl et al, 1990; Willing et al, 1990; Von Kalckreuth et al, 1991; LaFranchi et al, 1991). In addition data on segmental body proportion at final height (Crowne et al, 1990; Crowne et al, 1991) have also suggested that a moderate degree of spinal growth impairment may also occur. During normal pubertal development a dramatic increase in bone mineral density and mineral content (Bonjour et al, 1991) is observed from 11 to 14 yrs in girls and from 13 yrs to 17 years in boys. Osteopenia has been reported (Finkelstein et al; 1992) in
adult men who had untreated delayed onset of pubertal development. These data suggest that delayed onset of pubertal maturation may alter the normal bone accretion, predisposing to later osteoporosis.
Fig. 4 - Growth data from a boy with CDGP. Bone age is shown by the solid squares. Parental centiles are indicated on the right hand border. The onset of puberty occurred at the age of 13.8 years but the pubertal growth spurt was delayed until the age of 16 yrs.
<table>
<thead>
<tr>
<th>Authors</th>
<th>N</th>
<th>Predicted height</th>
<th>Mid-parental height</th>
<th>Final height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preece <em>et al</em>, 1980</td>
<td>40</td>
<td>161.7</td>
<td>172.8</td>
<td>164</td>
</tr>
<tr>
<td>Ranke <em>et al</em>, 1982</td>
<td>20</td>
<td>169.6</td>
<td>170.6</td>
<td>167.3</td>
</tr>
<tr>
<td>Volta <em>et al</em>, 1988</td>
<td>41</td>
<td>------</td>
<td>168.4</td>
<td>168.7</td>
</tr>
<tr>
<td>Holl <em>et al</em>, 1990</td>
<td>88</td>
<td>169.6</td>
<td>171.2</td>
<td>168.4</td>
</tr>
<tr>
<td>Willing <em>et al</em>, 1990</td>
<td>77</td>
<td>169.7</td>
<td>176.6</td>
<td>168.9</td>
</tr>
<tr>
<td>Crowne <em>et al</em>, 1990</td>
<td>43</td>
<td>166.1</td>
<td>170.6</td>
<td>164.1</td>
</tr>
<tr>
<td>Van Kalckreuth <em>et al</em>, 1991</td>
<td>20</td>
<td>173.9</td>
<td>173.9</td>
<td>171.3</td>
</tr>
<tr>
<td>LaFranchi <em>et al</em>, 1991</td>
<td>29</td>
<td>171.4</td>
<td>174.6</td>
<td>169.5</td>
</tr>
</tbody>
</table>

Table 1 - Mean value of predicted height, mid-parental height and final height in untreated boys with CDGP: review from the literature.
3.5- Endocrinology of CDGP

In CDGP the physical development is delayed and this is reflected in the retarded epiphyseal maturation. Endocrine investigations fail to show any endocrine pathology, because sex and gonadotrophin hormones concentrations are appropriate to the pubertal stage and GH and IGF-1 levels correlate to the reduced height velocity. Adrenal androgens, sex hormones and gonadotrophin values are generally low for chronological age but normal for bone age. Gonadotrophin response to a bolus intravenous GnRH injection will be indistinguishable from that obtained in normal prepubertal or pubertal children before and during puberty respectively. Nocturnal pulsatile gonadotrophin secretion will be the initial endocrine event of puberty as in normal pubertal development. Normal progression into puberty will then occur, with conservation of the normal harmony between growth and puberty. Biochemical assessment of GH secretion in this condition may be misleading, especially in boys. The physiological decrease in GH secretion seen in late prepuberty and early puberty, when growth decelerates, is amplified in delayed puberty, making this condition difficult to distinguish from true GH deficiency/insufficiency. However, in delayed puberty, a transient increase in GH secretion can be induced by priming with sex steroids prior to the test in both sexes. Low values of IGF-1 are also found reflecting the state of "GH insufficiency". During the spontaneous growth spurt both GH secretion and IGF-1 levels increase, reaching values similar to those seen during pubertal growth spurt in normal children.

Adrenal androgens do not appear to regulate growth or influence the onset of pubertal development directly. However, it has been hypothesised that an
interrelation between the hypothalamic-pituitary adrenal axis and melatonin may play a role in the regulation of growth and onset of puberty: high levels of melatonin occur before adrenarche and progressively decrease during late prepuberty and puberty (Waldhauser et al., 1984), but remain high in delayed puberty (Lee et al., 1975). Further studies are required to validate these hypotheses.

3.6- Diagnosis and Investigation of CDGP

The diagnosis of CDGP is usually made on clinical grounds and it is confirmed retrospectively by a normal progression through puberty. A careful history, physical examination, anthropometric measurements (standing height, sitting height, weight and pubertal rating) and bone age assessment are always the first steps and may suggest a diagnosis. Systemic and chronic disorders which cause secondary delayed puberty, should be identified and occult coeliac or inflammatory bowel diseases should be considered. Dysmorphic features may be suggestive of certain genetic conditions which are associated with delayed puberty, such as Noonan Syndrome, Prader-Willi Syndrome or Laurence-Moon-Biedl Syndrome. Turner syndrome in particular should be excluded in all short girls with delayed puberty. An initial identification of the necessity for endocrine investigation should be suggested by the pubertal maturation stage: the presence of early signs of pubertal maturation makes the diagnosis of CDGP suggestive, while complete absence of secondary sexual characteristics at ≥ 14 years in girls and 14.5 years in boys requires endocrine investigation. A further step is to examine the "consonance" or harmony between the pattern of acquisition of the different stages of
sexual maturation and growth spurt, as absence of the normal harmony points to an endocrinopathy and requires further investigation.

The most difficult task is to distinguish delayed puberty from hypogonadotrophic hypogonadism as, in the absence of pubertal signs, all available tests do not consistently distinguish between the two conditions. After GnRH stimulation tests there will be gonadotrophin release only if the pituitary has already been primed by endogenous GnRH secretion so that a rise in LH suggests the presence of endogenous puberty, but its absence may occur in children with either delayed puberty or hypogonadotrophic hypogonadism. The use of a GnRH agonist or prolactin response to TRH may improve the distinction between the two conditions but this is not widely recognised. Initial signs of pubertal development can be identified by the appearance of spontaneous sleep-associated nocturnal LH secretion but this is not a practical test as repeated overnight blood samplings are required. It has been suggested (Wu et al, 1993) that early morning testosterone measurement could instead be used as a predictor of the imminence of puberty. Clinically, the presence of normal or tall stature for chronological age and genetic target instead of short stature suggest hypogonadotrophic hypogonadism. The hypogonadism in Kallmann's syndrome (autosomal dominant disorders with variable penetrance, more prevalent in males) is associated with undescended testes, anosmia, colour blindness and other midline abnormalities, such as cleft lip and palate. A common approach is to exclude other causes of delayed puberty on clinical grounds and follow the patient's pubertal development. Monitoring of testicular size and ultrasound ovarian imaging represents a non invasive way to assess spontaneous pubertal progress: testicular volume > 4 ml and multicystic
ovarian morphology on ultrasound are the initial clinical features of spontaneous pubertal progression.

A basal gonadotrophin measurement will also help as high levels will be found in patients with hypergonadotrophin hypogonadism when older than 8-10 years. Acquired growth hormone and gonadotrophin insufficiency secondary to an intracranial tumour must always be considered in cases of recent marked growth deceleration and will require neuroradiological imaging. It has been suggested (Kaplowitz PB, 1989) that a short course of testosterone (100 mg/month, for 4 months) can help to differentiate CDGP from GH deficiency in boys: growth velocity will be increased to more than 8 cm/yr in boys with CDGP while it will only be around 4.5 cm/yr in case of GH deficiency. A skeletal survey may be required when segmental body disproportion (i.e. greater than the expected relatively short sitting height in delayed puberty) and/or a sub-optimal growth spurt occur.

3.7- Treatment of CDGP

Delay of growth and puberty may cause psychological sequelae especially in boys. Some patients will not perceive their short stature and lack of sexual development as a major problem while others will be extremely concerned. Once the diagnosis of CDGP has been made this should be carefully explained to both patients and parents and reassurance on almost "normal" final height outcome for genetic potential given. However, there is a significant number of patients, especially boys, who remained distressed. Deficit in emotional development, poor self identity, lack of independence, and antisocial behaviour can develop and their consequences can also persist when normal height and full sexual
maturation are attained (Crowne et al, 1990). Short stature often causes more concerns than lack of sexual development but sensitive enquiry may often reveal a hidden fear of failure to achieve normal future sexual function and fertility as well. Sometimes the medical referral is the consequence of parents' concerns or school doctors/nurses measurements, while the patient does not seem to perceive his/her delay as a major problem. It is always advisable to inquire who initially sought the medical referral as this may be relevant to therapeutic approach. Distressed patients, particularly boys, may need specialist psychological help as well as pharmacological intervention to improve patient's psychological well being. In addition, the recent association between delayed onset of puberty and an increased risk of developing osteoporosis in adulthood also suggest that a new approach in delayed puberty may be necessary not only for psychological reasons but also for optimising bone mass accretion.

Testosterone preparations or weakly anabolic steroids are the most suitable agents to treat boys with CDGP. The aims of the treatment are stimulating linear growth and inducing secondary sexual characteristics but without excessive stimulation of skeletal maturation. Testosterone can be used as oral (testosterone undecanoate, 40 mg a day for 3-6 months) or depot intramuscular (testosterone enanthate, 50-100 mg a month for 3-6 months) preparations. The latter has usually been preferred to the former even though supraphysiological levels of testosterone are found over the 48-72 hours after the injection, followed by gradual waning of effect over the next 3-4 weeks. In addition, there is a loss of the diurnal testosterone rhythm. The injections are painful, but, if accepted by the young patients, a record can be kept of when treatment is administered and this aids the assessment of compliance. On the other hand, peak testosterone and
Dihydrotestosterone levels after oral testosterone undecanoate administration are also excessive for prepubertal boys, even though after 3 to 6 months of treatment the total testosterone decreases together with a reduction in the sex hormone binding protein levels (Butler et al., 1992). Concerns have been expressed on the variable absorption of oral testosterone from the gastrointestinal tract which may cause reduction in its effectiveness. It has been suggested that its administration as a solution in arachis oil can improve its absorption. It is common practice to administer it in the morning after breakfast, to improve its absorption and mimic the diurnal pattern of morning testosterone rise. Further studies are required to establish its doses and effectiveness in boys with CDGP.

Oxandrolone is an anabolic steroid that is used (1.25-2.5 mg/day by oral preparations) in CDGP but due to its abuse in sport medicine it does not have a product licence in the United Kingdom and it can be prescribed only on a named patient basis. At the above doses it mainly induces a growth spurt, compared to testosterone which induces a more marked virilization. For this reason it is more suitable for treating growth delay in younger boys. The induced growth acceleration is usually sustained when the treatment is interrupted at the attainment of 4 ml testicular volume (Papadimitriou et al., 1991).

No short or long-term significant side effects or deterioration of final height attainment have been reported when sex/anabolic steroids are used at the above dose regimens (Joss et al., 1989; Wilson et al., 1988).

In girls low doses of ethinylestradiol (1-2 μg daily) can be administered for 3-6 months or until spontaneous sexual maturation has exceeded that produced by treatment.
Endogenous testosterone secretion from the testes can be induced by human chorionic gonadotrophin. This is an expensive treatment and requires frequent injections. It can not be used in girls because of development of ovarian hyperstimulation syndrome. Subcutaneous pulsatile GnRH can be used to mimic the exact sequence of normal pubertal maturation in boys and girls. It requires a pulsatile subcutaneous injection using a portable mini-pump and is expensive. Both human chorionic gonadotrophin and GnRH do not have any advantages over either testosterone or oxandrolone and are not used for the routine induction of secondary sexual characteristics and growth spurt in patients with CDGP.

Biosynthetic human growth hormone has also been used to treat growth delay in boys with CDGP (Bierich et al, 1992; Buyukgebiz et al, 1990). It is given by daily subcutaneous injection at the doses of 15-20 IU/m²/ week and is extremely expensive. It improves short-term growth rate in CDGP but it is to a lesser extent than that induced by oxandrolone (Buyukgebiz et al, 1990). Final height is not significantly improved (Bierich et al, 1992).
SECTION 4:

PATIENTS
4.1- Diagnostic Criteria for CDGP

- Absence of pubertal signs or delayed maturation at the chronological age of 13 years in boys and 12.5 in girls.
- Short stature compared to mid-parental centiles.
- Bone age delayed by more than 1.5 years.
- No clinical evidence of chronic disease or endocrinopathy.

The diagnosis of CDGP was made on clinical grounds and was retrospectively confirmed by the pattern of growth and by the spontaneous advancement through puberty. When the differential diagnosis between CDGP and growth hormone insufficiency was in doubt, pharmacological tests of pituitary function were performed, after priming with sex steroids.

4.2- Diagnostic Criteria for Prepubertal Growth Delay

Short stature compared to mid-parental centiles; bone maturation delayed by more than 1.5 years; absent of pubertal development; chronological age less than 13.0 in boys and 12.5 in girls; growth velocity above the tenth centile on the velocity standard chart.

4.3- Diagnostic Criteria for Growth Hormone Deficiency

Subnormal serum response of GH to pharmacological stimuli, GH peak values < 20 mU/l (Hughes A, 1989). If the patient was in late prepuberty the stimulation test was carried out after priming with stilboestrol.
4.4- General Criteria of Exclusion from Analysis

- Low birth weight
- Dysmorphic syndromes
- Abnormal karyotype (Turner's syndrome was excluded in all girls by karyotype study)
- Systemic chronic diseases
- Long-term treatment with corticosteroids.
SECTION 5:
METHODS
5.1- Anthropometric, Pubertal and Epiphyseal Assessment

Standing height was assessed by standard anthropometric techniques (Brook CGD, 1982) using an anthropometer. Sitting height (SH) was measured with an anthropometer (Harpenden), while sub-ischial leg length was estimated as standing height minus sitting height. All data were expressed as standard deviation score (SDS) in comparison to the English standards (Tanner et al, 1966 and 1976). The SDS was calculated by subtracting the population mean for a child’s peers from the child’s observed measurement and dividing by the standard deviation for that population. Therefore, considering the height SDS, a short child had a negative SDS value and a tall one positive. The formula sitting height SDS minus leg length SDS was used to obtain a disproportion score. Hence, a normally proportionate child was expected to have a disproportion score of 0, a child with short sitting height in comparison to leg length a negative score and a child with long spine a positive score. A disproportion score equal or greater than ±1 was chosen to indicate significant disproportion. Final height impairment was defined as the difference between corrected mid-parental height minus patient’s final height, both expressed as SDS.

Skeletal maturity, expressed as bone age (BA), was assessed by Tanner and Whitehouse method (TW2) on left hand and wrist radiography (Tanner et al, 1983). This method gives a series of standard appearances or stages through which radius, ulna, metacarpals, proximal, middle and distal phalanxes of the 1st, 3rd and 5th digits (radius, ulna and short bones, RUS) progress. Each bone is matched with the standard, and is then attributed a score which, summed, gives a bone maturity score. Standards are based on left hand and wrist radiography taken on 3000 healthy English children.
Bone age was determined by AA in the prospective studies and by two highly skilled paediatric endocrinologists at Institute of Child Health, London in the retrospective analysis.

Tanner and Whitehouse method (TW2) was used to calculate height prediction (Tanner et al, 1983). It was based on height, chronological age and bone age at the time of bone age determination. For girls, the presence or absence of menarche was also considered.

Parental heights were measured, mid-parental height was corrected for child's sex and defined according to Tanner’s criteria [(father's height, cm + (mother's height, cm +12.5 cm)) / 2 in boys; ((father's height, cm - 12.5 cm) + mother's height, cm) / 2 in girls (Tanner et al, 1970). Target height was obtained from the corrected mid-parental height ± 8.5 cm. Ninety-five per cent of offspring are normally expected to have a final height within parents’ target height, with values normally distributed around the corrected mid-parental height.

Staging of sexual maturation was done according to Marshall and Tanner standards (Marshall et al, 1969 and 1970). In table 2 and 3 the different stages for girls and boys respectively are summarised. Testicular volume was assessed using a Prader orchidometer.

A patient was considered as having attained final height when his/her growth rate was less than 2 cm/year, epiphyses were fused and adult secondary sexual characteristics were attained (Crowne et al, 1990).
<table>
<thead>
<tr>
<th>STAGE</th>
<th>BREAST DEVELOPMENT (B)</th>
<th>PUBIC HAIR (PH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prepubertal; no breast tissue</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Areolar enlargement</td>
<td>A few darker hairs along labia</td>
</tr>
<tr>
<td>3</td>
<td>Enlargement of breast and areola as single mound</td>
<td>Curly pigmented hairs across pubes</td>
</tr>
<tr>
<td>4</td>
<td>Projection of areola above breast as double mound</td>
<td>Small adult configuration</td>
</tr>
<tr>
<td>5</td>
<td>Mature adult breast with single contour</td>
<td>Adult pubic hair distribution</td>
</tr>
</tbody>
</table>

Table 2 - Tanner stages of puberty for girls (Marshall et al, 1969).
<table>
<thead>
<tr>
<th>STAGE</th>
<th>GENITAL MATURITY (G)</th>
<th>PUBIC HAIR (PH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prepubertal; testes 2 ml</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Enlargement of the testes &gt; 2 ml; reddening of the scrotum</td>
<td>A few darker hairs at the basis of the penis</td>
</tr>
<tr>
<td>3</td>
<td>Lengthening of the penis; further enlargement of testes to 6-10 ml</td>
<td>Curly pigmented hairs across pubes</td>
</tr>
<tr>
<td>4</td>
<td>Broadening of the glands penis; growth of testis to 10-15 ml</td>
<td>Small adult configuration</td>
</tr>
<tr>
<td>5</td>
<td>Genitalia adult in size and shape; testes 15-25 ml</td>
<td>Adult pubic hair distribution</td>
</tr>
</tbody>
</table>

Table 3 - Tanner stages of puberty for boys (Marshall et al, 1970).
5.2- Endocrinological Evaluation

- Overnight venous sampling for growth hormone, gonadotrophin and sex hormones. Each blood sample was withdrawn at regular interval (15 minutes) for 12 hours, starting at 7.00 p.m. from an heparinized catheter placed in a forearm vein. During the samplings the children were allowed normal activity and sleep. Blood samples were stored at room temperature and centrifuged within 15 hours. After centrifugation the plasma samples were frozen and stored until assayed.

- Pharmacological tests of GH secretion: GH peak value after insulin-induced hypoglycaemia or glucagon stimulation tests. If the patient was in late prepuberty the stimulation test was carried out after priming with stilboestrol (2 mg/day for the previous two days, in both sexes). The results were considered normal when peak GH value was >20 mU/l (Hughes A, 1989).

- Basal plasma IGF-1 and IGF-1 binding protein 3.

5.3- Hormone Assays

All hormonal assessment were measured by radioimmunological techniques.

Serum FSH and LH levels were measured using a solid-phase immunoradiometric assay. The sensitivity of the assay for both peptides was 0.5 U/l. The within-assay coefficients of variation for LH were 5.6, 3.6, 5.2 and 3.0 % at serum concentration of 2.9, 7.9, 18.3 and 35.8 U/l respectively. The between-assay coefficients of variation were 10.4, 3.1 and 5.4 % at serum concentrations of 4.7, 34.6 and 51.7 U/l. The within-
assay coefficients of variation for FSH were 10.7, 7.6 and 7.8% at serum concentration of 2.8, 5.8 and 13.2 U/l while the between-assay coefficients of variation were 8.1, 4.9 and 5.1% at serum concentrations of 2.9, 14.0 and 26.7 U/l.

GH concentration was measured using a solid-phase immunometric assay from NETRIA. The sensitivity of the assay was 0.2 mU/l. The mean within-assay coefficients of variation were 5.1, 2.4 and 2.6 % at serum concentrations of 0.8, 4.5 and 86.5 mU/l. The between-assay coefficients of variations were 3.3, 5.2 and 5.5 % at serum concentrations of 7.7, 21.7 and 45.8 mU/l.

IGF-1 concentration were measured using an in-house radioimmunoassay with acid/ethanol extraction. The sensitivity of the assay was 0.07 U/ml. The mean within-assay coefficients of variation were 11.3, 6.5 and 4.7 % at serum concentrations of 0.23, 1.23 and 3.53 U/ml. The between-assay coefficients of variations were 10.5, 12.1 and 5.1 % at serum concentrations of 0.38, 0.99 and 3.53 U/ml.

IGF BP-3 concentration were measured using a coated-tube immunoradiometric assay from Diagnostic System Laboratories. The sensitivity of the assay was 0.5 mg/l. The mean within-assay coefficients of variation were 3.9 and 0.76 % at serum concentrations of 7.3 and 27.3 mg/l. The between-assay coefficients of variations were 0.66 and 0.49 % at serum concentrations of 8.3 and 21.5 mg/l.

5.4- Others Investigations

- Pelvic ultrasound in girls to assess ovarian function and uterus development (Stanhope et al, 1985).
• Skeletal survey to exclude skeletal dysplasia, carried out if a discrepancy of more than 2 SDS was present between SH and SLL. The X-ray films were examined by an expert paediatric radiologist (Dr C. Hall, Great Ormond Street Hospital for Children, NHS Trust).

5.5- Treatment

• In patients with CDGP: boys were treated with either testosterone enanthate (50 mg/month by intramuscular injection) or testosterone undecanoate (40 mg/day, orally) or oxandrolone (1.25-2.5 mg/day, orally). Girls received ethinyloestradiol (2 µg/day, orally).

• In late prepubertal boys with growth delay: oxandrolone (1.25-2.5 mg/day, orally. This calculated at a mean dose of 0.05 mg/kg, range 0.03-0.18).

• In patients with growth delay in childhood: GH (30 IU/m²/week, by daily subcutaneous injection) in boys and girls.

• In boys with GH deficiency and growth delay/CDGP: GH (15 IU/m²/week, by daily subcutaneous injection) and either testosterone enanthate (50 mg/month by intramuscular injection) or oxandrolone (1.25-2.5 mg/day, orally).

The length of the treatment is specified elsewhere in the individual section.

The studies on short-term growth hormone treatment of growth delay in children during childhood and the randomised trial of anabolic steroid or testosterone undecanoate in CDGP were approved by The Standing Committee on Ethical Practice of Great Ormond Street Hospital, NHS Trust, London. Written parental consent was also obtained.
5.6- Statistical Analysis

Group data are presented as mean ± 1SD. Statistical methods were by the Student's paired and unpaired t test, one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls or Duncan procedures to assess the difference between groups. Pearson's test was used to determine significant correlation. Stepwise multiple regression analysis was used to identify predictive factors of final height outcome in CDGP. Statistical significance was accepted for p< 0.05. SPSS statistical computer package, release 6.0, was used for the analysis.

GH overnight neurosecretory profiles were assessed by Spectral Analysis (Chatfield C, 1984). Fourier transformation (Matthew DR, 1988), a form of time series analysis which deconvolutes complex signals into sine and cosine components, was applied to the data to examine the dominant periodicity and the amplitude of the pulses in each set of profiles. The method produces a power spectrum which gives a measure of the relative strengths of the signals at different pulse frequencies. Inspection of the spectrum, reveals its dominant periodicity, and its spectral power reflects the amplitude at that periodicity. Before the time series analysis all the data were stationarized to remove the trend from the data array, the differing stationarization technique was used. A graphical method of differences (Bridges et at, 1993) was used to test for any significant differences between the spectral powers of GH output for group contrasts of interest.
SECTION 6:

RESULTS
6.1- Final Height and Segmental Body Proportion in Boys and Girls with CDGP

6.1.1- Patients and Methods

The growth of 132 children (98 boys, 34 girls) with CDGP was retrospectively analysed at presentation and final height attainment to find out whether a delayed onset of pubertal maturation compromises final height and body proportion attainment. Clinical details of the patients are given in Table 4 and 5. All patients were in prepuberty or early pubertal maturation (testicular volume 4 ml in boys and breast stage 2 in girls). Clinical diagnosis of CDGP was made on clinical and anthropometric grounds. However, in order to exclude GH deficiency 51% of the patients were investigated by pharmacological tests of GH secretion, which were considered normal. 30% of the patients had skeletal surveys which failed to show any abnormalities. The number of patients who received therapeutic intervention is given in Table 4 and 5. Treatment in boys was either testosterone enanthate (50 mg, monthly) for 3-4 months or oxandrolone (1.25-2.5 mg, daily), for a mean of 4 months while in girls ethinylestradiol (2 µg, daily) was used for 4 months. In two boys and two girls parental height was not measured as the patients were adopted.

Statistical analysis was by paired t-test.

6.1.2- Results

Height SDS in the boys at presentation was -2.7 (0.7) which increased to -1.9 (0.9) at final height. A similar improvement in height prediction
occurred in the girls with height SDS -3.2 (0.8) at presentation and -2.3 (0.7) at final height. Height at presentation and at final height for boys and girls are illustrated in Fig. 5 and 6, respectively. Final height in both sexes was significantly less than either corrected mid-parental height (p<0.0001) or predicted adult height (p<0.0001). Final height achievement was a mean of 159.9 cm (6.7) in the boys and 147.6 cm (4.5) in the girls. Final height attainment and segmental body proportion in the boys (Fig. 5) appeared unaltered irrespective of whether treatment was administered. Data for girls were insufficient for further analysis as only two received treatment.

There was a significant difference between SH and SLL both at presentation (p<0.0001) and at final height (p<0.0001) for both boys and girls, as illustrated in Fig. 7. The mean difference was -1.2 (1.3) SDS in boys and -1.1 (1.2) SDS in girls at presentation and did not alter significantly at final height attainment [-1.3 (1.2) in boys and -1.2 (1.2) in girls] even though there was a significant growth in both SH and SLL (both p<0.0001) between the two measurements.
<table>
<thead>
<tr>
<th></th>
<th>At presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean chronological age, yr. (SD)</strong></td>
<td>14.1 (1.3)</td>
</tr>
<tr>
<td><strong>Mean bone age, &quot;yr.&quot; (SD)</strong></td>
<td>11.7 (1.5)</td>
</tr>
<tr>
<td><strong>Mean testicular volume, ml (SD)</strong></td>
<td>3.0 (0.5)</td>
</tr>
<tr>
<td><strong>Patients receiving treatment (%)</strong></td>
<td>30</td>
</tr>
</tbody>
</table>

Table 4 - Clinical data from 98 boys with CDGP, both at presentation and final height.

<table>
<thead>
<tr>
<th></th>
<th>At presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean chronological age, yr. (SD)</strong></td>
<td>13.0 (1.3)</td>
</tr>
<tr>
<td><strong>Mean bone age, &quot;yr.&quot; (SD)</strong></td>
<td>10.8 (1.1)</td>
</tr>
<tr>
<td><strong>Breast stage (B)</strong></td>
<td>B₁ : 74%</td>
</tr>
<tr>
<td></td>
<td>B₂ : 26%</td>
</tr>
<tr>
<td><strong>Patients receiving treatment (%)</strong></td>
<td>6</td>
</tr>
</tbody>
</table>

Table 5 - Clinical data from 34 girls with CDGP, both at presentation and final height.
Height expressed as SDS in 98 boys (Fig. 5) and in 34 girls (Fig. 6) with CDGP at presentation and final height as well as predicted and corrected mid-parental heights. Closed circles represent untreated patients, open circle, those treated. Mean and SD are shown by the horizontal bars.
Fig. 7 - Mean SH and SLL (shaded) SDS in 98 boys and 34 girls with CDGP. One standard deviation is illustrated by the horizontal bars.
6.2 - Predictive Factors at Presentation in the Determination of Final Height in CDGP

6.21 - Patients and Methods

The growth patterns of 78 boys with CDGP was retrospectively analysed until final height attainment. The aim of this study was to identify predictive factors of final height outcome at presentation, in order to select the patients who may need further investigations or benefit from alternative therapeutic approaches. Anthropometric data from the patients at presentation and at final height are shown in table 6. Final height impairment was defined as the difference between corrected mid-parental height minus patient’s final height, both expressed as SDS. The results were considered normal in all patients whose pituitary function was tested with pharmacological stimulation tests. A skeletal survey was carried out when more than 2 SDS differences between upper and lower segment were present. Sixteen boys were treated with depot testosterone (50 mg monthly for 3-4 months), six with oxandrolone (1.25 mg daily for a mean of 4 months) and one with both in sequence. Statistical analysis was by paired-sample t test. Pearson’s test was used to determine significant correlation between final height on one hand and standing height, difference between SH and SLL, chronological age, growth velocity and delayed bone maturation at presentation on the other in addition to a stepwise multiple regression. Treatment was not entered as variable as it is widely recognised that either testosterone or oxandrolone at the above doses do not influence final height outcome.
However, one-way analysis of variance followed by Duncan procedure was used to verify that final height outcome was not different among treated and not treated patients.

6.22- Results

At presentation height SDS was -2.7 (0.7) which increased to -2.0 (0.9) at final height. Final height was significantly less than either corrected mid-parental height (p<0.0001) or predicted height (p<0.0001). In figure 8 mean value ± 2 SD of final height, mid-parental height and predicted height SDS are illustrated. Considering the genetic target instead of mid-parental height, 58% of patients failed to achieve their full genetic potential while, among the remaining 42%, only 0.7% attained a final height above mid-parental corrected height. There was a significant difference between SH and SLL both at presentation (p<0.0001) and final height (p<0.0001). The mean difference between the SDS values of SH and SLL was -1.2 (±1.1) at presentation and remained unmodified at a value of -1.3 (±1.0) at final height attainment. At presentation in 19 patients (24%) the difference between SH and SLL was more than -2 SDS and in 9 of them (47%) this significant disproportion score was still present at final height attainment. In addition, 11 more patients (14%) had a disproportion score less than -2 SDS at presentation but it deteriorated to less than -2 SDS at final height. Hence, at final height 20 patients (26%) presented an eunuchoid habitus. All patients attained full sexual maturation, with testicular volume ≥ 15 mls, indicating normal gonadotrophin secretion. A significant negative correlation (r = -0.6, p<0.0001) was found between chronological age at presentation and degree of segmental body disproportion as represented on Fig. 9.
Multiple regression analysis, used to find the most suitable predictors of final height impairment, showed among the factors included in the analysis only standing height, growth velocity and degree of segmental proportion (difference between SH and SLL) were significantly associated with final height outcome. Therefore, patients who were taller, were growing at a faster rate and had a major degree of segmental body disproportion at presentation had less height impairment. The relationship was described by the regression equation:

\[
\text{Corrected mid-parental height SDS - Final height SDS} = -0.39 \text{ (Height SDS at presentation)} - 0.12 \text{ (growth velocity at presentation)} +0.22 \text{ (SH SDS - SLL SDS at presentation)} + 1.26
\]

\[ (R=0.49, R^2=0.24, \text{ adjusted } R^2=0.21; F=7.8, P=0.0001) \]

When we classified the patients according to the treatment received, (no therapy, oxandrolone or testosterone or both oxandrolone and testosterone), the oldest patients at presentation were those treated with testosterone \((p<0.05)\). Consequently, being the oldest they also had the most pronounced body disproportion \((p<0.05)\) with relatively long legs compared to a short spine. However, at final height, final stature, height impairment and body proportion was similar among groups \((p=ns)\) and the only significant difference among the boys in the three treatment options was in chronological age \((p<0.01)\), with, again, the patients receiving testosterone being the eldest.
<table>
<thead>
<tr>
<th><strong>Chronological age, yr.</strong></th>
<th>14.3 (12-18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bone age, &quot;yr.&quot;</strong></td>
<td>11.9 (7-15)</td>
</tr>
<tr>
<td><strong>Chronological age - bone age, yr.</strong></td>
<td>2.4 ± 1.3</td>
</tr>
<tr>
<td><strong>Height, SDS</strong></td>
<td>-2.7 ± 0.7</td>
</tr>
<tr>
<td><strong>Height, cm</strong></td>
<td>140.6 ± 8.6</td>
</tr>
<tr>
<td><strong>SH, SDS</strong></td>
<td>-3.5 ± 0.9</td>
</tr>
<tr>
<td><strong>SLL, SDS</strong></td>
<td>-2.3 ± 0.8</td>
</tr>
<tr>
<td><strong>SH - SLL, SDS</strong></td>
<td>-1.2 ± 1.1</td>
</tr>
<tr>
<td><strong>Growth rate, cm/yr.</strong></td>
<td>4.8 ± 1.6</td>
</tr>
<tr>
<td><strong>Height prediction, SDS</strong></td>
<td>-1.4 ± 0.8</td>
</tr>
<tr>
<td><strong>Mid-parental height, SDS</strong></td>
<td>-0.5 ± 0.6</td>
</tr>
<tr>
<td><strong>Testicular volume, ml</strong></td>
<td>3.3 (2-8)</td>
</tr>
<tr>
<td><strong>% of patients treated</strong></td>
<td>29.5 %</td>
</tr>
</tbody>
</table>

Table 6 - Clinical data from 78 patients with CDGP at presentation. Values are expressed as mean ± 1 SD or mean (range).
Fig. 8 - Mean height, expressed as SDS, ±2 SD in 78 boys with CDGP at final height as well as predicted and corrected mid-parental heights.

Fig. 9 - SH minus SLL, both expressed as SDS in 78 boys with CDGP at presentation plotted versus the chronological age.
6.3- Effect of Oxandrolone Treatment on Growth and Segmental Body Proportion in Prepubertal Boys with Growth Delay

6.31- Patients and Methods

The aim of this study was to examine the effect of oxandrolone on the growth of prepubertal boys with growth delay and short stature, before they had developed delayed puberty. Twenty-three prepubertal boys with growth delay were treated with low-dose oxandrolone (group A) and compared to 56 boys with CDGP who did not receive any treatment (group B). The clinical data from both groups are reported in Table 7. The anthropometric data at the last follow-up after the interruption of oxandrolone treatment are presented for the 20 out of 23 patients treated with oxandrolone while final height attainment is available for those with untreated CDGP. Oxandrolone was used in a dose regimen of 1.25-2.5 mg/day, calculated at a mean dose of 0.05 mg/kg, range 0.03-0.18. The mean duration of treatment was 1.2 yr. (0.7-1.8). Statistical analysis was by paired t test.

6.32- Results

At the cessation of anabolic steroid treatment (mean CA 13.1 yr., n=23), mean height SDS was -1.9 (0.5) (p< 0.0001) and body proportion was unchanged with a SH-SLL SDS mean value of -0.6 (p=NS). The induced growth acceleration was sustained when oxandrolone was interrupted and became indistinguishable from the spontaneous pubertal growth spurt. At
the last follow-up (CA 15.7 yr., range 13.1-19.4; growth velocity 6.0 cm/yr., range 0-12.9; n=20) height SDS mean value was -1.6 (p< 0.001 vs. pre-treatment value) and normal body proportion was still present (SH - SLL: -0.6 SDS). They all progressed into puberty (mean chronological age at pubertal onset 12.9, range 11.6-13.9 yr.), with spontaneous increase in testicular volume. Three patients had already attained final height. By contrast, in 56 boys with untreated CDGP height SDS was -2.7 (0.8) before the onset of pubertal growth spurt and became -2.1 (0.9) (p< 0.001) at final height attainment with, however, progressive deterioration in body proportion from value of SH-SLL of -1.0 to -1.2. In Fig. 10 the mean values (± 1SD) of standing height and difference between SH and SLL are represented prior to and at the end of oxandrolone treatment, and at the last follow-up in 20/23 patients with growth delay, while in Fig. 11 the standing height and the difference between SH and SLL (mean ± 1SD) are illustrated at presentation and at final height attainment in 56 boys with untreated CDGP. All values are expressed in SDS.

None of the patients experienced adverse side effects during their course of treatment.
Table 7 - Clinical data from 23 boys with growth delay before commencement of oxandrolone treatment (group A) and from 56 boys with untreated CDGP at presentation (group B).

<table>
<thead>
<tr>
<th></th>
<th>GROUP A</th>
<th>GROUP B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronological age, yr.</td>
<td>11.8 (range 9.0-12.8)</td>
<td>14.2 (range 12-17)</td>
</tr>
<tr>
<td>Bone age delay, yr.</td>
<td>2.3 (range 1.0-3.3)</td>
<td>2.3 (range 0.9-4.8)</td>
</tr>
<tr>
<td>Height SDS</td>
<td>-2.8 (1.5 SD)</td>
<td>-2.7 (0.8 SD)</td>
</tr>
<tr>
<td>SH - SLL, SDS</td>
<td>-0.5</td>
<td>-1.0</td>
</tr>
</tbody>
</table>

Fig. 10 - Mean values (±1 SD) of standing height and difference between sitting height (SH) and subischial leg length (SLL) before and after a course of oxandrolone treatment, and at the last visit are illustrated in 20 boys with growth delay. Values are expressed as SDS.
Fig. 11 - Mean values (±1 SD) of standing height and difference between sitting height (SH) and subischial leg length (SLL) at presentation and final height attainment are illustrated in 56 boys with untreated CDGP. Values are expressed as SDS.
6.4- Short-Term Growth Hormone Treatment of Growth Delay in Children during Childhood

6.41- Patients and Methods

In this study 24 (21 M, 3 F) short prepubertal children with growth delay were enrolled in a trial on short-term GH treatment. The aim was to improve short-term growth and therefore increase standing height before the onset of puberty. Two boys withdrew from the trial because of non-compliance and their data have not been included in the analysis. GH treatment was commenced at the chronological age of 8.4 (range, 5.3-10.6) years at the dose regimen of 30 IU/m²/week, by daily subcutaneous injection. GH doses was adjusted for alteration in surface area after 8 months. The treatment was administered for 12 months in all patients except in 2 who were treated for 18 months. Normal pituitary function was demonstrated using either pharmacological tests of stimulation (insulin-induced hypoglycaemia or glucagon) or an overnight profile of spontaneous GH secretion (repeated blood sampling every 15 minutes for 12 hours). Thyroid function was normal in all patients. The clinical data from 22 patients are reported in Table 8.

Statistical analysis was by paired-sample t test, Pearson’s correlation test and sign test. A stepwise multiple regression was used to determine the most suitable predictors of anthropometric response to GH. The study was approved by the Ethics Committee of Great Ormond Street Hospital, NHS Trust, London.
6.42- Results

Before GH treatment height SDS (-2.4±0.5) was significantly lower (p<0.0001) than corrected mid-parental height SDS (-0.5±0.6). However as bone maturation was delayed by a mean value of 2.4 (0.9) years, height SDS for bone age (0.0±1.2) was appropriated for mid-parental centile (p=ns). Mid-parental height, height SDS for bone age and height values at the commencement of GH treatment, all expressed as SDS, are plotted for each patient in figure 12. GH treatment induced a significant increase in growth velocity SDS (Fig. 13) which increased from a value of -0.6 (0.8), recorded the year before the commencement of treatment, to +4.7 (2.1). Catch-up growth was observed in all patients as shown in Fig. 14. Height SDS increased significantly (p<0.0001) to -1.6(0.7) while final height prediction, expressed as height SDS for bone age, remained unchanged. At 1 year follow-up, after the discontinuation of GH treatment, a profound growth deceleration was observed in all patients (Fig. 13 and 14) except in one girl who had, in the meantime entered puberty. All the other children remained prepubertal. Data from the girl who had started her pubertal development has been excluded from further analysis. Mean growth velocity SDS the year after GH treatment was significantly lower than during and prior to GH treatment (Fig.13). A significant improvement (p<0.002) in growth velocity SDS was seen during the second semester (-1.3±1.3) of follow-up compared to the first (-2.9±1.2), but its value was still significantly lower (p<0.05) than prior to GH treatment.

Growth velocity during GH treatment was not correlated with either growth velocity before or after GH therapy.

Multiple regression analysis, used to find predictive factors of response to GH treatment, showed among the factors included in the analysis
(chronological age at the beginning of treatment, degree of delayed bone maturation, sex, overall duration of treatment, mid-parental height and BMI) chronological age at the onset of GH treatment was the only predictor of response to GH. The younger the patient, the better the response to growth treatment. The relationship was described by the regression equation:

$$\Delta \text{ height gain SDS} = -0.1 \times (\text{Chronological age at the commencement of GH}) + 1.7$$

(R=0.5, \(R^2=0.3\), adjusted \(R^2=0.2\); \(F=7.0, P=0.01\))

In Fig. 15 the \(\Delta \text{ height gain SDS}\) is plotted versus chronological age of the patient at the commencement of GH treatment.

In Fig. 16 the anthropometric response of one patient treated with GH is illustrated.

Untoward side effects were not observed.
Fig. 12 - Mid-parental height, height for bone age and standing height at the commencement of GH treatment, all expressed as SDS, from 22 children with growth delay.

Fig. 13 - Mean growth velocity SDS (+1 SD) from 22 patients with growth delay prior to, during and 12 months after the discontinuation of GH treatment.
Fig. 14 - Height SDS from 22 patients with growth delay prior to, during, 6 and 12 months after the discontinuation of GH treatment. Broken lines represent girls while unbroken lines represent boys.

Fig. 15 - Correlation between chronological age and height increase after short-term GH treatment in 22 children with growth delay.
Fig. 16 - Growth data from a boy with growth delay treated with 1 year course of GH. Solid diamond represent bone age. Parental centiles are shown on the right hand border.
6.5- Oral Treatment of CDGP: a Randomised Trial of Anabolic Steroid or Testosterone Undecanoate

6.5.1- Patients and Methods

Thirty three boys with CDGP, mean chronological age 14.6 years (range 12.8-16.2), were randomised to receive either oxandrolone or testosterone as oral preparation to compare their effectiveness in inducing an acceleration in growth. Testosterone undecanoate (40 mg/day; group 1) was administered for a mean duration of 3.3 months, while oxandrolone (2.5 mg/day; group 2) for 3.7 months. The two drugs were given in the evening after evening meal, to mimic the normal pattern of androgen secretion in boys in early puberty. All boys were prepubertal or at an early stage of sexual maturation with a mean testicular volume of 4.6 mls (range, 3-8). Mean growth velocity was 4.2 cm/year during the 6 months before the commencement of treatment and bone age was delayed by 3.3 years. The anthropometric data from the patients in the two groups are reported in table 8. No cross over of treatment between the groups was possible because the induced growth spurt was sustained when treatment ceased.

Statistical analysis was by independent and paired t test, and Chi-test. The study was approved by The Standing Committee on Ethical Practice of the Great Ormond Street Hospital, London and written parental consent was obtained in all cases. In addition, ethics committee approval was also obtained from Crawley, Farnborough, Conquest and Royal Surrey County Hospitals.
6.5.2- Results

Both oral testosterone and oxandrolone induced growth acceleration. Mean growth velocity was 4.4 (1.8) cm/year in boys of group 1 (14 boys) and 4.1 (1.8) cm/year in those of group 2 (15 boys) for the 6 months before treatment. This increased to 10.1 (2.3) (p<0.0001) and 9.0 (2.0) (p<0.0001) respectively during treatment (figure 17). The induced growth acceleration was sustained at a mean value of 8.3 (2.9) (p>0.05) and 9.0 (2.5) cm/year (p>0.05) during the 6 months after the cessation of treatment. No significant difference was found in the anthropometric response to treatment between the two groups. The pattern of epiphyseal maturation during the sex or anabolic steroids treatments was also similar (p>0.05) between the two groups, with an unaltered height SDS for bone age after treatment compared to pre treatment values. All the boys treated with oral testosterone and those treated with oxandrolone presented a similar pattern of growth in response to either treatment, except four patients, three who initially received testosterone and one who received oxandrolone. The patients who did not respond to the initial 3 month courses of sex or anabolic steroids were then transferred to the alternative group and, therefore, treated with an additional 3 month course. The change of treatment was associated with a significant increase in growth rate. One of the non-responders to testosterone admitted poor compliance but a satisfactory compliance to oxandrolone. Poor compliance was denied by the other 3 non-responders. The percentage of non-responders in the two groups was not significantly different. Data from these 4 patients were excluded from analysis.

During treatment there was a spontaneous increase in testicular volume from a mean value of 4.6 mls (range, 3.0-6.0) in boys from group 1 and 4.6
mls (range, 3.0-8.0) in group 2 to 6.0 mls (range, 3.0-8.0) and 6.2 mls (range, 4.0-10.0) respectively followed by 8.0 mls (range 4.0-12) and 8.3 mls (range 4.0-12.0) at the end of the follow-up period. There was also a progressive development in secondary sexual characteristics in both groups with a similar rate of maturation between the two groups.

No adverse effects from the administration of either testosterone or oxandrolone were reported.
<table>
<thead>
<tr>
<th></th>
<th>Group 1 (testosterone)</th>
<th>Group 2 (oxandrolone)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>17</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Chronological age, yr.; mean (range)</td>
<td>14.5 (13.0-16.2)</td>
<td>14.6 (12.8-15.8)</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Height SDS</td>
<td>-1.97 (0.4)</td>
<td>-2.21 (0.7)</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Growth rate, cm/yr.</td>
<td>4.3 (1.7)</td>
<td>4.2 (1.7)</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Bone age, &quot;yrs&quot;</td>
<td>11.2 (1.6)</td>
<td>11.2 (1.0)</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Height SDS for bone age</td>
<td>0.70 (1.0)</td>
<td>0.40 (0.9)</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Mid-parental height SDS</td>
<td>-0.14 (0.6)</td>
<td>-0.08 (0.2)</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Testicular volume, ml; mean (range)</td>
<td>4.6 (3-6)</td>
<td>4.6 (3-8)</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>

Table 8 - Clinical data from 33 boys with CDGP at presentation, divided in two groups according to treatment received. Values are expressed as mean (SD) or mean (range).
Fig. 17 - Mean growth rate in 29 boys with CDGP treated with either oral testosterone or oxandrolone in pre-treatment, treatment and post treatment periods. SD are shown by horizontal bars.
6.6 - Treatment of Growth Delay in Boys with Isolated Growth Hormone Deficiency

6.6.1 - Patients and Methods

Thirteen prepubertal and 2 early pubertal boys with isolated GH deficiency (IGHD), while they were being treated with constant doses of GH, were included in a prospective study in order to study the effect of short-term treatment with either an anabolic steroid (oxandrolone) or testosterone on growth and puberty. The aim was to study the mechanisms by which the synergistic action between GH and sex steroids takes place during the pubertal growth acceleration. All patients expressed concerns about their physiological prepubertal or early pubertal deceleration in growth velocity, and for this reason androgen treatment was offered. The mean age at the commencement of GH treatment was 9.4 (2.5) years. All had subnormal serum response of GH to a pharmacological stimulus, GH response was < 20 mU/l to insulin-induced hypoglycaemia or glucagon. A standard physiological dose of GH was used (15 IU/m²/week, by daily subcutaneous injection) and was adjusted for alteration in surface area every six months. At a mean chronological age of 13.1 (1.1) secondary sexual characteristics and/or a growth acceleration were induced by either depot testosterone, 50 mg monthly for a mean of 9 months in 12 patients, in single or repeated 3 month courses of oxandrolone, 1.25 or 2.5 mg daily for a mean of 1 year in 3 patients, in single or repeated 4-6 month courses. The choice between the two treatments was mainly age-dependent, the youngest being treated with oxandrolone. It was also linked to the patients' wishes in having an induction of their secondary sexual characteristics, in addition to growth
catch-up, testosterone having a more viralization action at the used doses. Clinical data from the 15 patients at commencement of sex or anabolic treatment are reported in Table 9. Only 2 patients were in early puberty with a testicular volume > 4 ml. Statistical methods were by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls procedure to assess difference between groups and pair t test.

6.6.2- Results

During treatment with either sex or anabolic steroids there was a significant increase in height velocity ($F=8.86; p<0.0007$) (Fig. 18). Indeed, the mean growth rate was 5.7 (1.6) cm/year for the year before the induction of the growth spurt, increasing to 8.1 (1.2) cm/year during sex or anabolic steroid treatment ($p<0.05$), and sustained at 7.3(1.9) cm/year during the year after the cessation of treatment ($p<0.05$). The boys treated with oxandrolone or those with testosterone presented a similar pattern of growth in response to the treatment. Therefore, the two groups were analysed together. The relatively normal body proportion between sitting height and subischial leg length reported at the commencement of sex or anabolic steroid treatment did not alter significantly during the induced growth spurt. Indeed, both sitting height and subischial leg length demonstrated significant increase ($p<0.05$), from -2.3(0.7) and -2.4(0.7) SDS respectively at the commencement of androgen treatment to -2.1(0.7) and -2.1(0.8) SDS respectively at the cessation. There was no significant difference in height SDS for bone age (Fig. 17), which was -0.69(0.97) at the onset of treatment and -0.53 (0.84) after the cessation of sex/anabolic steroid treatment. During the induction of the growth acceleration, there was a progressive development in secondary sexual characteristics.
Indeed, there were a maturation from a mean genitalia stage 1.3 (1-2), pubic hair mean stage 1.1 (1-2) and axillary hair mean stage 1.1 (1-2) at the onset of testosterone treatment to a mean stage of 3.2 (3-4), 2.6 (2-4) and 1.4 (1-3) respectively. Patients treated with oxandrolone had both genitalia and pubic hair stage mean of 1.7(1-3) and axillary hair mean stage of 1.0(1) before steroid treatment and 3.3(3-4), 3.0(2-4) and 1.0(1) at the cessation of therapy. Testicular volume increased in all patients and a mean of 6.1 (range 4-10) mls was attained at the cessation of treatment, with a further increase to a mean of 9.4 (range 6-12) mls after 1 year. In this way a diagnosis of "isolated" GH deficiency was confirmed. The induced growth spurt occurred at too early a stage of sexual maturation to be due to the spontaneous growth spurt of puberty; the latter occurs at a 10 ml testicular volume. The boys treated with oxandrolone and those with testosterone presented a similar pattern of growth and sexual maturation in response to the treatment. Their data are reported in table 10 and illustrated in Fig. 16 and 17. There were no adverse effects from the administration of either depot testosterone or anabolic steroid treatment.
<table>
<thead>
<tr>
<th></th>
<th>Patients receiving oxandrolone</th>
<th>Patients receiving testosterone</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients</td>
<td>3</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Mean CA (yr.)</td>
<td>12.3 (1.6)</td>
<td>13.3 (0.9)</td>
<td>13.1 (1.1)</td>
</tr>
<tr>
<td>Mean BA delay (yr.)</td>
<td>1.7 (0.5)</td>
<td>2.7 (1.5)</td>
<td>2.5 (1.4)</td>
</tr>
<tr>
<td>Mean testicular volume and range (ml)</td>
<td>4 (2-8)</td>
<td>2.7 (2-8)</td>
<td>2.9 (2-8)</td>
</tr>
<tr>
<td>Mean growth rate (cm/yr.)</td>
<td>4.6 (0.6)</td>
<td>6.0 (1.7)</td>
<td>5.7 (1.6)</td>
</tr>
<tr>
<td>Mean height SDS for BA</td>
<td>-0.48 (0.2)</td>
<td>-0.74 (1.08)</td>
<td>-0.69 (0.97)</td>
</tr>
<tr>
<td>Mean SH-SLL SDS</td>
<td>0.13 (0.55)</td>
<td>0.03 (0.59)</td>
<td>0.05 (0.57)</td>
</tr>
</tbody>
</table>

Table 9 - Clinical data from 15 boys with isolated growth hormone deficiency on GH treatment at commencement of either sex or anabolic steroid treatment.
Fig. 18 - Yearly growth rates for 15 boys with IGHD during GH treatment receiving either oxandrolone (open circles) or testosterone (closed circles). Duration of treatment period reflects the interval between measurements within which treatment was administered. Mean and SD are shown by horizontal bars.

Fig. 19 - Height expressed as SDS for bone age in 15 boys with IGHD treated with oxandrolone (open circles) or testosterone (closed circles). Mean and SD are shown by horizontal bars.
6.7- Short-Term Growth Hormone Effect on Gonadotrophin and Endogenous Growth Hormone Secretion in Children with Growth Delay during Childhood

6.7.1- Patients and Methods

The effect of short-term GH treatment on the secretion of GH, IGF-1, IGF BP-3 and gonadotrophins (FSH and LH) was studied in 13 children (10 M, 3 F) by means of 12 hour overnight profiles. This formed part of a study of the pharmacological use of GH in growth delay in childhood (see section 6.4). The first overnight profile was carried out prior to the commencement of GH treatment and repeated after 2 to 3 weeks from the discontinuation of the treatment which lasted 1 year. For each child an indwelling intravenous cannula was inserted at 06.00 pm and after 30 minutes a sample of blood was drawn for the measurement of serum LH, FSH, GH, IGF-1 and IGF BP-3. Samples were then withdrawn at 15-minute intervals for the next 12 hours for the measurement of GH and gonadotrophins. Blood samples were spun and separated and stored at -20° C prior the assay. A pelvic ultrasound imaging was performed in each girls prior to and after GH treatment to assess ovarian function and uterus development.

Statistical analysis was by paired-sample t test and Pearson’s correlation test. GH data arrays were analysed by Fourier transformation to examine the dominant periodicity and amplitude of the pulse in each set of profiles. The data were stationarized before Fourier analysis. When the means and standard errors of two Fourier transforms were represented on a graph the
null hypothesis was rejected at p<0.025. A graphical method of differences was used to define any significant difference (Bridges et al, 1993). Period was plotted along the X-axis and the difference between the two transforms (with one transform chosen as the index) as the Y-axis. Post GH treatment transform was chosen as the index transform and its difference was always zero, following the X-axis. The standard error bars of the index transform at each period were plotted along this line. The difference at each period between the mean values for the index and for the second transform were calculated and plotted on the same graph, with the standard errors. A significant difference (p<0.025) existed when the areas on the graphs defined by the two sets of error bars at any individual periodicity did not overlap.

6.7.2- Results

Normal GH profile was observed in each patient prior to GH treatment. Mean 12-hour serum GH concentration increased slightly but not significantly prior to (8.4 ± 3.0 mU/l) and after GH treatment (11.5 ± 8.0 mU/l). There was no significant difference in the absolute spectral power between these two occasions suggesting similar amplitude pulses (Fig 18). The relative spectral power (Fig. 19), an indicator of pulse periodicity, showed a dominant pulse periodicity between 165 and 180 minutes (172.5 minutes) prior to GH treatment while after 1 year treatment the dominant periodicity was at 165 minutes. The graphical method of difference (Fig. 20) showed that a significant difference (p<0.025) was present at these periodicities as the area on the graph encompassed by the two sets of error bar between 165 and 180 minutes did not overlap.
Serum IGF-1 slightly increased from value of 0.58 (0.26) to 0.75 (0.32) U/ml during 1 yr. GH treatment and this was only in part due to the physiological increase in its level with age. By contrast, IGF BP-3 levels decreased from 2.83 (0.8) to 2.64 (0.85) mg/l during the year of treatment. The ratio IGF-1/BP-3 significantly increased from 0.8 to 1.1 (p<0.05) and it is shown in fig. 21.

FSH and LH profiles showed a prepubertal pattern of secretion with low values and absent pulsatility prior to GH treatment, in consonance with the prepubertal state of the children. The same pattern of secretion was seen after 1 year GH treatment in all patients except 1 girl who had onset of breast development during the following six months.

Pelvic ultrasound imagings showed that a physiological maturation in ovarian and uterus morphology occurred during the year of observation.
Fig. 20 - Fourier transform for GH, mean ± SEM for all 13 patients. Unbroken line: prior to GH treatment; broken line: after GH treatment.

Fig. 21 - Fourier transform for GH, mean ± SEM for all 13 patients. Unbroken line: prior to GH treatment; broken line: after GH treatment.
Fig. 22 - Plot of the differences between the Fourier transform. The shaded area is the index transform (post-GH treatment).
Fig. 23 - IGF-1 / BP-3 ratio prior to and after 1 year GH treatment in 13 children with growth delay.
SECTION 7:

DISCUSSION
7.1- Final Height and Segmental Body Proportion in Patients with CDGP

CDGP is the most frequent cause of delayed onset of puberty. It is not regarded as a disease but a condition, predominately because final height prognosis is appropriate for the parental centiles. However, data on final height attainment showed that CDGP is an heterogeneous condition in which final height outcome may be compromised (Preece et al, 1980; Ranke et al, 1982; Crowne et al, 1990; Crowne et al, 1991; Holl et al, 1990; Willing et al, 1990; Von Kalckreuth et al, 1991; LaFranchi et al, 1991). The data presented in this thesis confirm that both boys and girls with CDGP may not attain their genetic potential: 58% of boys failed to achieve the genetic target height, and only 4% were able to reach a final height greater than their mid-parental centiles. The absence of correlation between final height outcome and degree of delayed bone maturation suggested that final height prediction was not reliable for this condition. The actual standing height and relative growth velocity at presentation seemed to be much more important than the degree of delayed bone maturation. Consequently, patients who are extremely short in comparison to their parental centiles and are therefore growing slowly, need special attention as their final height prognosis may not be satisfactory.

A possible reason why there was a failure to achieve target height in 58% of patients may be partly due to the selection of patients who were referred to the endocrine centre and to the fact that long-term follow-up was mainly offered to those in whom height was more compromised. It can also be postulated that the group studied suffered from a variant of CDGP, in association with growth hormone insufficiency not revealed by pharmacological tests of GH secretion or an unclassified skeletal dysplasia.
In CDGP, when the pubertal growth spurt occurs, its duration, peak height velocity and consequently the total pubertal height gain are reduced. If this is counterbalanced by the increased prepubertal height gain (Bourguignon JP, 1988), final height should not be compromised. However, during late prepuberty or early puberty height gain is mainly due to growth in the lower segment since spinal growth is relatively delayed compared to leg length. Indeed, during the pubertal growth spurt normal children experience a transitional stage of development with relatively long legs (Nielson et al, 1986). As a result, if puberty is delayed, spinal growth is more compromised so that, when pubertal growth acceleration occurs, a segmental body disproportion with short SH and long SLL is already appreciable. A relatively short SH, compared to the SLL, was a consistent sign in the patients studied and it became of greater magnitude when puberty was severely delayed. Its absence in a boy older than 14 years, even in the presence of all other clinical signs of CDGP, should point to a different diagnosis while its minor appearance should be considered as an indicator of an inadequate final height outcome. A short SH was found in 68 patients (87%) at final height and in 20 of them (29%) there was a significant disproportion score greater than -2 SDS. The persistence of eunuchoid habitus at final height was also observed by other authors (Crowne et al, 1990; Crowne et al, 1991). This implied that, during the pubertal growth spurt in children with CDGP, impaired upper segment growth did not compensate for the previous deficit. Hence, a spinal growth failure was in part responsible for the failure to attain a final height appropriate to mid-parental centiles.

Historical data on body segmental proportion at final height in children with craniopharyngioma (Burns et al, 1981a), or idiopathic multiple pituitary hormone deficiency (Burns et al, 1981b), who had both late onset of
pubertal induction, have also shown typical eunuchoid proportions. On the contrary, the opposite body segmental disproportion, with a relatively short leg length, occurs in girls with central precocious puberty (Martinez et al, 1984). Furthermore, delayed puberty has also been associated with reduced skeletal mineralization of the spine in adult men who had untreated CDGP (Finkelstein et al, 1992). Consequently, a delay in the "tempo" of pubertal maturation may interfere with the growth and normal bone accretion of the spine causing an adult eunuchoid appearance and predisposing to later osteoporosis.

7.2- Therapeutic Intervention in CDGP

When for psychological reasons a therapeutic intervention is required in boys with CDGP, the traditional approach is the induction of growth spurt with either testosterone or anabolic steroid. Testosterone can be used as oral or depot intramuscular preparations. The latter has usually been preferred to the former as concerns have been expressed on the variable absorption from the gastrointestinal tract which may cause reduction in its effectiveness. Oxandrolone is the anabolic steroid mostly used (by oral preparations) in CDGP but due to its abuse in sports medicine it does not have a product licence in the United Kingdom and it can be prescribed only on a named patient basis. The results from the randomised trial of anabolic steroid or oral testosterone presented in this thesis indicates that oral testosterone (testosterone undecanoate) is an alternative to oxandrolone when oral treatment is required for boys with CDGP. In the UK this has the advantage of prescribing a drug which has a product licence. Both treatments induced a growth spurt of similar magnitude which was sustained when treatment was interrupted. As testosterone has a greater virilizating action in comparison to oxandrolone, a course of treatment
with testosterone would have been expected to induce or advance the development of secondary sexual characteristics. However, this was not found in this study, probably due to the small number of patients and the short duration of treatment. Spontaneous development in testicular volume was seen in all patients and this confirmed the diagnosis of CDGP, as well as indicating that neither treatment suppressed the hypothalamic-pituitary-gonadal axis. The induced growth acceleration occurred at too early a stage of sexual maturation to be due to the spontaneous pubertal growth spurt. Indeed, a pubertal growth spurt usually occurs in boys when 10 ml testicular volume is attained (Marshall et al, 1970).

It is recognised that traditional treatments of CDGP, if used at the appropriate doses, improve short-term growth rate, but do not alter final height (Wilson et al, 1988; Joss et al, 1989). Also in the patients studied, who received traditional interventions, final height or body proportion attainment were not modified. It can be hypothesised that this was mainly related to the late timing at which therapy was commenced: the patients had already had prolonged growth deceleration and therefore a significant degree of segmental body disproportion was already present. As reduced spinal growth seems to be related to delayed puberty, it may be postulated that if the delayed onset of puberty is anticipated, the marked spinal growth deceleration and reduced bone mineralization may be prevented. Boys with constitutional growth delay in childhood will almost certainly develop delayed puberty and therefore are at an increased risk of impaired final height associated to an eunuchoid appearance (Crowne et al, 1991) and to reduced bone mineralization in the spine (Finkelstein et al, 1992). In these circumstances low-dose anabolic steroids may be a therapeutic option in the treatment of growth delay during late pre-puberty/early puberty (11-12 years in boys) (Papadimitriou et al, 1991), when the marked spinal growth
deceleration has not occurred yet and therefore normal segmental proportion is still present. The aim of the treatment would be to bring forward the growth spurt into the normal age range, without deteriorating final height prognosis. Low-doses of oxandrolone were used in twenty-three prepubertal boys with growth delay to this effect. Oxandrolone treatment induced growth spurt and prevented deceleration in the upper segment in all boys, who otherwise were not expected to have their pubertal growth spurt until the attainment of 10 ml testicular volume (genitalia stage 3-4) (Marshall et al, 1970). The induced growth acceleration was sustained after the interruption of the treatment with maintenance of normal body proportion throughout puberty. It also allowed a normal pattern and rate of pubertal maturation. Final height attainment will confirm whether an improvement in final height has also occurred. These results suggest that treatment of growth delay with low-dose oxandrolone in late prepuberty/early puberty may be able to prevent spinal growth deceleration, with the presence of normal upper-lower body segmental proportion throughout puberty.

During the last 10 years biosynthetic GH has been used in short “non GH deficient” children in an attempt to improve final height outcome. Data so far published have shown that in these children GH treatment can significantly increase height velocity in the short-term, but in the long-term this is followed by attenuation of growth response, despite continuous treatment. In addition, puberty can be accelerated by GH treatment with progressive advancement in bone maturation and a consequent decrease in height prediction. The ultimate impact on final height remains to be established even though preliminary data suggest that a significant improvement is not likely. It can been hypothesised that short-term GH treatment may instead be advantageous in improving short-term growth
and therefore increasing height before the onset of puberty in prepubertal children with growth delay. High doses of human GH were used for a year in 22 younger children with growth delay to this effect. A significant growth acceleration was induced by GH in all children but this was followed by a marked growth deceleration the year after the interruption of treatment. Growth rate after GH treatment was significantly lower than the pre-treatment value with partial loss of the height gained during GH therapy. A longer follow-up will establish if a complete loss of the height gain following GH treatment occurs before the onset of puberty. Perhaps the most interesting finding will be the effect of GH treatment on the prepubertal gonads and its possible sequelae to the duration of pubertal maturation.

7.3- Treatment of Growth Delay in Boys with Isolated Growth Hormone Deficiency

At the time of onset of the growth spurt there is an increase in GH secretion (Albertsson-Wikland et al, 1994), which is amplitude modulated. It has been proposed that this increase in GH pulse amplitude, associated with the timing of the growth acceleration, is induced by the presence of sex steroids. It has also been suggested that the oxandrolone/testosterone induced growth spurt is mainly due to an increase in GH secretion rather than to a direct effect on the tissues (Stanhope et al, 1987b; Ulloa-Aguirre et al, 1990). However, it has been reported (Malhotra et al, 1993) that there is no effect of oxandrolone on the GH axis in boys with CDGP following oxandrolone administration. On the other hand, testosterone induces an increase in GH secretion by conversion to estradiol, even though an increase in GH secretion does not seem to be necessary for the pubertal growth spurt. Indeed, treatment with dihydrotestosterone, a non-
aromatizable androgen, induced an acceleration of growth rate without altering GH production (Keenan et al, 1993). The group of patients presented in this study, having IGHD and being on a constant GH dose regimen, offer an interesting clinical model to investigate the interaction between androgens and GH. The documented significant increase in growth rate, despite a constant GH dose regimen, suggests that the growth effect of androgens during the induced pubertal growth spurt is more likely to be due to a peripheral action, such as sensitisation of the cartilaginous growth plate to GH or an enhancement of the bioactivity of GH, rather than mediated via an increase in GH secretion. On the other hand, after the interruption of androgen therapy, a rebound in testosterone level and spontaneous pubertal progress may explain the sustained growth spurt. Interestingly, doubling the dose of GH during puberty in patients with IGHD does not seem to improve final height prognosis (Stanhope et al, 1992); the faster rate of progress through puberty is not associated with an increase in growth rate.

Both during and following treatment with oxandrolone or testosterone, the anthropometric response in boys with IGHD was similar to that of those with CDGP; this points to a more important role of sex steroids in spontaneous growth spurt than previously believed.

7.4- Short-Term Growth Hormone Effect on Gonadotrophin and Endogenous Growth Hormone Secretion in Children with Growth Delay.

It has been established that the effect on growth of GH treatment in patients with normal GH secretion wanes over time (Hindmarsh et al,
This slowing of growth velocity is irreversible, even with very high doses of GH. One explanation may be that a receptor down regulation occurs in response to continuous GH treatment, associated with a state of temporary pituitary suppression. When exogenous GH administration is suspended a reactivation of the endogenous GH secretion should take place followed by a transitional phase during which the all body system should readjust to the physiological lower plasma GH levels. Therefore, it might be postulated that soon after the discontinuation of GH therapy, higher GH serum concentration or changes in GH pulse frequency may be required to overcome the state of relative GH resistance.

The data presented in this study showed no statistically significant changes in spontaneous mean GH concentration 2 to 3 weeks after the cessation of exogenous GH treatment compared to pre-treatment values. The amplitude of the signal also remained unchanged while the frequency of the signal was significantly increased. The faster GH pulse release may therefore be a mechanism to overcome the state of peripheral GH resistance. However, even though there was no evidence of change in GH amplitude in response to pharmacological doses of GH, this does not exclude it completely as the pituitary gland may have recovered by the time the second profile was performed. Furthermore, the higher IGF-1 levels together with the reduced IGF BP-3 may also reflect the peripheral GH resistance. The temporary persistence of GH down regulation may, on the other hand, justify the marked growth deceleration observed after the interruption of GH treatment.

This hypothesis is supported by Laron's findings (Eshet et al, 1993). The Authors have suggested that GH treatment in children with CDGP increases the circulating levels of IGF-1 levels but it also induces a down-
regulation of IGF-1 receptors. They studied the IGF-1 binding sites on erythrocytes before and after 1-2 months of GH treatment in non-GH deficient short children and found an inverse correlation between plasma levels and the number of its binding sites, the latter partially compensated by an increase in the binding affinity.

Historical (Darendeliler et al, 1990) and randomised trial data (Stanhope et al, 1992) suggest that GH treatment of children with isolated growth hormone deficiency accelerates the progress of puberty with an overall reduction in the duration. This phenomenon appears to be dose dependent (Stanhope et al, 1992) with patients treated with higher dose regimens progressing through puberty faster, limiting therefore the efficacy of treatment. GH may influence pubertal development by increasing local production of insulin-like growth factors within the gonads (Tres et al, 1983; Davoren et al, 1986) and by modulating the gonadal response to gonadotrophin secretion (Adashi et al, 1985). The interaction between GH and gonad can also be the major limiting factor in the effectiveness of exogenous GH when used to improve final height in short patients not GH deficient/insufficient. The improved short-term growth rate induced by GH treatment in these patients may not result in an improvement of final height due to the reduction of height prognosis during puberty. In this study the prepubertal pattern of gonadotrophin secretion was not modified by exogenous GH treatment. However, clinical follow-up is required to detect if there is a long-term effect of GH treatment on the prepubertal gonad.
SECTION 8:

CONCLUSIONS
1. Final height outcome can be compromised in boys and girls with CDGP, with failure to attain their full genetic potential.

2. Height prediction is not reliable in this condition.

3. Standing height and growth velocity at presentation are predictive factors of final height outcome.

4. A relatively short sitting height is an important sign for the diagnosis of CDGP, especially when puberty is severely delayed. Its minor appearance is an indicator of inadequate final height outcome and it absence should point to an alternative diagnosis.

5. Impaired growth in upper segment during the pubertal growth spurt does not compensate for the previous deficit leading to an adult eunuchoid appearance.

6. Oxandrolone in prepuberty is effective at improving growth velocity and preventing body segmental disproportion. The attainment of final height will establish if an improvement in height prognosis has also occurred.

7. High dose GH treatment improved short-term growth in prepubertal children with growth delay, but this was followed by a significant growth deceleration when GH treatment ceased. Long-term follow-up is required to establish whether any effect of such treatments on the pubertal maturation has occurred.

8. Exogenous GH treatment did not alter the prepubertal pattern of gonadotrophin secretion but induced a state of resistance to GH action, with increase in spontaneous GH pulse frequency and increase IGF-1/BP-3 ratio when the treatment was suspended.

9. Oral testosterone can be used as a valid alternative to oral oxandrolone as both are effective at producing growth acceleration
and advancement in secondary sexual characteristics. This is important as oral testosterone has a product licence in UK while oxandrolone does not, due to its abuse in sports medicine and it can only be prescribed on a named patient basis.

10. The growth acceleration induced by either oxandrolone or testosterone in boys with isolated GH deficiency, receiving constant doses of GH, points to a more important role of sex steroids in the mechanism of the spontaneous growth spurt than previously believed.

11. The findings of this thesis suggest that a new approach to therapy in CDGP may be necessary. Treatment may be appropriate not only for psychological reasons but also for optimising final height. In addition, earlier therapeutic intervention may be indicated in order to prevent the eunuchoid appearance of a relatively short upper segment.
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APPENDICES
9.1- List of Tables

1. Mean value of predicted height, mid-parental height and final height in untreated boys with CDGP: review from the literature.


4. Clinical data from 98 boys with CDGP, both at presentation and final height.

5. Clinical data from 34 girls with CDGP, both at presentation and final height.

6. Clinical data from 78 patients with CDGP at presentation.

7. Clinical data from 23 boys with growth delay before commencement of oxandrolone treatment (group A) and from 56 boys with untreated CDGP at presentation (group B).

8. Clinical data from 33 boys with CDGP at presentation, divided in two groups according to the treatment received.

9. Clinical data from 15 boys with isolated GH deficiency on GH treatment at commencement of either sex or anabolic steroid treatment.
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6. Height expressed as SDS in 34 girls with CDGP at presentation and final height as well as predicted and corrected mid-parental heights.

7. Mean SH and SLL SDS in 98 boys and 34 girls with CDGP.

8. Height expressed as SDS in 78 boys with CDGP at final height as well as predicted and corrected mid-parental heights.

9. SH minus SLL, both expressed as SDS in 78 boys with CDGP at presentation plotted versus the chronological age.

10. Mean values (±1 SD) of standing height and difference between SH and SLL before and after a course of oxandrolone treatment, and 2 yrs after the interruption of treatment are illustrated in 20 boys with growth delay.

11. Mean values (±1 SD) of standing height and difference between SH and SLL at presentation and final height attainment are illustrated in 56 boys with untreated CDGP.

13. Mean growth velocity SDS (± 1SD) prior to, during and after 1 year GH treatment.

14. Height SDS in 22 children with growth delay before, during and 6 and 12 months after GH treatment.

15. Correlation between chronological age and height increase after short-term GH treatment in 22 children with growth delay.

16. Growth data from a boy with growth delay treated with a 1 year course of GH.

17. Mean growth rate in 29 boys with CDGP treated with either oral testosterone or oxandrolone in pre-treatment, treatment and post treatment periods.

18. Yearly growth rates for 15 boys with IGHD during growth hormone treatment receiving either oxandrolone or testosterone.

19. Height expressed as SDS for bone age in 15 boys with IGHD treated with anabolic or sex steroids.

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21. GH relative spectral power prior to and after 1 year GH treatment in 13 children with growth delay.

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23. IGF-1 / BP-3 ratio prior to and after 1 year GH treatment in 13 children with growth delay.
9.3- Abbreviations

CDGP : Constitutional Delay of Growth and Puberty
IGF BP-3 : IGF-1 Binding Protein 3
FSH : Follicle Stimulating Hormone
GH : Growth Hormone
GHD : Growth Hormone Deficiency
GHRH : Growth Hormone Releasing Hormone
GnRH : Gonadotrophin Releasing Hormone
IGF-I : Insulin-like Growth Factor I
IGHD : Isolated Growth Hormone Deficiency
LH : Luteinizing Hormone
SDS : Standard Deviation Score
SD : Standard Deviation
SEM : Standard Error of Mean
SH : Sitting Height
SLL : Subischial Leg Length
9.4- Appended Publications


9.5- Acknowledgements

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9.6- Declaration

1. The bulk of the work involved in this thesis was done in the three years that I was a Research Fellow at the Institute of Child Health, London (1992-1995).

2. Anthropometric measurement were performed at the growth clinic, Great Ormond Street Hospital for Children, NHS Trust, London. For the prospective studies pubertal ratings and ephiphyseal maturation scores were assessed by myself and Dr Stanhope independently.

3. All the blood samples involved in this thesis were taken by myself. Hormonal assays were performed by Ms J Janes at the Institute of Child Health, London, to whom I am most grateful.

4. Statistical analysis was performed by myself, using the computerised statistical programme SPSS.

5. Dr D Matthews, Radcliffe Hospital, Oxford, assisted with the analysis of growth hormone neurosecretory data.

6. My supervisor, Dr R Stanhope, Senior Clinical Lecturer at the Institute of Child Health, London, provided enormous encouragement and invaluable advice.
SECTION 10:

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