INFLUENCING THE EXTREMES OF GROWTH

too tall - too small

Cover illustration: Wedding of my grandparents

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INFLUENCING THE EXTREMES OF GROWTH
too tall - too small

BEINVLOEDING VAN EXTREME LENGTEGROEI
te groot, te klein

PROEFSCHRIFT

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groot klein

Ik zie een spin
Klein voor mij
Voor een vlieg een monster
Ik zie een buizerd
En ik denk wat klein
'Wat groot', dacht de muis
nog even

Peter van Ark, groep 6, Midwolde

Uit: De wereld om mij heen, dichtbundel verschenen onder auspiciën van de Stichting Werelddag voor Kinderen en Poëzie, ECI, Vianen 1993

CONTENTS

List of Abbreviations

ATT	Arginine tolerance test
AUC	Area under the curve
BA	Bone age
BMI	Body mass index
CA	Chronological age
CASAS	Computer aided skeletal age scoring system
CTS	Constitutionally tall stature
EE	Ethinylestradiol
FAI	Free androgen index
FH	Final height
FSH	Follicle stimulating hormone
GH	Growth hormone
GH-BP	Growth hormone binding protein
GHRH	Growth hormone releasing hormone
H	Height
HV	Height velocity
$HVSDS_{CA}$	Height velocity standard deviation score for chronological age
$HSDS_{CA}$	Height standard deviation score for chronological age
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
IPH	Index of potential height
IUGR	Intrauterine growth retardation
LH	Luteinizing hormone
M	Menarche
MS	Maturity score
oGTT	Oral glucose tolerance test
PHV	Peak height velocity
RH	Relative height
SD	Standard deviation
SDS	Standard deviation score
SDS_{CA}	Standard deviation score for chronological age
SE	Standard error
SHBG	Sex hormone binding globulin
SRIH	Somatotropin-release inhibiting hormone, somatostatin
SRS	Silver-Russell syndrome
T	Testosterone
TH	Target height
U-GH	Urinary growth hormone excretion

CHAPTER 1

General Introduction

NORMAL GROWTH AND EXTREMES OF GROWTH

INTRODUCTION

Growth is an important indicator of physical and emotional well-being in childhood. Deviations from the normal range both for height and for height velocity may indicate an underlying congenital or acquired problem. A thorough understanding of the factors influencing the process of normal growth is essential in order to understand the pathophysiology of extremes of growth.

What is normal growth and what are extremes of growth? This question can only be adequately addressed when related to population standards. It has been well established that populations of various ethnic origin may differ considerably in growth and development (1). Therefore, reference growth curves are obtained by measuring healthy individuals longitudinally or cross-sectionally (or both). In The Netherlands, such reference growth curves have been constructed (2). In fact, Dutch men and women belong to the world's tallest people. Knowing the normal variance of growth of the reference population, extremes of growth can be defined. Usually, an individual whose height differs more than 2 standard deviations from the population mean, i.e. a child with a height above the 97th percentile of the growth curve is considered too tall and a child growing below the 3rd percentile is considered too short. It needs to be emphasized though, that most of the children who grow beyond these percentiles are part of the continuum of a normal distribution curve and only a minority will have a defined abnormality. In addition, these cut-off points are quite arbitrary and other percentiles may be used.

Children growing at the extremes of height are assumed to encounter several psychosocial difficulties. In our culture, tallness is generally valued positive. An association between physical stature and achievement has been documented: tall individuals score higher on intelligence tests than short individuals (3,4) and persons who achieved higher social status tend to be taller than those of lower status (5). In contrast, short statured individuals are perceived to be less

competent, are seen less positively by peers, and are likely to be in lower positions within a given profession (6). Therefore, tall stature in childhood usually generates less anxiety initially than shortness. However, many tall adolescents feel different from their peers and may develop coping mechanisms such as kyphotic posture (in order to mask their tallness) and social withdrawal. In addition, practical problems such as clothing and shoeing, fear about future compatible partnering and careerplanning are also frequently reported problems faced by tall adolescents (7). In short stature, the main consequences are being infantilized and teased. In response the child may adopt several behavior patterns. Babyish behavior, becoming the clown or mascot, social withdrawal, low self-esteem and passiveness are just a grip of the problems described in children with short stature (8-10).

Growth is the result of complex processes. Multiple factors such as genetic constitution, nutrition, endocrine function and psychosocial well-being are involved in the processes of growth (11,12). The genetic component of height has been estimated to be 0.5 to 0.9; that is, 50% to 90% of the height variation is accounted for by genetic factors and therefore 50% to 10% is due to environmental factors (12). Assessment of the parental height as an indicator of the genetic component of the growth and development of a child is therefore of clinical interest (13). Important socio-economic factors that are associated with growth and development in children are social class, family size, birth rank, housing and crowding. Improved socio-economic conditions and more widespread health have lead to the manifestation of a positive secular trend in growth and development over the past few centuries (1,2,11). In 1865, the median height among Dutch recruits was 165 cm. One century later in 1965, the mean adult height in boys was 178 cm. Again, fifteen years later in 1980, mean adult height had increased by another 4 cm to 182 cm. In addition, in the middle of the 19th century, age of menarche in European girls was at about 16 to 17 years. Nowadays, the mean age of menarche is at 13 to 14 years. In contrast, deprivations and poor living conditions, stress, etc., could strongly affect the sensitive growing years in a negative way, causing an absence of a positive secular trend or even a negative tendency (1). Remarkably, studies of

fossil remains of our hominid ancestors demonstrate that the stature of individuals living during the last hundred-thousands of years reached the range of heights seen today: the mean stature of early anatomically modern *H.sapiens* in Europe (around 100,000 years ago) was 184 cm in males and 167 cm in females (14,15). Probably, similar phenomena responsible for positive and negative secular trends have affected human height throughout all our history.

Human growth appears to follow a typical pattern irrespective of ethnic origin or geographical region: from birth a high height velocity is observed until about 3 years of age; this is followed by a period with a lower and slowly decreasing velocity up to puberty. During puberty a sharp increase in height velocity occurs up to peak height velocity. Thereafter a decrease is noted until adult height is reached, in girls around 16 years and in boys around 18 years (16,17). Growth hormone (GH) and thyroid hormone are essential endocrine components for the regulation of physiological growth during childhood (16-18), whereas during pubertal growth independent and additional effects of gonadal steroid hormones are evident (19,20).

In the following paragraphs, we will discuss the endocrine regulation of normal growth during childhood and focus on the GH/IGF axis. Thereafter, a short synopsis of two examples of extremes of growth will be given: constitutionally tall stature and short stature after intrauterine growth retardation.

Growth hormone physiology

Human GH is synthesized by the anterior pituitary gland as a pre-GH molecule consisting of 217 amino acids. Subsequent cleavage yields the 191 amino acid peptide hormone with a molecular weight of 22 kDa, which forms about 80% of the secreted GH. The other 20% is a smaller variant of about 20 kDa (21). GH molecules circulate in both bound and free forms. The bound hormones are complexed to binding proteins (BP). These GH-BPs are identical to the extracellular domain of the GH receptor (22). They probably act as a modulator of release and

distribution of GH at tissue level.

GH is secreted in a pulsatile manner as a result of a complex of interacting neuroendocrine pathways (23). Among these are two antagonistic neurohormones: GH-releasing hormone (GHRH) and somatostatin (Somatotropin Release Inhibiting Hormone, SRIH). GHRH is synthesized in the hypothalamic arcuate and ventromedial nuclei and contains GH stimulatory activity. SRIH is produced in the periventricular and amygdaloid nuclei of the medial basal hypothalamus and has potent inhibitory properties on GH release. Axons from GHRH- and SRIH-containing neurons terminate in the median eminence and release their hormones in the vascular network of the portal system. The pulsatile pattern of GH secretion is orchestrated by episodic increases and decreases in the release of GHRH and somatostatin, respectively (24,25). In addition, many other neurotransmitters (such as adrenaline, acetylcholine) and neuropeptides (such as opioid peptides, galanin) are involved in the neural control of GH secretion with stimulatory or inhibitory effects on GH release (23).

Many physiological factors are known to affect GH secretion (24,26). These effects are achieved mainly by altering the secretion of GHRH and/or SRIH, rather than by direct action at the pituitary level. These physiological factors include endogenous rhythms, external stimuli and feedback by insulin-like growth factors (IGFs) and GH itself. Endogenous rhythms include surges of secretion during sleep and episodic secretion with peaks at 3 - 4 hours interval during day and night. The episodic nature of GH secretion varies considerably between physiological states, sexes and age. External stimuli for GH secretion include physical and emotional stress, exercise, starvation and metabolic substrates, including basic amino acids such as arginine and hypoglycaemia. Provocative tests based on these stimuli, and on administration of various stimulators or blockers of neurotransmitter action, have been developed to test GH secretion in children. Gonadal steroid hormones also play a critical role in the regulation of GH secretion by exerting their effects on multiple sites of the somatotrope axis (19,20). Feedback regulation of GH secretion is effected by IGF-I by inhibition at both hypothalamic and pituitary sites (27). There

is evidence for an inhibitory action of IGF-I on hypothalamic GHRH release and a stimulatory effect on the secretion of SRIH from the medial basal hypothalamus (19,24). In addition, local IGF-I production may also inhibit GH release from the pituitary via paracrine or autocrine mechanisms (28). GH can autoregulate its own secretion (short loop feedback) by inhibition of GHRH secretion rather than by enhancement of somatostatin release (29,30).

The GH/IGF axis

In 1957 Salomon and Daughaday demonstrated that GH stimulated sulphate incorporation into cartilage indirectly through a serum factor, initially termed sulphation factor, later designated somatomedin-C or IGF-I (31). This finding formed the basis of the so called somatomedin hypothesis, stating that circulating IGFs, produced at distant sites in response to GH, mediate the effect of GH by endocrine mechanisms (32). With advances in the understanding of biochemistry, physiology and clinical aspects of the IGFs (33), it is clear that this is an overly simplistic view. Various lines of evidence point to paracrine and/or autocrine actions for IGFs as well (34).

There are two different forms of IGF-peptides: IGF-I (70 aminoacids (aa)) and IGF-II (67 aa) with an aa-sequence homology of 60% (35). The IGFs interact with the cell by binding to specific receptors which have been well characterized (36). The type I IGF receptor is structurally related to the insulin receptor, binds IGF-I with higher affinity than IGF-II and binds insulin only weakly. The type II IGF receptor is structurally and functionally different from the insulin and type I IGF receptor. It is identical to the mannose-6-phosphate receptor, binds IGF-II with greater affinity than IGF-I and does not bind insulin. The IGFs are present in the circulation and throughout the extracellular space almost entirely bound to binding proteins, IGFBPs. To date, six IGFBPs have been characterized (37). The vast majority (approximately 90%) of total IGF circulates as a 150 kDalton complex, consisting of IGF-I or IGF-II plus IGFBP-3 and an acid labile subunit (38). The

concentration of IGFBP-3 has been found to be GH dependent, while levels of IGFBP-1 and IGFBP-2 are inversely correlated with GH status (39).

The IGFs have a wide array of biological effects in many tissues and cells. The best-known effects of IGFs relate to their acute anabolic effects on protein and carbohydrate metabolism and to their longer term effects on cell replication and differentiation (33,40,41). These biological actions may be influenced by the IGFBPs. Possible functions of the IGFBPs are 1) acting as transport proteins, 2) prolonging the half-lives of the IGFs, 3) providing a means of tissue- and cell type-specific localization, and 4) directly modulating the interaction of the IGFs with their receptors and thereby indirectly controlling their biological action (37,41,42).

Longitudinal bone growth

GH has a profound effect on longitudinal growth. With respect to the growth promoting effect of GH on the epiphyseal growth plates a dual effector theory of GH action has been advocated (43). GH stimulates bone growth directly by promoting the differentiation of precursor cells in the growth plate, and GH stimulates bone growth indirectly by inducing IGF-I responsive chondrocytes and by stimulating the local production of IGF-I. On the other hand, gonadal steroids also influence skeletal growth as shown by an increased growth rate at the time of gonadal maturation (19). Sex steroids have both indirect and direct effects on skeletal growth. The indirect action is mediated by GH and IGF-I, since sex hormones stimulate GH and IGF-I secretion (19,20). Evidence is present that sex steroids also have a direct growth effect, independent of IGF-I and GH (44,45).

The effects of GH and sex hormones on longitudinal bone growth become clear from clinical data of children presenting with growth disorders that can be conceived as experiments of nature (19,46). GH-deficient or -insensitive children are characterized by severe growth retardation. The long bones are relatively short compared to the spine length and head size. In contrast, children with hypogonadism have relatively long limbs. In this condition the growth plates of the

long bones remain open for GH action over a longer period of time due to lack of sex hormones. Patients with Laron dwarfism suffer from GH-insensitivity due to a functionally defective GH receptor gene. These patients have high circulating values of GH but no endocrine generation of IGF-I while a definite pubertal growth spurt is observed, suggesting a direct sex steroid effect on bone growth (19). Children with precocious puberty have elevated levels of sex hormones at a prepubertal age which cause early fusion of the epiphyseal growth plates. They will thus end up shorter than expected and show relatively short long bones (47).

Beside the effect of GH on longitudinal growth, it also exerts other metabolic effects on carbohydrate, lipid and mineral metabolism. These items have been extensively described in a number of reviews and will not be further discussed here (48-50).

Insight in the complex orchestration of physiological growth, mediated by variables such as GH secretion, IGFs and IGFBPs, which in turn have complex biological actions by itself, is essential for possible therapeutic intervention in children presenting with extremes of growth. In this thesis, we will focus on two examples of extremes of growth: constitutionally tall stature and short stature associated with intrauterine growth retardation. Both entities will be reviewed briefly.

CLINICAL ASPECTS OF THE EXTREMES OF GROWTH

Constitutionally tall stature

Natural growth pattern

Constitutionally tall stature (CTS) is a variant of the normal pattern of childhood growth and development and constitute 3 - 10 percent of the normal population, depending on the definition used. Usually, one or both parents are also tall, thus genetic and familial factors appear to play the most important role in

etiology and pathogenesis. Mean birthlength is at the 75th percentile, and tall stature becomes evident at the age of three to four years. Growth velocity is accelerated in early childhood but slows down after four or five years of age when the growth curve starts to parallel the normal curves (51). The diagnosis is generally made from family history, record of growth and physical examination. No apparent abnormalities are present at physical examination, which makes it possible to distinct from other excessive growth syndromes such as Marfan syndrome, homocysteinuria, and Klinefelter syndrome (52).

Endocrinology

Endocrinological studies of tall children indicate that tall stature is due, at least partly, to increased GH secretion. Studying children with various heights, a significant positive correlation was found between growth and GH secretion (18,53). Paradoxical GH responses to glucose loading and to administration of thyrotropin-releasing hormone similar to those seen in acromegaly have been observed in constitutionally tall children (54,55). Furthermore, elevated serum levels of IGF-I have been found (54,55). However, not all children show signs of relative hypersecretion of GH. A recent study showed a clear heterogeneity of GH secretion in tall children, with even the presence of low GH secretors (56). Therefore, other mechanisms than GH hypersecretion may play an important role in the etiology of tall stature, such as hypersensitivity to GH (56), enhanced pituitary responsiveness to GHRH (57) or a decreased inhibitory effect of somatostatin (58).

Height prediction

Height prediction plays a key role in the management of children with growth disorders and thus in children with CTS. In fact, possible therapeutic intervention is based on the estimated height prognosis: whenever the height prognosis exceeds a certain limit (usually 2 standard deviations above the mean of the population), treatment will be considered. Hence, accurate techniques for reliable height predictions are needed. In clinical practice various methods have

been developed of which the methods developed by Tanner et al (59) and Bayley and Pinneau (60) are most commonly used. They both share the use of bone age as an indicator of skeletal maturity in order to estimate final adult height. The first prediction method uses the bone age method developed by Tanner et al (59), whereas the latter utilizes the bone age method of Greulich and Pyle (61). This implies that many of the potential problems underlying the bone age determination methods have to be considered (62). For instance, both techniques make use of subjective processes and discontinuous scales which result in considerable inter- and intra-rater variability (63-65). In order to improve the objectiveness of bone age estimation the method of Tanner et al has recently been transformed into a computerized image analysis system using a continuous scale (66). However, before integrating this computer system into clinical practice, validation studies are needed. Thus far only a limited number of studies have been performed testing the reliability of height prediction methods in large groups of untreated children with tall stature (67-69). Nevertheless, knowledge about the specific advantages and disadvantages of the various methods is of utmost importance since it may influence clinical practice.

Short stature after Intrauterine growth retardation

Fetal growth

Fetal growth can be considered in two major phases: that relating to embryogenesis and organogenesis which is essentially the first half of gestation, and growth of the late gestation fetus. Growth in the first half of gestation shows minimal variation, except when associated with pathology e.g. chromosomal abnormalities or infections. Fetal growth in the second half of gestation is greatly constrained by the uterine environment and largely mediated by changes in fetal substrate supply (70). Therefore, reduction in fetal substrate supply by maternal disease or placental dysfunction causes a prompt reduction in fetal growth. This is accompanied by a redistribution of fetal blood flow to favour vital organs such as

the brain and heart. Important interactions are present between nutritional state and the interaction of maternal, placental and fetal hormones and growth factors (71-75). Most attention has been focussed on the role of insulin and insulin-like growth factors I and II as regulators of fetal somatic growth (76-81). Marked changes in insulin secretion are associated with clinical abnormalities of fetal growth: pancreatic agenesis leads to profound intrauterine growth retardation (IUGR) and fetal hyperinsulinemia due to maternal diabetes results in fetal overgrowth. In addition, levels of IGF-I in fetal and cord blood correlate with birth size. In fetuses with IUGR, circulating levels of IGF-I and IGF-II are reduced. GH on the other hand has a limited role in fetal growth. Thus, optimal growth can only occur if the nutritional and endocrine milieu is appropriate.

Postnatal growth

Children with intrauterine growth retardation (IUGR) comprise a heterogeneous group. As stated above, various causes may underlie the stunted growth at birth varying from chromosomal disorders, congenital infections, placental dysfunction to maternal disease (smoking, hypertension, alcohol abuse). However, in the majority of cases the etiology is not clear (idiopathic) (82). After IUGR, most of the children born without an underlying disorder do show catch-up growth within the first years of life. However, the percentage of children who fail to show catch-up growth after birth is different in various studies ranging from 10-30% (83-85). This variability is not only caused by the heterogeneity in etiology underlying the prenatal growth retardation, but also by the differences in definitions of IUGR. In practice various criteria have been used to define IUGR, including measures of absolute size, such as low birth weight (<2500 g) or very low birth weight (<1500 g), and measures of relative size (small for gestational age), variably defined as birthweight or birthlength less than the third or tenth percentile, and less than two SD below the population mean for gestational age and sex (68). However, since the postnatal growth of children with IUGR is usually related to height, it seems appropriate to define IUGR in terms of birthlength rather than birthweight. In a

recent Swedish study on postnatal growth in 5111 children 13% of the 111 children born with a short birthlength (<-2 SD below the mean for gestational age) showed insufficient catch-up growth in the first two years of life (86). Hokken et al (87) reported on the postnatal growth of 724 children born with IUGR (birthlength < P3 for gestational age) from three academic hospitals in The Netherlands and found a similar percentage of 15% of children without catch-up growth up to a height > P3 within the first two years of life. In this respect, they observed no significant difference between preterm and full-term babies. Therefore, one of the long-term effects of IUGR may be persistent short stature. Indeed, various studies on final adult height show that these children have shorter adult heights compared to the normal population and compared to their genetic potential (target height) (85,88-90).

Silver-Russell syndrome

The growth pattern of children with the Silver-Russell syndrome (SRS) shows a striking resemblance with that of IUGR children without dysmorphic signs (91,92). SRS is mainly characterized by short stature, low birth weight and dysmorphic features such as small triangular face, body asymmetry, clinodactyly, and down-curved corners of the mouth (93). Therefore, many studies on growth of children with short stature after IUGR usually include patients with SRS as well. There are indications that the natural history of children with IUGR includes an increase in skeletal maturation towards the latter half of the first decade of life (88,91,92). In addition, some patients with IUGR or SRS may develop an early puberty (91,93,94). Both phenomena may play an important role in the restriction of the final adult height of these children.

Endocrinology

The endocrine control of postnatal growth differs from that which applies prenatally. Whereas the intrauterine growth of the fetus is largely independent of the pattern of GH secretion, the role of the GH axis is dominant during childhood.

The pathophysiological mechanism underlying the failure to catch-up growth of children with short stature after IUGR is not completely understood. It is possible that persisting defects in the GH/IGF axis may be debit to the stunted growth. However, IUGR children, with and without dysmorphic signs, tend to be tiny and lack the typical signs of GH-deficient children such as abdominal fat distribution. Nevertheless, endocrinological studies did find evidence for disturbances in GH secretion: abnormal GH secretion patterns during 12 or 24 hours of physiological testing and/or insufficient GH responses to provocative tests have been observed (95-97). Few data are available on IGF-I and IGF-II levels in IUGR children during childhood. Normal IGF-I levels have been reported, but without correction for chronological age (96,98).

Other pathophysiological mechanisms

Factors involved in the pathophysiological state of IUGR itself, may also affect organ development. It is known that the brain undergoes a period of rapid weight gain during the later half of the fetal period with a climax at the time of birth (99). This growth spurt of the brain decreases during the first year of life. Besides, there are at least two periods of rapid brain cell multiplication. One occurs at 15-20 weeks of fetal development and concerns development of neuroblasts. A second period concerns glia cell multiplication, and starts at 25 weeks of gestation and ends probably in the second year of life (100). During these growth spurts, the brain is presumably vulnerable to disturbances in its nutritional supply. Thus, brain damage may occur due to the underlying factors responsible for the intrauterine growth retardation. In fact, animal studies gave arguments in support of this hypothesis (101). Other lines of evidence are derived from clinical studies in children with IUGR showing an association between IUGR and an increased incidence of mental handicap, lower intelligence, neurological deficits, poor academic performance and behavior problems (102-106). Therefore, the GH secretion disturbances observed in children with IUGR may reflect a form of brain dysfunction. More research is required to elucidate the pathophysiological

mechanism of the failure to catch-up growth in children with IUGR.

TREATMENT OPTIONS FOR THE EXTREMES OF GROWTH

Treatment of constitutionally tall stature

Psychology

Treatment of tall stature is generally based on psychological grounds. Some children with excessive growth may suffer considerably from being much taller than others. They feel different from their peers and are subject to hurtful remarks about their height. Coping mechanisms such as kyphotic posture (in order to mask their tallness), social withdrawal and even depression may develop. Practical problems concerning clothing and shoeing, fear about future partnering (especially in girls) and careerplanning are also frequently reported problems faced by tall children. This has lead to a search for height intervening therapy in children with CTS.

Sex hormone treatment

In 1956, Goldzieher used high doses of sex steroids in 14 adolescent girls with tall stature in order to reduce their final height (107). The basis for the use of sex hormones to limit adult height came from observations in children with precocious puberty. These children show early closure of the epiphyses due to premature secretion of gonadal steroids, which limit their eventual adult height (108,109). Since then, many reports have appeared describing the height reducing effect of administration of high doses of sex hormones in girls (110-128) and in boys (129-131). There is general agreement that a favorable effect on ultimate height results from such pharmacological therapy, with possible much greater effects in selected patients. Some studies have shown that the height reduction is greater when treatment has begun at a younger age and/or bone age (111,112,119,123,125,127,128). However, the establishment of the claimed effects is open to debate, since a well designed, prospective controlled study has never

been performed. This implies that the observed results may have been biased by several factors. For instance, results on height reduction had only been derived from comparison of the achieved height with the height prediction prior to treatment, and with no correction for the error of the prediction method used. In addition, when control groups had been used they tended to be small. Besides, while assessing the ultimate height reduction, differences in initial clinical data between treated children and controls such as age, bone age and height prediction, had not been taken into account. Furthermore, in many studies adult height had been assumed to be reached at a relatively young age. Therefore, interpretation of the height reducing effect of sex hormone treatment in children with CTS has to be seen with reservation.

Long-term side effects

An important issue concerning the treatment with high doses of sex steroids is the possibility of unwanted side effects. In this respect special attention has been focussed on hemostasis (132), lipid metabolism (133) and functioning of the hypothalamic-gonadal axis (134,135). So far, unwanted side effects have only been reported during treatment or shortly after discontinuation of therapy (92-131,136-140). Most side effects were found to be mild and reversible. Suppression of the hypothalamic-gonadal-axis induced by the pharmacological doses of sex steroids (via a negative feedback mechanism) was found to be reversible (134,135). However, the possibility of a long-term suppressive effect of sex hormone therapy on reproductive functioning in boys has been postulated (141,142). In girls, the ultimate 'proof' for complete reversibility of hypothalamic-gonadal suppression, pregnancy, has been reported in various single cases (111,118,120,124,127). However, systematic long-term follow-up studies on possible unwanted side effects are lacking. In this respect, the considerable amount of data on the association between long-term oral contraceptive use and possible health risks (reviewed in references: 143,144) are indicative as they may form a reflection of the prospective risks in estrogen-treated girls.

Other treatment modalities

Another approach in the management of tall stature relates to interference in the regulation of GH secretion. It is based on the assumption that tall stature is related to GH hypersecretion. In order to suppress endogenous GH secretion bromocriptine therapy has been used. Bromocriptine is a dopamine agonist and inhibits GH secretion in patients with acromegaly by binding to dopamine receptors at the pituitary level (145). In children with CTS, however, results are conflicting and the effectiveness of bromocriptine has not been substantiated (146-148). Recently, an alternative strategy has been proposed to limit growth with administration of a somatostatin-analogue. Preliminary studies showed an effective suppression of GH secretion and a significant reduction in growth rate by somatostatin therapy (149-151). However, final results on height reduction remains to be established. Moreover, the optimum mode of administration and the presence of serious side effects (such as asymptomatic gall bladder stones) need to be considered.

Treatment of short stature after IUGR

Psychology

Short stature, irrespective of the underlying cause, may encounter several psychological difficulties (6,8-10). A tendency to lower intelligence scores, behavior problems, low self-esteem, unemployment and lower social success have been reported (8,9,152-154). Apart from this, IUGR *per se* is associated with an increased prevalence of psychological disabilities. Several studies have been performed on this subject and associations have been found between IUGR and an increased incidence of mental handicap, lower intelligence, neurological deficits, poor academic performance and behavior problems (102-106).

GH treatment

In order to improve growth in children with short stature after IUGR, GH

treatment using human GH has been explored since the early 1970s (155-157). Initial results were disappointing probably due to the low dose and frequency of GH administration (2-3 times weekly). With the availability of biosynthetic GH, the efficacy and safety of daily treatment with GH has been tested in children with non-GH deficient short stature (158-160), inclusing in children with short stature after IUGR (95-97,160). In the study by Albertsson-Wikland (95) GH treatment with doses of 0.1 IU/kg/day of recombinant GH (≈0.7 IU/kg/week) resulted in a significant improvement of growth rate in five out of six children with SRS (height velocity standard deviation score (HVSDS) +0.85 during the first year and HVSDS +0.58 during the second year of treatment) and in seven out of ten IUGR patients (HVSDS +1.21 during the first year of treatment). Other studies showed similar results. In the study by Rochiccioli et al (96) nine children with IUGR were treated with a pituitary GH dose of 0.3 IU/kg/week for one year. The height velocity increased from a baseline level of 3.5 ± 0.8 cm/year to 7.0 ± 0.9 cm/year. The study by Stanhope et al (97,161) was a dose-response study comparing treatment with recombinant GH doses of 15 and 30 IU/m^2/week (≈ 0.5 and 1.0 IU/kg/week, respectively) in the first year of treatment. In the next two years of treatment all 24 children received the higher GH dose of 30 IU/m^2/week. The study included IUGR patients with and without dysmorphic features. In the first year of treatment a dose-dependent increase in height velocity was observed: HVSDS increased from -0.8 to +1.4 with the lower dose and from -0.8 to +3.6 with the higher dose. After three years of treatment, mean HVSDS was +1.1, irrespective of which initial treatment dose had been administered during the first year. There was no difference in the growth response of children with or without dysmorphic features. However, despite the sustained increase in growth rate, no significant change in height for bone age SD score was present. This may point to an unaltered height prognosis. Yet, in untreated IUGR patients, a natural decrease in height prognosis may be present associated with an inappropriate advance of epiphyseal maturation. Therefore it is possible, that GH treatment does lead to a greater adult height than would have been attained otherwise (161). Longer term studies with substantial greater

numbers of patients are required to answer the outstanding question whether GH treatment may be indicated for children with short stature after IUGR.

AIMS OF THE STUDY

Constitutionally tall stature

The studies presented in this thesis were undertaken to evaluate the value of height prediction methods and the effect of sex hormone treatment in children with CTS. In the Sophia Children's Hospital, constitutionally tall children have been treated with high doses of sex steroids since 1968. In girls oestrogens have been used (ethinyloestradiol 200 µg/day, orally, range 100-300µg/day) in combination with progestagens (medroxyprogesterone 5-10 mg/day, orally) every 5-10 days of the month. Tall boys have been treated with androgens: testosterone esters in various regimens with a total monthly dose up to 1000 mg).

The following questions were addressed:

- How reliable is adult height prediction in children with tall stature?
- What is the reliability of a computer aided skeletal scoring system in bone age assessment and is it applicable to bone age assessment in children with tall stature?
- What is the ultimate height reducing effect of sex hormone treatment in the management of tall stature?
- Are there long-term side effects of administration of high doses of sex steroids to pubertal children, especially with respect to gonadal function and fertility?
- Can we improve height prediction in tall children by using a prediction method based on growth data of tall subjects?
- Do previously treated children differ in psychosocial status from tall subjects who had not chosen for height reducing therapy?

Short stature after IUGR

The studies presented in the second part of this thesis were undertaken to obtain more insight in the pathophysiological mechanism of the stunted growth in children with IUGR. In addition, the efficacy and safety of recombinant GH therapy was evaluated.

The following questions were addressed:

- Are there disturbances in the GH/IGF axis explaining the growth retardation in children with short stature after IUGR?
- What is the effect of GH administration on linear growth and bone maturation in children with persistent short stature after IUGR?
- Is there a relationship between the GH secretory status and the growth response to GH treatment?
- Are there adverse effects of GH therapy on carbohydrate metabolism in children with IUGR?

Another important issue concerned the impact of GH treatment on various psychological aspects. Questions like: What is the psychological status of children with short stature after IUGR?, and: Does GH have a beneficial effect on psychological development? easily come into mind. These items will be subject of the thesis of Mrs. E.A. van der Reijden-Lakeman, psychologiste, entitled: "Growing pains: psychological evaluation of short stature in intrauterine growth retarded children, before and after two years of GH treatment" (162).

STUDY DESIGN

Constitutionally tall stature

To evaluate the questions raised above, all men and women, who had been seen at adolescence for evaluation of their constitutionally tall stature, and who reached the age of 18 were contacted by mail and asked to participate in a large follow-up study. In total, 247 men and 423 women were contacted, of whom 102 men and 249 women had received hormonal treatment for reducing their final adult height. Hundred and forty-five men and 174 women had not chosen for treatment and served as controls. Second mailings were sent to those who did not respond to the first mailing. Subjects were asked to participate in three substudies: 1) an auxological evaluation, 2) an evaluation on long-term effects, and 3) a psychosocial evaluation.

In the auxological part of the study, subjects were recalled to our outpatient clinic for final height assessment. In addition, auxological data were collected from the hospital charts and radiographs of the left hand and wrist were retrieved for bone age determinations and final height predictions.

To evaluate long-term effects of hormonal treatment, all subjects were interviewed in a standardized way. In women, this interview included questions about satisfaction with and possible side effects of hormone treatment, oral contraception, menstrual cycle characteristics, pregnancy and gynecological complaints. Men were asked about satisfaction with and possible side effects of hormone treatment and about their offspring. In addition, men were asked to participate in an andrological study. For this study, all participants were called in twice at the outpatient clinic of the Department of Andrology, Dijkzigt Hospital, Rotterdam. At these visits, every man underwent a medical history and a physical examination including testicular volume measurement and screening for varicocele. Finally, two semen samples were obtained for semen analysis and blood was taken for determination of plasma hormones.

To obtain insight in psychosocial aspects of constitutionally tall stature, five different, standardized questionnaires were sent to all participants by mail. Special areas of interest concerned self-esteem, social anxiety and assertiveness, general psychological well-being and body satisfaction.

The following inclusion- and exclusion criteria were used for these studies:

- constitutionally tall stature, defined as a height above the P90 according to Dutch references
- age of at least 18 years
- if treated, a treatment period of at least 6 months
- no endocrine or metabolic disorders, such as acromegaly or homocysteinuria
- no chromosomal abnormalities or syndrome, such as Klinefelter syndrome, Marfan syndrome or Sotos syndrome.
- no use of other growth intervening therapy

Table 1 presents the response rate of all subjects to the various substudies.

Subjects		Non-responders	Responders				
			Auxology	Psychology	Long-term effects		Non-participants
					Interview	Andrology	
Men							
Treated	102	7	65	76	64	43	20
Untreated	145	31	62	69	61	32	35
All	247	38	127	145	125	75	55
Women							
Treated	249	46	177	174	180		9
Untreated	174	51	95	106	92		16
All	423	97	272	280	272		25

Short stature after IUGR

To evaluate the questions raised above a multicenter study was set up in which four centers in the western part of The Netherlands participated: Sophia Children's Hospital, Rotterdam, Wilhelmina Children's Hospital, Utrecht, Academic Hospital of Free University, Amsterdam, and Juliana Children's Hospital, The Hague. The study population comprises 79 prepubertal children with short stature after IUGR. IUGR was diagnosed when birth length was 2 SD or more below the mean for gestational age according to the standards of Usher and McLean (163). In addition, the following inclusion- and exclusion criteria were used:

- no catch-up growth, defined as not obtaining a height equal to or above the P3
- growth rate equal to or below the P50 for chronological age
- uncomplicated neonatal period, *i.e.* without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), without complicated sepsis neonatorum and without long-term complications of respiratory ventilation such as bronchopulmonary dysplasia
- chronological age 3.00 to 8.99 in girls and 3.00 to 10.99 in boys
- prepubertal stage
- no chromosomal abnormalities or other organic causes for growth retardation, except for SRS
- no previous use of growth intervening therapy

To evaluate aspects of the GH/IGF axis, GH secretion was studied in a total of 40 IUGR children by 24-hour plasma GH profiles and standard arginine provocation tests. In addition, plasma IGF-I and IGF-II levels and urinary GH excretion were measured.

To assess the effects of GH therapy on linear growth, bone maturation, pubertal development and final height in children with short stature after IUGR, a randomized, double-blind, dose-response study was set up. After inclusion,

patients were randomly and blindly assigned to either 3 or 6 IU/m²/day (≈ 0.1 and 0.2 IU/kg/day) of recombinant GH (Norditropin®), administered by subcutaneous injection. In this ongoing study, patients have been examined at enrollment and subsequently every 3 months at the four participating centers. Measurements include auxological parameters, biochemical parameters and safety parameters.

To evaluate the psychological effects of GH treatment on children with short stature after IUGR, a parallel study was also performed. The results of this study before and after two years of GH treatment will be described in the thesis of Mrs. E.A. van der Reijden-Lakeman, psychologiste (163).

STRUCTURE OF THE THESIS

In *Chapters 2 to 8* studies on tall stature are discussed: *Chapter 2* describes the reliability of a recently developed computerized skeletal age scoring system and its applicability in children with CTS. This system can be used for assessment of bone age and final height predictions. The accuracy of various height predictions in the management of tall stature are addressed in the next two chapters (*chapters 3 and 4*). In addition, the effect of sex hormone therapy on height reduction in constitutionally tall children is evaluated in the same chapters (*chapter 3 and 4*), while adjusting for differences between treated and untreated tall children. *Chapters 5 and 6* describe the long-term effects of sex steroid treatment in pharmacological doses and focus on functioning of the hypothalamic-pituitary-gonadal axis. In *chapter 7* a new prediction model is presented to predict adult final height in children with tall stature based on growth data derived from a sample of untreated tall subjects. Finally, *chapter 8* describes psychosocial aspects in constitutionally tall stature.

Chapters 9 and 10 concern studies in children with short stature after IUGR. In *Chapter 9*, several aspects of the GH/IGF axis are studied. *Chapter 10* presents

the two-years results of the randomized, double-blind, dose-response multicenter trial in 79 prepubertal IUGR children comparing the efficacy and safety of two different doses of GH (3 and 6 IU/m^2/day).

Chapter 11 discusses the significance of the presented data. In addition, final conclusions and recommendations are made and suggestions for future research are given.

Chapters 12 and 13 present a summary of the thesis in the English and Dutch language, respectively.

REFERENCES

1. Eveleth PB, Tanner JM: Worldwide variation in human growth. London, Cambridge University Press, 1990
2. Roede MJ, Van Wieringen JC: Growth diagrams 1980. Netherlands third nation-wide survey. Tijdschr Soc Gezondheidsz 1985;63(Suppl):1-34
3. Humpreys LG, Davey TC, Park RK: Longitudinal correlation analysis of standing height and intelligence. Child Develop 1985;56:1465-1478
4. Taesdale TW, Owen DR, Sornssen TIA: Intelligence and educational level in adult males at the extremes of stature. Hum Biol 1991:63:19-30
5. Hensley WE, Cooper R: Height and occupational succes: a review and critique. Psychol Reports 1987;60:843-849
6. Underwood LE: The social cost of being short: social perceptions and biases. Review paper. Acta Paediatr Scand 1991;377(Suppl):3-8
7. Prader A, Zachmann M: Treatment of excessively tall girls and boys with sex hormones. Pediatrics 62:1202-1210
8. Law CM: The disability of short stature. Arch Dis Child 1987;62:855-859
9. Skuse D: The psychological consequences of being small. J Child Psychol Psychiatr 1987;28:641-650
10. Siegel PT, Clopper R, Stabler B: Psychological impact of significantly short stature. Review paper. Acta Paediatr Scand 1991;377(Suppl)14-18
11. Tanner JM: Growth at adolescence, 2nd edition. Blackwell, Oxford, 1962
12. Mascie-Taylor CGN. Biosocial influences on stature: a review. J Biosoc Sci 1991;23:113-128
13. Sorva R, Tolppanen EM, Lankinen S, Perheentupa J: Growth evaluation: parent and child specific height standards. Arch Dis Child 1989;64:1483 -1487
14. Garralda MD: Evolution of human height. In: Hernandez M, Argente J (eds): Human growth: basic and clinical aspects. Amsterdam, Elsevier Science Publishers B.V., 1992, p 135-142
15. Styne DM, McHenry H: The evolution of stature in humans. Horm Res 1993;39(Suppl):3-6
16. Karlberg J: A biologically-oriented mathematical model (ICP) for human growth. Acta Paediatr 1989;350(Suppl):70-94
17. Karlberg J: On the construction of the Infancy-Childhood-Puberty growth standard. Acta Paediatr 1989;356(Suppl):26-37
18. Albertsson-Wikland, Rosberg S: Analyses of 24-hour growth hormone profiles in children: relation to growth. J Clin Endocrinol Metab 1988; 67:493-500
19. Bourguignon JP: Linear growth as a function of age at onset of puberty and sex steroid dosage: therapeutic implications. Endocr Rev 1988;9:467-488
20. Kerrigan JR, Rogol AD: The impact of gonadal steroid hormone action on growth hormone secretion during childhood and adolescence. Endocr Rev 1992;13:281-298
21. Baumann G: Growth hormone heterogeneity: genes, isohormones, variants and binding proteins. Endocr Rev 1991;12:424-449
22. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, Barnard R, Waters MJ, Wood WI: Growth hormone receptor and serum binding protein: purification, cloning and expression. Nature 1987;330:537-543

23. Bertherat J, Bluet-Pajot MT, Epelbaum J: Neuroendocrine regulation of growth hormone. Minireview. Eur J Endocrinol 1995;132:12-24
24. Hartman ML, Veldhuis JD, Thorner MO: Normal control of growth hormone secretion. Horm Res 1993;40:37-47
25. Tannenbaum GS, Ling N: The interrelationship og growth hormone (GH)-releasing factor and somatostatin in generation of the ultradian rhythm of GH secretion. Endocrinology 1984;115:1952-1957
26. Johnston DG, Davies RR, Prescott RWG: Regulation of growth hormone secretion in man: a review. J Roy Soc Med 1985;78:319-327
27. Berelowitz M, Szabo M, Frohman LA, Firestone S, Chu L, Hintz RL: Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary, Science 1981;212:1279-1281
28. Fagin JA, Pixley S, Slanina S, Ong J, Melmed S: Insulin-like growth factor I gene expression in GH_3 rat pituitary cells: messenger ribonucleic acid content, immunochemistry, and secretion. Endocrinology 1987;120:2037-2043
29. Conway S, MaCann SM, Krulich L: On the mechanism of growth hormone autofeedback regulation: possible role of somatostatin and growth hormone-releasing factor. Endocrinology 1985;117:2284-2292
30. Müller EE: Neural control of somatotropic function. Physiol Rev 1987; 67:962-1053
31. Salomon WD, Daughaday WH: A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. J Lab Clin 1957;49:825-836
32. Gluckman PD, Douglas RG, Ambler GR, Breier BH, Hodgkinson SC, Koea JB, Shaw JHF: The endocrine role of Insulin-like growth factor-I. Review paper. Acta Paediatr Scand 1991;372(Suppl):97-105
33. Herington AC: Insulin-like growth factors: biochemistry and physiology. Ballière's Clin Endocrinol Metab 1991;5:531-551
34. Chatelain P, Naville D, Avallet O, Penhoat A, Jaillard C, Sanchez P, Saez J: Paracrine and autocrine regulation of insulin-like growth factor-I. Review paper. Acta Paediatr Scand 1991;372(Suppl):92-95
35. Daughaday WH, Rotwein P: Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. Endocr Rev 1989;10:68-91
36. Neely EK, Beukers MW, Oh Y, Cohen P, Rosenfeld RG: Insulin-like growth factor receptors. Review paper. Acta Paediatr Scand 1991;372 (Suppl):116-123
37. Drop SLS, Schuller AGP, Lindenbergh-Kortleve DJ, Groffen C, Brinkman A, Zwarthoff EC: Structural aspects of the IGFBP family. Mini review. Growth Regulation 1992;2:69-79
38. Baxter RC, Martin JL: Structure of the M, 140,000 of the growth hormone dependent insulin-like growth factor binding protein complex: determination by reconstitution and affinity-labeling. Proc Natl Acad Sci USA 1989;86:6896-6902
39. Blum WF: Insulin-like growth factors and their binding proteins. In:Ranke MB (ed.): Functional endocrinologic diagnostics in children and adolescence. Verlag, Mannheim 1992, pp102-117

40. Cohick WS, Clemmons DR; The insulin-like growth factors. Ann Rev Physiol 1993;55:131-153
41. Jones JI, Clemmons DR: Insulin-like growth factors and their binding proteins: Biological actions. Endocr Rev 1995;16:3-34
42. Rutanen EM, Pekonen F: Insulin-like growth factors and their binding proteins. Minireview. Acta Encodrinol 1990;123:7-13
43. Issakson OGP, Lindahl A, Nilsson A, Isgaard J: Mechanism of the stimulatory effect of growth hormone on longitudinal bone growth. Endocr Rev 1987;8:426-438
44. Corvol MT, Carrascosa A, Tsagris L, Blanchard O, Rappaport R: Evidence for a direct *in vitro* action of sex steroids on rabbit cartilage cells during skeletal growth: influence of age and sex. Endocrinology 1987; 120:1422-1429
45. Blanchard O, Tsagris L, Rappaport R, Duval-Beaupere G, Corvol M: Age-dependent responsiveness of rabbit and human cartilage cells to sex steroids *in vitro*. J Steroid Biochem Molec Biol 1991;40:711-716
46. Nilsson A, Ohlsson C, Isaksson OGP, Lindahl A, Isgaard J: Hormonal regulation of longitudinal bone growth. Eur J Clin Nutr 1994;48 (Suppl):S150-S160
47. Martinez L, Preece MA, Grant DB: Body proportions in precocious puberty. Acta paediatr Scand 1984;73:185-188
48. Davidson MB: Effect of growth hormone on carbohydrate and lipid metabolism. Endocr Rev 1987;8:115-131
49. Gustafsson J: Possible metabolic side effects of high dose growth hormone therapy. Acta Paediatr Scand 1989;362(Suppl):50-55
50. Ho, KKY, Kelly JJ, O'Sullivan AJ: Metabolic actions of growth hormone in man. In: Cowell CT, Ho KKY, Werther GA (eds): Growth and sexual development. Chur, Harwood Academic Publishers, 1993, p47-63
51. Dickerman Z, Loewinger J, Laron Z: The pattern of growth in children with constitutionally tall stature from birth to age 9 years. A longitudinal study. Acta Paediatr Scand 1984;73:530-536
52. Frasier SD: Tall stature and excessive growth syndromes. In: Pediatric Endocrinology. A clinical guide. Lifshitz F. (eds). Marcel Dekker Inc., New York, 1990
53. Rochiccioli P, Messina A, Tauber MT, Enjaume C: Correlation of parameters of 24-hour growth hormone secretion with growth velocity in 93 children of varying height. Horm Res 1989;31:115-118
54. Evain-Brion D, Garnier P, Schimpff RM, Chaussain JL, Job JC: Growth hromone response to thyrotropin-releasing hormone and oral glucose. Loading tests in tall children and adolescents. J Clin Endocrinol Metab 1983;56:429-432
55. Hindmarsh PC, Stanhope R, Kendall BE, Brook CDG: Tall stature: a clinical, endocrinological and radiological study. Clin Endocrinol 1986;25:323-331
56. Tauber M, Pienkowski C, Rochiccioli P: Growth hormone secretion in children and adolescents with familial tall stature. Eur J Pediatr 1994;153:311-316
57. Batrinos M, Georgiadis E, Panitsa-Fafila Ch, Stratigopoulos S: Increased GH response to GHRH in normal tall men. Clin Endocrinol 1989;30:13-17
58. Garcia Blanco M, Evain Brion D: Studies in constitutionally tall adolescents: somatostatin decrease associated with growth hormone increase after TRH injection. Clin Endocrinol 1984;21:459-463

59. Tanner JM, Whitehouse RH, Cameron N, Marshall WA, Healy MJR, Goldstein H: Assessment of skeletal maturity and prediction of adult height (TW2-method). Academic Press, London, 1983
60. Bayley N, Pinneau S: Tables for predicting adult height from skeletal age. J Pediatr 1952;40:423-441
61. Greulich WW, Pyle SI: Radiographic atlas of skeletal development of the hand and wrist, 2nd edition. Stanford, Stanford University Press, 1959
62. Preece MA: Prediction of adult height: methods and problems. Acta Paediatr Scand 1988;347(Suppl):4-11
63. Roche AF, Davila GH, Eyman SL: A comparison between Greulich-Pyle and Tanner-Whitehouse assessments of skeletal maturity. Radiology 1971;98:272-280
64. Kemperdick HF: Skelettalter-Bestimmung bei Kindern mit normalen und abweichendem Wachtumsverlauf. Fortschr Med 1981;99:152-156
65. Wenzel A, Melsen B: Replicability of assessing radiographs by the Tanner Whitehouse-2 method. Hum Biol 1982;54:575-581
66. Tanner JM, Gibbons RD: Automatic bone age measurement using computerized image analysis. J Pediatr Endocrinol 1994;7:141-145
67. Willig RP, Christiansen D, Kuhn N, Schaefer E, Stahnke N: Vorraussetzungen und ergebnisse der Östrogenbehandlung extrem großer Mädchen. Monatschr Kinderheilkd 1980;128:787-788
68. Brämswig JH, Hermeling W, von Petrykowski W, Schellong G: Comparison of height predictions with final adult height in boys with constitutional tall stature. In: Borms J, Hauspie R, Sand A, Suzanne C, Hebbelinck M (eds). Human growth and development. Plenum Press, New York, 1984; p 423-429
69. Joss EE, Temperli R, Mullis PE: Adult height in constitutionally tall stature: accuracy of five different height prediction methods. Arch Dis Child 1992;67:1357-1362
70. Gluckman PD, Breier BH, Oliver M, Harding JE, Bassett N: Fetal growth in late gestation. A constrained pattern of growth. Acta Paediatr Scand 1990;356(Suppl):105-110
71. Robinson JS, Falconer J, Owens JA: Intrauterine growth retardation: Clinical and experimental. Acta Paediatr.Scand. 1985;319(Suppl):135-142
72. Gluckman PD: Fetal growth: an endocrine perspective. Review paper. Acta Paediatr.Scand. 1989;349(suppl):21-25
73. Warshaw JB: Intrauterine growth restriction revisited. Growth, Genetics and Hormones 1992;8:5-8
74. Gluckman PD, Harding JE: The regulation of fetal growth. In: Hernandez M, Argente J (eds): Human growth: basic and clinical aspects. Amsterdam, Elsevier Science Publishers B.V., 1992, p 253-259
75. Gluckman PD, Harding JE, Oliver MH, Liu L, Ambler GR, Klempt M, Breier BH: Mechanisms of intrauterine growth retardation: role of fetal and maternal hormones. In: Müller EE, Cocchi D, Locatelli V (Eds): Growth hormone and somatomedins during lifespan. Berlin, Springer Verlag, 1993, p147-160
76. Underwood LE, D'Ercole AJ: Insulin and insulin-like growth factors/Somatomedins in fetal and neonatal development. Clin Endocrinol Metabol 1984;13:69-89

77. Bennett A, Wilson DM, Liu F, Nagashima R, Rosenfeld RG, Hintz RL: Levels of insulin-like growth factors I and II in human cord blood. J Clin Endocrinol Metab 1983;57:609-612
78. Ashton IK, Zapf J, Einsckenk I, MacKenzie IZ: Insulin-like growth factors (IGF) 1 and 2 in human foetal plasma and relationship to gestational age and foetal size during midpregnancy. Acta Endocrinol 1985;110:558 -563
79. Lasarre C, Hardouin S, Daffos F, Forestier F, Frankenne F, Binoux M: Serum insulin-like growth factors and insulin-like growth factor binding proteins in the human fetus. Relationship with growth in normal subjects and in subjects with intrauterine growth retardation. Pediatr Res 1991;29:219-225
80. Straus DS, Ooi GT, Orlowski CC, Rechler MW: Expression of the genes for insulin-like growth factor-I (IGF-I), IGF-II, and IGF-Binding Proteins-1 and -2 in fetal rat under conditions of intrauterine growth retardation caused by maternal fasting. Endocrinology 1991;128:518-525
81. Unterman TG, Simmons RA, Glick RP, Ogata ES: Circulating levels of insulin, insulin-like growth factor-I (IGF-I), IGF-II and IGF-Binding Proteins in the small for gestational age fetal rat. Endocrinology 1993; 132:327-337
82. Heinrich UE: Intrauterine growth retardation and familial short stature. Ballière's Clin Endocrinol Metab 1992;6:589-601
83. Tenovuo A, Kero P, Piekkala P, Korvenranta H, Sillanpaa, Erkkola R: Growth of 519 small for gestational infants during their first two years of life. Acta Paediatr Scand 1987;76:636-646
84. Fitzhardinge PM, Inwood S: Long-term growth in small-for-date children. Acta Paediatr Scand 1989;349(Suppl):27-33
85. Albertsson-Wikland K, Wennergren G, Wennergren M, Vilbergsson G, Rosberg S: Longitudinal follow-up of growth in children born small for gestational age. Acta Paediatr Scand 1993;82:438-443
86. Albertsson-Wikland K, Karlberg J: Natural growth in children born small for gestational age with and without catch-up growth. Acta Paediatr Scand 1994;399(Suppl):64-70
87. Hokken-Koelega ACS, de Ridder MAJ, van Lemmen RJ, den Hartog H, de Muinck Keizer-Schrama SMPF, Drop SLS: Children born small for gestational age: do they catch-up? Pediatr Res 1995;38:267-271
88. Job JC, Rolland A: Histoire naturelle des retards de croissance à debut intra-utérin. Arch Fr Pediatr 1986;43:301-306
89. Paz I, Seidman DS, Danon YL, Laor A, Stevenson DK, Gale R: Are children born for gestational age at increased risk for short stature? Am J Dis Child 1993;147:337-339
90. Chaussain JL, Colle M, Ducret JP: Adult height in children with prepubertal short stature secondary to intrauterine growth retardation. Acta Paediatr Scand 1994;399(Suppl):72-73
91. Tanner JM, Lejarraga H, Cameron N: The natural history of the Silver-Russell syndrome: A longitudinal study of thirty-nine cases. Pediatr Res 1975;9:611-623
92. Davies PS, Valley R, Preece MA: Adolescent growth and pubertal progression in the Silver-Russell syndrome. Arch Dis Child 1988;63:130-135
93. Marks LJ, Bergeson PS: The Silver-Russell syndrome. A case with sexual ambiguity, and a review of the literature. Am J Dis Child 1977; 131:447-451
94. Arisaka O, Arisaka M, Kiyokawa N, Shimizu T, Nakayama Y, Yabuta K: Intrauterine growth retardation and early adolescent growth spurt in two sisters. Clin Pediatr 1986;25:559-661

95. Albertsson-Wikland K: Growth hormone secretion and growth hormone treatment in children with intrauterine growth retardation. Acta Paediatr Scand 1989;349(Suppl):35-41
96. Rochiccioli P, Tauber M, Moisan V, Pinkowski C: Investigation of growth hormone secretion in patients with intrauterine growth retardation. Acta Paediatr Scand 1989;349(Suppl):42-46
97. Stanhope R, Ackland F, Hamill G, Clayton J, Jones J, Preece MA: Physiological growth hormone secretion and response to growth hormone treatment in children with short stature and intra-uterine growth retardation. Acta Paediatr Scand 1989;349(Suppl):47-52
98. Grunt JA, Howard CP, Daughaday WH: Comparison of growth and somatomedic C responses following growth hormone treatment in children with smal-for-data short stature, significant idiopathic short stature and hypopituitarism. Acta Endocrinol 1984;106:168-174
99. Dobbing J, Sands J: Timing of neuroblast multiplication in developing human brain. Nature 1970;226:639-640
100. Dobbing J, Sands J: Comparative aspects of the brain growth spurt. Early Hum Dev 1979;3:79-83
101 Thomas YM, Bedi KS, Davies RA, Dobbing J: A stereological analysis of the neuronal and synaptic content of the frontal and cerebellar cortex of weaning rats undernourished from birth. Early Hum Dev 1979;3:109-126
102. Fitzhardinge PM, Steven EM: The small for dates infant II: neurological and intellectual sequelae. Pediatrics 1976;50:30-57
103. Harvey D, Prince J, Bunton J, Parkinson C, Campbell S: Abilities of children who were small-for-gestational age babies. Pediatrics 1982;69:269-300
104. Westwood M, Kramer MS, Munz D, Lovett JM, Watters GV: Growth and development of full-term nonasphyxiated small-for-gestational-age newborns: follow-up through adolescence. Pediatrics 1983;71:376-382
105. Henrichsen L, Skinhoj K, Andersen GE: Delayed growth and reduced intelligence in 9-17 year old intrauterine growth retarded children compared with their monozygous co-twins. Acta Paediatr Scand 1986; 75:31-35
106. Matilainen R, Heinonen K, Siren-Tiusanen H: Effect of intrauterine growth retardation (IUGR) on the psychological performance of preterm children at preschool age. J Child Psychol Psychiat 1988;29:601-609
107. Goldzieher M: Treatment of excessive growth in the adolescent female. J Clin Endocrinol 1956;16:249-252
108. Schoevaart CE, Drop SLS, Otten BJ, Slijper FME, Degenhart HJ: Growth analysis up to final height and psychosocial adjustment of treated and untreated patients with precocious puberty. Horm Res 1990;34:197-203
109. Brauner R, Adan L, Malandry F, Zantleifer D: Adult height in girls with idiopathic true precocious puberty. J Clin Endocrinol Metab 1994;79: 415-420
110. Zachmann M, Ferrandez A, Mürset G, Prader A: Estrogen treatment of excessively tall girls. Helv Paediat Acta 1975;30:11-30
111. Wettenhall HNB, Cahill C, Roche AF: Tall girls: A survey of 15 years of management and treatment. J Pediatr 1975;86:602-610
112. Von Puttkamer K, Bierich JR, Brugger F, Hirche W, Schönberg D: Östrogentherapie bei Mädchen mit konstitutionellem Hochwuchs. Dtsch Med Wochenschrft 1977;102:983-988

113. Kuhn N, Blunck W, Stahnke N, Wiebel J, Willig RP: Estrogen treatment in tall girls. Acta Paediatr Scand 1977;66:161-167
114. Colle ML, Alperin H, Greenblatt RB: The tall girl. Prediction of mature height and management. Arch Dis Child 1977;52:118-120
115. Bierich JR: Estrogen treatment of girls with constitutional tall stature. Pediatrics 1978;62:1196-1201
116. Reeser HM, Heremans GFP, van Gelderen HH: Reduction of adult height in tall girls. Eur J Pediatr 1979;132:37-41
117. Willig RP, Christiansen D, Kuhn N, Schaefer E, Stahnke N: Voraussetzungen und Ergebnisse der Östrogenbehandlung extrem großer Mädchen. Monatsschr Kinderheilkd 1980;128:787-788
118. Andersen H, Jacobsen BB, Kastrup KW, Krabbe S, Peitersen B, Petersen KE, Thamdrup E, Wichmann R: Treatment of girls with excessive height prediction. Acta Paediatr Scand 1980;69:293-297
119. John G, Schellong G: Östrogentherapie hochwüchsiger Mädchen. Monatsschr Kinderheilkd 1980;128:545-550
120. Stöver B, Kollmann F: Ergebnisse der Behandlung hochwüchsiger Mädchen mit conjugierten Östrogenen. Monatsschr Kinderheilkd 1982; 130:36-40
121. Sorgo W, Scholler K, Heinze F, Heinze E, Teller WM: Critical analysis of height reduction in oestrogen-treated tall girls. Eur J Pediatr 1984;142: 206-265
122. Schambach H, Nitschke U: Die Behandlung des konstitutionellen Hochwuchses bei Mädchen mit physiologischen Östrogendosen in der Präpubertät. Monatsschr Kinderheilkd 1985;133:32-37
123. Bartsch O, Weschke B, Weber B: Oestrogen treatment of constitutionally tall girls with 0.1 mg/day ethinyl oestradiol. Eur J Pediatr 1988;147:59-63
124. Grüters A, Heidemann P, Schlüter H, Stubbe P, Weber B, helge H: Effect of different oestrogen doses on final height reduction in girls with constitutional tall stature. Eur J Ped 1989;149:11-13
125. Ignatius A, Lenko HL, Perheentupa J: Oestrogen treatment of tall girls: effect decreases with age. Acta Paediatr Scand 1991;80:712-717
126. Svan H, Ritzén EM, Hall K, Johansson L: Oestrogen treatment of tall girls: dose dependency of effects on subsequent growth and IGF-I levels in blood. Acta Paediatr Scand 1991;80:328-332
127. Normann EK, Trygstad O, Larsen S, Dahl-Jorgensen K: Height reduction in 539 tall girls treated with three different dosages of ethinyloestradiol. Arch Dis Child 1991;66:1271-1278
128. Joss EE, Zeuner J, Zurbrügg RP, Mullis PE: Impact of different doses of ethinyl oestradiol on reduction of final height in constitutionally tall girls. Eur J Pediatr 1994;153:797-801
129. Zachmann M, Ferrandez A, Mürset G, Gnehm HE, Prader A: Testosterone treatment of excessively tall boys. J Pediatr 1976;88:116-123
130. Brämswig JH, Schellong G, Borger HJ, Breu H: Testosteron-Therapie hochwüchsiger Jungen. Ergebnisse bei 25 Jungen. Dtsch Med Wschr 1981;106:1656-1661

131. Brämswig JH, von Lengerke HJ, Schmidt H, Schellong G: The results of short-term (6 months) high dose-testosterone treatment on bone age and adult height in boys of excessively tall stature. Eur J Pediatr 1988;148:104-106
132. Muntean W, Borkenstein M: Hemostatic changes in tall girls treated with high doses of ethinylestradiol. Eur J Pediatr 1980;134:245-248
133. Hinkel GK, Hanefeld M, Jaross W, Leonhardt W, Trübsbach A: Effect of high doses of oestrogens and androgens on lipoproteins: observations in the treatment of excessive growth with sexual hormones. Exp Clin Endocrinol 1985;86:17-25
134. Hanker JP, Schellong G, Schneider HPG: The functional state of the hypothalamo-pituitary axis after high dose oestrogen therapy in excessively tall girls. Acta Endocrinol 1979;91:19-29
135. Brämswig JH, Nieschlag E, Schellong G: Pituitary-gonadal function in boys after high dose testosterone treatment for excessively tall stature. Acta Endocrinol 1984;107:97-103
136. Trygstad O: Oestrogen treatment of adolescent tall girls; short term side effects. Acta Endocrinol 1986(Suppl)279:170-173
137. Werder EA, Waibel P, Sege D, Flury R: Severe thrombosis during oestrogen treatment for tall stature. Eur J Pediatr 1990;149:389-390
138. Panteon E, Loumaye E, Maes M, Malvaux P: Occurrence of prolactinoma after estrogen treatment in a girl with constitutionally tall stature. J Pediatr 1988;113:337-339
139. Traupe H, Mühlendahl von KE, Brämswig JH, Happle R: Acne of the fulminans type following testosterone therapy in three excessively tall boys. Arch Dermatol 1988;124:414-417
140. Commentz JC, Conrad C, Stahnke N, Willig RP: Priapism during testosterone treatment for reducing final height in tall stature. Horm Res 1994:41:103
141. Willig RP, Brod R, Stahnke N, Müller-Möhring G, Schirren C: Testicular function after testosterone treatment in tall adolescents. Ped Res 1988;24:537 (abstract)
142. Willig RP, Bettendorf M, Hinkel GK, Schwarz HP, Schulze W: Androgen treatment of tall stature during puberty may reduce sperm quality in adult life. Horm Res 1992;37(Suppl.4):3 (abstract)
143. Grimes DA: The safety of oral contraceptives: epidemiologic insights from the first 30 years. Am J Obstet Gynecol 1992;166:1950-1954
144. Kaunitz AM: Oral contraceptives and gynecologic cancer: an update for the 1990s. Am J Obstet Gynecol 1992;167:1171-1176
145. Melmed S: Acromegaly. Review. N Eng J Med 1990;322:966-977
146. Evain-Brion D, Garnier P, Blanco-Garcia M, Job JC: Studies in constitutionally tall adolescents. II. Effects of bromocryptine on growth hormone secretion and adult height prediction. J Clin Endocrinol Metab 1984;58:1022-1026
147. Schwarz HP, Joss EE, Zuppinger KA: Bromocriptine treatment in adolescent boys with familial tall stature: A pair-matched controlled study. J Clin Endocrinol Metab 1987;65:136-140
148. Schoenle EJ, Theintz G, Torresani T, Prader A, Illig R, Sizonenko PC: Lack of bromocriptine-induced reduction of predicted height in tall adolescents. J Clin Endocrinol Metab 1987;65:355-358
149. Tauber MT, Tauber JP, Vigoni F, Harris AG, Rochiccioli P: Effect of long-acting somatostatin analogue SMS 201-995 on growth rate and reduction of predicted adult height in ten tall adolescents. Acta Paediatr Scand 1990;79:176-181

150. Hindmarsh PC, Pringle PJ, Di Silvo L, Brook CGD: A preliminary report on the role of somatostatin analogue (SMS 201-995) in the management of children with tall stature. Clin Endocrinol 1990;32:83-91
151. Hindmarsh PC, Pringle PJ, Stanhope R, Brook CGD: The effect of continuous infusion of a somatostatin analogue (octreotide) for two years on growth hormone secretion and height prediction in tall children. Clin Endocrinol 1995;42:509-515
152. Lee PDK, Rosenfeld RG: Psychosocial correlates of short stature and delayed puberty. Pediatr Clin N Am 1987;34:851-863
153. Gordon M, Crouthamel C, Post EM, Richman RA: Psychosocial aspects of constitutional short stature: social competence, behaviour problems, self-esteem and family functioning. J Pediatr 1982;101:477-480
154. Westphal O: Is short stature a psychosocial handicap? Acta Peadiatr Scand 1989;362 (Suppl):24-26
155. Tanner JM, Whitehouse RH, Hughes PCR, Vince FP: Effect of human growth hormone treatment for 1 to 7 years on growth of 100 children eith growth hormone deficiency, low birthweight, inherited smallness, Turner's syndrome and other complaints. Arch Dis Child 1971;46:745-782
156. Grunt JA, Enriquez AR, Daughaday WH, Budd S: Acute and long-term responses to hGH in children with idiopathic small-for-dates dwarfism. J Clin Endocrinol Metab 1972;35:157-168
157. Foley TP Jr, Thompson RG, Shaw M, Baghdassarian A, Nissley SP, Blizzard RM: Growth responses to human growth hormone in patients with intrauterine growth retardation. J Pediatr 1974;84:635-641
158. Ad hoc committee on growth hormone usage, the Lawson Wilkins Pediatric Endocrine Society, and Committee on Drugs: Growth hormone treatment of children with short stature. Pediatrics 1983;72:891-894
159. Frasier SD, Lippe BM: Clinical review 11: The rational use of growth hormone during childhood. J Clin Endocrinol Metab 1990;71:269-273
160. Hindmarsh PC, Bridges NA, Brook CGD: Wider indications for treatment with biosynthetic human growth hormone in children. Review. Clin Endocrinol 1991;34:417-427
161. Stanhope R, Preece MA, Hamill G: Does growth hormone treatment improve final height attainment of children with intrauterine growth retardation? Arch Dis Child 1991;66:1180-1183
162. Van der Reyden-Lakeman EA: Growing pains? Psychological evaluation of short stature in intrauterine growth retarded children, before and after two years of GH treatment. Thesis, Rotterdam, 1996
163. Usher R, MacLean F: Intrauterine growth of liveborn caucasian infants at sea level: Standard obtained from measurements of infants born between 25 and 44 weeks of gestation. J Pediatr 1969;74:901-910

CHAPTER 2

Computer aided skeletal age scores (CASAS) in healthy children and in children with constitutionally tall stature.

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ABSTRACT

The manual Tanner-Whitehouse 2 method has recently been transformed into a computer-aided skeletal age scoring system (CASAS), which rates either the complete TW-RUS score (13b model) or a subset consisting of radius, ulna and the 4 bones of the third finger (6b model).

In this study the reliability of CASAS was evaluated in healthy children, the 13b model was compared with the manual ratings and with the 6b model in (subgroups of) 151 healthy children, and 362 children with constitutionally tall stature (CTS). In addition, reference curves for bone maturation in CTS are presented.

Some of mean differences in methods were statistically significant, however, since these mean differences were less than 0.4 BA 'year', they are clinically not significant. In all comparisons the range of the difference between the methods (either with the 6b or the 13b model) was considerable, but the combined within and between components of variance (0.7%) were in the same order of magnitude as reported for the manual readings. In general, the percentage of equal stage ratings on duplicate assessments was high (± 90%).

Our data indicate that this computerized method is applicable in these groups of children. The use of the 6b model seems preferable because it is less time consuming than rating 13 bones. In view of the percentages of manual insertions of a stage (up to 8% in all groups) the clinical use of this CASAS version (3.5) seems to be, in particular, with longitudinal studies. Manual substitution of a stage should be avoided and when performed its percentage and the limits for the acceptance of disagreement should be reported.

INTRODUCTION

It is common practice to determine the biological maturity of children by means of an estimation of the bone age (BA) from a radiograph of the hand and wrist. Tanner et al (1) described maturity indicators for the epiphyses of each bone of the hand and wrist (TW2-method). Each bone progresses through a series of specific stages with attributed weighted scores. These scores are summed to form the maturity score (MS), which in turn can be converted to a corresponding BA by means of a table. Although the TW2-method is widely used, the reliability is limited (2-5). Furthermore, this method uses an interval scale and consequently a difference of one stage in the rating of a particular bone may result in an increase of 0.3 BA *'years'*. In order to diminish the errors in the interpretation of maturity stages and to improve the BA ratings, the TW2-(RUS) method has recently been transformed by the original author into a computerized image-analysis system using a continuous scale (6,7). This system rates each of the 13 bones in the TW-RUS classification system (13b model). A shortened model, using a 6 bone subset consisting of radius, ulna and the 4 bones of the third finger, is also available (6b model).

This study evaluates [1] the reliability of a computer aided skeletal age scoring system (CASAS) in healthy children. A comparison is made with manual ratings in healthy children [2], as well as in children with a particular growth condition [3], i.e. children with constitutional tall stature (CTS). In order to determine whether the easier 6b model could substitute the more laborious 13b model, the 13b model of CASAS is compared with the 6b model in these groups of children. In addition, reference curves for bone maturation in CTS as determined by CASAS are presented [4].

POPULATIONS AND METHODS

Populations

Healthy children

81 boys and 70 girls with chronological age (CA) 2-22 years were recruited from the urban area of Rotterdam, the Netherlands. Children on medication or with conditions or disorders compromising growth and/or bone metabolism had been excluded from the study.

Constitutionally tall stature (CTS)

115 tall boys (aged 8.8-17.2 year) and 247 tall girls (aged 9.0-16.9 year) had visited our out-patient clinic for final height prediction. All children had heights at the 90th percentile or above. In total, 154 and 267 radiographs of the hand were gathered from boys and girls, respectively.

Study designs

1. *Reliability study in healthy children*

In order to determine between and within observer variance of CASAS (13b model) two observers made duplicate assessments of twenty blinded radiographs, which were selected by taking every first healthy child in a CA age-class of one year.

2. *CASAS in healthy children*

In a subgroup of 40 children (20 boys and 20 girls) radiographs rated with the 13b model of CASAS were compared for assessment of agreement with the 6b model. The radiographs of all 151 children were rated manually and with CASAS (6b model). For each sex the ratings were compared for assessment of agreement.

3. CASAS in CTS

Four-hundred twenty-one radiographs of all children with CTS were rated manually and with CASAS (6b model). In a subgroup of 40 children (20 boys and 20 girls) radiographs rated by the 13b model of CASAS were compared with the 6b model. Ratings were compared for assessment of agreement.

4. Bone maturation curves in CTS

In every yearly chronological age interval (rounded to the nearest integer year) only one X-ray of the hand from each patient was used, leaving 411 X-rays of all children with CTS (146 and 265 X-rays for boys and girls, respectively) for analysis with CASAS (6b model). Separate regression equations were estimated for boys and girls in CTS.

All protocols were approved by the Medical Ethical Committee of the Erasmus University Medical School and the Academic Hospitals involved in these studies, and informed consent was obtained from the participants or the guardians.

Methods

Radiographs and bone ratings

All radiographs have been taken by standard radiological techniques (TW2) and identification details were removed. Radiographs were rated manually (TW2 RUS method (1)) and by a computerized image analysis system (CASAS, version 3.5) (6,7). In combination with the
radius and ulna, the TW2-RUS score uses only three rays - each comprising a metacarpal and the accompanying phalanges - to avoid excessive weighting on the finger bones (13 bones-score). Apart from a 13 bones rating (13b), CASAS has a short model which analyses only 6 bones (6b; radius, ulna, and the bones of the third ray) to form a complete TW2-RUS score. The grades (A-I) from the third ray are then extrapolated to the first and fifth rays and the matching maturity scores

are calculated based on the original TW2 tables for that grade. If a grade allocated by CASAS does not conform to the expected grade of the observer it can be inserted manually. In our studies a manual insertion of a stage was only allowed after 3 attempts with a difference between expected and determined stage of two integer stages or more.

For each design comparing methods two different observers performed the readings, except for both the manual vs 13b and manual vs manual designs in TS, which were rated by one observer. Hence, inter-method and inter-observer variability may be inextricably compounded, as is often the real situation in practice.

Statistical analysis

Data are expressed as maturity scores (MS) or as RUS-BA *'years'*. In order to assess the degree of agreement between manual and CASAS ratings of maturity score (and subsequently BA) we used the approach described by Bland & Altman (8). In short, this method uses simple calculations and graphical techniques instead of correlation coefficients to describe the degree of agreement. Plots of the difference between methods against their average are given to illustrate the range of agreement by level.

Within and between observer variance of CASAS (13b model) was determined with an analysis of variance using the BMDP statistical package, module 8V. All other comparisons between methods were performed by paired Student's t-tests. Differences between sexes were tested with the Mann-Whitney-U test. P-values <0.05 were considered significant.

The regression equations for the CTS reference groups were calculated using a repeated measures ANOVA (BMDP, module 5V). The within-subject covariance matrix was assumed to have a first order autoregressive structure after reordering the data on an equidistant time axis.

RESULTS

1. Reliability study on 13 bones model

The estimated total component of between observer, within observer, and observer/radiograph interaction variance was, for both CASAS 13b and the manual ratings, only 0.7% of the total variance, leaving 99.3% for the between radiograph component of variance. The standard deviation of the within observer component of variance using CASAS was 0.36 BA *'years'*, for the manual ratings 0.25 BA *'years'*.

On average, a single observer using CASAS 13b gave the same stage rating (rounded to the integer stage) on two occasions in 88% of instances. For the manual readings the equal staging within observers was 90%. CASAS 13b showed most inconsistencies with regard to equal staging for the fifth metacarpal and proximal phalanx, and the fifth distal phalanx. Using CASAS 13b the average percentage of equal stage ratings between two observers was 88%, with the manual ratings 89%.

Most inconsistencies between observers were noted for the first proximal and distal phalanx. The CASAS 13b ratings resulted occasionally in a difference of 2 stages (1%), the manual ratings only showed differences between adjacent stages.

The percentage of manual insertions per radiograph was 8.1% and 5.6% for the first and second rating, respectively, which is equivalent to about one bone in every radiograph. In 89% of instances the same bone was manually inserted on both occasions. In the first rating period most manual insertions were performed for the radius, ulna, first metacarpal, and the distal phalanx of the fifth finger. In the second period only the first three bones accounted for most of the manual insertions. The bones which showed inconsistency with regard to manual insertions on either of the two rating sessions were the radius, ulna, the first metacarpal, and the distal phalanx of the fifth finger.

We then excluded X-rays of five fully matured children (manual MS=1000), because of the excess of extreme stages ('H' or 'I') which are more difficult to rate

by CASAS (see discussion). The percentages of manual insertions decreased only in the first rating period with 1.7%, mainly due to the radius and the distal phalanx of the fifth finger.

2. CASAS in healthy children

In a subgroup of 40 children (20 boys and 20 girls) the correlation coefficient between the 13b and 6b CASAS model was 0.99 in MS as well as in BA *'years'*. The mean difference between the models was only significantly different in boys and only when expressed as MS (P=0.002). This also resulted in a significantly smaller mean difference in MS in girls compared with boys (P=0.03), see Table 1.

Table 1. Mean difference and 95% limits of agreement between manual, 6b, and 13b CASAS ratings expressed in maturity scores (MS) and in bone age (BA) *'years'* from X-rays of healthy children and children with constitutionally tall stature (CTS).

			Maturity Scores		Bone Age *('years')*	
	Sex	N	Mean difference	95% Limits of agreement	Mean difference	95% Limits of agreement
Healthy children						
Manual vs 6b	M	81	-5.9	-84.3; +72.6	-0.10	-1.48; +1.28
	F	70	+17.3*&	-88.2;+122.8	+0.15*&	-1.08; +1.38
13b vs 6b	M	20	-24.4*&	-86.3; +37.5	-0.24	-2.00; +1.53
	F	20	+0.4	-77.0; +77.8	-0.17	-1.43; +1.09
CTS children						
Manual vs 6b#	M	154	-34.8*&	-176.6; +107.0	-0.31*&	-1.65; +1.03
	F	267	+4.0	-131.8; +139.8	+0.01	-1.33; +1.35
13b vs 6b	M	20	+2.1	-82.5; +86.7	-0.09	-1.09; +0.92
	F	20	-7.4	-67.8; +53.0	-0.09	-0.68; +0.50

sex: F=female, M=male
see also Figure 2
* significantly different between methods, P< 0.05
& significantly different between sexes, P< 0.05

In the X-rays of all 151 healthy children, the correlation coefficient between the manual and the CASAS 6b ratings was also 0.99 ($P<0.0001$). The mean difference between the two rating methods in MS was only significantly different in girls: on average, manual ratings were 17.3 points higher than CASAS ratings (or 0.15 BA *'years'*); 95% of the differences in MS in girls are expected to lie between -88.2 and +122.8 (or between -1.08 and +1.38 BA *'years'*). Since the mean difference between the methods was also significantly higher in girls compared with boys ($P=0.003$), values for boys and girls are given separately (see Table 1 and Figures 1a and 1b.

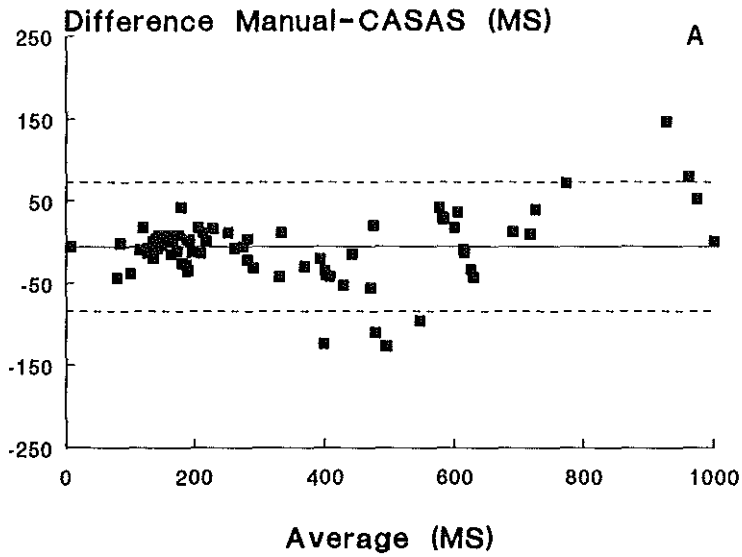
The percentage of manual insertions using CASAS 6b was 5.1%; the radius and ulna were manually inserted most frequently (74% of all manual insertions).

3. CASAS in CTS

In a subgroup of 40 CTS children (20 boys and 20 girls) the correlation coefficient between the 13b and 6b model of CASAS was 0.96 in MS as well as in BA *'years'*. The mean difference between the models expressed as MS and in BA *'years'* was not significant for both sexes (Table 1). When the manual ratings were compared with CASAS 6b in all children with CTS, the mean difference between the methods was significantly different in boys, but not in girls. In addition, the mean difference between the methods was smaller in boys compared with girls ($P<0.0001$), see Figures 2a and 2b.

The percentage of manual insertions using the 6b model (all CTS children) was low, 0.2% in boys and 1.9% in girls. Radius, ulna and the third metacarpal were most frequently manually inserted (81% of total manual insertions). In the 40 radiographs rated using the 13b model of CASAS the percentage of manual insertions was 7.7% for boys and 13.8% for girls. The difference in percentage of manual insertions between the 13b and 6b model was mainly explained by insertions of the first and fifth ray. Using the 13b model, manual insertions of the first and fifth ray accounted for 60% and 86% of all manual insertions in boys and girls, respectively.

Figure 1. Plot of the difference between manual and CASAS 6 bones ratings against the average of these methods expressed as maturity scores (MS) in healthy boys (A) and in healthy girls (B); mean difference (——), limits of agreement (·····).



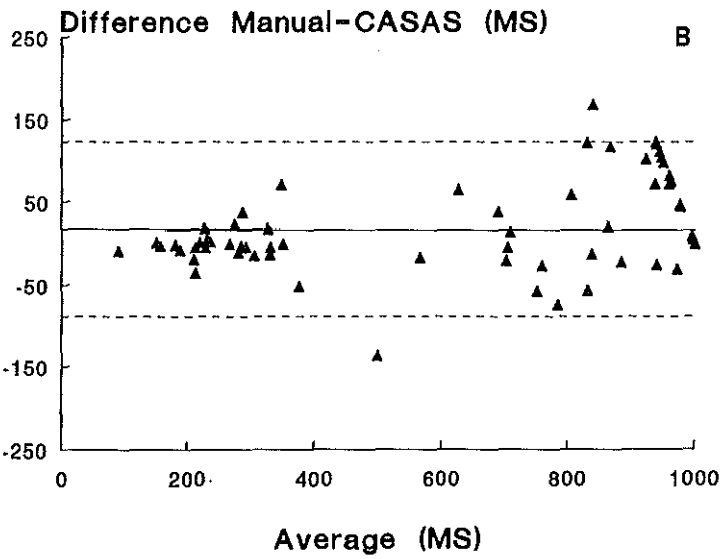
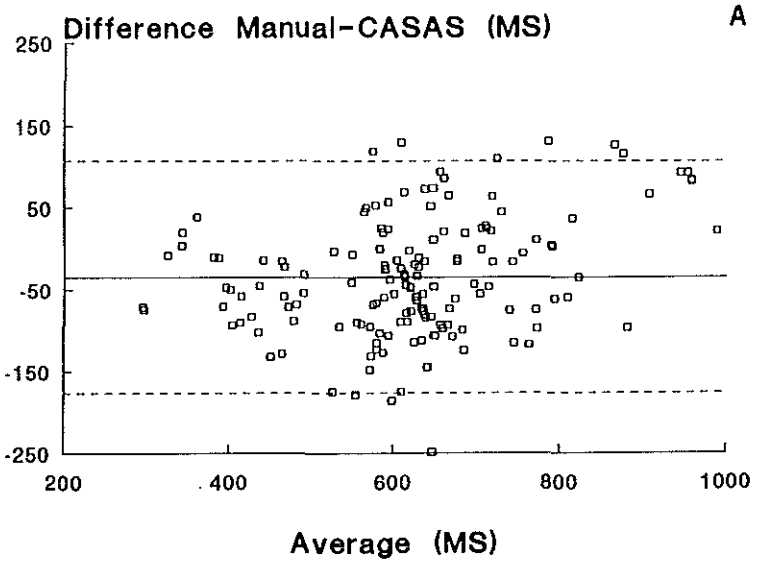
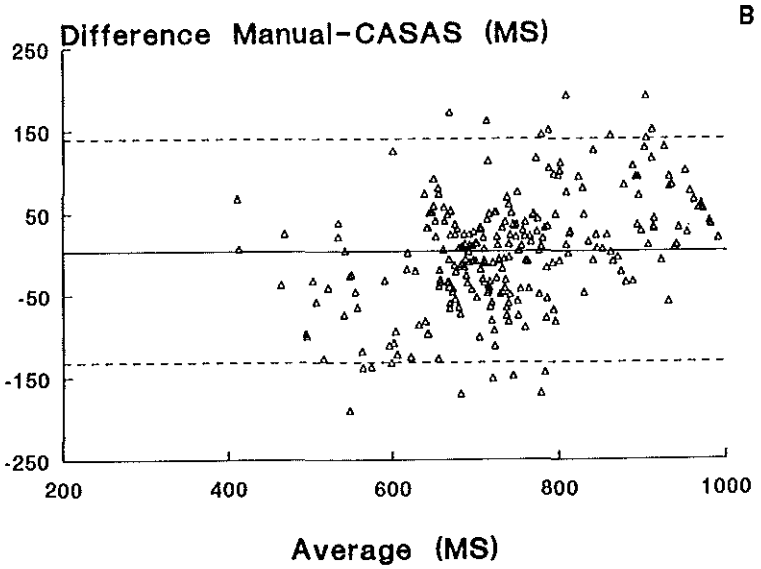


Figure 2. Plot of the difference between manual and CASAS 6 bones ratings against the average of these methods expressed as maturity scores (MS) in boys (A) and girls (B) with constitutionally tall stature; mean difference (——), limits of agreement (---------).





4. Bone maturation curves in CTS

Figures 3a and 3b depict the CASAS 6b ratings versus CA in CTS for boys and girls, respectively. The estimated regression models are given by the following equations:

For boys: BA (predicted)= $1.32*CA + 0.0002*CA^2 - 0.00009*CA^4$.

For girls: BA (predicted)= $1.46*CA - 0.023*CA^2 - 0.00005*CA^4$.

DISCUSSION

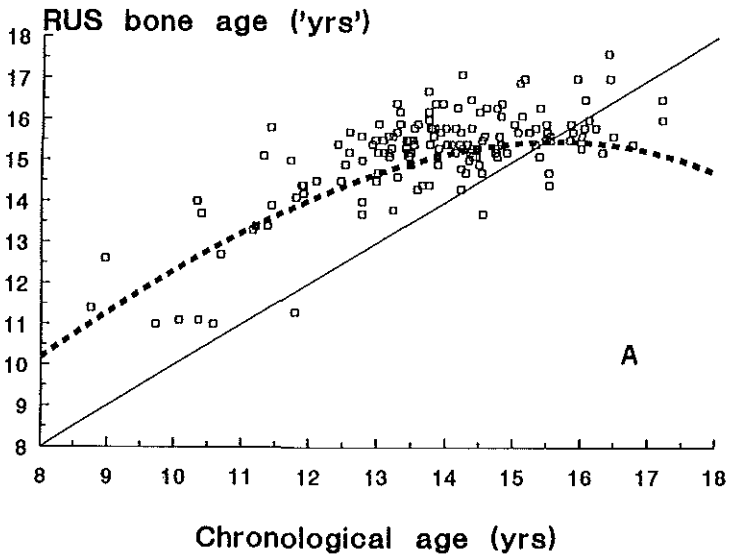
CASAS in healthy children

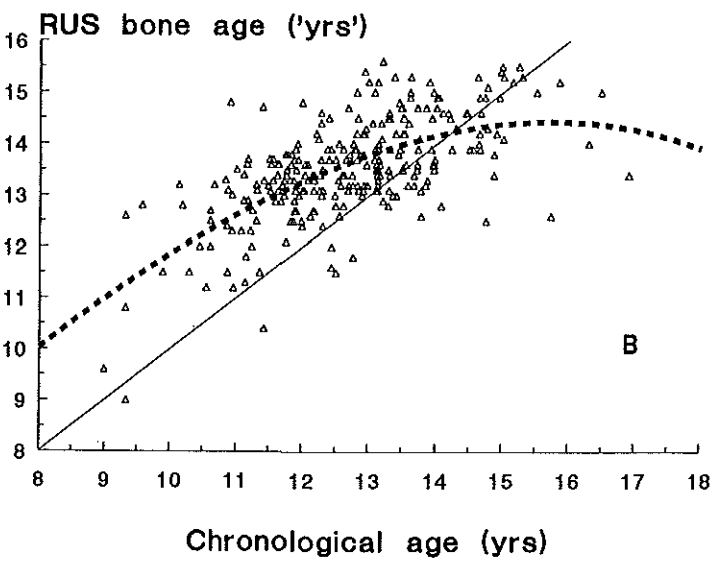
The reliability study of CASAS 13b showed very small estimated between and within observer components of variance. However, when expressed in BA *'years'* the range seems considerable and appears even slightly higher compared with the manual ratings. In an earlier

reliability study with the traditional manual X-ray ratings of healthy children from 7-17 years of age (3) the standard deviation of the within observer component of variance of two observers was similar (0.30-0.35 BA *'years'*) compared with those in our manual ratings (0.25 BA *'years'*) and with CASAS (0.36 BA *'years'*). It has been stated, though, that the reliability varies somewhat in different parts of the age span; as a result of a change of one stage of a single bone the BA may 'jump' by 0.3 *'years'* (1). The interval scale in the manual method has been replaced by a continuous scale in CASAS and, therefore, reliability should be better and equal at all ages. A further improvement would be the use of MS instead of BA. The correlation between biological maturation and CA varies between populations (9). Therefore, ideally every population should have its own standards for MS and BA. In fact, BA is a crude determination derived from the MS table.

A comparison of the staging in duplicate assessments by the same observers (within observer comparison) showed similar percentages for CASAS

Figure 3. Plot of bone age (BA) determined using CASAS 6 bones versus chronological age (CA) in boys (A) and girls (B) with constitutionally tall stature. The regression line (------) is depicted in comparison with the assumed y=x line for BA and CA (——).





13b (88%) and the manual ratings (90%). Ten percent of the ratings differed one stage and using CASAS occasionally a difference of two stages was observed. Noticeably, there were hardly any differences between the individual bones included in the 13b model of CASAS. Our percentage of different stages within observers using CASAS 13b is higher than reported by Tanner et al (2-5%) (5); however, in their study the CASAS 6b model was used. Using the manual method, Beunen et al (3) also found that a single observer gave the same stage rating on two occasions in about 90% of the cases (within observer comparison), whereas different observers gave the same stage in 75-85% of the cases. In our study, the latter (between observer comparison) percentage was similar for both CASAS and the manual ratings, 88% and 89% respectively. Again, using CASAS occasional differences of 2 stages were observed.

As a part of the CASAS grading process each bone is compared with reference standards which represent each stage of bone maturity. The best fitting stage together with the four adjacent stages are being compared with the standard by means of their root mean square errors. The extreme stages of each bone ('A' and 'H'/'I') do not have two adjacent stages on both sides. Although the analysis of covariance is adjusted for these extreme stages (personal communication), the extreme computer-rated stage scores, i.e. 1.0 and 8.0/9.0, are not always attained. To illustrate this, when all epiphyseal plates are visually closed and CASAS rates the stages with a deficit of minus 0.15, the difference with the expected MS of 1000 can mount to about 44 MS in boys and 33 MS in girls, i.e. 0.85 and 0.5 BA *'years'*, respectively. A comparable deficit of 0.15 of a grade to stage 'A' results in a difference of about 15 MS in boys and girls; at this very young CA also a substantial difference. It seems less important, however, since skeletal maturity is less often determined in the first years of life.

The percentage of manual insertions was slightly lower in the second rating period compared with the first period. The data are in good agreement with the CASAS 6b model ratings performed with X-rays from all healthy children (second study), in which 5% manual insertions were performed. There was a high degree of

consistency for each bone between the two rating sessions. When a smaller range was chosen for manual insertions, i.e. a difference between expected and determined stage of one integer stage or more, the percentage of manual insertions on the same X-ray series rated by the same observers increased to 11-12%, but the between and within observer variances did not show a marked change (data not shown).

The possibility of inserting a manual score with CASAS creates the problem of when to make use of this option. To what extent a difference between the expected and determined rating will be accepted is likely to depend on the experience of the user and on the reliability of the computer rating of the individual bones. In addition, the magnification of the individual bones using CASAS may induce distinct differences in expected ratings compared to manual ratings usually performed without magnification. Therefore, cut-off limits for such a manual expected rating remain very subjective, in particular when one keeps the evident within observer variability of manual ratings in mind. Ideally, one should not give a manual insertion at all and the computer should analyse the radiograph fully automatically. However, since CASAS is based on the same classification of developmental stages of each bone, one cannot omit to give a manual insertion whenever the computer rating is far from logical (e.g. stage 'B' instead of 'G') due to e.g. positioning, imaging problems, or software imperfectness. Consequently, CASAS is not yet completely independent of the observer. In addition, in our experience, individual CASAS ratings may sometimes vary considerably just by repositioning of the X-ray and without giving a warning to the user. The designers of the programme underlined this by stating that "correct assessment depends crucially on correct positioning" (5). The choice of acceptance of a single bone rating by the user is also dependent on the subjectiveness (experience) of the user. Therefore, in order to compare study results we consider that the percentage of manual insertions and the limits for the acceptance of disagreement must be reported, at least until the computer operating system is considered to be the gold standard.

The designers of CASAS suggest that prior knowledge is not needed; however, one should familiarize oneself with the sytem and some knowledge on the developmental stages of the various bones is needed to avoid evident mistakes in the rating. Although this is seldom due to the quality of the X-ray, it is obvious that the TW2 radiological technique (1) should be used, in particular with regard to the positioning of the hand.

The use of the 6b model seems preferable because it is less time consuming than rating 13 bones, 5-6 minutes versus 10 minutes or more, respectively. Although experienced TW2 'raters' might be able to perform the manual procedure quicker, it must be noted that after rating the stages data entry in the computer and subsequent calculations still have to follow, in contrast to CASAS.

The mean difference between the 13b and the 6b model of CASAS in healthy children also showed a significant gender difference only when expressed as MS; however, 95% limits of agreement were again wide.

The mean difference between the manual ratings and CASAS in healthy children was only significantly different in girls, although the 95% limits of agreement were considerable.

When X-rays of fully matured children were excluded from the analysis the 95% limits of agreement were only slightly 'narrower'. The mean difference between methods was significantly different between boys and girls. A comparable difference between the sexes was found in CTS children. We have no satisfying explanation for this phenomenon.

CASAS in CTS

On average, ratings of the 13b model were comparable with those of the 6b model in both boys and girls with CTS, although individual differences may be considerable. In girls with CTS the mean difference between the manual ratings and CASAS was also almost zero. In boys, ratings with CASAS were significantly higher compared with the manual ratings. These mean differences between

methods were also significantly different between the sexes. For both sexes, however, the 95% limits of agreement were of the same magnitude and comparable with that in healthy children.

The percentage of manual insertions in the 6b model of CASAS was remarkably low in CTS. Using the 13b model the percentage of manual insertions increased rapidly; it appeared that most of the manual insertions were due to the first and fifth rays, which are not included in the short 6b model. The use of the 6b model will thus reduce the number of difficulties in rating. This might explain the slight, but not significant difference in BA when the 6b model was compared with the 13b model.

Bone maturation curves CTS

Our mixed cross-sectional and longitudinal data of bone age in children with CTS show that BA is advanced at younger ages in boys as well as in girls. This discrepancy weakens as CA progresses. To our knowledge, there are no cross-sectional or longitudinal data on bone maturation in children with CTS. However, our findings are in agreement with our own clinical experiences. In addition, in various clinical studies the mean manual TW2 BA prior to the start of treatment was always higher compared with the mean CA of the patients, whereas the standard deviation of the BA was always smaller than the standard deviation of the CA (14-18). This seems to be in concert with our present findings.

CONCLUSIONS

In healthy girls and in boys with CTS the semi-automated CASAS 6b ratings were significantly different from manual ratings. The difference between the 13b and 6b ratings was only significant in healthy boys. In all comparisons the mean differences between methods (either with the 6b or the 13b model) were less than 0.4 BA 'year'. The range of the difference between methods was considerable, but

the within and between observer variations are in the same order of magnitude as reported for the manual readings. It should be noted, that both methods only estimate BA; a measure of the 'true' BA is unknown. Therefore, we think that this computerized method is applicable in these groups of children. The use of the 6b model seems preferable because it is less time consuming than rating 13 bones.

In view of the percentages of manual insertions the clinical use of this CASAS version seems to be with longitudinal studies of patients in particular. Manual substitution of a stage should be avoided and when performed its percentage and the limits for the acceptance of disagreement should be reported.

Our data indicate that CASAS is applicable in children with CTS. The bone maturation curves in boys and girls with CTS tend to be advanced at younger ages; however, as CA progresses this discrepancy weakens.

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REFERENCES

1. Tanner JM, Whitehouse RH, Cameron N, Marshall WA, Healy MJR, Goldstein H: Assessment of skeletal maturity and prediction of adult height (TW2-method). Academic Press, 1983, London
2. Taranger J, Bruning B, Claesson I, Karlberg P, Landström B: A new method for the assessment of skeletal maturity - the MAT method (mean appearance time of bone stages). Acta Paediatr Scand 1976;(Suppl)258:109-120
3. Beunen G, Cameron N: The reproducibility of TW2 skeletal age assessments by a self-taught assessor. Hum Biol 1980;7:155-162
4. Wenzel A, Melsen B: Replicability of assessing radiographs by the Tanner Whitehouse-2 method. Hum Biol 1982;54:575-581
5. Tanner JM, Gibbons RD: Automatic bone age measurement using computerized image analysis. J Pediatr Endocrinol 1994;7:141-145
6. Tanner JM, Gibbons RG, Bock DR: An image analysis system for TW2 skeletal maturity. Horm Res 1992;37 (Suppl 4):11
7. Tanner JM, Gibbons RD, Bock RD: Advantages of the computer-aided image analysis system for estimating tw skeletal maturity: increased reliability and a continuous scale. Pediatr Res 1993;33:s35
8. Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;I:307-310
9. Eveleth PB, Tanner JM: World-wide variation in human growth. University Press, 1990, Cambridge
10. Zachmann M, Ferrandez A, Murset G, Prader A: Estrogen treatment of excessively tall girls. Helv Paediatr Acta 1975;30:11-30
11. Zachmann M, Ferrandez A, Mürset G, Gnehm HE, Prader A: Testosterone treatment of excessively tall boys. J Pediatr 1976;88:116-123
12. John G, Schellong G: Oestrogen therapie hochwüchsiger Mädchen. Monatsschr Kinderheilk 1980;128:545-550
13. Stöver B, Kollmann F: Ergebnisse der Behandlung hochwüchsiger Mädchen mit conjuglerten Oestrogenen. Monatsschr Kinderheilk 1983;130:36-40
14. Brämswig JH, Hermeling W, Petrykowski WV, Schellong G: Comparison of height predictions with final adult height in boys with constitutional tall stature. In: Borms J, Hauspie R, Sand A, Susanne C, Hebbelinck M (eds) 1984, Human growth and development. Plenum Publishing Co, pp 423-429

CHAPTER 3

Accuracy of final height prediction and effect of growth reductive therapy in 115 constitutionally tall boys.

A comparison of nine prediction methods.

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ABSTRACT

Height reduction by means of treatment with high doses of androgens in constitutionally tall stature is a well-known, though still controversial therapy. The establishment of the effect of such therapy is dependent on the height prediction method applied. We evaluated the reliability of nine prediction methods in 55 untreated boys with constitutionally tall stature and the effect of height reductive therapy in 60 tall boys treated with high doses of androgens (cases). For this purpose we compared the predicted adult height with the attained height at a mean adult age of 25.1 yrs and adjusted the therapeutic effect for possible confounding variables.

At the time of the height prediction, controls were significantly shorter, had more advanced estimated bone ages (except for the bone age (BA) according to Greulich & Pyle (BA_{GP}), had lower target heights, and had smaller adult height predictions compared with cases (P-values <0.05). At the time of the follow-up cases were significantly taller than controls; mean (SD) final adult height was 199.7 (4.1) for cases and 196.0 (4.9) for controls (P<0.001).

In controls, a large variability was found for the errors of prediction of the various prediction methods and in relation to chronological age (CA). The *BP-*, *clinical-* and *RH-predictions** systematically overestimated adult height in constitutionally tall boys, whereas the other prediction methods sytematically underestimated final height. The mean (SD) absolute errors of the prediction methods varied from 2.3 (1.8) to 5.3 (4.3) cm. They were significantly negatively correlated with CA (r:-0.27 to -0.54, P-values<0.05), except for the *TW-*, and *CASAS-prediction**, indicating that height prognosis is more reliable with increasing CA. The extrapolation of the height SDS for BA (BA_{GP}) (*IPH_{GP}-prediction**) was found to be the most reliable method in predicting adult height. In addition, experienced clinicians gave an accurate height prediction by evaluating the growth chart of the child while taking into account various clinical parameters such as CA, BA and pubertal stage. * For explanation of abbreviations: see *Methods*.

The effect of androgen therapy was assessed by means of multiple regression analysis, while adjusting for the confounding variables height prediction, CA and BA at start of therapy. The mean (SD) adjusted effect varied from -1.7 (1.4) to 0.7 (1.4) cm. with ranges from -4.6 to 15.8 cm. The adjusted height reduction was dependent on the BA at the time of start of androgen therapy: the effect was more pronounced when treatment was started at a younger BA. The treatment effect was significantly negative at BAs exceeding 14 to 15 years. After cessation of therapy a mean (SD) additional growth of 2.4 (1.2) cm was observed. The mean (SD) BA_{GP} at that time was 17.1 (0.7) years.

These data demonstrate, that height prognosis in boys with constitutionally tall stature is rather inaccurate, the *IPH_{GP}-prediction* being the most reliable prediction method. Overall, the height reductive effect of treatment with high doses of androgens in tall boys is limited. However, a significant height reduction was found when treatment was started at BAs less than 14 to 15 years, depending on the method of BA assessment. Remarkably, at older BAs treatment was found to be contra-indicated, since androgen administration caused extra growth instead of growth inhibition. It is recommended that referral should take place early, preferably before puberty, for careful monitoring of growth and prediction. Moreover, when treatment is initiated, it is recommended not to discontinue therapy too early in order to avoid serious additional growth.

INTRODUCTION

Prediction of adult height is commonly used in the management of children with growth disorders. Over the years various methods have been developed to predict adult height of which the methods of Bayley and Pinneau (1) and of Tanner et al (2,3) are most commonly used. In general, height prediction models are based on growth data derived from normal growing children of normal stature. This implies that these methods may not give accurate results when applied to children

with growth disturbances. Tanner and co-workers have tried to overcome this problem and revised their original prediction model by including samples of children with growth disorders (2,3). With respect to constitutionally tall children, however, only a sample of 19 tall girls had been included.

In the management of children with constitionally tall stature, height prognosis plays a crucial role since it influences to a large extent the decision for therapeutic intervention with pharmacological doses of sex steroids. It is essential therefore, that the accuracy of the prediction technique applied is clinically acceptable. If treatment is applied, the effect of therapy is evaluated by subtracting the achieved final height from the predicted adult height. Therefore, the ultimate effect of the treatment relies on the reliability of the prediction method as well. In addition, it is to be expected that separate prediction models will provide different estimates of final adult height within the same person and thus a different result in height reduction. This is caused by the fact that the prediction methods refer to different norm populations and use different methodologies.

The height reducing effect of sex steroid therapy in constitutionally tall children has extensively been studied. However, to our knowledge, a well designed, prospective controlled study on the effect of such a therapy has never been performed. This implies that the reported results have been biased by several factors. For instance, results on height reduction have been derived from comparison of the achieved height with the height prediction prior to treatment without correction for the error of the prediction. In addition, when control groups have been used they tended to be small. Besides, while assessing the ultimate height reduction, differences in initial clinical data between treated children and controls such as age, bone age and height prediction, have not been taken into account. Furthermore, in many studies adult height have been assumed to be reached at a relatively young age. Therefore, we evaluated the applicability of nine prediction methods in untreated children with constitutionally tall stature and, with respect to these findings, the effect of height reductive therapy in children treated with high doses of sex steroids. For this purpose, we measured final adult height at

a mean age of 25 years and adjusted the effect of sex hormone therapy for possible confounding variables. In this article we describe our results in tall boys; the evaluation of girls with tall stature will be reported in an adjoining paper.

To date, only a few studies are available on the reliability of height predictions in boys with constitutionally tall stature (4-7). These studies show some conflicting results concerning the tendency to over- or underestimate adult height depending of the prediction method used. They indicate that there is no major advantage of any of the prediction methods studied and that there is considerable age and bone age related variation. Studies on the effect of androgen therapy on height reduction have shown different results using different prediction methods (4,8,9). Overall, these studies indicated that height reductive therapy was effective in tall boys irrespective of the prediction method used. However, considerable age and bone age related variation was present and individual effects overlapped the range of the prediction error.

In this study the reliability of various prediction methods and the effect of height reductive therapy in a total of 115 constitutionally tall boys was evaluated. For this purpose we compared the predicted adult height with the attained height at a mean adult age of 25.1 yrs (range 18.7 - 34.4 yrs.) and adjusted the therapeutic effect for possible confounding variables.

PATIENTS AND METHODS

Patients

Since the introduction of height reductive therapy in our institute in 1968 a total of 247 men, who were seen at adolescence for evaluation of their constitutionally tall stature, had reached the age of 18 at the time of our follow-up study. All were contacted by mail to participate in this study. Second mailings were send to those who did not respond to the first mailing. This study was part of a large follow-up study including psychosocial assessments and long-term sequelae.

Reports on psychology and long-term sequelae will be reported elsewhere.

During their puberty, 102 men had been treated for their tall stature (cases). Hundred and forty-five men had chosen not to undergo treatment for various reasons such as satisfaction with the given height prognosis or uncertainty about possible side-effects, and served as controls. Two hundred and nine men (95 cases and 114 controls) responded to our mailing. Of the responders, 127 men (65 cases and 62 controls) agreed to participate in this study. All participants gave informed consent.

We defined constitutionally tall stature as having a height equal to or above the 90th percentile (Dutch references (10)) at the time of referral. By this definition 5 boys were excluded as their heights were less than the 90th percentile. In addition, from 7 boys no radiograph could be retrieved from the archives for reassessment of bone age and height prediction. Therefore, 115 boys (60 cases and 55 controls) were included in this study.

In cases, most of the men (n=52) had received intramuscular preparations of testosterone ester mixtures (Sustanon® = testosterone proprionate, -fenylpropionate, -isohexanoate and -decanoate) 250 mg every week (n=45), 250 mg every two weeks (n=4) or 500 mg every two weeks (n=3). Four men used an oral testosterone ester (Andriol® = testosterone undecanoate) 240-320 mg per day; one of these patients switched to Sustanon® every two weeks after 8 months. Another four patients used daily injections of a single testosterone ester (Neo-hombreol® = testosterone propionate) 25-30 mg per day for 6 - 30 months after which they switched to Sustanon® 250 mg every two weeks. Treatment started at a mean (SD) age of 14.2 (1.3) yrs. with a range of 9.7 to 17.2 yrs. The mean duration of therapy was 1.4 (0.6) yrs. ranging from 0.7 - 3.7 yrs. In general, treatment was stopped when radiographs of hand and/or knee were considered to show nearly complete closure of the epiphyses. The mean (SD) length of the follow-up period after cessation of androgen therapy lasted 8.7 (4.1) years [range: 3.2 - 18.5 yrs].

METHODS

Auxology

Patients were recalled to our outpatient clinic for measurement of final height. Height measurements were performed by one investigator (WdW) using a Harpenden stadiometer. In addition, auxological data were collected from the hospital charts and radiographs of the left hand and wrist were retrieved and re-used for bone age determinations and final height predictions. For cases, only two radiograph were used: the one just before start of treatment and the one at the time treatment was stopped. For controls all radiographs were used from the age of 8, provided that the interval between every consecutive X-ray was at least 6 months.

Bone age

Bone age (BA) was rated manually according to the methods decribed by Greulich and Pyle (11) and Tanner et al (3)(TW2-RUS BA) by one investigator (MF). In cases, the BA at time of stop of therapy was rated according to Greulich and Pyle (11) by another investigator (WdW). In addition, TW2-RUS BA was determined by a third investigator using the short version (6 bones model) of a computer aided skeletal age scoring (CASAS) system, as described previously (12,13). BAs were rated unaware of the treatment design or the post treatment growth.

Final height predictions

Final height predictions were performed using the methods of Bailey and Pinneau (*BP-prediction*)(1) and Tanner et al (*TW-prediction*, mark II) (3). In addition, the relative height (*RH-prediction*) and the index of potential height (*IPH-prediction*)(14) were calculated.

BP-prediction: Using the BA as estimated by Greulich and Pyle (10), the percent of final height achieved was read in a table (1), and adult final height was calculated.

TW-prediction: From the regression equations provided by Tanner et al (3) final height was calculated using BA as well as height and chronological age (CA) as variables. For this study, we performed TW-predictions using the manual TW2-RUS bone ages (*TW-prediction*) as well as the TW2-RUS bone ages as determined by CASAS (*CASAS-prediction*) (12).

RH-prediction: Final height was calculated with the assumption that the height expressed as standard deviation score (SDS) for CA equals to adult height SDS.

IPH-prediction: Final height was predicted based on the assumption that the height SDS for BA remains constant up to final height. For this study we performed IPH-predictions using the bone ages as estimated by Greulich and Pyle (*IPH_{GP}-prediction*), Tanner et al (*IPH_{TW}-prediction*) and CASAS (*IPH_{CASAS}-prediction*). In addition, we predicted final height using a theoretical bone age (*$IPH_{modified}$-prediction*) derived from the observed relationship between BA (as estimated by CASAS) and CA, as described previously (13). For boys this relationship was $BA=1.32*CA + 0.0002*CA^2 - 0.00009*CA^4$.

RH-predictions and *IPH-predictions* were performed using Dutch references for height and SD-scores (10).

Beside these objective prediction methods we added a subjective *clinical-prediction*. These *clinical-predictions* were performed by two experienced pediatric endocrinologists separately (SDMKS, SD). For this purpose, a growth chart was constructed for each individual. The following data were extracted from the hospital records and, if available, plotted on the growth chart: height measurements available at time of referral, BA (estimated according to Greulich & Pyle by one investigator (MF) as stated above), father's height, mother's height, target height, and pubertal stage. Target height was calculated according to the formula: (Mother's height (cm) + Father's height (cm) + 12)/2 + 3 (15). Growth charts were then blindly and randomly rated for final height predictions. If separate final height predictions differed more than 2 cm among the raters, the prediction was repeated for consensus.

The study protocol was approved by the ethical committee of Academic Hospital, Erasmus University Rotterdam, The Netherlands.

Statistics

To assess the effect of treatment in cases all height predictions were used for analysis, since predictions were based on one radiograph per subject by inclusion. In controls, more than one radiograph was available for accuracy assessment of the various prediction methods. Using repeated predictions of one control subject for assessment of the accuracy of the prediction method and comparison with a treated group in which only one radiograph per subject was taken could induce bias. Therefore we used only one radiograph per control boy. The radiographs were selected such that the age distribution of treated and untreated boys were as alike as possible.

In controls, accuracy of the height prediction methods was expressed mathematically as predicted adult height minus actual adult height. Therefore, positive values indicate overestimation whereas negative values indicate underestimation of the predicted adult height. In addition, the reliability of each height prediction method was also expressed in absolute errors. The absolute errors demonstrate the method's overall predictive error and are independent of over- or underestimation. In cases, the calculation of predicted adult height minus actual final height was used to express the -uncorrected- effect of growth reductive therapy. Consequently, positive values indicate growth reduction whereas negative values indicate lack of growth reduction. Multiple regression analysis was used to assess the corrected height reducing effect of androgen therapy (see below). For the *clinical-prediction*, the mean of the two separate predictions was used for statistical analysis.

Differences between groups were tested with the Mann-Whitney-U-test. In controls, Friedman's test was used to test the difference in absolute errors of the various prediction methods. If this test was significant, Wilcoxon's matched pairs signed ranks test was used to test the difference in absolute errors between

groups and within each single prediction method. Throughout all age groups, the *BP-prediction* showed overestimation whereas the *IPH-methods* based on the bone age rated according to Tanner et al showed underestimation (IPH_{TW}-, IPH_{CASAS}-, and $IPH_{modified}$-prediction). The other methods varied in over- and underestimation within the age groups.

Table 2a. Bone age estimated by different methods in treated tall men and tall controls. Data expressed as mean (SD) and range.

Clinical data	Cases (n=60)	Controls (n=55)
Age at time of prediction (yrs)	14.20 (1.32) 9.72 - 17.18	13.90 (1.50) 10.36 - 17.17.
Height at time of prediction (cm)	189.3 (6.6) 166.0 - 202.8	183.8 (10.0)# 158.5 - 198.2
Height SDS at time of prediction	2.84 (0.86) 1.44 - 4.80	2.41 (0.69)# 1.33 - 4.44
BA - G&P (yrs)	14.0 (0.9) 9.0 - 15.5	14.2 (1.5) 11.0 - 18.5
BA - TW2-RUS (yrs)	14.8 (0.9) 10.7 - 16.2	15.2 (1.5)* 11.8 - 18.2
BA - CASAS (yrs)	15.2 (0.8) 11.0 - 16.4	15.4 (1.1)* 11.1 - 17.1

* Significant difference between cases and controls; $P<0.05$
\# Significant difference between cases and controls; $P<0.01$

The mean absolute errors varied from 2.3 (1.8) to 5.3 (4.3) cm. and were statistically significantly different (Friedman; $P<0.0001$). It appeared that the IPH_{GP}-*prediction* and the *clinical prediction* were most reliable in predicting adult height: their mean absolute errors were not significantly different from each other ($P=0.13$), but were significantly smaller compared to seven and five of the remaining prediction methods (P-values<0.05). On the other hand, the IPH_{CASAS}-, and *RH-prediction* showed most inaccuracy: their mean absolute errors were significantly larger compared to, respectively, seven and five of the other prediction methods

Table 2b. Adult height prediction (cm) according to various methods in treated tall men and tall controls. Data expressed as mean (SD) and range.

Prediction method	Cases (n=60)	Controls (n=55)
BP	204.5 (5.3) 193.5 - 220.7	198.8 (5.4)# 188.5 - 211.5
TW	200.3 (5.0) 191.0 - 210.8	195.1 (4.5)# 186.6 - 204.7
CASAS	199.0 (4.5) 190.3 - 210.0	194.3 (4.1)# 186.3 - 204.7
Clinical	201.6 (3.6) 195.0 - 209.0	197.1 (3.8)# 190.0 - 206.5
RH	201.0 (5.7) 191.6 - 214.2	198.1 (4.6)# 190.9 - 211.7
IPH_{GP}	200.3 (4.5) 191.6 - 217.8	196.0 (4.0)# 188.0 - 206.1
IPH_{TW}	196.6 (4.3) 187.4 - 213.1	192.4 (4.1)# 184.9 - 202.0
IPH_{CASAS}	195.1 (4.0) 185.2 - 204.2	190.8 (5.3)# 177.7 - 201.6
$IPH_{modified}$	195.5 (4.0) 186.2 - 205.5	192.1 (5.2)# 179.2 - 201.7
Final adult height	199.7 (4.1) 190.7 - 210.1	196.0 (4.9)# 186.6 - 209.3

Significant difference between cases and controls; P<0.005

(P-values<0.05). The remaining methods (the *BP-, TW, CASAS,* IPH_{TW}- and $IPH_{modified}$-*prediction*) had an intermediate position: they did not significantly differ from each other, but did from three or four of the remaining methods (P-values<0.05). In most methods applied, the mean absolute errors were significantly, negatively correlated with CA (r:-0.27 to -0.54; P-values<0.05). Only the *TW-prediction* and the *CASAS-prediction* failed to show a significant correlation with CA (r=0.11, P=0.41 and r=0.23, P=0.09, respectively).

Table 3a. Error of prediction (cm) of various prediction methods by chronological age in 55 controls

		Error of prediction (n=55)								
Age (yrs)	N	BP	Clinical	TW	CASAS	RH	IPH_{GP}	IPH_{TW}	IPH_{CASAS}	$IPH_{modified}$
≤ 12	7	4.2 (4.8)	0.6 (4.4)	0.3 (5.6)	-1.6 (6.8)	1.9 (5.9)	-0.1 (3.8)	-5.3 (4.5)	-11.3 (7.6)	-11.5 (5.7)
13	14	4.6 (4.3)	2.6 (4.0)	2.7 (4.7)	0.6 (4.3)	6.9 (6.1)	1.2 (4.0)	-4.0 (4.6)	-6.7 (3.1)	-5.2 (5.6)
14	16	2.7 (2.9)	1.4 (2.5)	0.2 (3.0)	-0.7 (2.6)	2.6 (4.1)	0.4 (2.2)	-3.0 (2.3)	-3.6 (1.9)	-2.0 (2.6)
15	12	0.6 (1.3)	-0.8 (1.9)	-5.8 (2.4)	-5.0 (2.0)	-2.2 (3.4)	-1.8 (1.6)	-3.9 (2.3)	-4.0 (2.8)	-2.9 (3.5)
≥ 16	6	1.2 (3.4)	0.2 (1.4)	-4.2 (2.4)	-3.6 (1.9)	-2.0 (3.2)	-0.6 (1.4)	-2.2 (2.0)	-2.2 (2.7)	-0.1 (3.2)
All	55	2.8 (3.6) [-6.0;11.6]	1.0 (3.2) [-8.3;7.4]	-0.9 (4.8) [-11.3;11.3]	-1.7 (4.2) [-15.1;6.4]	2.1 (5.7) [-9.8;15.2]	-0.1 (2.9) [-7.5;7.1]	-3.7 (3.3) [-13.6;8.4]	-5.3 (4.4) [-23.7;0.5]	-4.0 (5.2) -22.2;3.4]

Table 3b. Absolute error (cm) of various prediction methods by chronological age in 55 controls

		Absolute error (n=55)								
Age (yrs)	N	BP	Clinical	TW	CASAS	RH	IPH_{GP}	IPH_{TW}	IPH_{CASAS}	$IPH_{modified}$
≤ 12	7	4.8 (4.1)	3.8 (1.7)	3.9 (3.8)	4.4 (5.2)	4.9 (3.4)	2.9 (2.1)	5.4 (4.4)	11.3 (7.6)	11.5 (5.7)
13	14	5.7 (2.6)	3.9 (2.6)	4.3 (3.1)	3.0 (3.1)	8.4 (3.6)	3.3 (2.3)	5.3 (2.9)	6.7 (3.1)	5.4 (5.4)
14	16	2.9 (2.7)	2.5 (1.4)	2.1 (2.0)	2.1 (1.6)	3.8 (2.9)	1.8 (1.3)	3.1 (2.3)	3.6 (1.9)	2.3 (2.3)
15	12	1.0 (1.0)	1.6 (1.3)	5.8 (2.4)	5.0 (2.0)	3.1 (2.4)	1.8 (1.6)	3.9 (2.3)	4.0 (2.8)	3.5 (2.8)
≥ 16	6	1.9 (2.6)	1.0 (0.9)	4.2 (2.4)	3.6 (1.9)	2.9 (2.2)	1.2 (0.9)	2.2 (2.0)	2.5 (2.3)	2.6 (1.6)
All	55	3.3 (3.1) [0.0;11.6]	2.7 (2.0) [0.0;8.3]	3.9 (2.9) [0.4;11.3]	3.4 (2.9) [0.0;15.1]	4.9 (3.6) [0.1;15.2]	2.3 (1.8) [0.2;7.5]	4.0 (2.9) [0.3;13.6]	5.3 (4.3) [0.5;23.7]	4.6 (4.7) [0.2;22.2]

Table 4. Uncorrected effect of treatment (cm) according to various prediction methods by chronological age in 60 treated tall men.

		Effect of treatment (n=60)								
Age (yrs)	N	BP	Clinical	TW	CASAS	RH	IPH_{GP}	IPH_{TW}	IPH_{CASAS}	$IPH_{modified}$
≤ 12	3	10.6 (11.3)	6.1 (5.5)	5.7 (5.9)	4.4 (7.2)	8.6 (5.6)	7.3 (10.7)	-2.3 (5.8)	-6.1 (8.5)	-6.9 (4.9)
13	16	7.0 (5.4)	4.3 (2.8)	5.1 (3.7)	3.4 (3.3)	8.5 (3.5)	2.1 (4.9)	-3.3 (4.9)	-5.2 (2.9)	-3.8 (3.0)
14	16	4.2 (3.6)	1.9 (2.7)	1.8 (4.1)	0.0 (3.4)	0.6 (4.4)	-0.2 (3.1)	-3.8 (2.9)	-5.4 (2.2)	-5.1 (3.2)
15	14	2.9 (3.7)	-0.5 (2.3)	-3.6 (3.7)	-4.7 (2.7)	-3.3 (2.5)	-1.0 (3.4)	-2.5 (5.6)	-4.1 (2.7)	-4.2 (2.7)
≥ 16	11	3.2 (2.5)	-0.4 (1.9)	-4.1 (2.5)	-4.2 (2.9)	-4.3 (1.7)	-0.6 (2.6)	-2.9 (1.8)	-2.9 (2.2)	-2.8 (2.2)
All	60	4.8 (4.9) [-3.2;20.7]	1.7 (3.4) [-5.6;10.6]	0.5 (5.3) [-12.4;11.6]	-0.8 (4.7) [-9.9;9.0]	1.3 (5.2) [-8.3;15.1]	0.5 (4.5) [-6.8;17.8]	-3.1 (4.1) [-11.7;15.1]	-4.6 (3.0) [-15.0;1.9]	-4.2 (3.0) [-12.5;0.4]

Table 5. Estimated effect of androgen therapy on final height in 115 boys with constitutionally tall stature for nine prediction methods, adjusted for chronological age, bone age and height prediction.

Prediction method*	Effect of treatment	Effect > 0		P-value[1]	Mean effect[2]; range
BP	30.74 - 2.18 x BA_{GP}	BA_{GP}	< 14.10	0.0001	0.2 (2.0); -3.1 to 11.1
Clinical	22.30 - 1.64 x BA_{GP}	BA_{GP}	< 14.48	0.0024	0.7 (1.4); -1.6 to 8.4
TW	19.25 - 1.30 x BA_{TW}	BA_{TW}	< 14.81	0.037	0.0 (1.2); -1.8 to 5.3
CASAS	27.35 - 1.78 x BA_{CASAS}	BA_{CASAS}	< 15.37	0.013	0.3 (1.4); -1.8 to 7.8
RH	20.76 - 1.46 x BA_{GP}	BA_{GP}	< 14.22	0.018	0.3 (1.4); -1.9 to 7.6
IPH_{GP}	44.19 - 3.15 x BA_{GP}	BA_{GP}	< 14.03	<0.0001	0.0 (2.8); -4.6 to 15.8
IPH_{TW}	37.81 - 2.58 x BA_{TW}	BA_{TW}	< 14.66	<0.0001	-0.5 (2.4); -4.0 to 10.2
IPH_{CASAS}	32.56 - 2.17 x BA_{CASAS}	BA_{CASAS}	< 15.00	0.0012	-0.4 (1.7); -3.0 to 8.7
$IPH_{modified}$	38.97 - 2.72 x $BA_{modified}$	$BA_{modified}$	< 14.33	0.029	-1.7 (1.4); -2.7 to 6.2

* For explanation see *Methods*

[1] P-value for treatment effect modification by BA

[2] Mean of the individual treatment effects as calculated with the corresponding formulas

Effect of androgen therapy

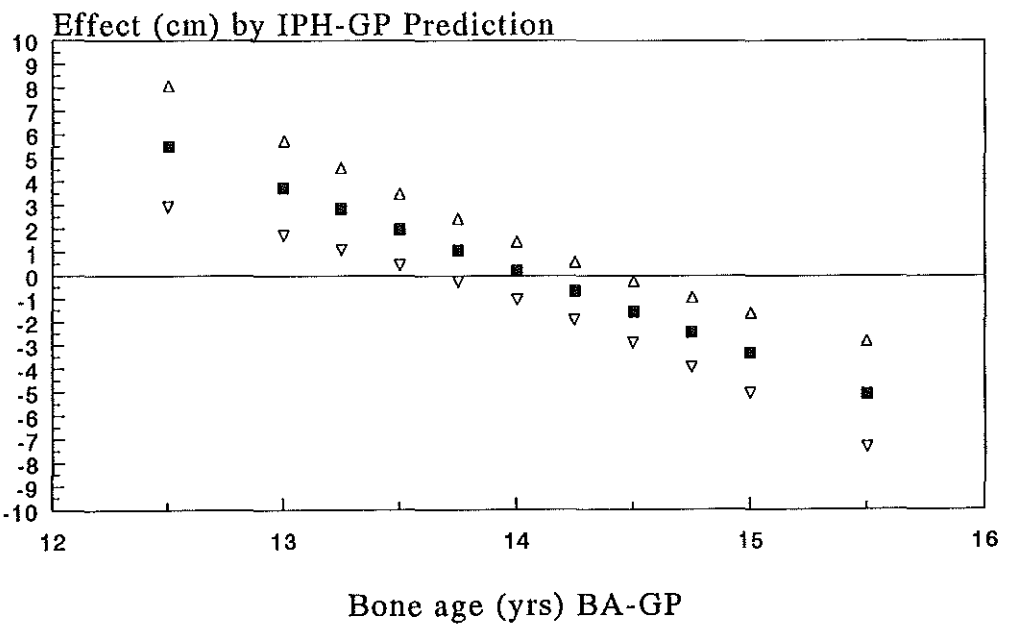
The -uncorrected- height reduction (i.e. height prediction minus actual adult height) in tall boys as determined by the various methods and in relation to CA is given in Table 4. From this table it is clear that the various methods showed large variability in calculating the effect of treatment; the *BP-prediction* showed the greatest mean effect. In general, the mean effect decreased with increasing age. In addition, its standard deviation also decreased. The *BP-prediction* was the only method calculating positive results throughout all age groups.

To adjust for possible confounding and to search for possible treatment effect modification, the above described multiple linear regression analysis was performed separately for each prediction method. In all methods there appeared to be a significant interaction between treatment and BA at start of treatment. In Table 5 the estimated treatment effect in relation to the appropriate BA at start of therapy is given for all prediction methods. For instance, for the *IPH_{GP}-prediction*, the adjusted effect of therapy was a linear function in the form: Effect (cm)= 44.19 - 3.15 x BA_{GP} (yrs). This effect is positive when the BA_{GP} is less than 14.03 yrs. The effect of therapy according to the *IPH_{GP}-prediction* and its 95% confidence interval are plotted in Figure 1. The mean adjusted effect, obtained after applying the appropriate BA into the equations summarized in table 5, varied from -1.7 (1.4) to 0.7 (1.4) cm with a range of -4.6 to 15.8 cm.

Post-treatment growth

In cases, the height at the end of therapy was 197.4 (4.0) cm. at a mean age of 15.69 (1.14) years, whereas adult height was 199.7 (4.1) cm., indicating an additional growth of 2.4 (1.2) cm after cessation of therapy with a range of -0.7 to 5.8 cm. No significant correlation was found between the post-treatment growth and either age at start of therapy, age at stop of therapy or the duration of therapy (r:-0.04 to -0.21; P-values >0.10). BA_{GP} at time of stop of therapy was available in 53 out of 60 patients and was 17.1 (0.7) yrs with a range from 14.5 to 18.5 yrs. We observed a significant relationship between post-treatment growth and the BA_{GP} at

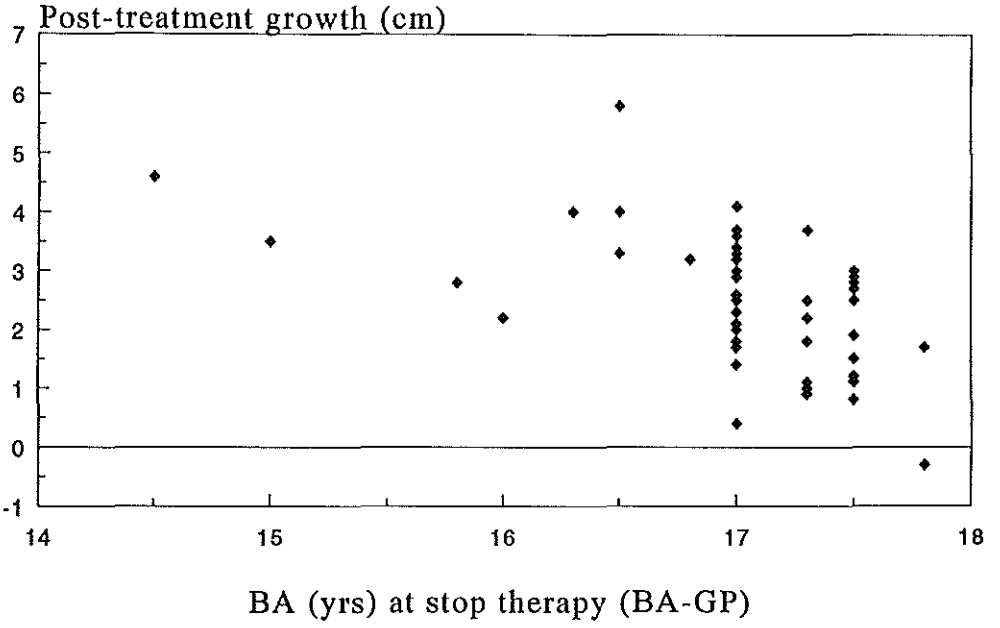
Figure 1. Adjusted effect of androgen therapy and its 95% confidence interval by bone age (BA_{GP}) in constitutionally tall boys.



Effect (cm) = 44.19 - 3.15 x BA_{GP} (yrs)
N.B. The adjusted effect of therapy of one boy with an estimated BA_{GP} of 9.0 years is not plotted for graphical reasons. Analyses with and without this case did not significantly influence the results.

time of stop of therapy ($r=-0.53$, $P<0.001$) (Figure 2). Multiple forward regression analysis, using the post-treatment growth as the dependent variable and CA at time of stop of treatment, BA_{GP} at time of stop of treatment and the duration of treatment as predictor variables, revealed, that both BA_{GP} and CA at time of cessation of treatment had a significant influence on the post-treatment growth ($P=0.0004$ and $P=0.041$, respectively), providing the following equation: Post-treatment growth (cm)= 19.19 - 0.75 BA_{GP}-stop (yrs) - 0.25 CA-stop (yrs) (R^2: 0.30; RSD: 0.98).

Figure 2. Post-treatment growth by bone age (BA_{GP}) at time of cesssation of androgen therapy in 53 tall boys (r=-0.53, P<0.001).



DISCUSSION

Accuracy of height prediction

Our study shows that there is no 'best' prediction model in tall boys. This is most clearly shown by the mean absolute prediction errors of the separate methods, which varied between 2.3 and 5.3 cm. With increasing age all methods became more accurate in predicting adult height as shown by the significant negative correlations between the absolute errors and CA and/or the decrease in the SD's of the absolute errors. Only the *TW-predictions*, whether based on manual rated- or computer rated bone age determinations, failed to follow this tendency. Overall, the *IPH_{GP}-prediction* and the *clinical-prediction* appeared to be the most accurate method: they had the lowest mean absolute errors. This implies that the most reliable prediction of adult height in tall boys can be performed by

extrapolation of the height SDS for the bone age according to Greulich and Pyle. In addition, it shows that experienced clinicians are capable of giving an accurate prediction by evaluating the growth chart of the child while taking into account age, bone age (G&P), target height and pubertal stage. This "weighing" mental process appears to be as reliable as a population based, mathematical prediction model. The *RH-prediction* and the remaining *IPH-predictions* (i.e. IPH_{TW}-, IPH_{CASAS}-, and $IPH_{modified}$-*prediction*) appeared to be of minor importance in constitutionally tall boys as their mean absolute errors were ≥ 4.0 cm.

As shown, every single method had a systematic tendency to over- or underestimate final height, which varied with CA as well. Knowledge of the method's tendency to over- or underestimate final height is of clinical importance since it contributes to the decision of possible therapeutic intervention in tall boys. In practice, one may increase the accuracy of a height prediction simply by correcting it for its systematic error as also suggested by Joss et al (6). However, there are considerable individual variations as shown by the large standard deviations of the mean errors of the prediction applied. This means that for instance for a tall boy of 13 years using the *BP-prediction*, the method gives a mean overprediction of 2.7 cm, but in individual cases this may vary from an underprediction of -2.9 cm (-2SD) to an overprediction up to 8.5 cm (+2SD).

To date, only a few studies have included a substantial number of subjects in order to assess the reliability of various prediction methods in tall boys. Brämswig and co-workers (5) reported a systematic overprediction of 2.1 (4.8) cm. of the *BP-prediction* and an absolute error of 4.0 (3.3) cm in 42 tall subjects. These are of the same order of magnitude as found by us. Joss et al (6) also described a systematic overprediction of the *BP-prediction* studying 32 tall boys. Unfortunately, exact data and absolute prediction errors were not given. In addition, they reported a systematic overprediction using the *TW-prediction*, which was even more pronounced at an older bone age. We also observed overprediction of the *TW-prediction* up to the age of 14 years. At older ages however, a clear underprediction was present in our study which is in contrast to the findings of

Joss et al (6), but in agreement with the findings of Brämswig et al (5) using the former TW-method (2). The relative inaccuracy of the *IPH_{TW}-prediction* was found by Joss et al (6) as well. Both studies used repeated predictions in the same subject for accuracy assessment, which may have induced bias in reported means and/or standard deviations. It is hard to judge the influence of the possible bias, but at least one could expect less variation in the accuracy of the prediction method tested reflected by smaller standard deviations of the estimates. This is due to the fact that the estimate of final height will be more accurate and less variable by increasing age.

Effect of androgen therapy

As expected the -uncorrected- effect of height reductive therapy (i.e. height prediction minus achieved adult height) varied with the prediction method applied (Table 4). Since every single prediction method had its own prediction error, the mean effect of treatment might be "corrected" by subtraction of the corresponding mean prediction errors, which has been done in the literature (4,8,9). In this way, in our study the *BP-prediction* showed the greatest mean "corrected" effect of 2.0 cm. The *IPH_{GP}-prediction* and the *clinical-prediction*, being the most accurate methods, calculated a mean "corrected effect" of only 0.6 and 0.7 cm, respectively. Overall, these results are much less than reported previously. Zachmann et al (8) reported an overall mean ("corrected") reduction of 5.4 cm. as determined by the former TW-method (2). Brämswig and co-workers (4) described an achieved mean ("corrected") height reduction varying from 5.3 cm. (former *TW-prediction*) to 4.7 cm. (*BP-prediction*). In a later paper (9), they reported a mean effect of 7.6 cm. using a short-term (6 months) treatment design and a mean effect of 9.6 cm. while using a long-term treatment design (mean 14.3 months) calculated with the *BP-prediction*. These results drop to 5.5 and 7.5 cm., respectively, when their reported overprediction of 2.1 cm. has been taken into account (5). Bettendorf et al (16) on the other hand, recently reported that short-term androgen therapy failed to reduce final adult height in constitutionally tall boys. These conflicting results may be due

to differences in CA at the time final adult height was measured and to differences in BA at the time of cessation of therapy. According to our own data, a mean post-treatment growth of 2.4 (1.2) cm. was observed. It appeared that at the time treatment was stopped the corresponding BA_{GP} was on average about 17 yrs. This means that at that point in time approximately 98.8% of the adult final height had been achieved (1), which mainly explained the observed additional growth. In addition, not surprisingly, post-treatment growth was more pronounced when treatment was stopped at an ealier bone age (Figure 2). At the time therapy had been stopped, the mean height reduction (after "correction" for the mean error of the prediction) was 4.4 cm according to the *BP-prediction* and 3.0 cm according to the *IPH_{GP}-prediction*. This is in the same order of magnitude as the results reported by Zachmann et al (8), and the first report of Brämswig et al (4), but is less compared to the latest study of Brämswig et al (9).

This approach of calculating the effect of treatment after "correcting" for the mean errors of the prediction will undoubtedly induce bias, because the control population on which the accuracy of the method is established is usually not strictly comparable to the treated cases on which the effect of treatment is assessed. We therefore chose for a more direct and conceptually simple approach using multiple linear regression. Basically in this approach the final adult height of treated and untreated boys is compared having equal CA, BA and height prediction. Separate analyses were done for each prediction method. All these multiple regression analyses revealed a significant treatment effect with a size depending on the BA at start of treatment. The mean adjusted effect for our treated boys varied from -1.7 to 0.7 cm. This means that on average the height reducing effect of androgen therapy on final height in our study is quite limited. However, the marked variation of the individual -uncorrected- effects of treatment as shown by its large standard deviations already suggested that there was certainly a subgroup of patients that has experienced more benefit from therapy than others. In fact, multiple regression analysis clearly showed that height reduction was more pronounced when treatment was started at a younger BA (Table 5). The finding of a bone age-related

effect is in concert with previous studies (5,9).

Beside the fact that the effect of androgen therapy was more pronounced at a younger BA, our study results also implicate that when treatment was started at a later BA final adult height significantly exceeded the height prognosis (Figure 1). It means that androgen therapy had induced *extra* growth instead of growth inhibition. Apparently, at an older BA the stimulating effect of androgens on bone maturation will not counterbalance its anabolic growth stimulating effect. We thought this to be quite an unexpected finding, but we believe that it limits the use of androgen therapy in the management of tall stature. The study of Blanchard et al (17) demonstrated that cartilage cells derived from 2-10 year old boys responded very well to the stimulating effect of sex steroids, while cells from children up to one year of age did not respond. Unfortunately, data from cartilage samples of older children were not available. It may be speculated that this age-dependent responsiveness of human cartilage cells to sex steroids may also be present during puberty and in some way be responsible for the net extra growth at older BAs.

We therefore believe that there is no *general* indication for height reductive therapy with high doses of androgens in constitutionally tall boys. But whenever considered, it should be restricted to boys with estimated BAs less than 14 (BA_{GP}) to 15 (BA_{CASAS}) yrs. In our study, treatment had been started at a BA_{GP} > 14 yrs in about 52% of the cases, indicating that on average boys had been treated at a rather late stage for optimal effect. It is recommended therefore to start treatment earlier and we stress the fact that referral should take place before the age of onset of puberty in order to be able to monitor growth and height prediction carefully. Furthermore, if treatment is initiated, it is essential not to discontinue therapy too early. Serious post-treatment growth can be expected when treatment is stopped at earlier (bone)ages: every additional treatment year in BA and CA will count for approximately 1 cm less additional growth. The latter is in contrast to the opinion of Brämswig and co-workers (9) who promoted short term (6-months) therapy and reported significant height reduction (uncorrected: 7.6 cm.) with a mean BA of 15.3 (0.8) yrs at stop of treatment. In our opinion, however, these results seem too

optimistic, since final height assessment was performed at a relatively young mean (bone) age. Others failed to show any height reducing effect using the same therapeutic strategy, but with assessment of final height at a definite later point in time (16).

Administration of high doses of androgens may lead to adverse effects, such as aggravation of acne and gynaecomastia, which are mostly mild in course (18,19). Occasionally, a serious complication such as priapism or acne fulminans has been reported. Up till now there is no evidence that pharmacological doses of sex steroids induce long-term side effects on reproductive functioning (18-20). It should be noted however, that in previously treated men slightly, though significantly, higher plasma levels of FSH were found compared with controls (mean (SD) plasma FSH: 3.3 (2.2) and 2.1 (0.8) IU/l, respectively). The meaning of this finding remains to be established since it coexisted in the presence of normal sperm quality, normal plasma testosterone levels and normal testes volume (19).

In summary, our study demonstrated that height prediction in constitutionally tall boys is rather inaccurate, the *IPH_{GP}-prediction* being the most reliable prediction method. Overall, the height reductive effect of high doses of androgens in tall boys is limited. However, a significant effect of treatment is expected when treatment is started at BA's less than 14 to 15 years, depending on the method of BA assessment. At older BAs, treatment was found to be contra-indicated, since androgen administration caused extra growth instead of growth inhibition. It is recommended that referral should take place early, preferably before puberty, for careful monitoring of growth and prediction. Moreover, when treatment is initiated, it is recommended not to discontinue therapy too early in order to avoid serious post-treatment growth.

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REFERENCES

1. Bailey N, Pinneau SR: Tables for predicting adult height from skeletal age, revised for Greulich and Pyle hand standards. J Pediatr 1952;40:423-441
2. Tanner JM, Whitehouse RH, Marshall WA, Healy MJR, Goldstein H: Assessment of skeletal maturity and prediction of adult height (TW2 method). Academic Press, New York 1975
3. Tanner JM, Whitehouse RH, Cameron N, Marshall WA, Healy MJR, Goldstein H: Assessment of skeletal maturity and prediction of adult height (TW2 method). Academic Press, London 1983
4. Brämswig JH, Schellong G, Borger HJ, Breu H: Testosteron-Therapie hochwüchsiger Jungen. Ergebnisse bei 25 Jungen. Dtsch Med Wschr 1981;106:1656-1661
5. Brämswig JH, Hermeling W, von Petrykowski W, Schellong G: Comparison of height predictions with final adult height in boys with constitutional tall stature. In: Borms J, Hauspie R, Sand A, Susanne C, Hebbelinck M (eds) Human growth and development. Plenum Press, New York 1984
6. Joss EE, Temperli R, Mullis PE: Adult height in constitutionally tall stature: accuracy of five different height prediction methods. Arch Dis Child 1992;67:1357-1362
7. Zachmann M, Sobradillo B, Frank M, Frisch H, Prader A: Bayley-Pinneau, Roche-Walner-Thissen, and Tanner height predictions in normal children and in patients with various pathologic conditions. J Pediatr 1978;93:749-755
8. Zachmann M, Ferrandez A, Mürset G, Gnehm HE, Prader A: Testosterone treatment of excessively tall boys. J Pediatr 1976;88:116-123
9. Brämswig JH, von Lengerke HJ, Schmidt H, Schellong G: The results of short-term (6 months) high dose-testosterone treatment on bone age and adult height in boys of excessively tall stature. Eur J Pediatr 1988;148:104-106
10. Roede MJ, van Wieringen JC: Growth diagrams 1980. Netherlands third nation-wide biometric survey. Tijdschr Soc Gezondheidszorg 1985;63 (Suppl):1-34
11. Greulich WW, Pyle SI: Radiographic atlas of skeletal development of the hand and wrist. Stanford University Press, Stanford 1959
12. Tanner JM, Gibbons RD: Automatic bone age measurement using computerized image analysis. J Pediatr Endocrinol 1994;7:141-145
13. Van Teunenbroek A, de Waal WJ, Roks A, Chinafo P, Fokker MH, Mulder P, de Muinck Keizer-Schrama SMPF, Drop SLS: Computer aided skeletal age scores (CASAS) in healthy children, girls with Turner syndrome and in children with constitutionally tall stature. Ped Res 1996; in press
14. Lenko HL: Prediction of adult height with various methods in Finnish children. Acta Paediatr Scand 1979;68:85-92
15. Van den Brande JVL, Degenhart HJ: Endocrinologie en groeistoornissen. In: Kindergeneeskunde, Van de Brande, Monnens (eds). Wetenschappelijke uitgeverij Bunge, Utrecht 1993
16. Bettendorf M, Grulich-Henn J, Schönberg D, Heinrich U: Failure of short-term, high dose testosterone treatment to reduce adult height in boys with constitutional tall stature. Horm Res 1994;41:102

17. Blanchard O, Tsagris L, Rappaport R, Duval-Beaupere G, Corvol M: Age-dependent responsiveness of rabbit and human cartilage cells to sex steroids in vitro. J Steroid Biochem Mol Biol 1991;40:711-716
18. Fyrand O, Fiskaadal HJ, Trygstad O: Acne in pubertal boys undergoing treatment with androgens. Acta Derm Venereol 1992;72:148-149
19. De Waal WJ, Vreeburg JTM, Bekkering F, Jong de FH, de Muinck Keizer-Schrama SMPF, Drop SLS, Weber RFA: High testosterone therapy for reduction of final height in constitutionally tall boys: does it influence testicular function in adulthood? Clin Endocrinol 1995;43:87-95

CHAPTER 4

Accuracy of final height prediction and effect of growth reductive therapy in 247 constitutionally tall girls.

A comparison of nine prediction methods.

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ABSTRACT

The administration of high doses of estrogens to constitutionally tall girls has been found to be effective in reducing final height. The establishment of the effect of such therapy, however, is open to debate. We evaluated the reliability of nine prediction methods in 88 untreated girls with constitutionally tall stature and the effect of height reductive therapy in 159 tall girls treated with high doses of estrogens (cases). For this purpose we compared the predicted adult height with the attained height at a mean adult age of 25.3 yrs and adjusted the therapeutic effect for possible confounding variables.

At the time of the height prediction, controls were significantly shorter, had more advanced estimated bone ages, had lower target heights, and had smaller adult height predictions compared with cases (P-values <0.05). At the time of the follow-up cases were significantly taller than controls; mean (SD) final adult height was 181.7 (3.2) for cases and 180.5 (3.8) for controls (P=0.02).

In controls, the mean (SD) errors of the single prediction methods varied from -3.3 (3.9) to 0.6 (3.9) cm. Six of the nine methods applied showed a systematic error within 1 cm of the adult final height. The mean (SD) absolute errors of the prediction methods varied from 1.9 (1.7) to 3.7 (3.5) cm. In all methods, we found a significant negative correlation between the absolute error and chronological age (CA) (*r*:-0.25 to -0.65, P-values<0.02), indicating that height prognosis is more reliable with increasing CA. The mean absolute errors were significantly higher in premenarcheal girls compared to postmenarcheal girls. This difference disappeared after correction for CA. Five prediction methods were found to be most reliable in predicting final height and were mutually comparable: the *BP-, TW-, CASAS-, clinical-* and *IPH_{GP}-prediction* (for explanation of abbreviations: see *Methods*).

The effect of estrogen therapy was assessed by means of multiple linear regression analysis, while adjusting for the confounding variables height prediction, menarche, CA and bone age (BA) at start of treatment. The mean (SD) adjusted effect varied from 1.1 (1.0) to 2.4 (1.4) cm with ranges from -2.6 to 6.2 cm. The

adjusted height reduction was mainly dependent on the BA at the time of start of estrogen therapy: the effect was more pronounced when treatment was started at a younger BA. After cessation of therapy a mean (SD) additional growth of 2.7 (1.1) cm was observed. The mean (SD) BA_{GP} at that time was 15.2 (0.6) years.

These data demonstrate that the accuracy of various methods to predict adult height in constitutionally tall girls is clinically acceptable. With increasing age, height prognosis became more accurate. We found a significant height reductive effect of high doses of estrogens in tall girls. This effect was more pronounced when treatment had been started at a younger BA. Serious additional growth was observed after cessation of therapy, which could be explained only partly by the BA_{GP} at that time; additional spinal growth may also account for part of the additional growth.

INTRODUCTION

Prediction of adult height is an important clinical tool in the management of children with tall stature. It not only forms the basis for therapeutic intervention with pharmacological doses of sex hormones, but it has also a key position in monitoring the ultimate effect of such a treatment. Therefore, secure techniques for reliable height predictions are needed. In general practice various prediction methods are being used (1-4). Since most methods are based on growth data derived from normal growing children, a critical appraisal of their qualities is required when applied to children with constitutionally tall stature. In chapter 3 we evaluated the reliability of nine prediction methods and the effect of height reductive therapy in tall boys (5). In this chapter we will discuss our findings in girls.

To date, the reliability of height prediction methods in constitutionally tall girls have been rarely studied in large untreated groups. Only Willig et al (6) and Joss et al (7) reported accuracy of height prediction methods in a substantial number of subjects (92 and 100 untreated tall girls, respectively). Others documented data on untreated groups varying from 9 to 28 tall girls in controlled studies on the effect of

estrogen therapy (8-16). In the various reports, the systematic errors of the prediction methods applied have been studied, which gave information about the tendency of the method to under- or overestimate final adult height. Unfortunately, absolute errors, which provide information about the reliability of the prediction method independently of its under- or overestimation, have never been reported. In addition, interpretation of the results is hampered by the fact that final adult height was measured at different points in time.

Studies on the effect of height reduction in tall girls have shown various results using different prediction models within the same study population (9-11,13,14,16-18). However, comparison of the ultimate height reducing effect between the numerous studies is limited; there are significant differences in study design, therapeutic regimen, initial clinical data and use of adult final height (11,13). Nevertheless, height reductive therapy in tall girls is considered to be effective irrespective of the prediction method used. However, it is acknowledged that considerable -age and bone age (BA) related- variation is present and individual effects may overlap the range of the prediction error (5,8-25). As in boys (5), claimed results on height reduction in tall girls are based on the prediction method itself or on comparison with relatively small control groups. Correction for differences between treated and untreated girls in for example chronological age, bone age and height prediction have never been done. We therefore evaluated the reliability of nine prediction methods in 88 untreated tall girls and calculated the ultimate effect of height reductive therapy in 159 tall girls after estrogen treatment. For this purpose we compared the predicted adult height with the attained height at a mean adult age of 25.28 yrs (range 18.65 - 35.47 yrs) and took into account possible confounding variables.

PATIENTS AND METHODS

Patients

Since the introduction of height reductive therapy in our institute in 1968 a

total of 423 women, who were seen at adolescence for evaluation of their constitutionally tall stature, had reached the age of 18 at the time of our follow-up study. All were contacted by mail to participate in this study. Second mailings were send to those who did not respond to the first mailing.

Two-hundred forty-nine women had undergone treatment for tall stature (cases). Hundred and seventy-four women had not chosen for hormonal therapy for various reasons such as satisfaction with the given height prognosis or uncertainty about possible side-effects, and served as controls. Three hundred twenty-six women (203 cases and 123 controls) responded to our mailing. Of the responders, 272 women (177 cases and 95 controls) agreed to participate in this study. All participants gave informed consent.

We defined constitutionally tall stature as having a height equal to or above the 90th percentile (Dutch references (26)) at time of referral. Therefore 13 girls were excluded. In addition, from 12 girls no radiographs could be retrieved from the archives for re-assessment of BA and height prediction. Thus, 247 girls (159 cases and 88 controls) were included in this study.

In cases, most of the women (n=143) had been treated with 200 μg ethinyl estradiol (EE)/day orally; thirteen women had received 300 μg EE/day and 3 women 100 μg EE/day. Every 5-10 days of the months progestagens (medroxy-progesterone 5-10 mg/day, orally) had been added. Treatment started at a mean (SD) age of 12.72 (1.22) yrs with a range of 9.88 to 16.91 yrs. The mean duration of therapy was 1.91 (0.63) yrs ranging from 0.60 - 3.62 yrs. In general, treatment had been stopped when radiographs of hand and/or knee were considered to show nearly complete closure of the epiphyses. The mean (SD) length of the follow-up period after cessation of estrogen therapy lasted 10.86 (4.76) years [range: 3.26 - 22.92 yrs].

Methods

Study protocol

The study protocol has been extensively described in the previous chapter (5). In short, patients were recalled to our outpatient clinic for assessment of adult final height. Auxological data were collected from the hospital charts and radiographs of the left hand and wrist were retrieved for BA determinations and

final height predictions. Bone age was rated manually according to the methods of Gruelich and Pyle (27) and Tanner et al (3) (TW2-RUS BA). In addition, a computer aided skeletal age scoring system (CASAS) was used for determination of TW2-RUS BA (28,29).

A detailed explanation of the various final height prediction methods has been given in chapter 3. In summary, final height predictions were performed using the well-known methods of Bailey and Pinneau (*BP-prediction*)(1) and Tanner et al (*TW-prediction*, mark II) (3). In addition, the relative height (*RH-prediction*) and the index of potential height (*IPH-prediction*)(4) were calculated. For the latter this was done based on all separate BA determination methods (IPH_{GP}-, IPH_{TW}-, IPH_{CASAS}- and $IPH_{modified}$-*prediction*). With regard to the $IPH_{modified}$-prediction, a theoretical BA was used derived from the observed relationship $BA=1.46*CA - 0.023*CA^2 - 0.00005*CA^4$ (29). *RH-predictions* and *IPH-predictions* were performed according to Dutch references (25). Finally, a subjective *clinical-prediction* was given by two experienced pediatric endocrinologists separately (SDMKS, SD). For this purpose, a growth chart was constructed for each individual containing height measurements available at the time of referral, BA (estimated according to Greulich & Pyle by one investigator (MF), father's height, mother's height, target height, and pubertal stage. Target height was calculated according to the formula: (Mother's height (cm) + Father's height (cm) - 12)/2 + 3 (30). Growth charts were blindly and randomly rated for final height predictions. If separate final height predictions differed more than 2 cm among the raters, the prediction was repeated for consensus.

The study protocol was approved by the ethical committee of Academic Hospital, Erasmus University Rotterdam, The Netherlands.

Statistics

In cases, all height predictions were used to assess the effect of estrogen treatment, since predictions were based on one radiograph per subject: the one prior to start of therapy. In controls, more than one radiograph was available for accuracy assessment of the various prediction methods. Using repeated

predictions of one control subject for assessment of the accuracy of the prediction method and comparison with a treated group in which only one radiograph per subject was taken could induce bias. Therefore we used only one radiograph per control girl. The radiographs were selected such that the age distribution of treated and untreated girls were as alike as possible.

In controls, accuracy of the height prediction methods was expressed mathematically as predicted adult height minus actual adult height. Therefore, positive values indicate overestimation whereas negative values indicate underestimation of the predicted adult height. In addition, the reliability of each height prediction method was also expressed in absolute errors. The absolute errors demonstrate the method's overall predictive error and are independent of over- or underestimation. In cases, the calculation of predicted adult height minus actual final height was used to express the -uncorrected- effect of growth reductive therapy. Consequently, positive values indicate growth reduction whereas negative values indicate lack of growth reduction. Multiple regression analysis was used to assess the corrected height reducing effect of estrogen therapy (see below). For the *clinical-prediction*, the mean of the two separate predictions was used for statistical analysis.

Differences between groups were tested with the Mann-Whitney-U-test. In case of a contingency distribution chi-square tests were used. In controls, Friedman's test was used to test the difference in absolute errors of the various prediction methods. If this test was significant, Wilcoxon's matched pairs signed ranks test was used to test the difference in absolute errors between separate prediction methods. Correlations between variables were tested with Spearman's correlation test. Multiple linear regression was used to adjust the treatment effect for possible confounding variables and to study treatment effect modification by other variables. In the analysis final height was the dependent variable, while treatment, CA, BA, height prediction and menarche (0=no, 1=yes) were independent variables. Interactions of CA, BA, height prediction and menarche with the treatment were added if significant. This approach was repeated for all prediction methods separately. In controls, multiple regression analysis was used to

evaluate the additional influence of menarche on the absolute error when corrected for CA. In addition, in cases, multiple forward regression was used to evaluate the additional influence of menarche on the -uncorrected- effect when corrected for CA and to identify the influence of various variables on the additional growth after cessation of therapy. No statistics were performed on the possible influence of the differences in dosage of EE because of the low numbers. Data are expressed as mean (SD) unless indicated otherwise.

RESULTS

Clinical data

Table 1 summarizes the clinical data of all patients. At the time of the follow-up controls were slightly, though not significantly, younger compared to cases.

Table 1 Clinical data of 159 treated tall women and 88 tall controls. Data expressed as mean (SD) and range.

Clinical data	Cases (n=159)	Controls (n=88)
Age (yrs)	25.59 (4.83) 18.69 - 35.47	24.73 (4.03) 18.65 - 34.21
height father (cm)	187.4 (7.0) (n=153) 170.0 - 205.0	183.9 (6.8) (n=86)# 170.0 - 204.0
height mother (cm)	174.7 (5.5) (n=153) 160.0 - 187.0	172.0 (4.7) (n=87)# 161.0 - 182.0
target height (cm)	178.0 (4.8) (n=153) 166.0 - 189.5	175.0 (4.4) (n=86)# 165.5 - 187.8
final adult height (cm)	181.7 (3.2) 174.0 - 190.5	180.5 (3.8)* 171.1 - 188.3
height at start of therapy (cm)	174.6 (4.7) 155.8 - 186.5	-
height at end of therapy (cm)	179.0 (3.3) 170.6 - 187.6	-
post-treatment growth (cm)	2.7 (1.1) 0.1 - 6.2	-

* Significant difference between cases and controls (P=0.02)
\# Significant difference between cases and controls (P<0.001)

Despite treatment, final adult height of the cases was significantly taller than controls (P=0.02). In addition, the heights of the cases' parents and thus their target heights were significantly taller (P<0.001). The bone age determinations and the final height predictions according to the various methods are given in Tables 2a and 2b. The age at time of the prediction was not significantly different between the groups, indicating that matching of the controls had appropriately been done. In contrast, the control group was significantly shorter at time of the referral, had higher estimated bone ages, and smaller adult height predictions (P<0.01). At the time of the prediction, 30 out of 159 cases and 31 out of 88 controls had already reached menarche, the proportions being significantly different (P=0.007). The age of the premenarcheal girls was significantly younger compared to the post-menarcheal girls in both cases and controls (P<0.01).

Table 2a. Bone age estimated by different methods in treated tall women and tall controls. Data expressed as mean (SD) and range.

Clinical data	Cases (n=159)	Controls (n=88)
Age at time of prediction (yrs)	12.72 (1.22) 9.88 - 16.91	12.75 (1.45) 8.99 - 16.49
Height at time of prediction (cm)	174.6 (4.7) 155.8 - 186.5	171.9 (7.3)# 146.5 - 184.5
Height SDS at time of prediction	2.39 (0.57) 1.33 - 4.05	2.07 (0.51)# 1.30 - 4.13
BA - G&P (yrs)	12.4 (1.0) 10.0 - 15.0	12.9 (1.4)# 9.0 - 16.0
BA - TW2-RUS (yrs)	13.3 (1.1) 10.2 - 16.0	13.8 (1.3)# 9.8 - 16.0
BA - CASAS (yrs)	13.4 (0.8) 11.2 - 15.6	13.7 (1.1)# 9.6 - 15.5

Significant difference between cases and controls (P<0.01).

Table 2b. Adult height prediction (cm) according to various methods in treated tall women and tall controls. Data expressed as mean (SD) and range.

Prediction method	Cases (n=159)	Controls (n=88)
BP	185.9 (3.9) 177.9 - 200.5	181.0 (3.9)# 172.1 - 189.4
TW	183.1 (3.5) 175.0 - 193.5	179.7 (3.6)# 170.6 - 188.3
CASAS	182.7 (3.2) 176.1 - 191.8	179.8 (3.9)# 170.9 - 189.1
Clinical	183.9 (3.0) 178.0 - 194.5	180.8 (2.7)# 174.0 - 189.0
RH	183.1 (3.5) 176.5 - 193.4	181.1 (3.1)# 176.4 - 193.9
IPH_{GP}	183.9 (4.3) 176.1 - 202.1	180.3 (3.6)# 169.9 - 188.4
IPH_{TW}	180.8 (3.7) 172.4 - 194.6	177.2 (4.2)# 165.0 - 186.8
IPH_{CASAS}	180.0 (3.5) 171.2 - 189.2	177.2 (4.9)# 161.7 - 188.6
$IPH_{modified}$	179.9 (3.3) 170.5 - 189.4	177.7 (4.5)# 167.4 - 188.0
Final adult height	181.7 (3.2) 174.0 - 190.5	180.5 (3.8)* 171.1 - 188.3

* Significant difference between cases and controls (P=0.02)
\# Significant difference between cases and controls (P<0.001)

Accuracy of prediction

The accuracy of the various final height predictions as determined in the control group and in relation to CA is summarized in Tables 3a and 3b. The mean errors of the single prediction methods varied from -3.3 (3.9) to 0.6 (3.9) cm. Six of the nine methods applied had a systematic error within 1 cm of the adult final height. On average, the *BP-, clinical-* and *RH-prediction* systematically over-

Table 3a. Error of prediction (cm) of various prediction methods by chronological age in 88 controls. Data expressed as mean (SD) and [range].

		Error of prediction (n=88)								
Age (yrs)	N	BP	Clinical	TW	CASAS	RH	IPH_{OP}	IPH_{TW}	IPH_{CASAS}	$IPH_{modified}$
≤ 11	16	-0.1 (4.2)	0.9 (3.9)	-1.7 (4.7)	-2.5 (4.6)	3.0 (7.1)	1.0 (4.8)	-6.6 (4.4)	-7.8 (5.0)	-7.5 (5.4)
12	21	1.4 (2.5)	0.5 (1.7)	-1.2 (2.5)	-1.0 (2.2)	1.1 (2.5)	-1.0 (2.6)	-4.6 (2.4)	-4.4 (2.2)	-3.8 (2.6)
13	26	0.5 (2.2)	-0.1 (2.2)	-0.3 (1.9)	-0.1 (2.6)	-0.2 (2.7)	-0.7 (1.9)	-2.3 (1.9)	-2.0 (2.9)	-1.8 (2.7)
14	13	0.5 (2.5)	0.2 (2.9)	-0.5 (3.4)	-0.4 (3.5)	-0.6 (2.8)	0.1 (2.7)	-1.3 (2.4)	-1.3 (2.4)	-0.7 (2.8)
≥ 15	12	-0.2 (1.5)	-0.2 (1.2)	-0.2 (3.0)	0.4 (2.4)	-0.4 (1.6)	-0.1 (1.4)	-0.7 (1.4)	-0.4 (1.4)	0.8 (1.5)
preM	57	0.5 (3.1)	-0.1 (2.7)	-1.3 (3.4)	-1.3 (3.5)	0.5 (4.7)	-0.6 (3.4)	-4.5 (3.3)	-4.6 (4.1)	-4.6 (4.3)
postM	31	0.5 (1.7)	0.9 (1.8)	0.9 (1.8)	0.2 (2.1)	0.9 (1.9)	0.3 (1.7)	-1.0 (1.7)	-0.8 (1.6)	0.5 (1.8)
All	88	0.5 (2.7) [-6.8;7.6]	0.3 (2.5) [-6.0;7.9]	-0.8 (3.1) [-10.1;7.7]	-0.7 (3.2) [-13.3;9.6]	0.6 (3.9) [-9.0;15.9]	-0.3 (2.9) [-7.6;9.7]	-3.3 (3.3) [-15.5;4.5]	-3.3 (3.9) [-18.0;7.9]	-2.8 (4.4) [-19.6;6.4]

Table 3b. Absolute error (cm) of various prediction methods by chronological age in 88 controls. Data expressed as mean (SD) and [range].

		Absolute error (n=88)								
Age (yrs)	N	BP	Clinical	TW	CASAS	RH	IPH_{OP}	IPH_{TW}	IPH_{CASAS}	$IPH_{modified}$
≤ 11	16	3.3 (2.5)	3.1 (2.3)	4.1 (2.9)	4.0 (3.2)	6.2 (4.4)	3.7 (3.2)	6.9 (3.8)	7.9 (4.8)	8.1 (5.6)
12	21	2.1 (1.9)	1.4 (1.0)	2.2 (1.7)	1.9 (1.5)	2.2 (1.5)	2.4 (1.4)	4.7 (2.3)	4.4 (2.2)	3.8 (2.6)
13	26	1.8 (1.4)	1.8 (1.3)	1.3 (1.4)	1.8 (1.8)	2.3 (1.4)	1.4 (1.4)	2.4 (1.7)	2.7 (2.2)	2.4 (2.2)
14	13	1.6 (2.0)	2.0 (2.1)	2.3 (2.4)	2.1 (2.7)	2.0 (2.0)	1.8 (2.0)	2.3 (1.4)	2.2 (1.6)	2.1 (2.0)
≥ 15	12	1.2 (0.7)	1.0 (0.7)	2.0 (2.2)	1.7 (1.6)	1.2 (1.0)	1.2 (0.7)	1.3 (0.9)	1.1 (0.9)	1.4 (0.9)
preM	57	2.4 (2.0)	2.1 (1.7)	2.7 (2.4)	2.7 (2.5)	3.5 (3.1)	2.6 (2.2)	4.7 (3.1)	5.0 (3.6)	4.9 (4.0)
postM	31	1.3 (1.2)	1.4 (1.4)	1.5 (1.5)	1.4 (1.6)	1.5 (1.4)	1.2 (1.3)	1.6 (1.1)	1.4 (1.2)	1.3 (1.3)
All	88	2.0 (1.9) [0.0;7.6]	1.9 (1.7) [0.0;7.9]	2.3 (2.2) [0.0;10.1]	2.3 (2.3) [0.0;13.3]	2.8 (2.8) [0.1;15.9]	2.1 (2.0) [0.0;9.7]	3.6 (3.0) [0.1;15.5]	3.7 (3.5) [0.1;18.0]	3.6 (3.8) [0.1;19.6]

preM = premenarcheal; postM = postmenarcheal

Table 4. Effect of treatment (cm) according to various prediction methods by chronological age in 159 treated tall women. Data expressed as mean (SD) and [range].

		Effect of treatment (n=159)								
Age (yrs)	N	BP	Clinical	TW	CASAS	RH	IPH_{GP}	IPH_{TW}	IPH_{CASAS}	$IPH_{modified}$
≤ 11	21	5.9 (3.0)	4.5 (2.7)	4.2 (2.2)	2.2 (2.7)	6.2 (3.1)	5.2 (4.3)	0.5 (3.2)	-2.9 (3.4)	-3.7 (3.3)
12	52	5.4 (2.8)	2.9 (2.6)	2.5 (2.9)	1.7 (2.2)	2.9 (3.0)	2.8 (4.1)	-1.1 (4.1)	-2.3 (2.2)	-2.3 (2.7)
13	48	3.6 (2.8)	1.7 (1.9)	1.6 (2.6)	1.2 (2.6)	0.0 (2.0)	1.4 (2.7)	-1.0 (2.0)	-1.2 (2.0)	-1.5 (1.9)
14	23	2.6 (1.7)	0.8 (1.4)	-1.7 (2.6)	-1.2 (2.3)	-1.2 (1.7)	1.0 (1.2)	-1.3 (1.5)	-1.0 (1.6)	-1.4 (1.6)
≥ 15	15	1.7 (2.2)	0.6 (1.0)	-1.9 (3.7)	-0.4 (3.2)	-2.1 (1.4)	0.9 (1.4)	-1.1 (1.4)	-0.3 (1.7)	-0.9 (1.5)
preM	129	4.5 (2.9)	2.4 (2.6)	1.7 (3.6)	1.1 (2.9)	1.4 (3.7)	2.4 (3.8)	-0.8 (3.2)	-1.9 (2.5)	-2.4 (2.5)
postM	30	3.0 (2.8)	1.5 (1.6)	0.2 (1.7)	0.4 (1.8)	1.4 (2.6)	1.4 (2.0)	-1.3 (1.3)	-0.8 (1.5)	-0.3 (1.5)
All	159	4.2 (3.0) [-1.2;12.3]	2.2 (2.5) [-2.5;11.8]	1.4 (3.3) [-7.6;12.6]	1.0 (2.7) [-6.8;8.1]	1.4 (3.5) [-4.5;11.9]	2.2 (3.5) [-4.1;14.8]	-0.9 (3.0) [-8.1;15.9]	-1.7 (2.4) [-8.9;5.3]	-2.0 (2.5) [-8.7;3.0]

preM = premenarcheal; postM = postmenarcheal

Table 5. Estimated effect of the estrogen treatment on final height in 247 girls with constitutionally tall stature for nine prediction methods, adjusted for chronological age, bone age (BA), menarche and height prediction.

Prediction method*	Effect of treatment	Effect > 0		P-value[1]	Mean (SD) effect[2]; range
BP	20.22 - 1.44 x BA_{GP}	BA_{GP}	< 14.04	<0.0001	2.4 (1.4); -1.4 to 5.8
Clinical	15.71 - 1.11 x BA_{GP}	BA_{GP}	< 14.15	<0.0001	1.9 (1.1); -0.9 to 4.6
TW	21.59 - 1.51 x BA_{TW}	BA_{TW}	< 14.30	<0.0001	1.5 (1.7); -2.6 to 6.2
CASAS	18.76 - 1.32 x BA_{CASAS}	BA_{CASAS}	< 14.21	0.0003	1.1 (1.0); -1.8 to 4.0
RH	18.46 - 1.27 x BA_{CASAS}	BA_{CASAS}	< 14.53	0.0003	1.4 (1.0); -1.4 to 4.2
IPH_{GP}	20.39 - 1.53 x BA_{GP}	BA_{GP}	< 13.33	<0.0001	1.4 (1.5); -2.6 to 5.1
IPH_{TW}	24.22 - 1.68 x BA_{TW}	BA_{TW}	< 14.42	<0.0001	-0.6 (2.1); -5.5 to 5.2
IPH_{CASAS}	9.88 - 0.70 x CA	CA	< 14.11	0.0046	1.0 (0.9); -2.0 to 3.0
$IPH_{modified}$	48.88 - 0.27 x prediction	prediction	< 181.0	0.0027	0.4 (0.9); -2.3 to 2.9

* For explanation see *Methods*

[1] P-value for treatment effect modification by BA

[2] Mean of the individual treatment effects as calculated with the corrresponding formulas.

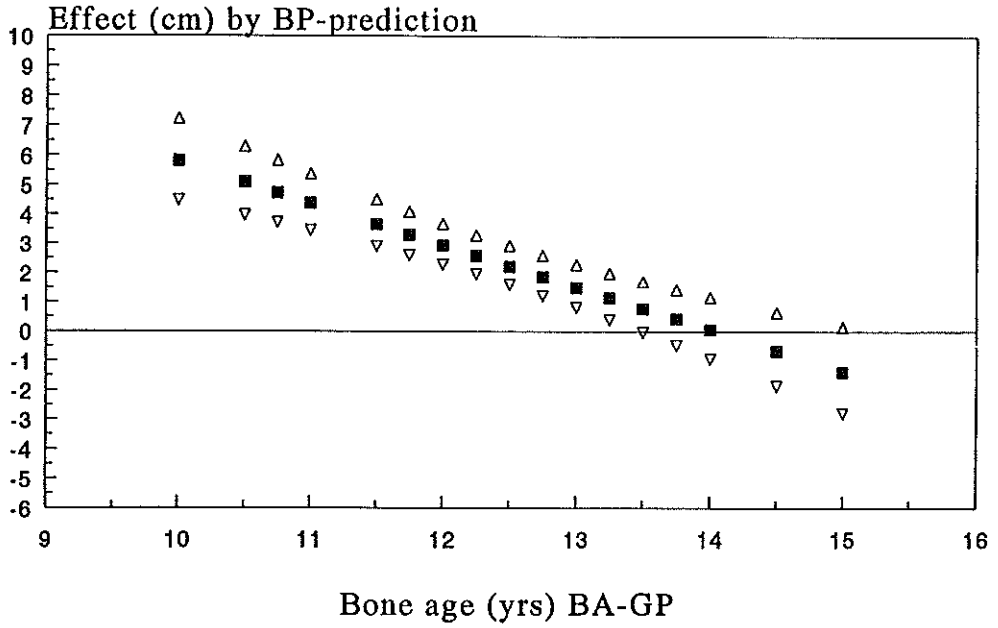
estimated adult height, whereas the other prediction methods systematically underestimated final height. Throughout all age groups, we found a large variability within each single prediction method in its tendency to over- or underestimate adult height.

The mean absolute errors varied from 1.9 (1.7) to 3.7 (3.5) cm and were significantly different (Friedman: P< 0.0001). It appeared that the *BP-, TW-, CASAS-, clinical-,* and *IPH_{GP}-prediction* were most reliable in predicting final height: their mean absolute errors were in the same order of magnitude (1.9 to 2.3 cm) and did not significantly differ from each other (P-values>0.05). Extrapolation of the height SDS for BA as estimated by Tanner-Whitehouse, CASAS or modification (*IPH_{TW}-*, *IPH_{CASAS}-*, and *$IPH_{modified}$-prediction*) showed the greatest inaccurary in predicting adult height: their mean absolute errors were mutually comparable, but were significantly larger than all other methods (P-values<0.05). The RH-prediction had an intermediate position: its mean absolute error was significantly larger than the *BP-, IHP_{GP}-*, and *clinical prediction* (P-values<0.05), significantly smaller compared to the *IPH_{TW}-*, *IPH_{CASAS}-*, and *$IPH_{modified}$-prediction* (P-values <0.05), but comparable to the *TW-*, and *CASAS-prediction* (P-values>0.15). In all methods applied, the mean absolute errors were negatively correlated with CA (r:-0.25 to -0.65; P-values<0.02). The mean absolute errors were significantly higher in premenarcheal girls compared to postmenarcheal girls for all methods (P-values<0.05), except for the *clinical-prediction* (P=0.06). These differences disappeared after correction for CA, except for the *IPH_{TW}-*, and the *$IPH_{modified}$-prediction* (regression coefficients (SE): -1.5 (0.7) cm, P=0.02 and -2.0 (0.9) cm, P=0.03, respectively).

Effect of estrogen therapy

The -uncorrected- effect of height reductive therapy in tall girls as determined by the various methods and in relation to CA is given in Table 4. The mean effect as calculated by the different methods showed large variability with the *BP-prediction* giving the greatest effect (4.2 (3.0) cm). The five most reliable prediction methods (*BP-, TW-, CASAS-, clinical-,* and *IPH_{GP}-prediction*) showed a greater height reducing effect in premenarcheal girls compared to postmenarcheal

Figure 1. Adjusted effect of estrogen therapy and its 95% confidence interval by bone age (BA_{GP}) in constitutionally tall girls.



Effect (cm) = 20.22 - 1.44 x BA_{GP} (yrs)

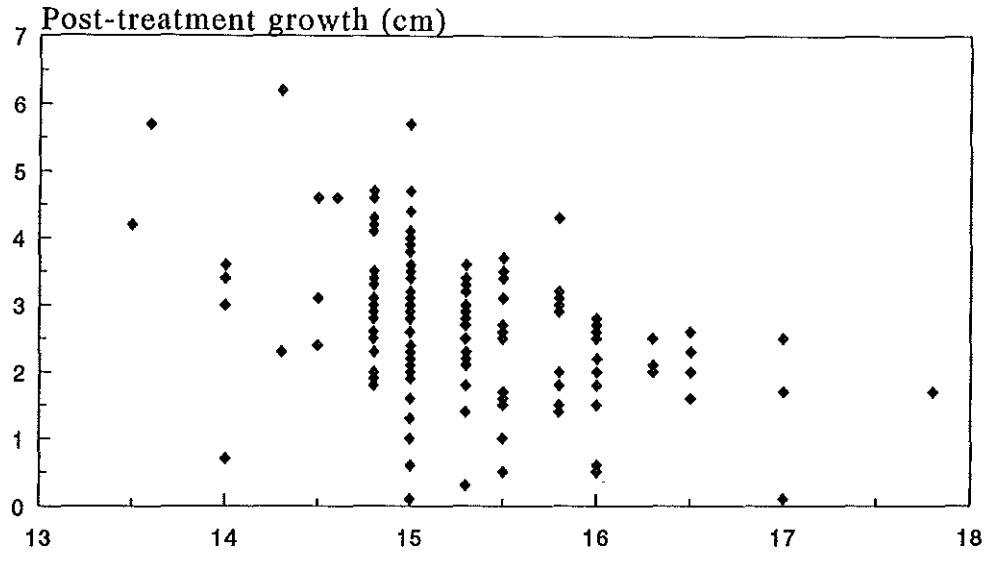
girls, which reached statistical significance only with the *BP-*, and the *TW-prediction* (P-values<0.02); these differences, however, dissappeared after correction for CA. The *IPH_{CASAS}-* and *$IPH_{modified}$-prediction* also calculated a significant difference in height reduction between pre- and postmenarcheal girls resulting in a less negative effect in postmenarcheal girls (P<0.01); this difference remained significant after adjustment for CA only for the *$IPH_{modified}$-prediction* (regression coefficient (SE):1.7 (0.5) cm, P=0.0003). This latter figure is more difficult to interpret, given the differences in mean errors in prediction between pre- and postmenarcheal girls, but indicate a more pronounced effect in premenarcheal girls. The remaining methods showed no significant difference in effect between pre- and postmenarcheal girls (P>0.05).

The above described multiple linear regression analysis was performed to adjust the effect of estrogen treatment for possible confounding and to search for possible treatment effect modification. This approach was repeated for each single prediction method. For all methods there appeared to be a significant interaction between treatment and either BA, CA or height prediction at the time of the start of estrogen therapy (Table 5). For the *BP-, TW-, CASAS-, clinical-,* and *IPH_{GP}-prediction*, which were found to be the most reliable prediction methods in tall girls, the adjusted effect was dependent on the BA at start of therapy. The ultimate adjusted height reducing effect of estrogen therapy according to the *BP-prediction* and its 95% confidence interval are plotted in Figure 1. The mean adjusted effect, obtained after applying the appropriate BA into the equations summarized in Table 5, varied from 1.1 (1.0) to 2.4 (1.4) cm and ranged from -2.6 to 6.2 cm.

Post-treatment growth

In cases, the height at the end of therapy was 179.0 (3.3) cm at a mean age of 14.73 (1.05) years, whereas adult height was 181.7 (3.2) cm, indicating an additional growth of 2.7 (1.1) cm after cessation of therapy with a range from 0.1 to 6.2 cm. Post-treatment growth was negatively correlated with the age at start of therapy (r=-0.28; $P<0.001$) and the age at stop of therapy (r= -0.35; $P<0.001$) but not with the duration of therapy (r=-0.09; P=0.29). BA_{GP} at time of stop of treatment was available in 151 out of 159 patients and was estimated 15.2 (0.6) yrs ranging from 13.5 to 17.8 yrs. We found a significant relationship between the additional growth after cessation of therapy and the BA at time of stop of therapy (r=-0.43, $P<0.001$) (Figure 2). Multiple forward regression analysis, using the post-treatment growth as the dependent variable and CA and BA at time of stop of treatment, menarche and the duration of treatment as predictor variables, revealed, that both BA and CA at time of cessation of treatment had a significant influence on the post-treatment growth (regression coefficients (SE): -0.62 (0.13), $P<0.0001$ and -0.20 (0.08), P=0.016, respectively), providing the following equation: Post-treatment growth (cm)= 15.15 - 0.62 BA_{GP}-stop (yrs) - 0.20 CA-stop (yrs) (R^2: 0.21, RSD: 0.98).

Figure 2. Post-treatment growth by bone age (BA_{GP}) at time of cessation of estrogen therapy in 151 tall girls (r=-0.43, P<0.001).



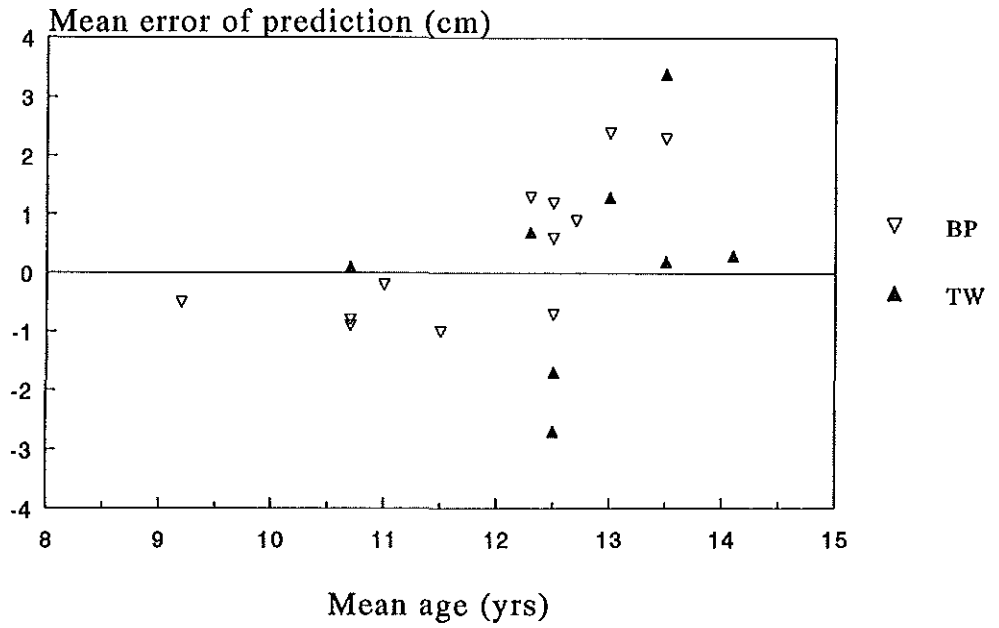
DISCUSSION

Accuracy of prediction

Our study shows that the accuracy of various methods to predict adult height in constitutionally tall girls is clinically acceptable. Five of the nine methods tested showed a mean absolute error ≤ 2.3 cm and a mean systematic error within 1 cm of the adult height. With increasing age all methods became more accurate in predicting adult height. This was illustrated by the significant negative correlations between the absolute errors and CA. The *BP-*, *TW-*, *CASAS-*IPH_{GP}- and *clinical-prediction* appeared to be the most reliable methods and were mutual comparable. The remaining models, i.e. the *RH-*, IPH_{TW}-, IPH_{CASAS}-, and $IPH_{modified}$-*prediction*, were of minor importance in predicting adult height in constitutionally tall girls. It is noteworthy, that experienced clinicians were capable of giving a height prediction

by evaluating the growth chart of the tall girl and taking into account clinical parameters such as age, bone age (G&P), target height and pubertal stage, which appeared as accurate as a population based, mathematically oriented height prediction methods.

Figure 3. Mean errors of *BP-*, and *TW-prediction* by mean age in untreated constitutionally tall girls as derived from the literature (references 6-16).



Reviewing the literature (6-16), data on the systematic tendency of the various prediction methods to over- or underestimate final adult height are not consistent (for summary see Figure 3). Variation in initial clinical data (CA and BA) and definition of adult height may account for this inconsistency. Some authors described an overall overestimation of adult height using the *BP-prediction* (7,10,11,13,15,16), whereas others reported an underestimation (6,9,12).

Nevertheless, the mean errors were rather accurate ranging from a mean underprediction of 0.9 cm to a mean overprediction of 2.4 cm. When the age of the various study populations was taken into account, it seemed that the *BP-prediction* underestimates adult height in tall girls at younger ages, whereas from the age of 11 a slight overestimation was present, which was confirmed by our study. Concerning the *TW-prediction*, most workers have used the older prediction method of Tanner et al (3) and described an overall overestimation of adult height ranging from 0.1 to 3.4 cm, which varied with the age of the study population (8-11). Only Willig et al (6) reported a mean underestimation of -1.7 to -2.7 cm Joss and co-workers (16), using the revised TW2 prediction method (4), calculated a mean overprediction of 1.3 cm. In contrast, we found a mean underestimation of -0.8 cm, which was more pronounced at younger ages. Knowledge of the systematic errors of the single prediction methods is of clinical importance since it may increase the accuracy of a height prediction by correcting it for its prediction error at a given CA, as also suggested by Joss et al (7). However, considerable individual variation will still be present as implied by the large standard deviations of the mean errors of the prediction method used. This can be illustrated by an example of a 12 year old girl using the *BP-prediction* in which the method, according to our study, revealed a mean overprediction of 1.4 cm, but which may vary in individual cases from an underprediction of -3.6 cm (-2SD) to an overprediction up to 6.5 cm (+ 2SD).

Absolute errors are independent of a method's tendency for under- or over-estimation and are more sensitive to deviations from the prediction. Therefore, it is more indicative to calculate the absolute error of a prediction model in a control population. Unfortunately, to our knowledge, absolute errors on the prediction methods applied have never been described in tall girls. Our study showed that the mean absolute errors of the *BP-, TW,- CASAS-, IPH*$_{GP}$- and *clinical-prediction* were clinical acceptable (≤ 2.3 cm). However, large errors in prediction are still possible in individual cases as the standard deviations were quite large. In addition, at ages younger than 11 years absolute errors were considerable indicating less reliability.

In our hands, height prognosis was more reliable in tall girls compared to tall

boys (5). This is illustrated by our findings that the mean errors were more close to zero and the mean absolute errors were smaller in girls than in boys. Differences in timing and intensity of the adolescent growth spurt between boys and girls (31,32) may be a possible cause for this finding; in our study height prediction was performed at about the expected age of the peak height velocity (PHV) in the untreated tall boys, whereas in the untreated girls a height prognosis was calculated after the expected age of PHV. For both boys and girls, the *IPH_{GP}-prediction* gave a satisfying height prognosis; this method can easily be implemented in clinical practice. In addition, experienced clinicians may also rely on their own judgement as the *clinical-prediction* showed acceptable reliability in both boys and girls.

Effect of estrogen therapy

The present study showed that the effect of estrogen treatment in tall girls varied with the prediction method applied (Table 4). One may "correct" the mean calculated effect of therapy by substraction of the systematic prediction errors, which has been commonly done in the literature. In this way, all prediction methods calculated a positive mean effect, suggesting that, overall, treatment was effective. The five most reliable methods (i.e. the *BP-, TW,- CASAS-, IPH_{GP}-* and *clinical-prediction*) showed a mean "corrected" height reduction varying from 1.2 to 3.7 cm. These findings support previously published data in which the mean reported height reduction ("corrected" and uncorrected) ranged from 2.1 cm to 10.0 cm (6,8-25). A clear comparison, however, is hampered by differences in initial clinical data (especially CA and BA), duration of treatment, therapeutic regimen (different doses and estrogen preparations), and the point in time of adult height assessment. For example, in our study a mean additional growth of 2.7 (1.1) cm was observed after cessation of therapy, which is in the same order of magnitude as reported by others (13,18,21). It implies that at the time therapy was stopped the mean "uncorrected" height reduction amounted to 6.4 cm using the *BP-prediction*, stressing the importance of defining adult height. The cause of the observed additional growth is not quite clear. In our study, the BA_{GP} at time of cessation of

estrogen therapy was estimated on average at about 15 years, indicating that approximately 99.3% of the adult final height had been achieved (1). It explains therefore only part of the post-treatment growth. Since sex steroids in tall girls reduce spinal growth to a much lesser extent than long bone growth (32,33), it is plausible that part of the remaining post-treatment growth at a mean age of 14.73 (1.05) years reflects additional spinal growth.

As discussed previously in chapter 3, evaluating the effect of estrogen treatment after "correction" for the mean errors of the separate prediction methods will induce bias. We therefore chose for a more direct and conceptually simple approach using multiple linear regression. In this way, analyses revealed a significant treatment effect with a size depending on either BA, CA or the height prediction (Table 5). In the five most reliable prediction methods (i.e. the *BP-, TW,- CASAS-, IPH_{GP}-* and *clinical-prediction*) the treatment effect was related to the corresponding BA, resulting in a mean height reduction varying from 1.1 to 2.4 cm and ranging from -2.6 to 6.2 cm. These mean results are less than claimed in the literature (6,8-25). However, this is probably due to differences in statistical approach and differences in study design, as explained above. Our study clearly indicated that the effect of treatment was more pronounced when treatment had been started at a younger BA (see Figure 1), which is in concert with previous studies (10,14,19,21,24,25), although others could not find such relationship (8). Inconsistency is present whether or not premenarcheal girls may experience more height reduction compared to postmenarcheal girls. Some reports are in favour with this finding (16,20-22), while others observed no difference (8,13,23,24). In the present study, premenarcheal girls seemed to benefit more from therapy than postmenarcheal girls. However, this was likely to be due to the differences in CA between these groups since we found no additional effect of menarche over CA explaining the variability in the -uncorrected- effect of treatment. This may, at least partly, explain the contradictive results.

In our experience, tall girls benefited more from sex steroid therapy than tall boys (5). In fact in boys, even a significant growth induction was observed at an older BA (5). It is possible that this was due to the timing of the start of therapy in

the adolescent growth phase. Our treated tall girls had started therapy at a mean age of 12.7 yrs, which is about 1.2 to 0.7 yrs after the age of PHV. In contrast, our treated tall boys started therapy at a mean age that was only 0.5 to 0.2 yrs after the age of PHV (31,32). Another explanation might be differences in mechanism of action by which estrogens and androgens limit linear growth. The mechanisms by which sex steroids act, however, are not fully understood. Part of the growth reduction may be mediated via the GH/IGF-axis (35,36). Indeed, in tall girls a dose dependent reduction of IGF-I plasma levels had been observed during estrogen treatment (15,20), although this decrease was not significant using a dosage of 250 µg EE/day (15). Therefore, the decrease in growth velocity observed after start of treatment with doses ≤ 200 µg EE (24,25,37) is hard to relate to changes in IGF-I concentration, but suggests that other factors are involved. Another important factor playing a role in the height reducing process is the influence of sex hormones on bone maturation. In fact, the observation that premature secretion of gonadal steroids in precocious puberty cause acceleration of bone maturation which leads to short adult stature due to early fusion of the epiphyseal growth plates (38-40), is one of the rationales for sex hormone therapy in tall stature. Indeed, in the management of tall stature sex steroid treatment resulted in an advanced bone maturation. The regulation of bone growth is very complex and is beyond the scope of this article. It is generally agreed, however, that the steroid hormone effects on bone maturation are due to an indirect action mediated by the GH/IGF-I axis combined with a direct effect at tissue level (35,41,42). In addition, several studies point to the possibility that there are sex-specific and age-dependent responses to sex steroids present in cartilage cells *in vitro* (43-45). Moreover, it is possible that in males androgens may partly act on cartilage after their metabolic conversion into estrogens (35,43). A recent report of a 28-year old adult man with absence of functional estrogen receptors together with unfused epiphyseal growth plates (46) confirms the need of estrogens for bone maturation in the male. All these aspects may underlie the differences in therapy response between boys and girls, although the precise mechanism is far from clear.

High dose estrogen therapy may cause minor side effects, which are likely

to be dose related (47,48). Occasionally a serious complication as thrombosis has been reported. Up till now there is no evidence that there are long-term side effects on reproductive function (48). However, concerning a possible association between estrogen therapy and malignancy based on results in oral contraceptive studies (49), definite conclusions cannot be drawn at this point in time. It is important therefore to balance all pros and cons in dialogue with the tall girl and her parents in order to come to a careful decision regarding possible therapeutic intervention.

In summary, these data demonstrate that the accuracy of various methods to predict adult height in constitutionally tall girls is clinically acceptable. With increasing age, height prognosis became more accurate. A significant height reductive effect of high doses of estrogens was found in tall girls. This effect was mainly dependent on the BA at the start of therapy: the effect was more pronounced when treatment had been started at a younger BA. Serious additonal growth was observed after cessation of therapy, which could be explained only partly by the BA_{GP} at that time; additional spinal growth may have also accounted for part of the post-treatment growth.

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REFERENCES

1. Bailey N, Pinneau SR: Tables for predicting adult height from skeletal age, revised for Greulich and Pyle hand standards. J Pediatr 1952;40:423-441
2. Tanner JM, Whitehouse RH, Marshall WA, Healy MJR, Goldstein H: Assessment of skeletal maturity and prediction of adult height: TW2 method. Academic Press, New York 1975
3. Tanner JM, Whitehouse RH, Cameron N, Marshall WA, Healy MJR, Goldstein H: Assessment of skeletal maturity and prediction of adult height (TW2 method). Academic Press, London 1983
4. Lenko HL: Prediction of adult height with various methods in Finnish children. Acta Paediatr Scand 1979;68:85-92
5. De Waal WJ, Fokker MH, van Gurp EAFJ, Stijnen Th, de Muinck Keizer-Schrama SMPF, Aarsen RSR, Drop SLS: Accuracy of final height prediction and effect of growth reductive therapy in 115 constitutionally tall boys. A comparison of nine prediction methods. Chapter 3, thesis 1996.
6. Willig RP, Christiansen D, Kuhn N, Schaefer E, Stahnke N: Voraussetzungen und Ergebnisse der Östrogenbehandlung extrem großer Mädchen. Monatsschr Kinderheilkd 1980;128:787-788
7. Joss EE, Temperli R, Mullis PE: Adult height in constitutionally tall stature: accuracy of five different height prediction methods. Arch Dis Child 1992;67:1357-1362
8. Zachmann M, Ferrandez A, Mürset G, Prader A: Estrogen treatment of excessively tall girls. Helv Paediat Acta 1975;30:11-30
9. Reeser HM, Heremans GFP, van Gelderen HH: Reduction of adult height in tall girls. Eur J Pediatr 1979;132:37-41
10. John G, Schellong G: Östrogentherapie hochwüchsiger Mädchen. Monatsschr Kinderheilkd 1980;128:545-550
11. Sorgo W, Scholler K, Heinze F, Heinze E, Teller WM: Critical analysis of height reduction in oestrogen-treated tall girls. Eur J Pediatr 1984;142:206-265
12. Schambach H, Nitschke U: Die Behandlung des konstitutionellen Hochwuchses bei Mädchen mit physiologischen Östrogendosen in der Präpubertät. Monatsschr Kinderheilkd 1985;133:32-37
13. Bartsch O, Weschke B, Weber B: Oestrogen treatment of constitutionally tall girls with 0.1 mg/day ethinyl oestradiol. Eur J Pediatr 1988;147:59-63
14. Ignatius A, Lenko HL, Perheentupa J: Oestrogen treatment of tall girls: effect decreases with age. Acta Paediatr Scand 1991;80:712-717
15. Svan H, Ritzén EM, Hall K, Johansson L: Oestrogen treatment of tall girls: dose dependency of effects on subsequent growth and IGF-I levels in blood. Acta Paediatr Scand 1991;80:328-332
16. Joss EE, Zeuner J, Zurbrügg RP, Mullis PE: Impact of different doses of ethinyl oestradiol on reduction of final height in constitutionally tall girls. Eur J Pediatr 1994;153:797-801
17. Colle ML, Alperin H, Greenblatt RB: The tall girl. Prediction of mature height and management. Arch Dis Child 1977;52:118-120
18. Andersen H, Jacobsen BB, Kastrup KW, Krabbe S, Peitersen B, Petersen KE, Thamdrup E, Wichmann R: Treatment of girls with excessive height prediction. Acta Paediatr Scand 1980;69:293-297

19. Wettenhall HNB, Cahill C, Roche AF: Tall girls: A survey of 15 years of management and treatment. J Pediatr 1975;86:602-610
20. Von Puttkamer K, Bierich JR, Brugger F, Hirche W, Schönberg D: Östrogentherapie bei Mädchen mit konstitutionellem Hochwuchs. Dtsch Med Wochenschrft 1977;102:983-988
21. Kuhn N, Blunck W, Stahnke N, Wiebel J, Willig RP: Estrogen treatment in tall girls. Acta Paediatr Scand 1977;66:161-167
22. Bierich JR: Estrogen treatment of girls with constitutional tall stature. Pediatrics 1978;62:1196-1201
23. Stöver B, Kollmann F: Ergebnisse der Behandlung hochwüchsiger Mädchen mit conjugierten Östrogenen. Monatsschr Kindrheilkd 1982;130:36-40
24. Grüters A, Heidemann P, Schlüter H, Stubbe P, Weber B, helge H: Effect of different oestrogen doses on final height reduction in girls with constitutional tall stature. Eur J Ped 1989;149:11-13
25. Normann EK, Trygstad O, Larsen S, Dahl-Jorgensen K: Height reduction in 539 tall girls treated with three different dosages of ethinyloestradiol. Arch Dis Child 1991;66:1271-1278
26. Roede MJ, van Wieringen JC: Growth diagrams 1980. Netherlands third nation-wide biometric survey. Tijdschr Soc Gezondheidszorg 1985;63(Suppl):1-34
27. Greulich WW & Pyle SI: Radiographic atlas of skeletal development of the hand and wrist. Stanford University Press, Stanford 1959
28. Tanner JM, Gibbons RD: Automatic bone age measurement using computerized image analysis. J Pediatr Endocrinol 1994;7:141-145
29. Van Teunenbroek A, de Waal WJ, Roks A, Chinafo P, Fokker MH, Mulder P, de Muinck Keizer-Schrama SMPF, Drop SLS: Computer aided skeletal age scores (CASAS) in healthy children, girls with Turner syndrome and in children with constitutionally tall stature. Ped Res 1996; in press
30. Van den Brande JVL, Degenhart HJ: Endocrinologie en groeistoornissen. In: Kindergeneeskunde, Van de Brande, Monnens (eds). Wetenschappelijke uitgeverij Bunge, Utrecht 1993
31. Preece MA: Prediction of adult height:methods and problems. Acta Paediatr Scand 1988;347(Suppl):4-11
32. Buckler J: A longitudinal study of adolescent growth. Springer-Verlag, London 1990
33. Hermanussen M, Geiger-Benoit K, Burmeister J: Differential effects of oestrogen treatment on the proportionality of growth in tall girls. Eur J Pediatr 1989;149:14-17
34. Brinkers JM, Lamoré PJ, Gevers EF, Boersma M, Wit JM: The effect of oestrogen treatment on body proportions in constitutionally tall girls. Eur J Pediatr 1994:153:237-240
35. Bourguignon JP: Linear growth as a function of age at onset of puberty and sex steroid dosage: Therapeutic implications. Endocr Rev 1988;9:467-488
36. Kerrigan JR, Rogol AD: The impact of gonadal sterold action on growth hormone secretion during childhood and adolescence. Endocr Rev 1992;13: 281-298
37. Van der Werff ten Bosch JJ, Bot A: Growth of tall girls without and during oestrogen treatment. Neth J Med 1981;24:52-57
38. Schoevaart CE, Drop SLS, Otten BJ, Slijper FME, Degenhart HJ: Growth analysis up to final height and psychosocial adjustment of treated and untreated patients with precocious puberty. Horm Res 1990;34:197-203

39. Kletter GB, Kelch RP: Effects of gonadotropin-releasing hormone analog therapy on adult stature in precocious puberty. J Clin Endocrinol Metab 1994;79:331-334
40. Brauner R, Adan L, Malandry F, Zantleifer D: Adult height in girls with idiopathic true precocious puberty. J Clin Endocrinol Metab 1994;79:415-420
41. Turner RT, Riggs BL, Spelsberg TC: Skeletal effects of estrogen. Endocr Rev 1994;15:275-300
42. Nilsson A, Ohlsson C, Isaksson OGP, Lindahl A, Isgaard J: Hormonal regulation of longitudinal bone growth. Eur J Clin Nutr 1994;48(Suppl.1): S150-S160
43. Blanchard O, Tsagris L, Rappaport R, Duval-Beaupere G, Corvol M: Age-dependent responsiveness of rabbit and human cartilage cells to sex steroids in vitro. J Steroid Biochem Molec Biol 1991;40:711-716
44. Nasatzky E, Schwartz Z, Boyan BD, Soskolne WA, Ornoy A: Sex-dependent effects of 17ß estradiol on chondrocyte differentiation in culture. J Cell Physiol 1993;154:359-362
45. Schwartz Z, Nasatzky E, Ornoy A, Brooks BP, Soskolne WA, Boyan BD: Gender-specific, maturation-dependent effects of testosterone on chondrocytes in culture. Endocrinology 1994;134:1640-1647
46. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS: Estrogen resistance caused by a mutation in the estrogen receptor gene in a man. N Engl J Med 1994;331:1056-1061
47. Trygstad O: Oestrogen treatment of adolescent tall girls; short term side effects. Acta Endocrinol 1986;279(Suppl):170-173
48. De Waal WJ, Torn M, de Muinck Keizer-Schrama SMPF, Aarsen RSR, Drop SLS: Long term sequelae of sex steroid treatment in the management of constitutionally tall stature. Arch Dis child 1995;73:311-315
49. Kaunitz AM: Oral contraceptives and gynecologic cancer: an update for the 1990's. Am J Obstet Gynecol 1992;167:1171-1176

CHAPTER 5

Long term sequelae of sex steroid treatment in the management of constitutionally tall stature.

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ABSTRACT

We evaluated possible long term side effects of high doses of sex steroids in the management of constitutionally tall stature and paid special attention to hypothalamic-pituitary-gonadal functioning. For this purpose, 64 tall adult men and 180 tall adult women, who received supraphysiological doses of sex hormones during puberty, were interviewed in a standardized way at a mean follow-up period of 10 years after cessation of treatment. Sixty-one untreated tall adult men and 94 untreated tall adult women served as controls. The majority of the subjects were satisfied with their decision regarding hormonal therapy. Seventy-seven percent of the women and 78 percent of the men reported one or more side effects during therapy. Most side effects were mild. In women, only 3% stopped treatment because of an adverse event; in men, the reported side effects were no reason to stop treatment. The frequency of reported side effects in women was higher during treatment with high doses of estrogens than during oral contraceptive use, indicating a dose dependent relationship. Amenorrhoea of longer than 6 months after cessation of therapy was found in 5%. Menstrual cycle characteristics of previously treated women were comparable with controls. Overall, malignancy was not reported. Information about a total of 127 pregnancies was obtained and revealed no distinct differences in details and outcome between previously treated women and men, and controls. In conclusion, at a mean follow-up of 10 years there is no evidence that pharmacological doses of sex hormones may have a long-term effect on reproductive function. However, this period is still too short to draw definite conclusions.

INTRODUCTION

Treatment of tall adolescent boys and girls with high doses of sex steroids in order to reduce their final height is a widely used method (1-10). In our hospital we

have been treating constitutionally tall children since 1968. In girls, estrogens (ethinylestradiol (EE) have been used (200 μg/day, range:100 - 300 μg/day) in combination with progestagens (medroxyprogestrone 5-10 mg/day, orally) every 5-10 days of the month; boys have been treated with androgens (testosterone esters in various therapeutic regimens with a total monthly dosage up to 1000 mg.). Over the years height reduction by means of supraphysiological doses of sex hormones has been a matter of discussion, not only because of the problems in assessing the real growth reductive efficacy and the lowest effective dosage, but also because of the possibility of unwanted side effects. In this respect special attention focussed on haemostasis (11), lipid metabolism(12) and functioning of the hypothalamic-gonadal axis (13,14). In addition, the impressive bulk of available data on the association between long term oral contraceptive use and possible health risks (reviewed in 15,16) are indispensable as they form an excellent reflection of the prospective risks in estrogen-treated girls.

So far, unwanted side effects have only been reported during treatment or shortly after discontinuation of therapy (1-10,17-21). Most side effects were found to be mild and reversible. Supression of the hypothalamic-gonadal-axis induced by the pharmacological doses of sex steroids was found to be reversible (13,14). However, the possibility of a long-term suppressive effect of sex hormone therapy on reproductive functioning in boys has been postulated (22). In a recent study in 43 previously treated tall men and 30 untreated tall controls we could not confirm this postulation (23). In girls, the ultimate 'proof' for complete reversibility of hypothalamic-gonadal suppression, pregnancy, has been reported in various single cases (1-4,6,8,9).

To offer further data on acceptance and possible long term effects of high doses of sex steroids in the management of constitutionally tall stature we interviewed tall adult men and women at a mean follow-up period of 10 years after discontinuation of height reductive therapy by means of a self-constructed questionnaire. Our main field of interest was the functioning of the hypothalamic-gonadal axis. Since our study was intended to be non-invasive, this was covered

by questions about menstruation, gynaecological complaints and childbearing. Tall adult men and women who had not received treatment during their pubertal period served as controls.

PATIENTS AND METHODS

Patients

Since the introduction of height reductive therapy in our institute in 1968 a total of 247 men and 423 women, who were seen at adolescence for evaluation of their constitutionally tall stature, had reached the age of 18 at the time of our follow-up study. All were contacted by mail to participate in this study. Second mailings were sent to those who did not respond to the first mailing. This study was part of a large follow-up study in constitutionally tall stature, including auxological and psychosocial assessments. Reports on auxology and psychology will be reported elsewhere.

During their puberty, 102 men and 249 women had been treated for their tall stature (cases). Usually, the indication for the therapy was a predicted final height > 2,5 SD (> 180 cm for girls and > 200 cm for boys). Occasionally, therapy was initiated in idiopathic scoliosis. Hundred and forty-five men and 174 women had not chosen to undergo treatment for various reasons such as satisfaction with the given height prognosis or uncertainty about possible side-effects, and served as controls. Two hundred and nine men (95 cases and 114 controls) and 326 women (203 cases and 123 controls) responded to our mailing. Of the responders, 125 men (64 cases and 61 controls) and 272 women (180 cases and 92 controls) agreed to participate in this study. Clinical data of the participants are summarized in table 1. All participants gave informed consent.

Table 1. Clinical data of 125 adult men and 272 adult women with constitutionally tall stature. Data expressed as mean (SD) and range.

	WOMEN		MEN	
	Cases (n=180)	Controls (n=92)	Cases (n=64)	Controls (n=61)
Age at time of FU (yrs)	25.7 (4.9) 18.7 - 35.9	24.8 (4.1) 18.7 - 34.2	26.1 (4.3)* 18.7 - 34.4	4.3 (4.2) 18.6 - 34.1
Final adult height (cm)	181.6 (3.4)$ 173.8 - 190.5	180.4 (3.7) 171.1 - 188.3	199.8 (4.1)# 190.7 - 210.1	196.2 (5.0) 186.6 - 209.3
Length of the FU (yrs)	10.9 (4.9) 3.1 - 22.9	-	8.6 (4.0) 3.2 - 18.5	-
Duration of therapy (yrs)	1.91 (0.61) 0.60 - 3.62	-	1.36 (0.56) 0.67 - 3.68	-
Treatment regimen	100 µg EE/day: n= 9 200 µg EE/day: n=157 300 µg EE/day: n= 14	-	T-ester mixture[a], i.m. (Sustanon®): 250 mg/week: n= 50 250 mg/2 wks: n= 2 500 mg/2 wks: n= 4 T-undecanoaat, oral (Andriol®): 240-320 mg/day : n= 4 T-propionate, i.m. (Neo-Hombreol®): 25-30 mg/day n= 4	-

a Mixture of T-propionate, T-fenylpropionate, T-isohexanoate and T-decanoate.
* Significant difference between cases and controls, P=0.02
$ Significant difference between cases and controls, P=0.01
# Significant difference between cases and controls, P=0.0001

Methods

All participants were interviewed in a standardized way by one investigator (WdW). In women, the standardized interview included questions about satisfaction and possible side effects of hormonal therapy, oral contraception (OC), menstrual cycle characteristics (without OC use), pregnancy and gynaecological complaints. Men were asked about satisfaction and possible side effects of hormonal therapy and about offspring. The standardized possibilities of adverse effects during sex hormone therapy were extracted from the literature. Most of the participants were interviewed in an outpatient clinic setting. Five women and one man (all cases) were interviewed by telephone.

If a subject had sought gynaecological consultation because of suspected subfertility additional information was asked for after informed consent was obtained.

The protocol was approved by the ethical committee of Academic Hospital, Erasmus University, Rotterdam, The Netherlands.

Statistics

Differences between groups were tested using Wilcoxon's two sample test. In women, cases were grouped according to their total daily EE dosage. Differences between these 3 groups were tested with the Kruskall-Wallis test. In men, no statistics were performed on the various therapeutic regimens because of the low numbers in the groups. Chisquare tests (with Yates' correction when appropriate) were used in case of a contingency distribution. Incidence rates of adverse events, pregnancy outcome and gynaecological consultation were compared using Fisher's exact test.

RESULTS

GIRLS

Therapy and adverse effects

In the control group, four women (4%) regretted their decision not to have undergone hormonal treatment for height reduction. Ninety-three percent of the cases were satisfied with the result of their treatment; 7 % showed dissatisfaction. The main reason for dissatisfaction was the little effect of the therapy compared with the given height prediction. Eleven percent of the cases would not apply for height reductive therapy again; they were worried about uncertainties of long term side effects or thought that tall stature in girls is less a problem nowadays.

Separate possible adverse effects during hormonal therapy and their reported frequency are listed in table 2. Hundred thirty-nine women (77%) gave notice being troubled by one or more side-effect during treatment. Forty-one women (21%) could not remember any adverse effect. Five women (3%) stopped treatment because of an adverse event: persisting nausea, suspected ovarian cyst, enlarged uterus, amaurosis fugax, and headache. We found no relationship with the total daily dosage of EE and the number of reported adverse effects.

Menstruation and oral contraception (OC)

Reported recurrence of menstruation after cessation of therapy is listed in table 3. Eighty-two percent of the cases reported a regular menstrual cycle compared to 74 percent of the controls (P=0.15). The reported duration of the menstrual cycle was 31.6 (12.2) days for cases and 32.4 (11.3) days for controls (P=0.11). When we calculated the reported cycle length of the regularly menstruating women only, the mean duration became shorter: 28.6 (2.2) days and 29.3 (5.3) for cases and controls, respectively (P=0.42) This was due to the finding that women with irregular menstrual cycles had a higher frequency of very long menstrual cycles. The reported duration of the menses was slightly, though significantly, different between cases and controls: 5.3 (1.5) days and 6.1 (1.7) days, respectively (P<0.01). When we calculated the duration of the menses of the regular menstruating women only, this difference became smaller, but was still significant (5.3 (1.4) days and 5.9 (1.4) days for cases and controls, respectively, P<0.01). No difference was found in the proportion of intermenstrual bleedings

Table 2. Number of reported adverse effects (%) during estrogen therapy and OC use in constitutionally tall women.

Type	EE therapy	OC use	
	Cases (n=180)	Cases (n=160)	Controls (n=87)
- headache/migraine	23 (13)	22 (14)	7 (8)
- nausea/vomitus	25 (14)	4 (3)	5 (6)
- fluor vaginalis	24 (13)	9 (6)	10 (11)
- pigmentation of areola and nipples	48 (27)	-	-
- weight gain	74 (41)	34 (21)	26 (30)
- leg cramps at night	36 (20)	5 (3)	6 (7)
- change in psychological or sexual behavior	6 (3)	15 (9)	12 (14)
- galactorrhoea	8 (4)	-	2 (2)
- hypertrichosis	6 (3)	-	-
- thrombosis	-	2 (1)	-
- hypertension	3 (2)	1 (0.6)	1 (1)
- bleeding disturbances	17 (9)	31 (21)	14 (16)
- interval bleedings	10 (6)	25 (16)	12 (14)
- no bleedings	2 (1)	3 (2)	1 (1)
- metrorrhagia	4 (2)	1 (0.6)	1 (1)
- oligomenorrhoea	1 (0.5)	-	-
- dysmenorrhoea	-	2 (1)	-
- cysts or tumours in mammae	2 (1)	2 (1)	2 (2)
- cysts or tumours in uterus	1 (0.5)	2 (1)	1 (1)
- cysts or tumours in ovariae	1 (0.5)	1 (0.6)	2 (2)
- other	27 (15)	8 (5)	3 (3)
-accelerated pubertal development	10 (6)	-	-
-polyfagia (boulemia?)	7 (4)	1 (0.6)	1 (1)
-striae	5 (3)	-	-
-dizziness	2 (1)	-	-
-amaurosis fugax	1 (0.5)	-	-
-loss of taste	1 (0.5)	-	-
-M.Pfeiffer	1 (0.5)	-	-
-candidiasis	-	3 (2)	-
-fluid retention	-	2 (1)	1 (1)
-dry eyes	-	1 (0.6)	-
-gall stones	-	1 (0.6)	-
-endometriosis	-	1 (0.6)	-
-exanthema	-	-	1 (1)

between regularly menstruating cases and controls.

The use of OC's and the proportion of reported side effects was not significantly different between cases and controls. The separate possible adverse effects during OC use and their reported frequency in cases and controls are listed in Table 2. There was no significant difference in the distribution of each of the separate reported adverse effects between the groups (P-values> 0.05).

Table 3. Reported recurrence of menstruation after cessation of therapy in 180 treated constitutionally tall women.

Recurrence	Number
After 1 month	114
After 1 - 6 months	51
After 6 months - 1 year	5
More than 1 year	4
Unknown (went unto oral contraceptives)	3
Cannot remember	3

Pregnancy

Details and outcome of 100 pregnancies are listed in table 4. There was no significant difference between cases and controls regarding the distribution of the number of miscarriages, the time before realisation of pregnancy and the number of pregnancies after fertility induction.

Fifty-seven cases and 20 controls had ever consulted a gynaecologist for various reasons, the proportions being not significantly different (P=0.11). Subfertility, gynaecological infections and menstrual cycle disturbances were most frequently reported as the reason for consultation. Eleven cases and one control consulted a gynaecologist for undesired childlessness. This difference appeared to be not statistically significant between the groups (P=0.07). The various causes for subfertility in these women were endometriosis, uterine- or tubal abnormalities, hyperprolactinaemia, myoma uteri and ovalutory dysfunction. Of the 12 women, five

(4 cases and 1 control) succeeded to become pregnant by means of fertility induction (table 4).

Table 4. Details and outcome of pregnancies in constitutionally tall men and women.

WOMEN

		Cases (n=40)	Controls (n=16)
Number of pregnancies:		63	27
Outcome	- Abortus provocatus	4	1
	- Miscarriage	8	1
	- Extra uterine pregnancy	3	-
	- Mola hydatidosa	-	1
	- Pregnant at time of study	4	1
	- Normal birth	43	23
	- 1 child	22	7
	- 2 children	10 (1 twin)	5
	- 3 children	1	2
Details	- Toxicosis	4	-
	- Symphysiolysis	-	1
	- Prenatal blood loss	1	-
	- Manual placenta removal	-	1
	- Fluxus post partem	1	-
	- Premature contractions	1	-
	- Fertility induction	4	1
Duration of realisation of pregnancy			
	- less than 1 year	51	25
	- between 1-2 years	2	1
	- more than 2 years	8	1

Table 4 (continued)

MEN		Cases (n=6)	Controls (n=10)
Number of pregnancies:		11	16
Outcome	- Miscarriage	1	1
	- Normal birth	10	15
	- 1 child	10	15
Details	- Asphyxia durante partem	-	2
Duration of realisation of pregnancy			
	- less than 1 year	10	15
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	- more than 2 years	-	1

In addition, two cases spontaneously became pregnant. The remaining four cases were still under gynaecological control at the time of the interview. In cases, women who consulted a gynaecologist did not receive a significantly different total daily dosage of EE compared to women who had not consulted a gynaecologist (P=0.11). In addition, the total daily dosage of EE in women who sought gynaecological consultation because of subfertility did not differ from that of women who consulted a gynaecologist for other reasons.

BOYS

Therapy and adverse effects

In the control group, 6 men (10%) regretted their decision not to have undergone androgen therapy for height reduction. They appeared to be significantly taller than those who showed no regret (mean (SD) final height 202.8 (5.4) versus 195.5 (4.4), P=0.004).

Eighty-six percent of the cases were satisfied with the result of their treatment; 14 % showed dissatisfaction. The main reason for dissatisfaction was the little effect of the therapy compared with the given height prediction. Eight percent would not apply for height reductive therapy again, because of treatment discomfort or uncertainty of long term side effects.

Fifty men (78%) gave notice being troubled by one or more side-effect during treatment. Forteen men (22%) could not remember any adverse effect. Aggravation of acne was most frequently reported (39%), followed by painfulness of the injection (16%), weight gain (14%), gynaecomastia (13%), muscle ache (13%), oedema (9%) and change in psychological or sexual behavior (5%).

Pregnancy

Details and outcome of 27 pregnancies are listed in table 4. Men who had no children yet also had no wish for children at the time of the interview. There was no significant difference between cases and controls regarding outcome of pregnancy and the time before realisation of pregnancy.

DISCUSSION

Long term follow-up data of sex steroid therapy of constitutionally tall adolescents are lacking. In order to obtain more insight in possible long term side effects we performed a non-invasive controlled study and focussed on the functioning of the hypothalamic-gonadal axis. In spite of the non-invasive character of our study, cases were more willing to participate in this follow-up study than controls. It is possible that this may have biased the results. Cases would probably have more linked particular conditions with their past hormonal treatment what may have lead to over-reporting. Therefore results must be interpreted with some reserve.

Our study showed, that many patients experienced one or more side effects during therapy. Fortunately, most of them were mild and led to cessation of therapy in only 3% of the women. In men, therapy was not discontinued because of adverse effects. Our findings are in concert and additional to the side effects reported by others (1-10,17-21).

In girls, adverse effects of estrogens occurred more frequently during therapy than during OC use. This reflects a dose dependent effect of estrogens on the incidence of adverse events. The scala of reported side effects reported during OC use was not significantly different between cases and controls and was of the same order as reported in the literature (24,25). Two cases reported thrombosis during OC use. Despite the fact that the risk of thromboembolism increases with the estrogen dose (26), we did not find thrombosis during therapy. One girl who had a history of amaurosis fugax might have had thromboembolism. However, no haemostatic changes were found at that time. In general, thrombosis is found to be an uncommon side effect of height reductive therapy (1,17,18); whenever thrombosis ocurred it mostly coincided with other risk factors for thromboembolism such as immobilisation. Amenorrhoea of longer than 6 months after cessation of height reductive therapy was reported in about 5 %. The incidence of amenorrhoea following cessation of pill intake is about 0.5% (27). In addition, the overall prevalence of secondary amenorrhoea of more than 6 months in women aged 15 - 34 is about 1.3% (28). This may suggest an increase of amenorrhoea after height reductive therapy. It should be noted however, that there are no convincing data that OC use is causally related to amenorrhoea and that other risk factors for amenorrhoea, such as smoking, nutrition and exercise, were not adequately adressed (27,29). We found no important differences in menstrual cycle characteristics between cases and controls. This implies that no important hormonal imbalance is present in previously treated tall women. Malignancy was not reported in our study. The possibility of a dose-dependent effect and a relationship with OC use at a young age and duration of OC use with increased risks on breast cancer (30) raises the need for long term follow-up in patients

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treated with pharmacological doses of estrogens. In our study, only 49 of the 180 cases were older than 30 years at time of the follow-up. Therefore, our mean follow-up period in women of almost 11 years is still too short to give any answer to this important issue.

In boys, aggravation of acne was by far the most reported side effect. This is in agreement with others (3,5). A causal relationship with androgen therapy is likely, as shown by Fyrand et al (31). Gynaecomastia occurred in 13 % of the cases, probably due to the increased peripheral aromatization of androgens into estrogens. Since gynaecomastia is rather prevalent in population studies in boys (32), it is hard to say whether this condition is increased. In one patient, surgical correction was necessary several years after cessation of therapy.

Thus far, only casuistic data have been available on successful pregnancies after height reductive therapy (1-4,6,8,9). In our follow-up study information about a total of 127 pregnancies was obtained and revealed no distinct differences in details and outcome of pregnancies between treated women and men and controls. These results indicate, that long term effects of high doses of sex hormones on fertility are unlikely. It is tempting to draw definite conclusions at this time, but one should not forget the social trend to have children at a later age and the fact that the control group in men was significantly younger. In addition, the finding in women that a higher, though not significantly, proportion of cases had sought gynaecological consultation because of subfertility should not be ignored. However, this latter finding is hard to interpret. There is always the possibility of bias by over-reporting in cases, as discussed above. Furthermore, additional information about the causes for subfertility in these women showed no clear systematical "pattern", indicating that a certain pathophysiological mechanism is unlikely. And finally, the prevalence of infertility in the normal population is in the same order of magnitude (10-25%, depending on the definition used (33)). Besides, the meaning of significantly higher plasma levels of FSH in previously treated tall adult men in the presence of normal sperm quality, normal plasma testosterone levels and normal testes volume, remains to be established (23).

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Therefore, definite conclusions on the influence of pharmacological doses of sex hormones on fertility cannot be drawn yet. But for now, our data are reassuring since there is no clear evidence that treatment with high doses of sex steroids does induce any harmful effect on reproductive function in man or woman.

In summary, treatment of tall adolescent boys and girls with high doses of sex hormones in order to reduce their final height is satisfying to most of the patients, despite the fact that many patients experience side-effects during therapy. Most side-effects during therapy are mild. The incidence of side-effects is probably dose-dependent. Post-treatment amenorrhoea of longer than 6 months was found in 5% of the women. No important differences in menstrual cycle characteristics were found between cases and controls. Information about a total of 127 pregnancies was obtained and revealed no distinct differences in details and outcome between treated men and women and controls. At a mean follow-up period of 10 years, there is no evidence that pharmacological doses of sex hormones may have a long-term effect on reproductive function or cancer risks. However, this period is still too short to draw definite conclusions.

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REFERENCES

1. Wettenhall NH, Cahill C, Roche AF: Tall girls: A survey of 15 years of management and treatment. J Pediatr 1975;86:602-610
2. Prader A, Zachmann M: Treatment of excessively tall girls and boys with sex hormones. Pediatrics 1978;62:1202-1210
3. Crawford JD: Treatment of tall girls with estrogen. Pediatrics 1978;62:1189-1195
4. Andersen H, Jacobsen BB, Kastrup KW, Krabbe S, Peitersen B, Petersen KE, Thamdrup E, Wichmann R: Treatment of girls with excessive height prediction. Acta Paediatr Scand 1980;69:293-297
5. Brämswig JH, Schellong G, Borger HJ, Breu H: Testosteron-Therapie hochwüchsiger Jungen. Dtsch Med Wschr 1981;106:1656-1661
6. Stöver B, Kollmann F: Ergebnisse der Behandlung hochüchsiger Mädchen mit conjugierten Ostrogenen. Monatsschr Kinderheilkd 1982;130:36-40
7. Brämswig JH, Lengerke v. HJ, Schmidt H, Schellong G: The results of short-term (6 months) high-dose testosterone treatment on bone age and adult height in boys of excessively tall stature. Eur J Pediatr 1988;148:104-106
8. Grüters A, Heidemann P, Schlüter H, Stubbe P, Weber B, Helge H: Effect of different oestrogen doses on final height reduction in girls with constitutional tall stature. Eur J Pediatr 1989;149:11-13
9. Normann EK, Trygstad O, Larsen S, Dahl-Jorgensen K: Height reduction in 539 tall girls treated with three different dosages of ethinyloestradiol. Arch Dis Child 1991;66:1271-1278
10. Joss EE, Zeuner J, Zurbrügg RP, Mullis PE: Impact of different doses of ethinyl oestradiol on reduction of final height in constitutionally tall girls. Eur J Pediatr 1994;153:797-801
11. Muntean W, Borkenstein M: Hemostatic changes in tall girls treated with high doses of ethinylestradiol. Eur J Pediatr 1980;134:245-248
12. Hinkel GK, Hanefeld M, Jaross W, Leonhardt W, Trübsbach A: Effect of high doses of oestrogens and androgens on lipoproteins: observations in the treatment of excessive growth with sexual hormones. Exp Clin Endocrinol 1985;86:17-25
13. Hanker JP, Schellong G, Schneider HPG: The functional state of the hypothalamo-pituitary axis after high dose oestrogen therapy in excessively tall girls. Acta Endocrinol 1979; 91: 19-29
14. Brämswig JH, Nieschlag E, Schellong G: Pituitary-gonadal function in boys after high dose testosterone treatment for excessively tall stature. Acta Endocrinol 1984;107:97-103
15. Grimes DA: The safety of oral contraceptives: epidemiologic insights from the first 30 years. Am J Obstet Gynecol 1992;166:1950-1954
16. Kaunitz AM: Oral contraceptives and gynecologic cancer: an update for the 1990s. Am J Obstet Gynecol 1992;167:1171-1176
17. Trygstad O: Oestrogen treatment of adolescent tall girls; short term side effects. Acta Endocrinol 1986;(Suppl)279:170-173
18. Werder EA, Walbel P, Sege D, Flury R: Severe thrombosis during oestrogen treatment for tall stature. Eur J Pediatr 1990;149:389-390
19. Panteon E, Loumaye E, Maes M, Malvaux P: Occurrence of prolactinoma after estrogen treatment in a girl with constitutionally tall stature. J Pediatr 1988;113:337-339

20. Traupe H, Mühlendahl von KE, Brämswig JH, Happle R: Acne of the fulminans type following testosterone therapy in three excessively tall boys. Arch Dermatol 1988;124:414-417
21. Commentz JC, Conrad C, Stahnke N, Willig RP: Priapism during testosterone treatment for reducing final height in tall stature. Horm Res 1994:41:103
22. Willig RP, Bettendorf M, Hinkel GK, Schwarz HP, Schulze W: Androgen treatment of tall stature during puberty may reduce sperm quality in adult life. Horm Res 1992;37 (Suppl.4):3
23. De Waal WJ, Vreeburg JTM, Bekkering F, de Jong JH, de Muinck Keizer-Schrama SMPF, Drop SLS, Weber RFA: High dose testosterone therapy for reduction of final height in constitutionally tall boys: does it influence testicular function in adulthood? Clin Endocrinol 1995;43:87-95
24. Hillard PJA: The patient's reaction to side effects of oral contraceptives. Am J Obstet Gynaecol 1989;161:1412-1415
25. Akerlund M, Rode A: Comparative profiles of reliability, cycle control and side effects of two oral contraceptive formulations containing 150 µg desogestrel and either 30 µg or 20 µg ethinyl oestradiol. Br J Obstet Gynaecol 1993;100:832-838
26. Iman WHW, Vessey MP, Westerholm B, et al: Thromboembolic disease and the steroidal content of oral contraceptives: a report to the committee on safety of drugs. Br Med J 1970;2:203-209
27. Hull MGR, Bromham DR, Savage PE, Barlow TM, Hughes AO, Jacobs HS: Post-pill amenorrhea: a causal study. Fertil Steril 1981;36:472-476
28. Münster K, Schmidt L, Helm P: Secondary amenorrhoea: prevalence and medical contact-a cross-sectional study from a Danish county. Br J Obstet Gynaecol 1992;99:430-433
29. Gold EB, Bush T, Chee E: Risk factors for secondary amenorrhea and galactorrhea. Int J Fertil 1994;39:177-184
30. Rushton L, Jones DR: Oral contraceptive use and breast cancer risk: a meta-analyses of variations with age at diagnosis, parity and total duration of oral contraceptive use. Br J Obstet Gynaecol 1992;99:239-246
31. Fyrand O, Fiskaadal HJ, Trygstad O: Acne in pubertal boys undergoing treatment with androgens. Acta Derm Venereol 1992;72:148-149
32. Braunstein GD: Gynecomastia. N Engl J Med 1993;328:490-495
33. Greenhall E, Vessey M: The prevalence of subfertility: a review of the current confusion and a report of two new studies. Fertil Steril 1990;54:978-983

CHAPTER 6

High dose testosterone therapy for reduction of final height in constitutionally tall boys: does it influence testicular function in adulthood?

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ABSTRACT

We have studied the effect of treatment with high doses of androgens during puberty on testicular function in adult men with constitutionally tall stature, taking into account confounding factors interfering with sperm quality, since existing published data do not include whether testicular function is impaired by such treatment. Forty three previously androgen treated tall men (cases) and thirty non-treated tall men (controls) agreed to participate in our study. The study work-up comprised physical examination, semen analysis and measurements of plasma hormone levels (LH, FSH, testosterone (T), sex steroid binding globulin (SHBG) and inhibin). It appeared that sperm quality and testis volume were comparable between cases and controls. Mean sperm concentration was 66.4 x 10^6/ml in cases and 66.2 x 10^6/ml in controls. A left-sided varicocele was found in 45% of the cases and 37% of the controls. In cases we observed a significant effect of the age at start of androgen therapy on sperm motility (regr. coeff. (SE): 4.92 (2.41%), P=0.048). In addition, testis size at start of therapy had a significant effect on sperm concentration (regr. coeff. (SE): 5.57 (1.54 x 10^6/ml), P=0.0012) and on total sperm count (regr. coeff. (SE): 43.1 (7.73 x 10^6), P=0.0001). Plasma levels of T, SHBG and inhibin were not statistically different between both groups. Cases had significantly higher FSH levels (mean (SD): 3.3 (2.2) versus 2.1 (0.8) IU/l, P=0.004) and significantly lower LH levels (mean (SD): 2.3 (0.9) versus 3.1 (1.4) IU/l, P=0.019). We found a significant effect of the age at start of therapy on plasma FSH level in the treated men (regr. coeff. (SE): -0.73 (0.18 IU/l), P=0.0003]. In conclusion, treatment with high doses of androgens for reduction of final height in constitutionally tall stature has no long-term side-effect on sperm quality, testicular volume and plasma testosterone levels. However, treated men had significantly higher plasma levels of FSH compared with controls. The meaning of this difference remains to be established. Varicocele in adult tall men is present in 42%.

INTRODUCTION

Treatment of tall adolescent boys with high doses of testosterone is a widely used method to reduce their final height (1,2,3,4). This therapy induces suppression of the hypothalamic-pituitary-gonadal axis (1,3). In adult men, low levels of gonadotropins caused by high doses of androgens are known to reduce sperm production to oligospermia or azoospermia (5). Contraceptive studies have shown that androgen-induced suppression of spermatogenesis is reversible in adult men (6,7). However, extrapolation of these data to the management of tall stature in pubertal boys must be viewed with caution since factors which regulates spermatogenesis in the normally functioning adult testis may not be the same as during puberty (8). Androgen therapy in tall boys is usually initiated with the first signs of puberty and it is in this peripubertal period that important maturational processes are taking place in the testis (8-13). Influenced by complex hormonal actions, these maturational processes eventually lead to initiation of spermatogenesis. Onset of spermatogenesis (spermarche) as detected by urine analysis (spermaturia) appears to be an early pubertal event: the median age of spermarche has been estimated to be 13-14 years (14-17). In addition, it is noteworthy that administration of testosterone esters at high doses can cause morphological and cytological changes, as shown in rat and human adult testes (18-20).

It has not been known whether the suppressive effects of high dose testosterone therapy on maturing gonads in puberty may affect pituitary-gonadal functioning in adulthood. Brämswig et al (1) demonstrated normalization of the gonadotropin levels in 100 tall boys after discontinuation of treatment with follow-up periods up to 48 months, although transient hypergonadotropic LH- and FSH-secretory patterns occurred. Zachmann, Prader and co-workers (3,4) briefly reported that testicular volume and sperm count were normal 1.5 years after discontinuation of treatment, although in individual cases recovery periods up to five years had been observed. In contrast, Willig et al (21,22) recently reported

reduced sperm concentration in treated tall men compared to untreated controls and postulated the possibility of long-term suppresive effects of high doses of androgens on reproductive function. Unfortunately, however, none of the latter reports corrected for confounding factors interfering with parameters of sperm quality. It has been well established that varicocele (23-26), smoking (27), sexually transmitted diseases (28) and cryptorchism (29) are likely to affect sperm quality and/or plasma hormone levels. The purpose of this study was to investigate whether treatment with high doses of androgens in tall boys during puberty may induce long-term effects on pituitary-gonadal functioning while taking into account interfering confounding factors.

MATERIALS AND METHODS

Patients

Two hundred and forty-seven adult men, who were seen at adolescence for evaluation of tall stature in the Sophia Children's Hospital, Rotterdam, were contacted by mail to participate in an andrological follow-up study. Of the 247 men, 102 had been treated for their tall stature during puberty (cases). The 145 non-treated men served as controls. Ninety-five out of 102 cases responded to our mailing (response rate (RR):93%) and 43 out of 95 (45% of the responders) participated in the andrological study. For controls, the RR was 79% (114/145) and 32 out of 114 participated in this study (28% of the responders). All participants gave informed consent.

Androgen therapy started at a mean (SD) age of 14.4 (1.2) years with a range of 12.1 to 17.2 years. Pubertal stages (according to Tanner (55)) and testicular volume (mean of the two testes) at the time of start of treatment were recorded. In 9 cases, testicular volume could not be retrieved (Table 1). The duration (SD) of therapy was 17.1 (7.2) months, ranging from 8.7 to 44.2 months. Most patients used intramuscular preparations of testosterone ester mixtures

(Sustanon '250'® =testosterone propionate, -fenylpropionate, -isohexanoate and -decanoate) 250 mg every week (n=32), 250 mg every two weeks (n=2) or 500 mg every two weeks (n=4). Three patients used an oral testosterone ester (Andriol® =testosterone undecanoate) 240-320 mg per day; one of these patients switched to Sustanon '250'®, 500 mg every two weeks after 8 months. Two patients used daily injections of the testosterone ester testosterone propionate (Neo-hombreol®) in a dosage of 30 mg per day for at least one year after which they switched to Sustanon '250'®, 250 mg every two weeks. The mean (SD) age at follow-up was 24.2 (4.1) years (range 18.2-33.0 years), i.e. a mean (SD) follow-up period of 8.3 (3.9) years (range 2.8-17.2 years) after discontinuation of treatment. Data on the growth reductive efficacy of the androgen therapy will be published elsewhere.

Two controls were excluded from the study because of known testicular impairment. Mean (SD) age at follow-up of the 30 control subjects was 26.4 (4.4) years with a range from 19.2 to 34.0 years.

All participants were called in twice at the outpatient clinic of the department of Andrology. Clinical assessment at the first visit comprised medical history and physical examination. Height was measured by a Harpenden Stadiometer. Testicular volume was estimated using Prader's orchidometer (30). The diagnosis of varicocele was made by palpation (all subjects) and/or Doppler measurement (Doppler stethoscope: 8.2 MHz blood flow detector) (67/73 patients). We graded the varicocele as follows: grade 0: subclinical varicocele only detectable by Doppler stethoscope, grade 1: varicocele detectable by palpation with Vasalva's manoeuvre, grade 2: varicocele detectable by palpation but not visible, and grade 3: visible varicocele. In addition, blood was taken for determination of plasma hormones and the first semen sample was obtained. A second semen sample was obtained during the last visit.

The study protocol was approved by the ethical committee of Erasmus University Hospital, Rotterdam.

Semen analysis

All semen samples were collected after a period of sexual abstinence of at least three days. Semen was obtained at the hospital by masturbation. The mean (SD) time interval between the two semen samples was 1.4 (0.5) months (range 0.9-3.0 months).

Semen analysis was performed within 30 minutes after ejaculation according to standard World Health Organisation (WHO) procedures (31). Volume, sperm concentration, total sperm count, motility and morphology were assessed. Sperm concentration was separately determined by two experienced technicians using the Makler chamber (32). The mean of the two counts was used, provided that the difference between the two counts did not exceed 20% of the mean; otherwise, the counting was repeated. In 108 samples (26 controls and 28 treated men) the concentration of sperm was also determined using the improved Neubauer haemocytometer as described in the WHO manual (31). Sperm motility, calculated as the percentage of progressive motile spermatozoa (classes a + b, WHO manual (31)), was determined by the same technicians in all semen samples. If the difference between the two observations exceeded 20%, motility assessment was repeated. Sperm morphology was evaluated after Diff-Quick staining using strict criteria (33).

Hormone measurements

LH and FSH were measured using a commercially available enhanced luminescence immunometric method (Kodak Clinical Diagnostics, Amersham, UK). The intra- and interassay variability were < 6.2% and < 12.6% for LH and < 5.2% and < 12.7% for FSH, respectively. Testosterone (T) was measured by radio-immunoassay, using the antiserum described previously by Verjans et al (34). The intra- and interassay variability were < 9.2% and < 10.0%, respectively. Sex hormone binding globulin (SHBG) was measured using a commercially available immunoradiometric assay (Diagnostic Products Corporation, Los Angeles, USA). The intra- and interassay variability of this kit were < 5.3% and < 10.8%,

respectively. The ratio of T to SHBG was calculated in order to obtain an estimate of the free androgen fraction (FAI). Inhibin was measured by radioimmunoassay as described by Robertson et al (35), using an antiserum against purified 32 kDa bovine follicular fluid (bFF) inhibin and iodinated 32 kDa bFF inhibin. All samples were measured in one assay. The intra-assay variability was 16.6%. This relatively high value was likely to be caused by the fact that all concentrations were read in the lower range of the standard curve for which CV values of 12% were reported by others using the same assay (36).

Statistical analysis

The agreement of the sperm concentration as determined by the Makler chamber and by the improved Neubauer haemocytometer was evaluated using the method described by Bland & Altman (37). The sperm concentration as measured by the Makler chamber was used for further statistical analysis. For parameters of sperm quality, the mean of the two semen samples was used for statistical analysis; in two previously treated tall men only one semen sample was obtained and used for statistical analysis. Differences between cases and controls were tested with Wilcoxon's two sample test. Cases were divided into five different groups according to the therapeutic regimen used: group 1 (testosterone esters 250 mg/week, n=32), group 2 (testosterone esters 250 mg/2 weeks, n=2), group 3 (testosterone esters 500 mg/2 weeks, n=4), group 4 (testosterone-undecanoate, n=3), and group 5 (testosterone-propionate, n=2). Differences between these groups were tested with the Kruskall-Wallis test. Chi-square tests were used in case of a contingency distribution. Correlations between variables were tested with Spearman's rank correlation test. Multiple linear regression analysis was used to correct for confounding factors (analysis of covariance). For this purpose we created two groups: one group included subjects who had at least one of the following interfering conditions: varicocele, smoking, cryptorchism or venereal disease; the other including subjects with no confounding factor at all. When correlation analysis between variables showed a significant association, multiple

linear regresssion analysis was used to analyse the effect of variables after correction for interfering conditions using the same grouping variable.

RESULTS

Response

The response rate to our mailing of the treated men was significantly higher than for the controls (P=0.003). Non-responding was due to change of address or to assumed lack of interest even after a second mailing. Of the responders, a significantly higher percentage of cases participated in this andrological study (P=0.015).

Clinical data

Clinical data of the treated men and the controls at the time of follow-up are summarized in Table 1. Compared to the control group, cases were significantly taller and younger (P=0.021 and P=0.044, respectively). Age at the time of start of androgen therapy was not correlated with the length of the follow-up period.

Table 1. Clinical data expressed as mean (SD) and [range].

	Treated men (n=43)	Controls (n=30)	P-value
Height (cm)	199.4 (4.0) [190.7 - 208.3]	196.9 (6.0) [188.3 - 209.3]	0.021
Age (yrs)	24.2 (4.1) [18.2 - 33.0]	26.4 (4.4) [19.4 - 34.0]	0.044
Testicular volume (ml) (mean of two testes)	22.1 (4.1) [9.5 - 25.0]	23.1 (2.7) [15.0 - 25.0]	0.448
Pubertal stages* at time of start of androgen therapy	P1: 6 G1: 4 P2: 3 G2: 2 P3: 7 G3: 4 P4: 18 G4: 23 P5: 10 G5: 10	-	-
Testicular volume (mean of two testes) at start of androgen therapy	≤ 10 ml: 7 11-15 ml: 9 >15 ml: 18 unknown: 9	-	-

* Pubertal stages according to Tanner (32)

No statistical difference was found with respect to the presence of varicocele, smoking habits, previous venereal disease or operative correction for cryptorchism before puberty between both groups (Table 2). All varicoceles were left-sided. The size of the varicocele did not different between cases and controls.

Table 2. Occurrence of factors which may confound with parameters of semen quality.

Confounding factor	Treated men (n=43) Percentage (n)	Controls (n=30) Percentage (n)	P-value#
Varicocele (left-sided)	45 (18) (n=40)*	37 (10) (n=27)*	0.69
Grade 0	12.5 (5)	11 (3)	
Grade 1	5 (2)	0 (0)	
Grade 2	10 (4)	19 (5)	
Grade 3	17.5 (7)	7 (2)	
Smoking	40 (17)	43 (13)	0.93
Venereal disease	5 (2)	3 (1)	1.00
Cryptorchism	9 (4)	3 (1)	0.64

* In 3 treated men and in 3 controls no additional Doppler measurement was performed. All 6 patients had at least no clinical signs of varicocele at physical examination.

\# Statistics based on absolute numbers, not on percentages.

At the time of the study 5/43 cases and 6/30 controls had fathered one or more children. All 11 men reported pregnancy after less than one year of unprotected coitus. Abortion was mentioned by one previously treated man and one control. Men without children had no wish for children yet.

Testicular volume

Mean testicular volume at the time of follow-up did not differ between the groups (Table 1). This was also the case after adjustment for interfering factors (difference (SE) 0.67 (0.75 ml), P=0.3747). Adult mean testicular volume was

moderately correlated with parameters of semen quality (sperm density and motility) for both cases and controls (r:0.39-0.52, P<0.05). In cases, adult mean testicular volume was not significantly correlated with either the age at start of androgen therapy, duration of therapy, length of the follow-up period or testicular volume at time of start of androgen therapy. However, adult testis size significantly correlated with the level of plasma FSH in the treated group (r=-0.33, P=0.031). This association was still significant after adjustment for interfering conditions (regression coefficient (SE): -0.1 (0.06 IU/l), P=0.03).

Semen analysis

For the sperm concentration data, the linear correlation coefficient (r) between the Makler chamber and the Neubauer haemocytometer was r=0.94 (P<0.001). The mean difference between both methods (Makler minus Neubauer) was -3.9 x 10^6/ml. Ninety-five percent of the differences were between -44.1 x 10^6/ml and 36.3 x 10^6/ml.

The results of the semen analyses are summarized in Table 3. We found no significant difference for any of the semen parameters between cases and controls. Six out of 43 cases and 4 out of 30 controls had oligozoospermia (sperm concentration less than 20 x 10^6/ml, WHO 1992) in both semen samples (NS). An additional 4 cases and 2 controls showed oligozoospermia in one semen sample.
In cases, we found no significant correlations between parameters of semen quality and the age at start of treatment, except for sperm motility (r= 0.34; P=0.025). Duration of treatment and the length of the follow-up period showed no significant correlation with sperm quality. A significant correlation was found between mean testicular volume at start of therapy and sperm concentration as well as total sperm count (r=0.46, P=0.007 and r=0.45, P=0.007, respectively). Age at start of therapy was correlated with testicular volume at that time (r=0.34, P=0.048). Semen quality did not differ statistically between the five therapeutic regimen groups. We did not observe any significant difference in semen parameters between men with or without varicocele for both cases and controls.

Analysis of the data after correction for interfering clinical conditions also revealed no significant differences between cases and controls in parameters of sperm quality or adult testicular volume. In cases, age at start of therapy still had a significant effect on sperm motility (regression coefficient (SE):4.9 (2.4%), P=0.048). Adding testicular volume at start of therapy to the model showed no additional effect on sperm motility. Testicular volume at start of therapy still showed a significant effect on sperm concentration and on total sperm count (regression coefficients (SE): 5.6 (1.5 $x10^6$/ml), P=0.0012 and 34.1 (7.7 $x10^6$/ml), P=0.0001, respectively). Adding age at start of treatment to these regression models showed no additional effect on either sperm concentration or total sperm count. However, plasma FSH level did show an additional significant effect on sperm concentration over testis size at start of therapy (regression coefficient (SE):-14.6 (4.4 x 10^6/ml), P=0.0027).

Table 3. Results of semen analysis in 43 treated and 30 untreated tall men. Data expressed as mean (SD) [range].

Parameters of semen quality#	Treated tall men (n=43)	Controls (n=30)	P-value
Volume (ml)	4.3 (1.7) [1.5 - 7.8]	4.0 (1.1) [2.1 - 7.3]	0.79
Sperm Concentration (10^6/ml)	66.4 (59.1) [1.5 - 315.0] median: 47.5	66.2 (43.6) [0.1 - 170.0] median: 62.5	0.56
Total sperm count (10^6/ejaculate)	290.5 (263.0) [5.9 - 960.0] median: 191.3	250.8 (157.6) [0.7 - 586.0] median: 243.6	0.87
Motility (%)	44.6 (18.7) [7.5 - 80.0]	48.0 (19.1) [0.0 - 80.0]*	0.44
Morphology (%)	20.8 (6.5) [6.0 - 32.0]	18.5 (8.5) [0.0 - 34.0]*	0.21

\# Mean results of two samples

* Including two men showing very low sperm concentration (<0.1 x 10^6/ml) in which sperm motility and sperm morphology could not be assessed

Plasma hormone levels

The results of the plasma hormone levels for all men are summarized in Table 4. We found no difference in T- and SHBG-levels and FAI between cases and controls. Cases had significantly higher FSH levels (P=0.004) and lower LH levels (P=0.0187) compared with controls. Fifteen out of 43 cases had plasma FSH levels higher than the mean + 2 SD of the controls compared with 1 out of 30 controls. To verify the observed difference in gonadotropins in our study we additionally determined plasma LH and FSH levels of a group of 72 normal healthy men with the same age range (18-34 years) who visited a blood bank. Mean (SD) plasma LH and FSH values of these 'normal' men were 3.6 (1.9) IU/l [range: 0.2-10.7] and 2.5 IU/l (1.3) [range: 0.6-6.4], respectively. These values were comparable with our tall control group, but again, were significantly different from our treated group (P< 0.03).

Table 4. Plasma hormone levels in treated and untreated tall men. Data expressed as mean (SD) and range.

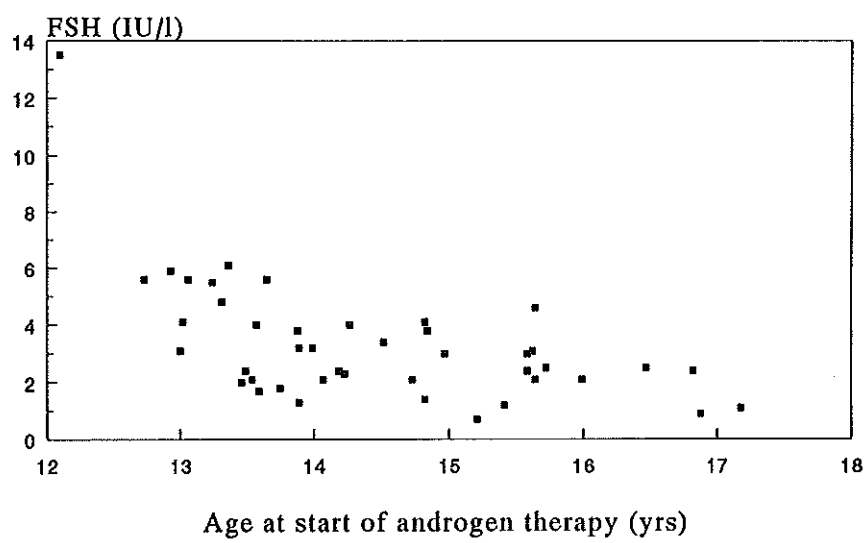
Plasma hormone	Treated tall men (n=43)	Controls (n=30)	P-value
LH (IU/l)	2.3 (0.9) 0.2 - 5.4	3.1 (1.4) 1.2 - 6.0	0.0187
FSH (IU/l)	3.3 (2.2) 2.6 - 13.5	2.1 (0.8) 0.3 - 4.0	0.004
T (nmol/l)	16.8 (4.6) 8.3 - 29.5	17.6 (6.8)δ 5.7 - 34.1	0.79
SHBG (nmol/l)	39.8 (11.7) 18.0 - 69.0	36.4 (10.5) 12.0 - 57.7	0.28
FAI	0.44 (0.13) 0.17 - 0.77	0.53 (0.28)δ 0.13 - 1.5	0.29

FAI= Free Androgen Index (T/SHBG)
δ In one control T determination was erroneously not performed (n=29)

Gonadotropin levels in cases and controls did not significantly differ between men with oligozoospermia or normospermia or between men with or without a varicocele. In general, levels of plasma hormones were not significantly correlated with parameters of semen quality; however, we observed a significant negative correlation between sperm concentration and plasma FSH level in cases (r=-0.34, P=0.024).

In the androgen treated tall men there was a significant negative correlation between the age at start of treatment and the level of FSH at time of follow-up (r=-0.49; P=0.001) (Figure 1). We also found a negative correlation between mean testicular volume at start of therapy and plasma FSH levels (r=-0.43, P=0.011). We observed a trend towards higher levels of FSH in men who had been treated for a longer period, although not statistically significant (r=0.28; P=0.096). The five treatment groups showed differences in plasma FSH levels with the highest FSH levels in patients who had been treated with testosterone-propionate. These differences however, did not reach statistical significance (P=0.098). For LH levels there was no such relationship.

Figure 1. Relationship between FSH and age at start of therapy (r=-0.49;P=0.001)



The difference (SE) in mean plasma FSH between cases and controls, corrected for interfering factors, was 1.1 (0.3) IU/l, P=0.0014; the difference (SE) in mean plasma LH was -0.8 (0.3) IU/l, P=0.0071. Testosterone, SHBG and FAI were not significantly different between cases and controls. We found a significant effect of the age at start of therapy on plasma FSH level of the treated men (regression coefficient (SE): -0.7 (0.2 IU/l), P=0.0003) after correction for interfering conditions. Adding testicular volume at start of therapy or testicular size at adulthood to the regression model showed no significant additional effect over age at start of therapy on plasma FSH level.

In a subgroup of 30 men (18 cases and 12 controls) we had the opportunity to measure inhibin levels in saved blood samples. We did not find a significant difference in inhibin levels between cases and controls in this subgroup (mean (SD) inhibin levels: 2.10 (0.73 U/ml) and 2.53 (1.27 U/ml) for cases and controls, respectively, P=0.27). This was also the case after adjustment for confounding conditions (difference (SE): -0.42 (0.36 U/ml), P=0.26). Inhibin levels did not significantly correlate with FSH levels.

DISCUSSION

Our study shows that sperm quality in previously androgen treated tall men is comparable with a control group of untreated tall men. In our control population, mean sperm concentration as determined by the Makler chamber was 66.2 x 10^6/ml. That is in the same range of the normal population as reported recently by Carlsen et al (38). The considerable variability in semen quality seen in our control group confirms the work of other studies in 'normal' men (39,40). The fact that previously treated men showed semen quality comparable to controls, even after correction for possible interfering conditions, implies that severe long-term complications on spermatogenesis are unlikely, at least after a mean follow-up period of 8 years.

The selection of our population may have biased the results; treated tall men were more willing to participate in this follow-up study than untreated tall men. However, none of the men participated because of suspected infertility. Therefore, we have no reason to believe that a structural bias has occurred.

Our findings on sperm quality are in agreement with the experiences briefly reported by Prader et al (3,4). They found that most of their tall patients had normospermia (sperm counts more than 20 x 10^6/ml) between a few months and a few years after treatment. In addition, studies in hypogonadotrophic hypogonadism of prepubertal onset have shown that androgen treatment for viralization induction did not impair subsequent spermatogenesis (41,42) although one may argue that the recommended dose for this replacement therapy is much less. Our results are in contrast with the findings of Willig and co-workers (21,22), who found reduced sperm concentration in previously treated tall men compared to controls. Their control group, however, showed a mean value of sperm concentration of 120.2 ± 111.9 x 10^6/ml. Their treated group showed a mean sperm concentration of 63.4 ± 50.6 x 10^6/ml, which is comparable with the normal population (38) and with our study. In an attempt to stress the possibility of unwanted long-term side effects of androgen treatment, Willig et al (22) subsequently reported that 16/60 of their patients showed abnormal sperm quality (considering not only sperm concentration but also sperm morphology and motility) in contrast to 2/20 of their controls. In our opinion this difference is not statistically significant (Fisher's exact test: $P>0.10$). Moreover, it is known that even in normal fertile men abnormal semen results can be found in a large proportion of samples (39). It is possible that differences in patient selection, semen analysis methodology and treatment regimens may account for the observed differences. In addition, the extent in which interfering conditions are present may cause important bias as well.

We found an overall prevalence of varicocele of 42% (12% subclinical and 30% clinical). It is difficult to interpret these figures as there is no consensus about the definition of a varicocele and various epidemiological studies have used different methods to diagnose this condition (42). Nevertheless, our finding of

clinical varicocele is higher than reported in the normal population (12.4-25.8 %) but more or less in the same range as in men being evaluated for infertility (19-41%) (43,44). A relation with androgen treatment is unlikely since no difference in prevalence of varicocele was observed between cases and controls. One could speculate about the impact of stature on the pathogenesis of varicocele as our study subjects have heights in the upper range of the normal Dutch population.

We observed different levels of gonadotropins in previously treated tall men compared to controls (tall and 'normal' men). How can this observation be explained considering the comparable semen output? In the management of tall stature, androgen treatment usually starts after the onset of puberty. It is known that spermarche is an early pubertal event that can occur without other signs of puberty and with small testicular volume (15,45,46). In the pubertal period, the complex maturational processes that take place within the gonads are not fully completed (9,12,47). In our study group, the mean age at the start of treatment was 14.4 years. At that time about 15% of the patients had a pubertal Tanner stage less than G3 and/or less than P3. In addition, 21% of the boys had a mean testicular volume $\leq$ 10 ml. This means that supraphysiological doses of androgens have been given in a period of testicular maturation and maybe even prior to spermarche. Theoretically, these high levels of exogenous androgens may have influenced intratesticular maturational processes directly or via the suppression of gonadotropins. In fact, it is known that FSH plays an important role in the initiation of spermatogenesis by effects on Sertoli cell replication and function, the regulation of the replication of differentiated spermatogonia, and on the degeneration process of germ cells (reviewed in: Sharpe, 1994 (8)). In addition, morphological and cytological changes have been described after androgen administration in adult testes of rat and human (18,19,20), and it has not extensively been studied whether these changes are reversible. Obviously, our study gives no insight into the nature of the dynamic processes during androgen therapy or into the presence of any intratesticular, morphological or histological changes. It is possible, however, that the higher levels of FSH reflect intratesticular changes (48), due to androgen

treatment received in a period of maturation. These increased FSH levels may compensate for partially disturbed germinal function in order to maintain normal sperm quality (49). On the other hand, the difference in gonadotropins may also reflect a change in responsiveness at the hypothalamic-pituitary level. It is known that steroids exert negative feedback effects at the level of the hypothalamus as well as the pituitary (50,51). At least part of this negative feedback is mediated by aromatization of testosterone to oestradiol (52). Animal models show regulating effects of oestradiol on neuronal development (53). Although conclusive data are lacking it may be possible that the high levels of steroids during puberty have induced alterations at the hypothalamic-pituitary level. In an attempt to get more insight into the underlying mechanism we determined inhibin levels in a small subgroup of 30 subjects. We found no significant difference in inhibin levels between these groups and observed no significant inverse relationship between inhibin and FSH concentration. Therefore the exact pathophysiological mechanism explaining the observed differences in gonadotropins remains to be verified.

One might surmise that treatment started at an early pubertal stage has more effect on testicular function than treatment started at a later point. Some of our findings are in concert with this theory as we observed a significant effect of the age at start of therapy on both the level of FSH and sperm motility. In addition, testicular size at start of treatment significantly influenced sperm concentration and total sperm count. On the other hand, semen quality and plasma hormone levels were not significantly different between the pubertal stages (Tanner stages P and G) at the start of therapy (data not shown).

Treatment with high doses of androgens induces reduction in testicular volume in adult men (5) as well as in tall adolescent boys (3,4,54). This implies major intratesticular changes during therapy such as a decrease in seminiferous tubule size (18,19). These processes are likely to be reversible as testicular volume normalizes after discontinuation of therapy (3-5,54). In our study, there was no difference in mean testicular volume between cases and controls. This is in contrast with the observations of Willig et al (21,22), who reported significantly smaller

testicular sizes in previously treated men.

In summary, our study shows that treatment with high doses of androgens for reduction of final height in constitutionally tall stature has no long-term side-effect on sperm quality, testicular volume and plasma testosterone levels. However, treated men had higher plasma FSH levels compared with controls; the meaning of this finding remains to be established. A left-sided varicocele was prevalent in 42% of the tall men.

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REFERENCES

1. Brämswig JH, Nieschlag E, Schellong G: Pituitary-gonadal function in boys after high dose testosterone treatment for excessively tall stature. Acta Endocrinol 1984;107:97-103
2. Brämswig JH, Von Lengerke HJ, Schmidt H, Schellong G: The results of short term (6 months) high-dose testosterone treatment on bone age and adult height in boys of excessively tall stature. Eur J Ped 1988;148:104-106
3. Prader A, Zachmann M: Treatment of excessively tall girls and boys with sex hormones. Pediatrics 1978;62:1202-1210
4. Zachmann M, Ferrandez A, Mürset G, Gnehm HE, Prader A: Testosterone treatment of excessively tall boys. J Pediatr 1979;88:116-123
5. Mauss J, Börsch G, Bormacher K, Richter E, Leyendecker G, Nocke W: Effect of long-term testosterone oenanthate administration on male reproductive function: clinical evaluation, serum FSH, LH, testosterone, and seminal fluid analysis in normal men. Acta Endocrinol 1975;78:373-384
6. Matsumoto AM: Effects of chronic testosterone administration in normal men: safety and efficacy of high dosage testosterone and parallel dose-dependent suppression of luteinizing hormone, follicle-stimulating hormone and sperm production. J Clin Endocrinol Metab 1990;70:282-287
7. WHO: Task force on methods for the regulation of male fertility: Contraceptive efficacy of testosterone-induced azoospermia in normal men. Lancet 1990;336:955-959
8. Sharpe RM: Regulation of spermatogenesis. In: Knobil E, Neill JD, eds The physiology of Reproduction. 2nd ed. New York: Raven Press 1994:1363-1434
9. Cortes D, Müller J, Skakkebaek NE: Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. Int J Androl 1987;10:589-596
10. Kretser de DM, McLachlan RI, Robertson DM, Wreford NG: Control of spermatogenesis by FSH and testosterone. Baillière's Clin Endocrinol Metab 1992;6:335-354
11. Matsumoto AM, Bremner WJ: Endocrinology of the hypothalamic-pituitary-testicular axis with particular reference to the hormonal control of spermatogenesis. Baillière's Clin Endocrinol Metab 1987;1:71-88
12. Müller J, Skakkebaek NE: Quantification of germ cells and seminiferous tubules by stereological examination of testicles from 50 boys who suffered from sudden death. Int J Androl 1983;6:143-156
13. Steinberger E: Hormonal control of mammalian spermatogenesis. Physiol Rev 1971;51:1-22
14. Kulin HE, Frontera MA, Demers LM, Bartholomew MJ, Lloyd TA: The onset of sperm production in pubertal boys: Relationship to gonadotropin excretion. Am J Dis Child 1989;43:190-193
15. Nielsen CT, Skakkebaek NE, Richardson DW, Darling JAB, Hunter WM, Jorgenson M, Nielsen A, Ingerslev O, Kelding N, Müller J: Onset of the release of spermatozoa (spermarche) in boys in relation to age, testicular growth, pubic hair, and height Journal of Clin Endocrinol Metab 1986;62:532-535
16. Richardson DW, Short RV: Time of onset of sperm production in boys. J Biosoc Science 1978;Suppl 5:15-25

17. Schaefer F, Marr J, Seidel C, Tilgen W, Scharer K: Assessment of gonadal maturation by evaluation of spermaturia. Arch Dis Child 1990;65:1205-1207
18. Barham SS, Berlin JD: Fine structure and cytochemistry of testicular cells in men treated with testosterone propionate. Cell and Tissue Research 1974;148:159-182
19. Heller CG, Nelson WO, Hill IB, Henderson E, Maddock WO, Jungck EC Paulsen CA, Mortimore GE: Improvement in spermatogenesis following depression of the human testis with testosterone. Fertil Steri 1950;1:415-422
20. Jezek D, Simunic-Banek L, Pezerovic-Panijan R: Effects of high doses of testosterone propionate and testosterone enanthate on rat seminiferous tubules-a stereological and cytological study. Arch Toxicol 1993;67:131-140
21. Willig RP, Hinkel GK, Brod R, Stahnke N, Müller-Möhring G, Horn K, Schirren C: Long-term side effects on testicular and pituitary function following testosterone treatment for tall stature in boys. Acta Endocrinol 1991;36(Suppl 1) (abstract)
22. Willig RP, Bettendorf M, Hinkel GK, Schwarz HP, Schulze W: Androgen treatment of tall stature during puberty may reduce sperm quality in adult life. Horm Res 1992;37(Suppl 4) :3 (abstract)
23. Chehval MJ, Purcell MH: Deterioration of semen parameters over time in men with untreated varicocele: evidence of progressive testicular damage. Fertil Steril 1992;57:174-177
24. Gorelick JI, Goldstein M: Loss of fertility in men with varicocele. Fertil Steril 1993;59:613-616
25. Haans LCF, Laven JSE, Mali WPTH, te Velde ER, Wensing CJG: Testis volumes, semen quality and hormonal patterns in adolescents with and without a varicocele. Fertil Steril 1991;56:731-736
26. WHO: The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. Fertil Steril 1992a;57:1289-1293
27. Vine MF, Margolin BH, Morrison HI, Hulka BS: Cigarette smoking and sperm density: a meta-analysis. Fertil Steril 1994;61:35-43.
28. Purvis K, Christiansen E: Infection in the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. Review. Int J Androl 1993;16:1-13
29. Yavetz H, Harash B, Paz G, Yogev L, Jaffa AJ, Lessing JB, Homonnai ZT: Cryptorchism: Incidence and sperm quality in infertile men. Andrologia 1992;24:293-297
30. Prader A: Testicular size: assessment and clinical importance. Triangle 1966;7:240-243
31. WHO: WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge, University Press 1992b
32. Makler A: The improved ten-micrometer chamber for rapid sperm count and motility evaluation. Fertil Steril 1980;33:337-338
33. Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Oehninger S: Predictive value of abnormal sperm morphology in in-vitro fertilization. Fertil Steril 1988;49:112-117
34. Verjans HJ, Cooke BA, de Jong FH, de Jong CMM, van der Molen HJ: Evaluation of a radioimmunoassay for testosterone estimation. J Steroid Biochem 1973;4:665-676

35. Robertson DM, Hayward S, Irby DC, Jacobson J, Clarke L, McLachlan RI, de Kretser DM Radioimmunoassay of rat serum inhibin: changes after PMSG stimulation and gonadectomy. Mol Cell Endocrinol 1988;58:1-8
36. McLachlan RI, Matsumoto AM, Burger HG, de Kretser DM, Bremner WJ: Follicle-stimulating hormone is required for quantitatively normal inhibin secretion in men. J Clin Endocrinol Metab 1988;67:1305-1408
37. Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;i:307-310
38. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE: Evidence for decreasing quality of semen during past 50 years. Br Med J 1992;305:609-613
39. Barratt CLR, Dunphy BC, Thomas EJ, Cooke ID: Semen characteristics of 49 fertile males. Andrologia 1988;20:264-269
40. Hommonnai ZT, Patz G, Weiss JW, David MP: Quality of semen obtained from 627 fertile men. Int J Androl 1980;3:217-228
41. Burger HG, de Kretser DM, Hudson B, Wilson JD: Effects of preceding androgen therapy on testicular response to human pituitary gonadotropin in hypogonadotropic hypogonadism: a study of three patients. Fertil Steril 1981:35:64-68
42. Delemarre-Van de Waal HA: Induction of testicular growth and spermatogenesis by pulsatile, intravenous administration of gonadotrophin-releasing hormone in patients with hypogonadotrophic hypogonadism. Clin Endocrinol 1993;38:473-480
43. Hargreave TB: Varicocele - A clinical enigma. Review. Br J Urol 1993;72:401-408
44. Laven JSE: The varicocele. Experimental animal models and a randomized clinical study in adolescents. 1991 - Thesis.
45. Janczewski Z, Bablok L: Semen characteristics in pubertal boys. III. Semen quality and somatosexual development. Arch Androl 1985b;15:213-218
46. Nysom K, Pedersen JL, Jorgensen M, Nielsen CT, Müller J, Keiding N, Skakkebaek NE: Spermaturia in two normal boys without other signs of puberty. Acta Paediatr 1994;83:520-521
47. Janczewski Z, Bablok L: Semen characteristics in pubertal boys: I. Semen quality after first ejaculation. Arch Androl 1985a;15:199-205
48. Bergmann M, Behre HM, Nieschlag E: Serum FSH and testicular morphology in male infertility. Clin Endocrinol 1994;40:133-136
49. Karpas AE, Matsumoto AM, Paulsen CA, Bremner WJ: Elevated serum follicle-stimulating hormone levels in men with normal seminal fluid analyses. Fertil Steril 1983;39:333-336
50. Finkelstein JS, O'Dea LL, Longcopes C, Schoenfeld DA, Crowley WF: Sex steroid control of gonadotropin secretion in the human male. I. Effects of testosterone administration in normal and gonadotropin-releasing hormone-deficient men. J Clin Endocrinol Metab 1991a;73:609-620
51. Finkelstein JS, O'Dea LL, Whitcomb RW, Crowley WF: Sex steroid control of gonadotropin secretion in the human male. II. Effects of estradiol administration in normal and gonadotropin-releasing hormone-deficient men. J Clin Endocrinol Metab 1991b;73: 621-628

52. Bagatell CJ, Dahl KD,, Bremner WJ: The direct pituitary effect of testosterone toinhibit gonadotropin secretion in men is partially mediated by aromatization to estradiol. J Androl 1994;15:15-21
53. Yang SL, Hsu C, Hsu HK, Liu KM, Peng MT: Effects of long-term estradiol exposure on the hypothalamic neuron number. Acta Endocrinol 1993;129:543-547
54. Brämswig JH, Schellong G, Borger HJ, Breu H: Testosteron-Therapie hochwüchsiger Jungen. Deutsche Med Wochenschr 1981;106:1656-1661
55. Tanner JM, Whitehouse R: Longitudinal standards for height, weight-height, height velocity and stages of puberty. Arch Dis Child 1976;51:170-179

CHAPTER 7

A new model to predict final height in constitutionally tall children.

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ABSTRACT

In order to develop new height prediction models for children with constitutionally tall stature (CTS) 55 boys and 88 girls with CTS were recalled to our outpatient clinic for measurement of adult final height (FH). Auxological data, including height (H), age (CA), and target height (TH), were collected from the hospital charts and radiographs of the left hand and wrist were retrieved and used for bone age (BA) determination (BA according to the methods of Greulich and Pyle (BA_{GP}) and Tanner and Whitehouse (BA_{RUS})). Standard multiple regression techniques were used to develop prediction equations for FH. In addition, to test the predictive capability of the newly derived prediction models, FH was measured in a second group of constitutionally tall children (n=32) and compared with the predicted FH according to our models. In addition, a comparison was made with other existing prediction methods. Mean (SD) FH was 196.0 (4.9) cm in boys and 180.5 (3.8) cm in girls. The ultimate regression equation for boys was: FH (cm) = 216.07 + 0.75 x H + 0.25 x TH - 11.09 x CA - 14.02 x BA_{GP} + 0.74 x (CA x BA_{GP}). For girls the final regression equation was: FH = 161.42 + 0.73 x H + 0.15 x TH - 8.41 x CA - 8.83 x BA_{RUS} - 2.45 x M + 0.55 x (CA x BA_{RUS}). The models showed satisfying accuracy in height prognosis: the mean (SD) error of prediction were -1.4 (3.2) cm in boys and -0.5 (3.1) cm in girls with corresponding mean (SD) absolute errors of 2.7 (2.2) cm and 2.0 (2.4) cm, respectively. Compared to the currently available prediction methods, the newly derived models were found to be quite promising. Their clinical additional usefulness has to be ascertained in larger groups of tall children.

INTRODUCTION

Constitutionally tall stature is a variant of the normal pattern of childhood growth and constitutes 3-10 percent of the normal population, depending on the

definition used. Prediction of adult height is an important clinical tool in the management of tall children. In fact, therapeutic intervention is usually based on the estimated height prognosis. Hence, accurate techniques for reliable height predictions are needed. In clinical practice various prediction methods are being used (1-4). Most existing methods are based on growth data derived from normal growing children. Only the revised prediction equations described by Tanner et al (3) included longitudinal growth data of a sample of 19 tall girls. Thus applying height predictions to children with excessive growth may give inaccurate results. Recently, we have described the accuracy of various height prediction methods in tall statured boys and girls (5). It appeared that the current prediction models give more accurate results in tall girls compared to tall boys. Nevertheless, the mean absolute errors of the single prediction methods were still considerable, varying from 2.3 to 5.3 cm in boys and from 1.9 to 3.7 cm in girls with individual errors up to 20 cm, reflecting the relativity of its accuracy.

In this study we describe new prediction models for constitutionally tall children based on a dataset of 55 untreated tall boys and 88 untreated tall girls. In addition, we tested the reliability of these models in 32 children (16 boys/16 girls) and compared them with other existing prediction methods.

PATIENTS AND METHODS

Study protocol

Study sample

Selection of the study population has extensively been described previously (5). In short, 145 men and 174 women (date of birth before 1975), who were seen at adolescence for evaluation of constitutionally tall stature at the Sophia Children's Hospital, Rotterdam, were contacted by mail to participate in a follow-up study. No subject had received treatment interfering with longitudinal growth. Ultimately, 62 men and 95 women participated in the study after informed consent was obtained.

Auxological data including growth data, pubertal stage and parental heights were collected from the hospital charts. Radiographs of the left hand and wrist were retrieved and used for bone age (BA) determinations. For each subject every first available radiograph was used from the age of 8 onwards, providing only one measurement per subject for analysis.

Constitutionally tall stature was defined as a height equal to or above the 90th percentile according to Dutch references (6) at the time of referral. Seven men and 7 women were excluded from analysis because their heights were less than the 90th percentile according to their hospital charts and/or because their corresponding radiographs could not be retrieved from the archives for re-assessment of skeletal age. Therefore, data of in total 55 men and 88 women were included in this study.

Subjects were recalled to our outpatient clinic for measurement of final height (FH). Height measurements were performed by one investigator using a Harpenden stadiometer. The mean (SD) age at follow-up was 26.0 (4.4) years (range: 18.7 - 34.4 years) for men and 24.7 (4.0) years (range: 18.7 - 34.2 years) for women. BA was rated according to the methods described by Greulich and Pyle (8) and Tanner et al (3) (TW2-RUS BA) by one investigator.

Validation sample

In a second step, another group of untreated tall subjects (n=55, date of birth between 1975-1976, i.e. 18 years or older at time of final height measurement) was contacted by mail to participate in a validation study of our newly developed regression models. All subjects had visited our institute for constitutionally tall stature and had eventually received no hormonal treatment. In total 34 patients (16 boys/18 girls) agreed to participate after obtained informed consent. Final height was measured as described above and radiographs of the hand and wrist were retrieved for BA-assessment. Again, every first available X-ray was used from the age of 8 yrs onwards providing one measurement per subject for analysis. Two girls were excluded from analysis: one because her height at time of referral was

less than the 90th percentile according to Dutch references (6) and another because her age at time of referral was less than 8 years. Therefore, data of 16 boys and 16 girls were used for this validation study. Final adult height was predicted using our newly developed regression models and using the methods of Bailey and Pinneau (1) (*BP-prediction*), and Tanner et al (3) (TW Mark II, *TW-prediction*). In addition, the index of potential height (4) (*IPH_{GP}-prediction*) was calculated based on the assumption that the height SDS for BA remains constant up to final height. The BA according to Greulich and Pyle was used for the IPH-prediction since we have shown previously that this method was quite reliable in tall children (5).

The study protocol was approved by the ethical committee of Academic Hospital, Erasmus University Rotterdam, The Netherlands.

Statistical procedure

Study sample

Statistical analysis involved the use of standard multiple regression techniques. Since growth was expected to be different for males and females, men and women were analyzed separately. Separate regression models for boys and girls were obtained in which the dependent variable Y (= adult final height (FH in cm)) was expressed as a combination of predictor variables. In advance we decided to include the following possible predictor variables: chronological age (yrs) at time of measurement (CA), BA (yrs) according to Greulich and Pyle (BA_{GP}) and Tanner et al (BA_{RUS}) at time of measurement, height (cm) at time of measurement (H), target height (cm) (TH), and menarche (only girls) at time of measurement (M;1=yes, 0=no). The TH was calculated according to the formula's: (Mother's height + Father's height +12)/2 + 3 for boys and (Mother's height + Father's height -12)/2 + 3 for girls (8). Age at time of referral ranged from 8.75 to 17.17 yrs in boys and from 8.99 to 16.49 yrs in girls. Since BA determinations are *estimates* of skeletal maturation we included two different techniques of BA assessment assuming that two estimates will approximate the real value more

reliably.

The first step of the analysis involved a backward procedure on all predictor variables. We did not investigate every possible interaction between the included variables because it would greatly increase the risk for spurious findings. However, interaction terms between age and the remaining variables were thought to be of importance. Therefore, in the second step, the model was extended in a stepwise, forward manner adding these interaction terms to the model. The cut-off level for significance was set at P=0.05. The residuals of the models were analyzed in order to check its validity. Too small numbers per age-class of a year were present in order to be able to construct age-class specific equations as done by Tanner and co-workers (2,3). The prediction equations were in the form:

FH a function of CA, BA_{GP}, BA_{RUS}, H, TH, (M in girls), and interactions with CA (CA x BA_{GP} etc.).

Puberty was not entered as a predictor variable since data on pubertal stages as derived from the hospital records were incomplete.

Validation sample

In a second analysis, data of 32 untreated tall subjects (16 boys/16 girls) were used to test the predictive capability of the newly derived prediction models. In addition, the accuracy of the new models to predict FH in these subjects was compared with that of the *BP-, TW*, and *IPH_{GP}-prediction* as described above. For this purpose, the accuracy of the height prediction was expressed mathematically as predicted height minus observed FH (in cm). Therefore, positive values indicate overestimation whereas negative values indicate underestimation of the adult height by the method applied. In addition, the reliability of each height prediction method was also expressed in absolute errors. The absolute error demonstrates the method's overall predictive error ignoring over- or underestimation. Wilcoxon's matched pairs signed rank test was used to test the differences in absolute errors between the separate prediction methods.

RESULTS

Prediction model

BOYS

Mean FH of the 55 untreated tall men was 196.0 (4.9) (range: 186.6 - 209.3 cm). Their mean TH was 187.6 (5.4) cm, ranging from 174.5 to 200.5 cm.

Backward multiple regression analysis revealed that all included variables had a significant predictive effect on FH ($P<0.001$), except for CA at time of measurement ($P=0.30$) and BA_{RUS} ($P=0.43$) (Table 1a). In the next procedure BA_{RUS} was removed from analysis. We decided, however, not to exclude CA from the model since we thought CA to be an important objective parameter and we wanted to look for its interactions. Forward, stepwise multiple regression analysis including the interaction terms between CA and the remaining variables, revealed a significant additional effect of the interaction CA x BA_{GP} ($P<0.001$) (Table 1b), providing the following regression equation for FH prediction: $FH= 216.07 + 0.75 \times H + 0.25 \times TH - 11.09 \times CA - 14.02 \times BA_{GP} + 0.74 \times (CA \times BA_{GP})$. In this model, the percentage explained variability of FH (R^2) was 77% and the residual standard deviation (RSD) was 2.5 cm. Analysis of the residuals revealed no evidence for violations of the model.

GIRLS

Mean FH of the 88 untreated tall women was 180.5 (3.8) cm ranging from 171.1 to 188.3 cm. Their mean TH was 175.0 (4.4) cm (range: 165.5-187.8 cm).

Backward multiple regression analysis on FH including all variables revealed that TH, BA_{RUS} and H had a significant contribution to the model (P-values <0.01), whereas CA ($P=0.87$), BA_{GP} ($P=0.16$) and M ($P=0.08$) had not. After removal of BA_{GP}, the remaining variables showed a significant predictive effect on final adult height ($P<0.05$), except for CA at time of measurement ($P=0.71$) (Table 2a). For the same reason as mentioned in boys we decided not to exclude CA from the model. Subsequent forward stepwise multiple regression analysis including the

Table 1a Results of backward, stepwise multiple regression analysis on final height in boys with tall stature.

Variable	Regression coefficient	SE	P-value
Height	0.56	0.11	<0.0001
Target height	0.34	0.09	0.0003
BA_{GP}	-3.11	0.87	0.0008
CA	-0.68	0.64	0.30
BA_{RUS}	-0.56	0.71	0.43
Constant	91.48		

RSD: 3.1 cm; R^2:0.63

Table 1b Results of stepwise forward multiple regression analysis on interaction terms on final height in boys with tall stature.

Variable	Regression coefficient	SE	P-value
Height	0.75	0.09	<0.0001
BA_{GP}	-14.02	1.93	<0.0001
CA	-11.09	1.94	<0.0001
CA x BA_{GP}	0.74	0.13	<0.0001
Target height	0.25	0.07	0.0007
Constant	216.07		

Final equation: FH = 216.07 + 0.75 x H + 0.25 x TH -11.09 x CA - 14.02 x BA_{GP} + 0.74 x (CA x BA_{GP})
RSD: 2.5 cm; R^2: 0.77

BA_{GP} = Bone age according to Greulich and Pyle (yrs)
BA_{RUS} = Bone age according to Tanner et al (yrs)
CA = Chronological age at time of measurement (yrs)
SE = Standard error

Table 2a Results of backward multiple regression analysis on final height in girls with tall stature.

Variable	Regression coefficient	SE	P-value
Height	0.53	0.09	<0.0001
BA_{RUS}	-1.93	0.40	<0.0001
Target height	0.24	0.07	0.0012
Menarche	-1.85	0.89	0.041
CA	-0.18	0.48	0.71
Constant	77.76		

RSD: 2.8 cm; R^2: 0.52

Table 2b. Results of stepwise forward multiple regression analysis on interaction terms on final height in girls with tall stature.

Variable	Regression coefficient	SE	P-value
Height	0.73	0.08	<0.0001
BA_{RUS}	- 8.83	1.60	<0.0001
CA	- 8.42	1.90	<0.0001
CA x BA_{RUS}	0.55	0.12	<0.0001
Menarche	- 2.45	0.81	0.0035
Target height	0.15	0.07	0.029
Constant	161.42		

Final equation: FH = 161.42 + 0.73 x H + 0.15 x TH - 8.42 x CA - 8.83 x BA_{RUS} - 2.45 x M + 0.55 x (CA x BA_{RUS})
RSD: 2.5 cm; R^2: 0.61

BA_{RUS} = Bone age according to Tanner *et al* (yrs)
CA = Chronological age at time of measurement (yrs)
SE = Standard error

interaction terms between CA and the remaining variables, revealed a significant additional effect of the interaction CA x BA_{RUS} ($P<0.001$). Results of this multiple regression model, given in Table 2, provided the following equation for predicting FH: FH= 161.42 + 0.15 x TH + 0.73 x H - 8.41 x CA - 8.83 x BA_{RUS} -2.45 x M + 0.55 x (CA x BA_{RUS}). The percentage explained variability of FH (R^2) was 61% and the RSD was 2.5 cm. Analysis of the residuals revealed no evidence for violations of the model.

Accuracy

Clinical data of the 32 subjects of the validation sample are summarized in Table 3. The error of prediction and the absolute errors of the newly derived regression equations in comparison with those of the *BP-, TW-*, and *IPH-predictions* are given in Table 4.

Table 3 Clinical data of 32 tall children (16 boys/16 girls) at time of height prediction. Data expressed as mean (SD) and [range].

Clinical data	Boys (n=16)	Girls (n=16)
CA (yrs)	13.14 (1.56) [10.09 - 15.74]	11.73 (1.46) [8.39 - 14.02]
Height (cm)	181.0 (12.8) [155.5 - 198.1]	166.8 (8.9) [144.6 - 177.0]
Height SDS_{CA}	2.75 (0.70) [1.79 - 3.83]	2.13 (0.66) [1.28 - 3.62]
BA_{GP} (yrs)	13.8 (2.0) [10.0 - 18.0]	11.9 (1.4) [8.5 - 13.8]
BA_{RUS} (yrs)	14.5 (2.3) [8.8 - 18.2]	13.0 (1.2) [9.3 - 14.6]
Target height (cm)	190.2 (3.1) [183.9 - 195.0]	176.4 (6.4) [165.5 - 187.1]
Final height (cm)	197.6 (2.6) [192.3 - 202.0]	180.9 (4.5) [174.4 - 190.8]

CA = Chronological age at time of measurement
BA_{GP} = Bone age according to Greulich and Pyle
BA_{RUS} = Bone age according to Tanner et al

In boys, the *BP-prediction* overestimated FH by a mean of 2.3 cm, whereas the *TW-*, and *IPH_{GP}-prediction* gave an underestimation of 0.7 and 0.2 cm, respectively. The SD's of the mean errors of these methods ranged from 3.8 to 4.7 cm. The mean error of our model was -1.4 cm, but showed the smallest SD of 3.2 cm. The mean absolute error of our model was 2.7 (2.2) cm. In comparison, the BP- and TW-prediction had a mean absolute error of more than 4 cm. The IPH_{GP}-prediction showed a comparable absolute error of 2.7 cm, but had a slightly larger SD. The difference in absolute errors between our model and the others reached significance only for the the *TW-prediction* ($P<0.04$).

In girls, all prediction methods underestimated FH by 0.2 to 2.5 cm with corresponding SD ranging from 2.8 to 3.7 cm. The mean absolute errors varied from 2.0 to 2.9 cm, with our our model showing the smallest error. However, no significant differences were found between the absolute errors of the four prediction methods (P-values> 0.05).

Table 4. Accuracy of height prediction (error and absolute error in cm) as estimated by our regression equations in comparison with the *BP-, TW-, and IPH_{GP}-prediction* in 32 tall children. Data expressed as mean (SD) and [range].

	Error of prediction (cm)				Absolute error of prediction (cm)			
Prediction method	New Model	BP	TW	IPH_{GP}	New Model	BP	TW	IPH_{GP}
Boys (n=16)	-1.4 (3.2) [-7.3; 5.6]	2.3 (4.7) [-6.6;10.6]	-0.7 (5.2) [-8.8; 9.6]	-0.2 (3.8) [-7.8; 7.4]	2.7 (2.2) [0.3; 7.3]	4.1 (3.2) [0.0;10.6]	4.2 (2.9)* [0.3; 9.6]	2.7 (2.6) [0.0; 7.8]
Girls (n=16)	-0.5 (3.1) [-8.0; 5.8]	-0.2 (3.5) [-6.4; 5.4]	-2.5 (2.8) [-10.8; 0.3]	-0.6 (3.7) [-7.5; 5.7]	2.0 (2.4) [0.3; 8.0]	2.7 (2.1) [0.3; 6.4]	2.6 (2.7) [0.3; 10.8]	2.9 (2.3) [0.1; 7.6]

* Significantly different with the regression model ($P<0.04$)

DISCUSSION

It is generally agreed, that prediction models derived from a population of normal growing, normal statured children should be applied to a group children with growth disorders with considerable caution (3,9). Hence, for children growing at the upper extremes of normal, that is beyond +2 SD above the mean of the normal population, it seems to be far better to use prediction models based on growth data derived from a sample of tall children. So far, only the regression equations described by Tanner et al have been constructed from data with inclusion of a small sample of tall children (3). Unfortunately, only girls had been been included. We developed a new prediction model for boys and girls with tall stature based on a set of auxological data derived from 55 tall boys and 88 tall girls. Our sample of untreated, healthy tall children came from a clinical population who sought medical advice with respect to their tallness at adolescence. Therefore, it comprises an excellent reference group for clinical practice. It should be noted, however, that this group of untreated patients was not strictly comparable with patients who had ultimately received therapeutic intervention; this latter group had a significantly higher height prognosis and a significantly younger BA, as shown previously (5).

The accuracy of a regression model is reflected in its residual standard deviation (RSD); the smaller the RSD the better the model predicts the dependent variable (in our case: FH) by the combination of predictor variables. In our final models, the RSD was 2.5 cm for both boys and girls. This implies that about 95% of the predictions lie within approximately 5 cm of the real value (± 2 RSD). From a practical point of view, this seems to be a rather large range. However, compared to other prediction models it is quite acceptable. Tanner et al (3), constructing regression equations in an age-specific manner, reported RSDs up to 4.1 cm in boys and 3.6 cm in girls for the same age ranges as in our population; the RSD's decreased with increasing age. In addition, Karlberg and co-workers recently described age-dependent regression models based on growth data from birth to

final height of 3650 children (9). Their smallest RSD was 0.69 height SD, which represents approximately 4 cm.

The explained variability of FH (R^2) in our models was 0.77 and 0.61 in boys and girls, respectively. This is in the same order of magnitude as reported by others (3,9). We used simple anthropometric data and included BAs as estimators for skeletal maturation in order to predict FH. It must be remembered however, that linear growth is the resultant of very complex processes influenced by a number of exogenous and endogenous factors including social class, nutrition and hormones (10,11). Many of these influencing factors have not been included in our model, nor in others. In addition, the timing or the intensity of the adolescent growth spurt may also contribute to the source of prediction error (12). A reliable variable expressing this phenomenon however, is lacking. Unless substantial predictor variables are identified, which, preferably, can be easily obtained in an outpatient clinical setting, it is unlikely that significant improvement will be made in the prediction of adult height.

We tested the reliability of the prediction models in a separate group of 32 patients and compared it with three other, widely used prediction methods: the *BP-*, *TW-*, and *IPH_{GP}-prediction* (1-4). In this sample, height prediction in tall girls was more accurate than in tall boys. This is in concert with our previous report on height prognosis in children with constitutionally tall stature (5). Our regression models slightly underestimated FH by approximately 0.5 cm in girls and 1.5 cm in boys. The absolute errors of the models were 2.0 cm for girls and 2.7 cm for boys. In comparison with the currently available prediction methods, our model certainly has a contributive value in height prediction in tall children as shown by the small absolute errors. In addition, especially in boys, the SDs of both the mean error and the absolute error of our newly developed models were smaller (or comparable in girls) compared to the other prediction methods, indicating less individual variation. Although the reliability of our models had only been tested in a small group of tall children, these preliminary data are quite promising. They justify the idea that height prognosis in children with tall stature is more accurate when based on

growth data derived from tall children. Applying the models to a larger group of children with excessive growth is recommended to ascertain its clinical additional use. In addition, combining the dataset of both the study sample and the validation sample will enlarge the sample size and increase its predictive capability (see note).

In summary, new prediction models are presented for boys and girls with constitutionally tall stature based on a dataset of 143 untreated tall children. The reliability of these models was tested in a separate group of 32 patients and compared to three other prediction methods. The models showed satisfying accuracy in height prognosis and were found to be quite promising compared to the currently available prediction methods. Their clinical additional usefulness has to be ascertained in larger groups of tall children.

Note: the regression equations based on the combined samples of untreated tall children were:

Boys (n=71): FH= 213.66 + 0.62 x H + 0.29 x TH - 10.49 x CA - 12.98 x BA_{GP} *+ 0.72 x (CA x* BA_{GP}*); RSD = 2.6 cm.*

Girls (n=103): FH= 130.53 + 0.76 x H + 0.15 x TH - 5.63 x CA - 1.51 x BA_{RUS} *- 6.19 X* BA_{GP} *+ 0.39 x (CA x* BA_{GP}*); RSD = 2.6 cm.*

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REFERENCES

1. Bailey N, Pinneau SR: Tables for predicting adultheight from skeletal age, revised for Greulich and Pyle hand standards. J Pediatr 1952;40:423-441
2. Tanner JM, Whitehouse RH, Marshall WA, Healy MJR, Goldstein H: Assessment of skeletal maturity and prediction of adult height: TW2 method. Academic Press, New York 1975
3. Tanner JM, Whitehouse RH, Cameron N, Marshall WA, Healy MJR, Goldstein H: Assessment of skeletal maturity and prediction of adult height (TW2 method). Academic Press, London 1983
4. Lenko HL: Prediction of adult height with various methods in Finnish children. Acta Paediatr Scand 1979;68:85-92
5. De Waal WJ, Greyn-Fokker MH, van Gurp EAFJ, Toolens AMP, Stijnen Th, de Muinck Keizer-Schrama SMPF, Drop SLS: Accuracy of final height and effect of growth reductive therapy in 362 constitutionally tall children. J Clin Endocrinol Metab 1996 (March); in press
6. Roede MJ, van Wieringen JC: Growth diagrams 1980, Netherlands. Third nation-wide survey. Tijdschr Soc Gez 1985;62(Suppl):1-34
7. Greulich WW, Pyle SI: Radiographic atlas of skeletal development of the hand and wrist. Stanford University Press, Stanford 1959
8. Van den Brande JVL, Degenhart HJ: Endocrinologie en groeistoornissen. In: Kindergeneeskunde, van den Brande, Monnens (eds). Wetenschappelijke Uitgeverij Bunge, Utrecht 1993
9. Karlberg J, Lawrence C, Albertsson-Wikland K: Prediction of final height in short, normal and tall children. Acta Paediatr Scand 1994;406(Suppl):3-9
10. Mascie-Taylor CGN: Biosocial influences on stature: a review. J Biosoc Sci 1991;23:113-128
11. Loveridge N, Farquharson C, Scheven BAA: Endogenous mediators of growth. Proc Nutr Soc 1990;49:443-450
12. Preece MA: Prediction of adult height: methods and problems. Acta Paediatr Scand 1988;347(Suppl):4-11

CHAPTER 8

Psychosocial aspects in constitutionally tall stature.

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ABSTRACT

Data on psychological aspects in constitutionally tall stature are scarce. We studied psychosocial status in 424 adult men and women with constitutionally tall stature (height > P90). Two hundred forty-three subjects (69 boys and 174 girls) had been treated for their tallness during childhood with high doses of sex hormones (cases). Hundred eighty-two tall subjects (76 boys and 106 girls) had not chosen for treatment and served as controls. Psychosocial information was obtained via five different questionnaires. Special areas of interest concerned self-esteem, social anxiety, psychological well-being and body cathexis. In addition, educational level, employment, marital status and specific height related aspects were studied. No distinct differences between previously treated tall subjects and tall controls were found in self-esteem, social anxiety, psychological well-being and body perception. However, in tall men self-esteem was significantly higher than the test norms for both cases and controls ($P<0.02$ and $P<0.001$, respectively). In addition, previously treated tall men had less social anxiety and showed more assertiveness compared to tall controls and the norm population (P-values<0.02). In retrospect, previously treated tall men and women experienced more problems related to their height than tall controls; they had been teased more frequently and experienced more remarks or jokes about their stature (P-values<0.03). Tall subjects did very well in terms of education and employment: they showed higher levels of education and had a higher percentage of students compared to the Dutch population of the same age range (P-values <0.01). In conclusion, no major psychological maladjustment was found in previously treated tall children compared to tall controls. It is speculative whether the administration of high doses of sex steroids or possible psychological mechanisms such as denial may have contributed to these favourable outcomes. Height related problems such as teasing and hurtful remarks may have played a role for the choice in favour of height reductive therapy.

INTRODUCTION

Psychosocial problems in tall adolescent boys and girls have been recognized by pediatricians and endocrinologists. This has led to intervening therapies with high doses of sex steroids in order to reduce their final adult height (1-4). Although psychosocial factors form the main reason for treatment extensive psychological investigation before or during height reductive therapy has never been performed. Despite this, many clinicians share their experiences that some children with excessive growth may suffer considerably from being much taller than others. A commonly heard concern is that these children feel different from their peers and are subject to hurtful remarks about their height. As a consequence, coping mechanisms such as kyphotic posture (in order to mask the tall stature), social withdrawal and even depression have been noticed. Practical problems concerning clothing and shoeing, fear about future compatible partnering (especially in girls) and careerplanning are also frequently reported problems faced by tall children (1-4).

There is no doubt however, that many tall adolescents have no concern at all about their height. In fact, in our culture, tallness is generally valued positively. An association between physical stature and achievement has been documented: taller individuals score higher on intelligence tests, even when various interfering factors are taken into account (5,6). Individuals who have achieved higher social status tend to be taller than those of lower status (7,8). In addition, experimental studies have shown that tall individuals are regarded to be more competent. For instance, in the study of Dannenmaier and Thumin (9) individuals with higher assigned status were ascribed greater heights. Furthermore, competence expectations from parents and adults may vary with the physical stature of the child assigning more difficult tasks to taller children (10). In self-report scales of 82 9-11-year-old girls, height was linked to superior adjustment and career importance (11).

Typically, many tall children and their parents seek for medical attention at the time of puberty. It is commonly believed that the process of pubertal

development is interrelated with changes in psychological functioning (12,13). Therefore, changes in physical appearance may affect self-perception. Indeed, various studies in adolescent boys and girls have shown associations between physical status and self-esteem or body satisfaction (11,14-16). Studies relating height and self-esteem, however, showed conflicting results. Coopersmith (17) found no significant relationship between the actual height and self-esteem in 85 pre-adolescent and adolescent males. Prieto and Robbins (18) on the other hand, studying 69 young adolescent males, found positive and significant relationships between their own, peers' and teacher's perceptions of their height and self-esteem, although no relation was observed between the actual height and self-esteem. Nottelman and Welsh (19) examined the relationship between stature and competence perceptions in 126 sixth- and seventh-grade children. They found that tall girls in their last year of elementary school rated themselves lowest on general competence. In secondary school however, short girls rated themselves lowest and tall stature was regarded more favorably. Results in boys were less pronounced, but showed that tall boys regarded themselves less competent than short boys in secondary school. In addition, Hensley (20) studied 85 male and 125 female undergraduates and revealed no distinct pattern for height and self-esteem for either gender.

Our study was set out to obtain more insight into the psychosocial status of a group of constitutionally tall adult men and women, part of whom had been treated for their tallness during childhood. Main goal of the study was to investigate whether or not previously treated tall subjects differed in psychological functioning from tall subjects who had not chosen for any intervening therapy. For this purpose, psychological questionnaires were send to the participants and comparisons were made between treated and untreated tall subjects. Special areas of interest concerned self-esteem, social anxiety and assertiveness, general psychological well-being and body-cathexis. The latter refers to the degree of (dis)satisfaction with parts of the body. In addition, we evaluated the influence of height on socioeconomic status and relationships by comparison with data from

the Dutch population.

METHODS

Subjects

Since the introduction of height reductive therapy in our institute in 1968, 247 men and 423 women, who were seen at adolescence for evaluation of their constitutionally tall stature, had reached the age of 18 at the time of our follow-up study. All were contacted by mail to participate in the study. Second mailings were send to those who did not respond to the first mailing. This study was part of a large follow-up study in constitutionally tall stature, including auxological assessments and long-term sequelae. Reports on auxology and long-term sequelae are reported elsewhere (21-23).

During their puberty, 102 men and 249 women had been treated for their tall stature (cases). The indication for treatment was usually a predicted final height > 2.5 SD above the mean (> 180 cm for girls and > 200 cm for boys). Occasionally, treatment was initiated in idiopathic scoliosis. Treatment consisted of high doses of sex steroids. In girls, estrogens have been used (ethinylestradiol 200 μg/day orally, range: 100-300 μg/day), while boys have been treated with androgens (testosterone esters in various regimens with a total monthly dosage up to 1000 mg intramuscular). Hundred and forty-five men and 174 women had not chosen to undergo treatment for various reasons such as satisfaction with the given height prognosis or uncertainty about possible side-effects, and served as a comparison group (controls). Two hundred and nine men and 326 women responded to our mailing. Of the responders, 145 men (69 cases and 76 controls) and 280 women (174 cases and 106 controls) agreed to participate in this part of study (see Table 1). All participants gave informed consent.

In women, the mean (SD) age at the time of the study was 25.5 (5.0) yrs [range: 18.7 - 35.8 yrs] for cases and 24.6 (4.0) yrs [range: 18.7 - 34.2 yrs] for controls (P=0.14). Part of the women had also agreed to participate in the

auxological part of our follow-up study in which final adult height was measured (21). Therefore, final height was available in 158 cases and 92 controls and was 181.7 (3.3) cm and 180.5 (3.8), respectively ($P<0.02$).

In men, the mean (SD) age at the time of the study was 24.3 (4.1) yrs. [range: 18.7 - 34.4 yrs.] for cases and 25.7 (4.3) yrs. [range: 18.7 - 34.4 yrs] for controls ($P=0.06$). Like in the women, part of the men had participated in the auxological study so that measured final height was available (21). In 61 cases mean (SD) final height was 199.7 (3.9) and in 62 controls 196.3 (5.1) ($P=0.0001$).

Table 1. Response rate of 247 tall males and 423 tall females.

Subjects		Number	Response (%)	Participants (%)*
MALE	Cases	102	95 (93)	69 (73)
	Controls	145	114 (79)	76 (67)
FEMALE	Cases	249	203 (82)	174 (86)
	Controls	174	123 (71)	106 (86)

* Percentage of responders

Procedure

In order to obtain psychosocial information five different questionnaires were sent to the participants by mail. Completion of the questionnaires took approximately 45 minutes. Most of the subjects returned their questionnaires by hand, while visiting our outpatient clinic for final height measurement as part of the auxological study. The remainder was returned by mail. If no reply was received within two months, subjects were contacted by phone for reminding. Once the questionnaires were returned, no action was undertaken to fill in missing data.

Measures

1. The "Delft Questionnaire" (24) is a screening instrument for general unspecified psychological problems and was used to gain a global

psychological impression. It contains 33 items, giving a maximum total score of 33 and a minimum of zero. A score of 21 or higher is suggested by the test as a cut-off point for further psychological investigation. The test is normed according to a Dutch sample of 665 men and 1167 women.

2. Social anxiety and assertiveness was measured by the "Inventory List on Association with Others" (25). This Dutch questionnaire is a self-reported inventory and contains two scales of social activities: overall social discomfort during contact with others (Discomfort) and overall social frequency of this contact (Frequency). The scales contain five subscales each: concerning passing criticism, expressing personal opinions, paying compliments to others, initiating conversations, and stating positive self-assertiveness. The scales Discomfort and Frequency contain 35 items on a 5 point Likert scale. Total scores of the two scales are obtained by addition of all 35 items (minimum: 35, maximum: 175). The total scores of the various subscales are scored with use of a test-key. The test is supplied with a norm-table of a Dutch reference population of 130 men and 146 women with a mean (SD) age of 38.6 (13.4) yrs.
3. Self-esteem (positive attitude towards work, flexibility and well-adaptedness) was measured by a subscale of the "Dutch Personality Questionnaire" (26), which is a translated and shortened version of the California Psychological Inventory. The subscale contains 19 items on a three points scale. After addition, a total score is obtained (maximum: 38, minimum:0) that can be compared to a Dutch norm scale based on a sample of 2730 men and 2956 women with a mean (SD) age of 34.6 (13.6) years.
4. The degree of satisfaction with various parts of the body was measured by a Body Cathexis List. This list was originally developed by Secord and Jourard (27) but it was translated and modified for this study. The list consists of various body features (25 in boys and 24 in girls) which the subject is asked to rate on a 5-point Likert scale varying from 'very satisfied (1)' to 'very dissatisfied (5)'. The total score is calculated by addition of all items divided

by its number.

5. A self-constructed, prestructured questionnaire (Standard Questionnaire) with open and multiple choice answers was used to gather information about present socio- economic status including education, employment and marital status. In addition, specific height related questions had been included to obtain information about possible psychological distress of height in the social context. For this purpose we questioned the subjects about teasing and hurtful remarks, careerplanning, partnering and perception of height. Most of these questions were put in a categorical manner and subjects were asked to choose only one possibility to answer (*e.g.* 'yes/no' or 'never/sometimes/often/always').

The protocol was approved by the ethics committee of Academic Hospital, Erasmus University, Rotterdam, The Netherlands.

Reference group

For the Body Cathexis List and the self-constructed Questionnaire no normative data were available. Therefore, a group of 73 students (37 males/36 females) were asked to fill in these questionnaires and were used as a reference group. Mean (SD) age of the students was 22.2 (2.0) [range: 19.0 - 26.0] for men and 22.2 (2.0) [range: 19.0 - 27.0] for women, being significantly different compared to cases and controls for the separate sexes (P-values$<$0.05). Mean (SD) adult height of the students was 186.1 (6.7) and 168.2 (7.2) for men and women, respectively. This was quite comparable to the mean adult height for the Dutch population (28), but it was significantly shorter compared to our study population for both sexes (P-values$<$0.05).

For evaluation of socio-economic status (education, employment and marital status), reference data of the Dutch population derived from the Central Bureau of Statistics, The Hague, The Netherlands (29,30), were additionally used for comparison.

Statistics

Since incomplete questionnaires were present in a very small percentage of the subjects (<1%), only complete questionnaires were used for statistical analysis. Differences in psychological test variables between groups were tested non-parametrically (two groups: Mann-Whitney test, three groups: Kruskall-Wallis test). Comparison with normative data of the various instruments was done using the two sample t-test, since only mean and SD was available. In case of a contingency distribution chisquare tests were used. Differences between cases, controls and students in the height related questions of the Standard Questionnaire were tested with the Kruskall-Wallis test in case of ordinal data. If this test was significant, the Mann-Whitney test was used to test the differences between groups. In case of binary data, chisquare tests were used. Comparison of socioeconomic data with reference data of the Dutch population was done using the Kruskall-Wallis test in case of ordinal data (educational level) and using chisquare tests in case of binary data (concerning marital status ("married - unmarried") and employment status ("student - no student")). Correlations between variables were tested with Spearman's rank correlation test.

RESULTS

Psychological well-being and social anxiety

Results of assessment of psychological well-being and social anxiety are listed in Table 2. Psychological well-being as determined with the "Delft Questionnaire" was not significantly different between cases, controls and the norm population for both men and women. The proportion of subjects with scores ≥ 21 was equal in cases and controls for males (11.5% and 18.9%, respectively; P=0.33) and females (19.9% and 24.8%, respectively; P=0.42), which was not significantly different to the norm population (17.1% for males and 20.6% for females).

Male cases scored significantly lower on overall social discomfort as

measured with the "Inventory List on Association with Others" in comparison with the norm population (P<0.02), but not with their tall controls (P>0.05). In addition, they scored higher on overall frequency of the social discomfort but this did not reach statistical significance. With respect to the various subscales, analysis revealed that the difference in overall social discomfort was particularly due to the scores of the subscale stating positive self-assertiveness: cases scored significantly lower on this subscale compared to controls as well as the norm population (P-values <0.02). In addition, the subscale scoring the frequency of this social discomfort was significantly higher in cases compared to both controls and test-norms (P-values <0.01). In women, total scores on social discomfort and its contact frequency were comparable between cases and controls and the norm population. Subscales analysis revealed no significant difference between cases, controls and norm population.

Table 2. Outcome of 4 psychological questionnaires: Dutch Personality Questionnaire, Delft Questionnaire, Body-Cathexis List, and Inventory on Association with Others.

	MALE				FEMALE			
Questionnaire	Cases	Controls	Norms	Students	Cases	Controls	Norms	Students
DPQ	29.7# (5.1)	30.1* (5.5)	28.0 (5.6)	-	28.4 (6.4)	29.0 (5.9)	28.0 (5.6)	-
DQ	12.4 (6.4)	12.4 (7.4)	12.6 (7.4)	-	13.9 (7.4)	14.3 (7.5)	14.3 (7.2)	-
BCL	2.22 (0.29)	2.25 (0.42)	-	2.22 (0.36)	2.28 (0.35)	2.28 (0.35)	-	2.40 (0.31)
IAO								
-Discomfort	60.9@ (15.0)	67.4 (21.7)	66.8 (16.5)	-	67.4 (16.6)	67.6 (18.4)	66.8 (16.5)	-
-Frequency	116.6 (16.6)	112.4 (17.9)	113.0 (16.3)	-	114.1 (14.6)	114.0 (14.9)	113.0 (16.3)	-

DPQ = Dutch Personality Questionnaire
DQ = Delft Questionnaire
BCL = Body Cathexis List
IAO = Inventory List on Association with Others
# P<0.01 compared to the norm population
@ P<0.02 compared to the norm population
* P<0.001 compared to the norm population

Self-esteem and body cathexis

Results on assessment of self-esteem and body cathexis are summarized in Table 2. In tall men and women, self-esteem as measured with the "Dutch Personality Questionnaire" was comparable between cases and controls. However, in tall men, self-esteem was significantly higher compared to the normative data for both cases and controls ($P<0.01$ and $P<0.001$, respectively). Self-esteem in tall women was not different from the normative data. Body cathexis was not significantly different between cases and controls and comparable to students for both men and women. We did not find any significant correlation between height and self-esteem or height and body cathexis for either treated and untreated tall men and women, or students. However, self-esteem was moderately correlated with body cathexis in tall women ($r=-0.34$, $P<0.001$ and $r=-0.39$, $P<0.001$ for cases and controls, respectively) and treated tall men ($r=-0.26$, $P=0.04$), but not in male controls ($r=-0.19$, $P=0.12$).

Socio-economic aspects

Tables 3a and 3b summarize data of the level of education and employment status of the treated and untreated tall subjects and in comparison with a sample from the Dutch population of the same age range (18-34 yrs) (30). In females, the educational level was significantly different between cases, controls and the normal population ($P<0.0001$): cases and controls had a higher levels of education compared to the Dutch population of the same age range. In males, no difference in educational level was found between the groups. With respect to employment status, a significant difference was found in the proportion of subjects being student at time of the study between cases, controls and the normal population for both men and women ($P=0.0014$ and $P<0.0001$, respectively): more tall subjects were student compared to a representative sample of the Dutch population of the same age range.

Table 3a. Educational level in treated tall males and females compared to tall controls and the normal population.

	MALE (%)			FEMALE (%)		
	Cases n=69	Controls n=76	Population[1] n=887	Cases n=174	Controls n=106	Population[1] n=1144
Primary school	3	4	5	1	-	8
Secondary school	32	21	22	22	21	27
High school	51	54	58	56	56	49
University	15	21	15	21	24	15

1 = Central Bureau for Statistics (ref. 30)

Table 3b. Employment status of treated tall males and females compared to tall controls and the normal population.

	MALE (%)			FEMALE (%)		
	Cases n=69	Controls n=76	Population[1] n=889	Cases n=174	Controls n=106	Population[1] n=1144
Employed	59	64	73	61	54	39
Unemployed	3	4	4	2	4	2
Student	36	26	19	29	36	12
Houswive/-man	-	-	1	7	7	46
Retired	-	-	1	0.6	-	1
Other	1	5	2	0.6	-	1

1 = Central Bureau for Statistics (ref. 30)

Relational aspects

The marital status of cases and controls and compared to that of the Dutch population of the same age range (18-34 yrs) (31) are given in Table 4. A significant difference was found in marital status between cases, controls and the normal population for both men and women (P-values <0.01); less tall subjects were married at the time of the study compared to the Dutch population. Upon the

question whether or not one has ever had a serious relationship, has had intercourse and has left one's parental home, an equal proportion of cases and controls answered confirmative. The mean age at the time these events occurred for the first time are listed in Table 5 and were not significantly different between cases and controls for both men and women.

Table 4. Marital status of treated tall males and females compared to tall controls and the normal population.

	MALE (%)			FEMALE (%)		
	Cases n=69	Controls n=75*	Population[1] n=2.181.076	Cases n=174	Controls n=106	Population[1] n=2.088.158
Married	14	24	32	28	25	44
Unmarried	86	76	66	70	74	53
Divorced	-	-	2	2	2	3
Widowed	-	-	<1	-	-	<1

1 = Central Bureau for Statistics (ref. 31)

Table 5. Relational aspects of treated and untreated tall males and females. Data expressed as mean (SD) and [range].

	MALE				FEMALE			
	Cases		Controls		Cases		Controls	
	n	Age	n	Age	n	Age	n	Age
Age first serious partner	61	18.0 (3.2) [14.0-32.0]	58	18.5 (2.8) [14.0-28.0]	157	17.5 (2.3) [13.0-25.0]	98	17.8 (2.2) [14.0-26.0]
Age first coitus	59	18.2 (2.7) [15.0-32.0]	56	18.1 (2.4) [12.0-25.0]	145	17.8 (2.2) [10.0-25.0]	95	18.1 (2.0) [14.0-26.0]
Age leaving parental home	34	20.2 (2.2) [18.0-25.0]	46	21.4 (2.7) [17.0-29.0]	120	19.8 (2.2) [15.0-27.0]	77	20.1 (1.9) [18.0-27.0]

Height related aspects

Previously treated tall men and women had been teased more frequently about their stature at school compared to their tall controls (P=0.0009 and P=0.0006 for men and women, respectively): in total, only 19% of the previously treated tall men and 20% of the treated women had never been teased at school compared to 39% in both tall control groups. In contrast, 70% of the male students and 69% of the female students reported that they had never been teased about their stature at school, which was significantly different to both cases and controls for men as well as women (P-values <0.001).

In men, cases experienced more remarks or jokes about their stature than controls or students (P=0.0006 and P=0.0082, respectively). In women, cases were also more subject to jokes and/or remarks about their height, which was significantly different to tall controls (P=0.029), but not to students (P=0.10).

With respect to occupation, an equal proportion of cases, controls and students reported to be limited by their height to choose a job they wanted. In addition, there was no difference in the proportion of subjects that, despite suitability, had ever been rejected for application because of their stature. Height limited occupations, as recalled by men, varied from aircraft-personnel (pilot/steward), army-personnel to racing driver. In women, professions like stewardess, ballet-dancer, nurse, police-officer and jockey had been mentioned.

The question whether or not height had ever played a role in the finding of a partner was answered confimative by an equal proportion of tall females (49% and 38% for cases and controls, respectively; P=0.10) in contrast to 14% of the controls (cases vs. students:P=0.0003; controls vs. students:P=0.014). In males, 25% of the cases confirmed that height had played a role in partnering, which was higher than controls (8%, P=0.013), but not significantly different to students (19%, P=0.67).

Previously treated tall subjects recalled to be more dissatisfied with their height as a child than students and/or controls. In females, 64% of the cases and 47% of the controls were dissatisfied with their height during childhood in contrast

to 39% of the students (cases vs. students and controls:P<0.0001 and P=0.0004, respectively; controls vs. students:P=0.071). In males, this percentage was 46 for cases and 26 for controls, while only 19% of the students recalled dissatisfaction (cases vs. students and controls:P=0.0072 and P=0.15, respectively; controls vs. students:P=0.071). At the time of the study, male cases were still more dissatisfied with their stature than controls and students (cases vs. controls and students:P=0.020 and P=0.0036, respectively; controls vs. students:P=0.21). Upon the question whether they would prefer to have a different stature, 46 percent of the cases answered confirmative, in contrast to 21% of the controls and 22% of the students (P-values<0.01); cases (and controls) preferred to be shorter, while students preferred to be slightly taller. In females, cases were as satisfied as tall controls with their present height (P=0.39), while students appeared to be more dissatisfied than cases and controls (P=0.027 and P=0.0086, respectively). In addition, 45% of the female cases preferred to have a different height, which was a significantly higher proportion than controls (25%, P=0.0028). In contrast, 55% of the female students preferred to have a different height, which was in the same order of magnitude as in cases, but significantly higher than controls (P=0.0021). It appeared that cases (and controls) would have liked to be shorter, while female students preferred to be slightly taller.

DISCUSSION

Data on psychosocial aspects in constitutionally tall children are scarce. We obtained psychosocial information from a total of 145 tall adult men and 280 tall adult women, part of whom had received height reductive therapy during childhood. Our main objective was to look for possible differences in psychological functioning between previously treated tall subjects and tall controls. In addition, we evaluated the influence of height on various psychosocial aspects by comparing data from tall subjects with that from the Dutch population.

Screening for psychological well-being by means of the "Delft Questionnaire" revealed no evident abnormalities in cases and controls when compared to the normative data. With respect to social anxiety and assertiveness, as determined by the "Inventory List on Association with Others", previously treated women reported to experience no more problems in interpersonal contact than tall controls or the norm group. Previously treated tall men however, showed less social anxiety and were more assertive than the norm population and their tall controls, although in the latter this difference did not reach statistical significance. Subscale analysis revealed that this was particularly due to the fact that they had less difficulty in stating positive self-assertiveness. In addition, they tended to avoid this behavior to a lesser extent than controls and the norm population. It may be speculated that these differences point into the direction of a psychological mechanism such as denial. Usually, low scores on self-criticism are interpreted as evidence of defensiveness saying negative things about oneself. Therefore denial and its counterpart "accentuating the positive", psychological mechanisms which have been reported to occur in short statured adult populations (31,32), might explain the results in tall men. Another explanation could be that the observed differences may be related to the androgen therapy. Androgens are known to affect human behaviour. Individual differences in testosterone levels have been linked to externalizing behaviors such as aggression and assertiveness (13,33-35). Results, however, are not consistent. Mattson et al (33) found higher plasma levels of testosterone in forty 14 to 19 years old male delinquents compared to a control group of fifty-eight 15 to 17 years old healthy boys. The group of Nottelmann (13,34) on the other hand, found lower rather than higher sex-steroid levels to be associated with higher degrees of self-image and behavior problems. In addition, they stated that asynchrony between different aspects of development (e.g. between hormone levels and age or pubertal stage) may have implications for adjustment of boys (and girls). These studies concerned physiological plasma testosterone levels. To our knowledge, there are no studies on the influence of pharmacological doses of androgens on adolescent behavior. In adult men,

administration of high doses of androgens did not affect aggressive behavior (36,37). Therefore, the possibility of any causal relationship between androgen therapy during puberty and adult assertiveness remains speculative.

Self-esteem as measured with a subscale of the "Dutch Personality Questionnaire" revealed no significant differences between cases and controls. However, compared to the norm population, tall males scored slightly, though significantly, higher. In women no such difference was observed. The meaning of these results is unclear. In adolescents and adults with various chronic diseases, the finding of a higher or even a normal self-esteem has been linked with denial of their ilness (38,39). This does also apply for adults with short stature (31,32). It is possible that such a defense mechanism might be present in tall adults. However, additional data supporting this assumption are lacking. We found no relationship between the actual height and self-esteem in tall men and women. This is in concordance with others (17,18,20). It is conceivable therefore, that a person's self-esteem may be not so much related to one's actual height but more to one's perception of height, as suggested by Prieto and Robbins (18).

Tall subjects and students were equally satisfied with their body. We observed no relationship between the actual height and body satisfaction in tall men and women and in students. Our findings are in agreement with the study of Shim and others (40) in adult men, who reported that body satisfaction in tall men was comparable to average- and big men; short men were found to have the lowest body satisfaction. Gupta and coworkers (41) selected 174 subjects from a Canadian shopping mall and revealed an inverse correlation between height and body dissatisfaction in men, though not in women. In view of the heights of their study population however, this observation is probably more related to shorter people. Our finding that self-esteem was positively correlated with body image supports the theory that body perception is related to self-concept (27).

Since our study was retrospective, it is possible that what we measure now as aspects of psychosocial status in adulthood may have been different from what we would have measured in adolescence. For instance, studies in adolescent boys

and girls do indicate that height (and weight) relative to same-age or same-grade peers may have important implications for self-esteem and self-concept during the pubertal years (11,14-16,18). In fact in our study, previously treated men and women recalled to be more frequently teased at school and to be more subjected to remarks or jokes about their stature than controls and students. In addition, they reported to be more dissatisfied with their stature as a child. Unfortunately, psychological evaluation of adolescent tall children prior and during sex steroid treatment in a controlled manner has never been performed. It remains speculative therefore, whether the height reductive therapy may have had a positive influence on the development of psychosocial functioning. Other adaptive mechanisms, such as denial as discussed above, could have played a contributive role as well. In addition, transient effects of physical stature on self-regard in tall adolescent children without height intervening therapy have been described (19).

Several studies point to the possibility that tall people may have more occupational succes than shorter people, especially in men (6-8). Our study seems to confirm this finding: a large percentage of tall subjects was still student at the time of the study, while in a representative sample of the Dutch population of the same age range most of the people were employed (29). It suggests that taller people would be able to achieve higher academic positions. In our study, this was particularly the case in females where the difference in employment was most strikingly: most of the tall women were employed or still student, while in the normal population most women run the household at these ages.

The marital status of tall people was different compared to the normal population: less tall subjects were married. This might be due to the fact that many of the tall subjects were still students; generally as a student, one keeps from marriage until one's study is finalized. On the other hand, a low percentage of marriage might also be seen as a sign of a delayed maturation process resulting in an unsatisfactory psychosocial maladjustment or social isolation as suggested in studies from adult patients with short stature (42,43). The possibility of a delayed psychosocial maturation, however, is contradicted by other findings in our study.

For instance, information about the psychosexual maturation revealed a quite normal pattern: the age of the first sexual intercourse was comparable to that reported for the Dutch population (44). In addition, the process of leaving the parental home (which could also be conceived as a maturational sign) was quite the same as the trend observed in the Dutch population (45-47) nowadays.

One may argue that the study population could have been biased by selection. It appeared that more cases responded to our call for the follow-up study than controls. However, of the responders, an equal proportion of subjects was willing to participate in this follow-up study. It is possible that this procedure has caused some bias, but it is hard to estimate the effect of this bias on the results as the reason for non-responding was not known. It might also be possible that for instance more students had been included explaining the differences in employment and marital status between tall subjects and the normal population. However, the patients had only been selected by their diagnosis of constitutionally tall stature unaware of any other demographic or socio-economic data. It is true, though, that our study concerned referred subjects, who might be different to tall people from the normal population. To what extent this may account for the observed differences remains speculative.

In summary, our study revealed no major psychological maladjustment in previously treated tall children compared to an untreated tall control group. No distinct differences were found in psychological well-being, social anxiety, self-esteem and body perception. However, tall men showed slightly higher scores on self-esteem than the norms. In addition, previously treated tall men had less social anxiety and showed more assertiveness compared to tall controls and the norm population. It is speculative whether the administration of high doses of sex steroids or possible psychological mechanisms such as denial may have contributed to these favourable outcomes. In retrospect, previously treated tall men and women experienced more problems related to their height than tall controls, especially during childhood. This may have played a role for the choice in favour of height reductive therapy. Compared to the normal population tall subjects did very

well in terms of education and employment confirming the idea that tallness is linked to occupational succes. Further research is needed to establish the psychological problems in constitutionally tall children and to study the possible beneficial effects of height intervening therapy.

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REFERENCES

1. Wettenhall HNB, Cahill C, Roche AF: Tall girls: a survey of 15 years of management and treatment. J Pediatr 1975;86:602-610
2. Prader A, Zachmann M: Treatment of excessively tall girls and boys with sex hormones. Pediatr 1978;62:1202-1210
3. Bailey JD, Park E, Cowell C: Estrogen treatment of girls with constitutional tall stature. Ped Clin North Am 1981;28:501-512
4. Zachmann M: Use and risks of androgen therapy: replacement, constitutional delay, and tall stature. In: Forest MG (ed). Androgens in childhood. Pediatric Adolescent Endocrinology. Karger, Basel 1989;19:247-263
5. Humphreys LG, Davey TC, Park RK: Longitudinal correlation analysis of standing height and intelligence. Child Development 1985:56:1465-1478
6. Taesdale TW, Owen DR, Sorenssen TIA: Intelligence and educational level in adult males at the extremes of stature. Hum Biol 1991;63:19-30
7. Schumacher A: Zur bedeutung der Körperhöhe in der menschlichen Gesellschaft. I. Untersuchungen zur sozialen Körperhöhedifferenzierung. Zeit Morphol Antropol 1981;72: 213-228
8. Hensley WE, Cooper R: Height and occupational succes: a review and critique. Psychological Reports 1987;60:843-849
9. Dannenmaier WD, Thumin FJ: Authority status as a factor in perceptual distortion of size. J Soc Psychol 1964;63:361-365
10. Brackbill Y, Nevill DD: Parental expectations of achievement as affected by children's height. Merrill-Palmer Quarterly 1981;27:429-441
11. Brooks-Gunn J, Warren MP: The psychological significance of secondary sexual characteristics in nine- to eleven-year-old girls. Child Development 1988;59:1061-1069
12. Petersen A: Pubertal development as a cause of disturbance: Myths, realities and unanswered questions. Genetic, Social and General Psychology Monographs 1985;111: 205-232
13. Nottelmann ED, Inoff-Germain G, Susman EJ, Chrousos GP: Hormones and behavior at puberty. In: Adolescence and puberty. Bancroft, McChover Reinisch (eds). Oxford University Press, New York 1990
14. Blyth DA, Simmons RG, Bulcroft R, Felt D, Van Cleave EF, Bush DM: The effect of physical development on self-image and satisfaction with body-image for early adolescent males. Res Comm Health 1982;2:43-73
15. Davies E, Furnham A: Body satisfaction in adolescent girls. Br J Med Psychol 1986; 59:279-287
16. Martin S, Housley K, McCoy H, Greenhouse P, Stigger F et al: Self-esteem of adolescent girls as related to weight. Perceptual and Motor Skills 1988;67:879-884
17. Coopersmith S: The antecedent of self-esteem. Freeman, San Francisco 1969
18. Prieto AG, Robbins MC: Perceptions of height and self-esteem. Perceptual and Motor Skills 1975;40:395-398

19. Nottelmann ED, Welsh CJ: The long and the short of physical stature in adolescence. J Early Adolesc 1986;6:15-27
20. Hensley WE: Gender, self-esteem and height. Perceptual Motor Skills 1983;56:235-238
21. De Waal WJ, Fokker MH, Stijnen Th, van Gurp EAFJ, Toolens AMP, de Muink Keizer-Schrama SMPF, Drop SLS: Accuracy of final height and effect of growth reductive therapy in 362 constitutionally tall children. J Clin Endocrinol Metab 1996, accepted for publication (march)
22. De Waal WJ, Torn M, de Muinck Keizer-Schrama SMPF, Aarsen RSR, Drop SLS: Long term sequelae of sex sterold treatment in the management of constitutionally tall stature. Arch Dis Child 1995;73:311-315
23. De Waal WJ, Vreeburg JTM, Bekkering F, de Jong FH, de Muinck Keizer-Schrama SMPF, Drop SLS, Weber RFA: High dose testosterone therapy for reduction of final height in constitutionally tall boys: does it influence testicular function in adulthood? Clin Endocrinol 1995;43:87-95
24. Appels A: Provisional Manual for the Delft Questionnaire. Voorlopige handleiding bij de Delftse Vragenlijst. Swets & Zeitlinger, Amsterdam 1985
25. Van Dam-Baggen CMJ, Kraaimaat FW: Manual for the Inventory List on Association with Others. Inventarisatielijst Omgaan met Anderen. Handleiding. Swets & Zeitlinger, Lisse 1987
26. Lutelijn F, Starren J, van Dijk H: Manual for the Dutch Personality Questionnaire. Nederlandse Persoonlijkheids Vragenlijst, Handleiding. Swets & Zeitlinger, Amsterdam 1985
27. Secord PF, Jourard SM: The appraisal of body cathexis: body cathexis and the self. J Consult Psychol 1953;17:343-347
28. Roede MJ, van Wieringen JC: Growth diagrams 1980, Netherlands. Third nation-wide survey. Tijdschrift Sociale Gezondheidszorg 1985;62(Suppl):1-34
29. Central Bureau for Statistics. Life style of the Dutch population 1991. Figures. De leefsituatie van de nederlandse bevolking 1991. Kerncijfers. SDU/Uitgeverij, CBS publicaties, 's Gravenhage 1992
30. Central Bureau for Statistics. Annual statistics. Population of the Netherlands by marital status, sex, age and country of nationality, 1 January 1991. Jaarcijfers. Bevolking van Nederland naar burgerlijke staat, geslacht, leeftijd en land van nationaliteit, 1 januari 1991. Maandstatistiek der Bevolking 1991;9:40-41
31. Rotnem D, Cohen D, Hintz R, Genel M: Psychological sequelae of relative "treatment failure" for children receiving human growth hormone replacement therapy. J Am Acad Child Psych 1979;18:505-520
32. Mitchell CM, Joyce S, Johanson AJ, Libber S, Plotnisck L, Migeon CJ, Blizzard RM: A retrospective evaluation of psychosocial impact of long-term growth hormone therapy. Clin Pediatr 1986;25:17-23
33. Mattsson A, Schalling D, Olweus D, Löw H, Svensson J: Plasma testosterone, agressive behavior and personality dimensions in young male delinquents. J Am Acad Child Psych 1980;19:476-490

34. Nottelmann ED, Susman EJ, Inoff-Germain G, Cutler Jr. GB, Loriaux DL, Chrousos GP: Developmental processes in early adolescence: relationships between adolescent adjustment problems and chronological age, pubertal stage, and puberty-related serum hormone levels. J Pediatr 1987;110:473-480
35. Mooradian AD, Morley JE, Korenman SG: Biological effects of androgens. Endocrine Rev 1987;7:1-28
36. Anderson R, Bancroft J, Wu F: The effects of exogenous testosterone on sexuality in normal men. J Clin Endocrinol Metab 1992;75:1503-1507
37. Bagatell CJ, Heiman JR, Matsumoto AM, Rivier JE, Bremner WJ: Metabolic and behavioral effects of high-dose, exogenous testosterone in healhy men. J Clin Endocrinol Metab 1994;79:561-567
38. Kellerman J, Zeltzer L, Ellenberg L, Dash J, Rigler D: Psychological effects of illness in adolescence. I. Anxiety, self-esteem and perception of control. J Pediatr 1980;97:126-131
39. Utens EMWJ, Verhulst FC, Erdman RAM, Meijboom FJ, Duivenvoorden HJ, Bos E, Roeland RTC, Hess J: Psychosocial functioning of young adults after surgical correction for congenital heart disease in childhood; a follow-up study. J Psychosomatic Res 1994;38:745-758
40. Shim S, Kotsiopulos, Knoll DS: Short, average-height, tall, and big men: Body cathexis, clothing and retail satisfactions, and clothing behavior. Perceptual Motor Skills 1990;70:83-96
41. Gupta MA, Schork NJ, Dhaliwal JS: Stature, drive for thinness and body dissatisfaction: a study of males and females from a non clinical sample. Canad J Psych 1993;38:59-61
42. Dean HJ, McTaggart TL, Fish DG, Friesen HG: The educational, vocational and marital status of growth hormone deficient adults treated with growth hormone during childhood. Am J Dis Child 1985;139:1105-1110
43. Sartorio A, Morabito F, Peri G, Conti A, Faglia G: The social outcome of adults with constitutional growth delay. J Endocrinol Invest 1990;13:593-595
44. Van Zessen G, Sandfort Th (red): Sex in the Netherlands: sexual behavior, risk and prevention of AIDS. Seksualiteit in Nederland: seksueel gedrag, risico en preventie van aids. Swets & Zeitlinger, Amsterdam 1991
45. Central Bureau for Statistics. Visser H: Girls leave home at a younger age than boys. Meisjes eerder het huis uit dan jongens. Maandstatistiek der Bevolking 1988;5:7
46. Central Bureau for Statistics. Latten JJ: In the eighties young people stayed longer with their parents again. Development since the sixties. Jongeren bleven in jaren '80 weer langer bij hun ouders. Ontwikkelingen sinds de jaren '60. Maandstatistiek der Bevolking 1990;10:14-24
47. Central Bureau for Statistics. De Jong AH: Boys live two years longer in the parental home than girls. Jongens wonen twee jaar langer thuis dan meisjes. Maandstatistiek der Bevolking 1993;1:8

CHAPTER 9

Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation (IUGR).

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ABSTRACT

In order to obtain insight in the pathophysiological mechanism underlying the failure to catch up growth in children with short stature after intrauterine growth retardation (IUGR) we investigated the GH/IGF axis, as GH secretion disturbances might play a role in the growth retardation of these children. In total, 40 prepubertal children (15 girls/25 boys; mean age [range] 7.5 yr. [3.4 - 10.8 yr.]) with short stature (height below the P3) after IUGR, defined as a birth length below the P3 for gestational age, were studied. GH secretion was determined by a 24-hour plasma GH profile test (sampling every 20 minutes) and, on a separate occasion, by a standard arginine provocation test (ATT). Plasma IGF-I and -II levels were measured at start of the profile test. Urine was collected to measure urinary GH (U-GH) levels. Plasma and urinary GH were determined by double antibody RIA. IGF-I and -II were determined by specific RIA after acid chromatography. The 24-h GH profiles were analyzed using PULSAR. We found similar endogenous GH secretion in boys as in girls. Boys had significantly lower mean GH levels compared to healthy controls. Forty percent of the children met our criteria of a *normal* 24-h GH profile (group A; n=16). Sixty percent (n=24) did not fulfil these criteria. We subdivided these 24 children into two groups: group B (n=14): children with either mean GH levels less than controls but with at least one spontaneous GH peak above 20 mU/L, ór children with normal mean GH levels but with no GH peak above 20 mU/L (*subnormal* 24-h GH profile) and group C (n=10): children with mean GH levels less than controls and no GH peak above 20 mU/L (*low* 24-h GH profile). The GH secretory abnormalities were due to a decrease in pulse amplitude, not in pulse frequency. Mean (SD) maximal GH response during ATT was 22.3 (12.1) mU/L. Nineteen children (47.5%) had a maximal GH value <20 mU/L. Moderate, but significant correlations were found between several 24-h GH profile characteristics and the maximal GH response during ATT (r= 0.31-0.35; P<0.05). Mean (SD) overnight U-GH excretion was 3.8 (2.1) and 4.4 (3.5) μU/night for boys and girls, respectively. Compared to healthy schoolchildren overnight U-

GH was lower in boys, but not in girls. Mean (SD) IGF-I and IGF-II SDS for chronological age (CA) levels was -0.88 (1.40) and -0.64 (1.48), respectively. Plasma IGF-I and -II levels were significantly reduced compared to controls. Height SDS_{CA} or height velocity SDS_{CA} did not correlate with either spontaneous or stimulated GH secretion, U-GH excretion or plasma IGF-I and -II levels. In conclusion, our study indicates that 50-60% of children with short stature after IUGR have 24-h GH profile abnormalities and/or subnormal responses to arginine provocation, while mean plasma IGF-I and -II levels are significantly reduced, indicating GH insufficiency. U-GH excretion is lower in boys, but not in girls. The precise mechanism of the failure to catch up growth needs further elucidation. It seems justified to start clinical trials in order to investigate whether treatment with exogenous GH will be beneficial to these children.

INTRODUCTION

Short stature after intrauterine growth retardation (IUGR) is a well known phenomenon. Whereas most children born after IUGR do show extensive catch-up growth within the first two years of life, a certain percentage, up to 30% fail to do so (16). IUGR is the result of many different disturbances. Conditions interfering with fetal growth have commonly been differentiated into fetal, placental, maternal and environmental factors. In many cases no underlying condition can be elucidated (20). A special group of IUGR-children are children with Silver-Russell Syndrome (SRS) (44). These children have phenotypical features such as a triangular face, body asymmetry and clinodactyly of the fifth finger. They are short at birth, show minimal catch-up growth during infancy and reach a mean adult height of about -3.6 height SD score, corresponding to 142.0 cm for girls and 150,7 cm for boys (13). The underlying mechanism of the stunted growth in IUGR children is poorly understood. In order to find an explanation for this failure to catch up growth some investigators have studied GH secretion during childhood in both

dysmorphic and non-dysmorphic IUGR children (1,2,33). Abnormal GH secretion during 12 or 24 hours of physiological testing and/or insufficient GH response to various provocative stimuli have been described (1,2,33). Interpretation of these results however, is difficult as the various study populations are differently defined. In addition, little is known about the plasma IGF-levels and urinary GH excretion (U-GH) in these children. We therefore studied spontaneous plasma GH secretion during 24 hours as well as maximal plasma GH response after arginine stimulation in 40 prepubertal children with short stature after IUGR. In addition, we measured U-GH levels during day- and nighttime as well as plasma IGF-I and -II levels.

SUBJECTS AND METHODS

Study group

Forty prepubertal children (25 boys/15 girls) with short stature after IUGR were studied. The mean (SD) age was 7.5 (2.0) yr, with a range of 3.4-10.8 yr. All patients fulfilled the following inclusion criteria: 1. IUGR defined as a birthlength below the third percentile for gestational age (48), 2. an uncomplicated neonatal period, i.e. without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without complicated sepsis neonatorum and without long-term complications of respiratory ventilation such as broncho-pulmonary dysplasia or pneumothorax, 3. no catch-up growth above the P3 for chronological age (Dutch references (34)) within the first two years of life, 4. height velocity for chronological age $\leq$ P50 (Dutch references (31)), 5. no signs of puberty defined as Tanner stage M/G 1 or testicular volume $<$ 4 ml. (45), 6. chronological age $<$ 9 yrs for girls and $<$ 11 yrs for boys, and 7. no chromosomal abnormalities or other organic causes for growth retardation, except for Silver-Russell syndrome (SRS).

Clinical details of the patients are given in Table 1. In 2 boys the diagnosis SRS was made. Body mass index (weight(kg)/height(m^2))(BMI) was calculated and expressed in SD scores for chronological age ($BMI\text{-}SDS_{CA}$) using a logarithmic

transformation (35).

Table 1. Clinical data of 40 prepubertal children with short stature after IUGR. Data expressed as mean (SD).

Clinical data	Boys	Girls
Number	25#	15
Birth length (cm)	41.9 (4.7)	41.5 (6.5)
Birth length (SDS)	-3.34 (1.24)	-3.88 (2.07)
Birth weight (g)	1970 (745)	1850 (720)
Birth weight (SDS)	-2.42 (1.15)	-2.88 (1.02)
Gestation (weeks)	37 (4)	37 (4)
Chronological age (yrs)	8.2 (1.8)*	6.3 (1.8)*
Height SDS_{CA}	-3.07 (0.63)	-2.74 (0.58)
Height Velocity (cm/yr)	4.9 (1.0)	5.5 (1.1)
$HV\text{-}SDS_{CA}$	-1.08 (0.89)	-1.07 (1.12)
$BMI\text{-}SDS_{CA}$	-1.24 (0.83)	-1.86 (1.34)

SDS = standard deviation score
CA = chronological age
BMI = body mass index
HV = height velocity
*: P = 0.004; boys compared to girls
#: 2 boys had Silver Russell Syndrome (SRS)

Study protocol

Children were admitted to the hospital on the morning of the test. A nonthrombotic catheter was inserted in the anticubital vein with a heparin lock. Blood sampling started directly afterwards. Every 20 minutes 0.5 ml blood was taken for a period of 24 hours for measurement of plasma GH. At start blood was also taken for IGF-I and -II determination. During their stay, children moved freely about, slept at night and had a normal pattern of eating. During the blood sampling urine was collected for measurement of U-GH. Urine was collected as day and night urine. Night urine consisted of urine produced during sleep, including early morning urine.

On a separate occasion a standard arginine tolerance test was performed after an overnight fast. Arginine 0.5 g/kg was infused in 30 minutes. Blood samples were taken at T=-15, 0, 15, 30, 45, 60, 90 and 120 minutes for plasma GH determination.

Blood samples were stored on ice for no longer than 3 hours. After centrifugation, plasma samples were frozen (-20° C) and stored until assayed. During collection urine was stored in the refrigerator. After collection, the total amount of urine was measured and urine samples were frozen (-20° C) until assayed.

The protocol was approved by the Ethics Committee of each participating centre.

Hormone assays

All GH assays in plasma and urine were performed in the same laboratory. All GH samples were analyzed in duplicate in the same assay. Plasma GH was measured by a double antibody RIA, as described previously (23). Urinary GH was measured by the same RIA as that described above with a preincubation technique and an antiserum dilution of 1:500,000. For the isolation of GH, Millipore filters (UFPI-LGC-BK) with a mol wt discrimination of 10,000 were used. Overnight U-GH levels were compared with the mean of 5 consecutive overnight urine samples of a group of 93 prepubertal healthy schoolchildren aged 4-11 years (own data, unpublished). Mean (SD) overnight U-GH level of the controls was 4.8 (2.6) μU/night.

Plasma IGF-I and -II concentrations were determined by specific RIA after acid chromatography in order to remove interfering binding proteins, as published previously (23). Since both growth factors are dependent on age and sex, values were transformed to SD scores for chronological age using reference values based on 600 samples from a healthy Dutch population, as described previously (23). Reference data for IGF-II were only available from the age of 7.

Analysis of 24-h GH profiles

The 24-h GH profiles were analyzed using the Pulsar program (29) and adapted for Quick Basic by Rosberg and Albertsson-Wikland (PC-Pulsar, 1987). The Pulsar program identifies secretory peaks by height and duration from a smoothed baseline, using the assay SD as scale factor. Peak selection criteria appropriate for our own assay conditions and data set were established before the 24-h GH profiles were analyzed (23). With these settings the Pulsar program did not detect any peaks when 72 consecutive samples from each of 3 different plasma pools were assayed. From the Pulsar program the following profile characteristics were extracted: area under the curve above zero (AUC_0) and above baseline (AUC_B), total number of peaks, mean and maximal value, mean peak amplitude. The sum of the amplitude was calculated by multiplying the mean of the peak amplitude with the number of peaks.

Obtaining control data from a group of healthy children was considered unethical, as information on 24-h GH profiles in healthy prepubertal children was available (4). We have previously shown that the methodology and GH measurements as performed in this control study are comparable with ours (24). We therefore compared the mean plasma GH levels of the 24-h GH profiles of our study patients with the mean GH levels of 62 healthy prepubertal children (18 girls/44 boys) as reported by Albertsson-Wikland and Rosberg (4). Mean (SD) of mean plasma GH levels of these controls were 5.9 (0.6) mU/L for boys and 4.8 (0.6) mU/L for girls (4). To correct for sex we also expressed the mean GH values of our study group as SD scores.

Based on descriptions in the literature (3,4,49) we defined a normal 24-h GH profile as a profile with: 1. well defined GH peaks returning to baseline levels between the pulses, 2. at least one major GH peak above 20 mU/L, 3. a mean GH level over 24 hours within the range of 4.8 ± 2 SD mU/L for girls and 5.9 ± 2 SD mU/L for boys, and 4. a normal diurnal rhythm.

Statistical analysis

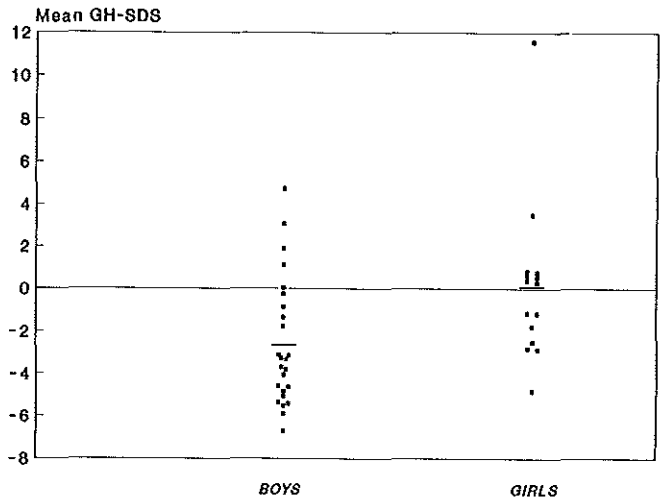
Differences between two groups were tested with Wilcoxon's two sample test. The Kruskall-Wallis test was used to test differences between the three GH profile subgroups. If this test was significant, Wilcoxon's two sample test was used to differentiate between the groups. Correlations were tested with Spearman's rank correlation test. Comparisons with the control group were performed using a simple approximate Z-test, since only mean and standard deviation were available for the control group. Means of variables expressed as standard deviation scores were tested with the one-sample t-test.

RESULTS

GH secretion: 24-h GH profile and arginine tolerance test

24-h GH profile. Mean (SD) values of the GH profile characteristics for boys and girls are given in Table 2. No significant difference was found between boys and girls for any of the 24-h GH profile characteristics. Compared to the healthy control group mean GH levels were significantly lower in boys ($P<0.0001$) but not in girls. The mean GH values expressed as SD scores are given in Figure 1.

Figure 1. Mean GH-SDS during 24-h plasma GH profiles for boys and girls.



The 24-h GH profiles showed considerable variation: mean values of GH over the 24-h period of all children ranged from 1.8 - 11.8 mU/L (mean 4.5 ± 2.0 mU/L), the number of peaks ranged from 4- 17 (mean 10.6 ± 2.9) and the maximum GH peak during the 24-h period ranged from 7.0 to 68.0 mU/L (mean 29.3 ± 14.9 mU/L). The GH peaks of 3/40 children failed to return to baseline levels between the pulses; their calculated baseline GH level was > 2 mU/L.

16 children (8 boys/8 girls) showed a pattern of GH secretion that met all our criteria of a normal GH profile [as described under *Analysis of 24-h GH profiles* above]. The remaining 24 children (60%) had a GH profile that did not meet these criteria. Yet these remaining GH profiles showed distinct differences in GH secretory pattern mainly marked by the absence of spontaneous GH peaks above 20 mU/L. Therefore we decided to divide the 24-h GH profiles in three subgroups: group A, B and C (see Figure 2). Group A includes children with a *normal* GH profile, defined as stated above. Group B consists of children with either a mean plasma GH level of more than 2 SD below the mean plasma GH values of the controls and with at least one spontaneous GH peak above 20 mU/L, ór with a normal mean plasma GH value and with no spontaneous GH peak above 20 mU/L (*subnormal* GH profile). Group C includes children with GH profiles with a mean plasma GH level of more than 2 SD below the mean plasma GH values of the controls and no spontaneous GH peak above 20 mU/L (*low* GH profile). According to these criteria 40 % of the patients had a normal GH profile, 35% showed a subnormal GH profile and 25% a low GH profile. From the 3 patients with elevated baseline GH levels, two were found to have a subnormal GH profile and 1 a low GH profile. There was no difference in sex between the three groups. The 24-h GH profile characteristics of the groups A - C are summarized in Table 2. The numbers of peaks did not statistically differ between the subgroups. However the mean peak amplitude was statistically different between the three groups ($P<0.01$). On the other hand, age, height SDS_{CA}, and HV-SDS_{CA} were not statistically different between the groups A, B and C.

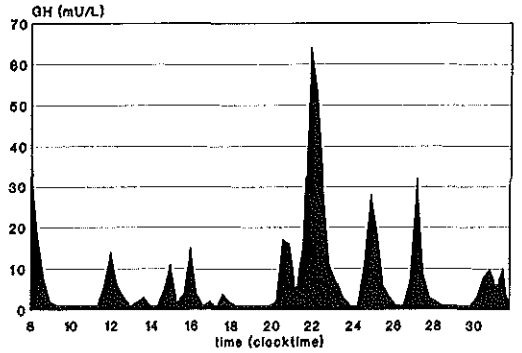
Table 2. Characteristics of the 24-h plasma GH profiles and maximal plasma GH response during provocation (ATT) in 40 IUGR children. Data expressed as mean (SD).

	24-GH profile characteristics							Maximal GH response during ATT (mU/L)
	Mean GH (mU/L)	Maximal GH (mU/L)	No. of peaks	AUC_O (mU/L.24h)	AUC_B (mU/L.24h)	Sum of amplitudes (mU/L)	mean peak amplitude (mU/L)	
Sex								
Boys (n=25)	4.3	30.6	10.2	304.7	224.0	100.2	10.6	21.4
	(1.8)	(15.6)	(3.1)	(126.2)	(128.5)	(60.3)	(5.9)	(12.3)
Girls (n=15)	4.8	27.0	11.3	338.7	251.4	111.5	9.7	23.7
	(2.3)	(14.0)	(2.6)	(163.1)	(154.3)	(77.9)	(5.1)	(12.0)
All (n=40)	4.5	29.3	10.6	317.5	234.3	104.4	10.2	22.3
	(2.0)	(14.9)	(2.9)	(140.1)	(137.4)	(66.7)	(5.6)	(12.1)
Classification of 24-h GH profile								
Group A (n=16)	6.2*	41.6#	11.1	438.6°	356.0§	160.4§	14.8§	26.1
-Normal-	(2.0)	(15.0)	(2.8)	(140.0)	(135.9)	(72.1)	(5.3)	(12.1)
Group B (n=14)	3.8*	25.4#	9.9	259.8°	181.1§	80.4§	8.8§	21.0
-Subnormal-	(0.9)	(15.6)	(3.2)	(57.5)	(47.3)	(20.1)	(2.7)	(12.8)
Group C (n=10)	2.9*	15.0#	11.0	206.4°	116.2§	50.6§	5.0§	17.9
-Low-	(0.7)	(4.7)	(3.1)	(49.0)	(37.0)	(14.8)	(2.3)	(10.0)

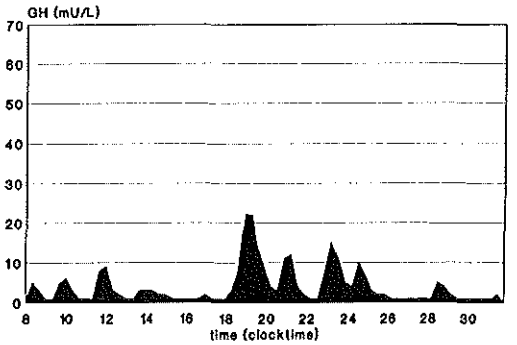
AUC_O = calculated area under the curve above zero
AUC_B = calculated area under the curve above baseline
ATT = arginine tolerance test

#: difference between each pair of groups (P<0.001)
§: difference between each pair of groups (P<0.01)
*: difference between each pair of groups (P<0.02)
o: difference between each pair of groups (P<0.05)

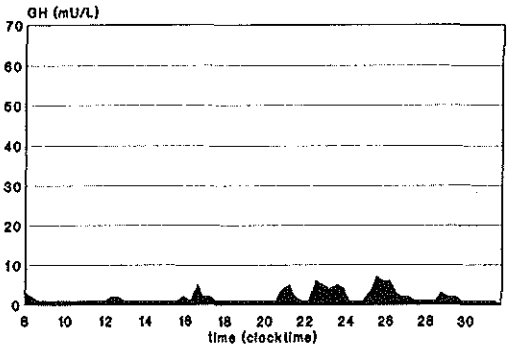
Figure 2. Representative examples of 24-h plasma GH profiles.



Group A: Normal 24-h GH profile



Group B: Subnormal 24-h GH profile



Group C: Low 24-h GH profile

Arginine tolerance test (ATT). The mean (SD) maximal plasma GH response during ATT was 22.3 (12.1) mU/L. with a range from 4.5 to 56 mU/L. There was no difference between the sexes. Nineteen out of 40 children (47.5%) had a maximal GH-response less than 20 mU/L. The response during ATT was not statistically different between the groups A - C (see Table 2). In group A, 10 out of 16 patients showed a normal GH response (maximal GH peak ≥ 20 mU/L) during ATT. In group B and C a normal response was found in 11/24 cases.

We found a moderate but significant correlation between the endogenous GH secretion and the stimulated GH secretion. The following 24-h GH profile characteristics correlated with the maximal GH response during ATT: mean GH value, number of peaks > 20 mU/L, AUC_B, mean peak amplitude, and the sum of the amplitudes (r_{Sp}= 0.31-0.35; $P<0.05$). On the other hand no correlation was found between parameters of GH secretion and parameters of growth (expressed as H-SDS_{CA} or HV-SDS_{CA}). In addition there was also no correlation between parameters of GH secretion and severity of IUGR (expressed as birthlength SDS).

24-h urinary GH excretion

Results of urinary GH excretion are shown in Table 3. There was no difference in U-GH excretion between boys and girls. Compared to controls, boys, in contrast to girls, had lower mean overnight U-GH levels ($P<0.05$). U-GH excretion was not different between the GH profile groups A, B and C.

U-GH levels did not significantly correlate with either stimulated or endogenous GH secretion. In addition, no correlation was seen between the urinary GH excretion and the growth parameters (H-SDS_{CA} and HV-SDS_{CA}) or the severity of IUGR (birthlength SDS).

Expressing the data corrected for creatinine excretion in U-GH/ng creatinine did not change any of these findings.

IGF-I and IGF-II levels

Plasma IGF-I and -II levels are given in Table 3. Mean (SD) SDS_{CA} for plasma IGF-I was -0.88 (1.40). For IGF-II, SD score could only be calculated for 29/40

Table 3. Age, plasma IGF-I and -II levels and urinary GH excretion (U-GH) in 40 IUGR children. Data expressed in mean (SD).

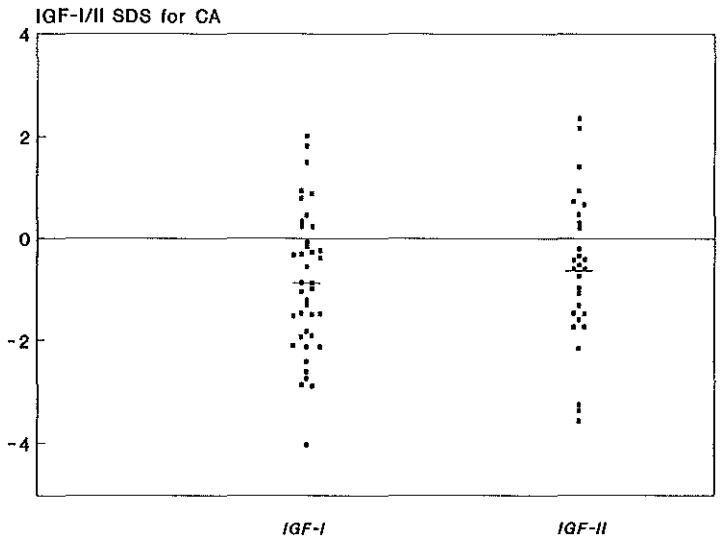
	Age (years)	IGF-I SDS_{CA} (n=29)	IGF-II SDS_{CA} (μU/24h)	U-GH/24h (μU/day)	U-GH day (μU/night)	U-GH night
Sex						
Boys (n=25)	8.2 (1.8)	-0.74 (1.43)	-0.64 (n=21) (1.54)	9.3 (4.3)	5.5 (3.5)	3.8 (2.1)
Girls (n=15)	6.3* (1.8)	-1.10 (1.35)	-0.65 (n=8) (1.39)	9.5 (6.4)	4.9 (3.3)	4.4 (3.5)
Classification of 24-h GH profile						
Group A (n=16) *-Normal-*	7.4 (2.1)	-0.55 (1.28)	-0.11 (n=11) (1.10)	11.1 (6.5)	6.0 (3.9)	4.9 (3.5)
Group B (n=14) *-Subnormal-*	7.7 (2.2)	-1.23 (1.36)	-1.18 (n=10) (1.46)	7.3 (4.6)	4.0 (3.4)	3.5 (1.6)
Group C (n=10) *-Low-*	7.3 (1.9)	-0.91 (1.61)	-0.69 (n=8) (1.84)	9.1 (3.4)	5.9 (2.0)	3.2 (1.9)

* P = 0.004 girls versus boys

patients (children > 7 years). Mean (SD) SDS_{CA} for IGF-II was -0.64 (1.48). Mean values of IGF-I SDS_{CA} and IGF-II SDS_{CA} were significantly different from zero ($P<0.001$ and $P<0.05$, respectively), i.e. the IGF-levels were significantly reduced compared to controls (see Figure 3). IGF-I and -II levels were not significantly different between boys and girls. Furthermore IGF-I SDS_{CA} as well as IGF-II SDS_{CA} did not significantly differ between the three GH profile groups (A-C).

Plasma IGF-I expressed as SDS_{CA} was correlated with the maximal plasma GH peak during ATT ($r_{sp}=0.43$; $P<0.01$) but not with endogenous GH secretion. Interestingly, the IGF's did not correlate with the parameters of growth (H-SDS_{CA} and HV-SDS_{CA}) or severity of IUGR (birthlength SDS).

Figure 3. IGF-I and -II SDS for chronological age.



DISCUSSION

Our study shows that many prepubertal children with short stature after IUGR have 24-h GH profile disturbances. Forty percent of the children had a 24-h GH profile that met all our criteria of a normal profile. Consequently, sixty percent of the children showed an abnormal 24-h GH profile. These findings are comparable with previous studies (1,33), despite differences in patient selection. The finding of

such a high percentage of GH secretory disturbances in these children is surprising, because they lack clinical features of GH deficiency. In fact, these children had a low BMI, thus the low spontaneous GH levels in plasma were not due to a high BMI as reported in healthy children (27,36). Ten out of the 24 children with an abnormal 24-h GH profile (25% of all patients) secreted a very low amount of GH and failed to produce a GH peak greater than 20 mU/L, indicating a marked GH insufficiency. This is in accordance with the studies of Albertsson-Wikland and Ackland et al, who found percentages of 37.5 % and 12.9 %, respectively (1,2). Assuming a spectrum in GH secretion (4,30), we divided all 24-h GH profiles into a kind of gliding-scale, ranging from normal -via subnormal- to low GH secretion (group A, B and C, respectively). By this division it was clear, that this gradual reduction in GH secretion was caused by a decrease in GH pulse amplitude, not in pulse frequency. It is known that GH pulse amplitude increases with age (28,36), however, in our study age did not differ between the groups. The high percentage of GH secretory abnormalities in our patient group may be attributed to a disturbance in GH pulse amplitude.

47.5 % of the children showed a subnormal response during arginine provocation (< 20 mU/L). This percentage is somewhat higher than reported previously (2,33). This difference is probably caused by the pharmacological stimulation tests used, as it has been shown that different provocation tests have different intensities of response (32). Several studies have shown that stimulated GH hormone levels do not completely reflect endogenous GH levels in children with short stature and that it is not uncommon for stimulation tests to overestimate spontaneous GH secretion (7,15,39), although others have argued for the contrary (37). In our study we found a significant, though moderate, correlation between various 24-h GH profile characteristics and the maximal GH response during ATT. When we combined the results of the 24-h GH profiles and the pharmacological stimulation tests, the accuracy of the ATT to categorize the children correctly according to their 24-h GH profile was only 57.5%. Conversely, stimulated GH secretion did not reflect endogenous GH secretion in 42.5% of the patients:

underestimation in 6 (15%) and overestimation in 11 patients (27.5%). In addition, 11/24 patients with an abnormal GH profile (groups B and C) showed a normal GH response to arginine provocation indicating a neurosecretory dysfunction (NSD), as described by Spiliotis et al (40). Thus NSD accounted for 46% of the GH secretory abnormalities. This finding is higher than reported by others (33).

Compared to healthy schoolchildren mean overnight U-GH excretion was lower in boys, but not in girls. The reason for this finding is not clear. It might be related to the fact that mean plasma GH levels in boys were lower than controls, but on the other hand, we found no significant correlation between U-GH levels and parameters of endogenous GH secretion. In addition, measurement of U-GH levels (over 24 hours as well as during day- or nighttime) did not enable us to distinguish between the different GH profile groups. Our finding is in contrast with other studies in short children where urinary GH levels were found to be well correlated with endogenous GH secretion (12,25,43). Difference in statistical approach may account for this discrepancy, because in these studies parametric correlations tests were used. We also found good correlations between parameters of endogenous GH secretion and U-GH levels by use of a parametric correlation test (*r*-values up to 0.60), but we considered it better to use a non-parametric correlation test as U-GH levels were not normally distributed.

During fetal growth IGFs play an important role (14) and birth size is correlated with IGF-I (but not with IGF-II)(5,6,18). In addition, decreased IGF-I and -II plasma levels are described in IUGR, in man as well as in animal models (26,42,47). Catch-up growth in IUGR children has also been associated with normalization of IGF-I levels (46). Few data are available on IGF-I or -II levels in IUGR children during childhood. Grunt et al and Rochiccioli et al reported normal IGF-I levels in IUGR children (19,33). In these studies, however, IGF-I levels were not corrected for age. To our knowledge, there are no data on IGF-II levels during childhood in IUGR children. In our study, we found significantly reduced mean levels of IGF-I and -II SDS for CA. 9/40 children had an IGF-I SDS_{CA} value more than 2 SD below the mean. As IGF-I levels are at least in part GH-dependent, the

reduced IGF-I levels reflect our findings of disturbances in GH secretion in these children. Indeed, we found a significant correlation (r_{sp}=0.43; P<0.01) between the maximal GH response during ATT and IGF-I SDS_{CA} values. Surprisingly, IGF-I levels did not correlate with endogenous GH secretion. This is in contrast with healthy children in whom IGF-I levels do reflect endogenous GH secretion (8). We cannot give a satisfacory explanation for this discrepancy. The cause of the decreased mean IGF-II levels is less clear, as IGF-II is found to be less GH-dependent. Reduced levels of IGF-II have also been described in GH-deficient children and children with normal short stature (38). The physiological meaning of IGF-II, however, is still unclear, and thus the interpretation of this finding is speculative.

We could not find any relation between the growth status of the child and the GH profiles, U-GH excretion or the growth factors. This is in accordance with Ackland et al, while others do not mention any relationship (1,2,33). This might be due to the little variance in growth status of these children. Several studies indicate the presence of a relationship between the growth during childhood and the level of GH secretion in as much it concerns the extremes in height (3,22,49); however, within the normal range of growth rates, this relation is less clear cut (11). The severity of IUGR was also not related to any of the above parameters. For GH, this finding was not completely unexpected as the influence of GH on birthlength is at least of minor importance. Apparently, the IGF-levels at childhood do not reflect birthlength. As IGF becomes GH dependent, postnatal adaptive mechanisms may be responsible for this. The heterogeneity of IUGR may also play a role.

The findings outlined do not enable us to elucidate the complete pathophysiological mechanism in IUGR children. Certainly, we have shown that many children have GH secretion disturbances, reduced IGF-levels and/or reduced U-GH excretion, but this GH/IGF axis disorder can only in part explain the fact of their growth failure. There are several children with no distinct GH/IGF axis abnormalities, who still failed to show postnatal catch up growth. One could speculate about abnormalities of the growth hormone receptor, the IGF-binding proteins or second messenger systems. In addition, the heterogeneity of the

etiology of IUGR may also play a role in some unexplained findings. However, supportive data are lacking.

With the availability of biosynthetic GH the efficacy and safety of GH treatment in children with non-GH deficient short stature is explored (17,21). This also accounts for children with short stature after IUGR (2,9,10,41). Our study results give support to the intention to treat these children in view of the 24-h GH profiles abnormalities, the subnormal GH responses during ATT and the reduced growthfactors. However, whether treatment will be beneficial to all children or only to those with suspected GH insufficiency can only be investigated within clinical trials including all children regardless of their GH secretion, as the complete pathophysiological mechanism is unclear and as there seems to be no clear relationship between GH secretion status and growth in these children.

In summary, our results indicate that many children with short stature after IUGR have 24-h GH profile abnormalities and/or subnormal responses to arginine provocation indicating a GH insufficiency. Mean serum levels of IGF-I and -II are significantly reduced. Compared to controls mean plasma GH levels and U-GH excretion are lower in boys, but not in girls. The precise mechanism of the failure to catch up growth needs further elucidation. It seems justified to start clinical trials in order to investigate whether treatment with exogenous GH will be beneficial to these children.

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REFERENCES

1. Ackland FM, Stanhope R, Eyre C, Hamill G, Jones J, Preece MA: Physiological growth hormone secretion in children with short stature and intra-uterine growth retardation. Horm Res 1988;30:241-45
2. Albertsson-Wikland K: Growth hormone secretion and growth hormone treatment in children with intrauterine growth retardation. Acta Paediatr Scand 1989;349(Suppl):35-41
3. Albertsson-Wikland K, Rosberg S: Analyses of 24-hour growth hormone profiles in children: relation to growth. J Clin Endocrinol Metab 1988;67:493-500
4. Albertsson-Wikland K, Rosberg S: Methods of evaluating spontaneous growth hormone secretion. In: Functional endocrinologic diagnostics in children and adolescents (ed. Ranke M.B.) 1992:76-101. J & J Verlag, Mannheim
5. Ashton IK, Zapf J, Einsckenk I, MacKenzie IZ: Insulin-like growth factors (IGF) 1 and 2 in human foetal plasma and relationship to gestational age and foetal size during midpregnancy. Acta Endocrinol 1985;110:558-563
6. Bennett A, Wilson DM, Liu F, Nagashima R, Rosenfeld RG, Hintz RL: Levels of insulin-like growth factors I and II in human cord blood. J Clin Endocrinol Metab 1983;57:609-612
7. Bercu BB, Shulman D, Root AW, Spillotis BE: Growth Hormone (GH) provocative testing frequently does not reflect endogenous GH secretion. J Clin Endocrinol Metab 1986;63:709-716
8. Blum WF, Albertsson-Wikland K, Rosberg S, Ranke MB: Serum levels of insulin-like growth factor I (IGF-I) and IGF Binding Protein 3 reflect spontaneous growth hormone secretion. J Clin Endocrinol Metab 1993;76:1610-1616
9. Chaussain JL, Landier F: Growth hormone treatment of short children born small for gestational age: a multicentre study. Ped Res 1993;33(Suppl.5):62
10. Colle M, Maes M, Chatelain P, Job JC, Vanderschueren-Lodeweyck M, Job B: Three years results of two dose-regimens of growth hormone in prepubertal children with idiopathic intra-uterine growth retardation. Ped Res 1993;33(Suppl.5):66
11. Costin G, Kaufman FR, Brasel JA: Growth hormone secretory dynamics in subjects with normal stature. J Pediatr 1989;115:537-544
12. Crowne EC, Wallace WHB, Shalet SM, Addison GM, Price DA: Relationship between urinary and serum growth hormone and pubertal status. Arch Dis Child 1992;67:91-95
13. Davies PSW, Valley R, Preece MA: Adolescent growth and pubertal progression in the Silver-Russell syndrome. Arch Dis Child 1988;63:130-135
14. D'Ercole AJ: The insulin-like growth factors and in utero growth. Growth Genet & Horm 1992;8:1-5
15. Donaldson DL, Pan F, Hollowell JG, Stevenson JL, Gifford RA, Moore WV: Reliability of stimulated and spontaneous Growth Hormone (GH) levels for identifying the child with low GH secretion. J Clin Endocrinol Metab 1991;72:647-652
16. Fitzhardinge PM, Inwood S: Long-term growth in small-for date children. Acta Paediatr Scand 1989;349(Suppl):27-33
17. Frasier SD, Lippe BM: Clinical review 11: The rational use of growth hormone during childhood. J Clin Endocrinol Metab 1990;71:269-273
18. Gluckman PD, Brinsmead MW: Somatomedin in cord blood: relationship to gestational age and birth size. J Clin Endocrinol Metab 1976;43:1378-1381

19. Grunt JA, Howard CP, Daughaday WH: Comparison of growth and somatomedin C responses following growth hormone treatment in children with small-for-date short stature significant idiopathic short stature and hypopituitarism. Acta Endocrinol 1984;106:168-174
20. Heinrich UE: Intrauterine growth retardation and familial short stature. Baillière's Clin Endocrinol Metab 1992;6(3):589-601
21. Hindmarsh PC, Bridges NA, Brook CGD: Review: Wider indications for treatment with biosynthetic human growth hormone in children. Clin Endocrinol 1991;34:417-427
22. Hindmarsh PC, Smith PJ, Brook CGD, Matthews DR: The relationship between height velocity and growth hormone secretion in short prepubertal children. Clin Endocrinol 1987;27:581-591
23. Hokken-Koelega ACS, Hackeng WHL, Stijnen Th, Wit JM, de Muinck Keizer-Schrama SMPF, Drop SLS: Twenty-four-hour Growth Hormone (GH) profiles urinary GH excretion and plasma Insulin-like Growth Factor-I and -II levels in prepubertal children with chronic renal insufficiency and severe growth retardation. J Clin Endocrinol Metab 1990;71:688-695
24. Hokken-Koelega ACS, Stijnen Th, de Muinck Keizer-Schrama SMPF, Blum WF, Drop SLS. Levels of growth hormone IGF-I and -II and cortisol in prednisone treated children with growth retardation after renal transplantation. J Clin Endocrinol Metab 1993;77:932-938
25. Kida K, Ito T, Hayashi M, Kaino Y, Goto Y, Ikeuchi M, Matsuda H: Urinary excretion of human growth hormone in children with short stature: Correlation with pituitary secretion of human growth hormone. J Ped 1992;120:223-237
26. Lassarre C, Hardouin S, Daffos F, Forestier F, Frankenne F, Binoux M: Serum insulin-like growth factors and insulin-like growth factor binding proteins in the human fetus Relationships with growth in normal subjects and in subjects with intrauterine growth retardation. Ped Res 1991;29:219-225
27. Martha PM, Gorman KM, Blizzard RM, Rogol AD, Veldhuis JD: Endogenous growth hormone secretion and clearance rates in normal boys as determined by deconvolution analysis: relationship to age pubertal status and body mass. J Clin Endocrinol Metab 1992;74:336-344
28. Mauras N, Blizzard RM, Link K, Johnson ML, Rogol AD, Veldhuis JD: Augmentation of growth hormone secretion during puberty: evidence for a pulse amplitude-modulated phenomenon. J Clin Endocrinol Metab 1987;64:596-601
29. Merriam GR, Wachter KW: Algorithms for the study of episodic hormone secretion. Am J Physiol 1982;243:E310-318
30. Ranke MB, Haber P: Growth Hormone stimulation tests. In: Functional endocrinologic diagnostics in children and adolescents (ed. Ranke MB) 1992;61-75. J & J Verlag Mannheim
31. Rikken B, Wit JM: Prepubertal height velocity references over a wide age range. Arch Dis Child 1992;67:1277-1280
32. Rochiccioli P, Enjaume C, Tauber MT, Plenkowski C: Statistical study of 5473 results of nine pharmacological stimulation tests: a proposed weighting index. Acta Paediatr Scand 1993;82:245-248
33. Rochiccioli P, Tauber M, Moisan V, Plenkowski C: Investigation of growth hormone secretion in patients with intrauterine growth retardation. Acta Paediatr Scand 1989;(Suppl) 349:42-46

34. Roede MJ, van Wieringen JC: Growth diagrams 1980 Netherlands. Third nation-wide survey. Tijdschrift Sociale Gezondheidszorg 1985;62(Suppl):1-34
35. Rolland-Cachera MF, Sempé M, Guilloud-Bataille M, Patois E, Péquignot-Guggenbuhl F, Fautrad V: Adiposity indices in children. Am J Clin Nutr 1982;36:178-184
36. Rose SR, Municchi G, Barnes KM, Kamp GA, Uriarte M, Ross JL, Cassorla F, Cutler GB: Spontaneous growth hormone secretion increases during puberty in normal girls and boys. J Clin Endocrinol Metab 1991;73:428-435
37. Rose SR, Ross JL, Uriarte M, Barnes KM, Cassarla FG, Cutler GB: The advantage of measuring stimulated as compared with spontaneous growth hormone levels in the diagnosis of growth hormone deficiency. N Engl J Med 1988;319:201-207
38. Rosenfeld RG, Wilson DM, Lee PDK, Hintz RL: Insulin-like growth factors I and II in evaluation of growth retardation. J Pediatr 1986;109:428-433
39. Seigel SF, Becker DJ, Lee PA, Gutai JP, Foley TP, Drash AL: Comparison of physiologic and pharmacologic assessment of growth hormone secretion. Am J Dis Child 1984;138: 540-543
40. Spiliotis BE, August GP, Hung W, Sonis W, Mendelson W, Bercu BB: Growth hormone neurosecretory dysfunction: a treatable cause of short stature. JAMA 1984;251:2223-2230
41. Stanhope R, Preece MA, Hamill G: Does growth hormone treatment improve final height attainment of children with intrauterine growth retardation? Arch Dis Child 1991;66:1180-1183
42. Straus DS, Ooi GT, Orlowski CC, Rechler MW: Expression of the genes for insulin-like growth factor-I (IGF-I) IGF-II and IGF-Binding Proteins-1 and -2 in fetal rat under conditions of intrauterine growth retardation caused by maternal fasting. Endocrinology 1991;128:518-525
43. Sukegawa I, Hizuka N, Takano K, Asawaka K, Horikawa R, Hashida S, Ishakawa E, Mohri ZI, Murakami Y, Shizume K: Urinary Growth Hormone (GH) measurements are useful for evaluating endogenous GH secretion. J Clin Endocrinol Metab 1988;66:1119-1123
44. Tanner JM, Lejarraga H, Cameron N: The natural history of the Silver-Russell syndrome: a longitudinal study of thirty-nine cases. Ped Res 1975;9:611-623
45. Tanner JM, Whitehouse R: Longitudinal standards for height weight-height height velocity and stages of puberty. Arch Dis Child 1976;51:170-179
46. Thieriot-Prevost G, Boccara JF, Francoual C, Badoual J, Job JC: Serum insulin-like growth factor 1 and serum growth promoting-activity during the first postnatal year in infants with intrauterine growth retardation. Ped Res 1989;24:380-383
47. Unterman TG, Simmons RA, Glick RP, Ogata ES: Circulating levels of insulin insulin-like growth factor-I (IGF-I) IGF-II and IGF-Binding Proteins in the small for gestational age fetal rat. Endocrinology 1993;132:327-337
48. Usher R, MacLean F: Intrauterine growth of liveborn caucasian infants at sea level: Standard obtained from measurements of infants born between 25 and 44 weeks of gestation. J Pediatr 1969;74:901-910
49. Zadik Z, Chalew SA, Raiti S, Kowarski AA: Do short children secrete insufficient growth hormone? Pediatrics 1985;76:355-360

CHAPTER 10

GH-therapy in prepubertal children with short stature after intrauterine growth retardation: two years results of a randomized, double-blind, dose-response trial.

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ABSTRACT

To evaluate the effect of growth hormone (GH) on linear growth, bone maturation, pubertal development and final height (FH) in children with short stature after intrauterine growth retardation (IUGR) a multicenter, randomized, double-blind, dose-response study is being carried out in 79 prepubertal IUGR children (52 boys/27 girls). IUGR was diagnosed when birth length was 2 SD or more below the mean for gestational age according to the standards of Usher & McLean. Patients have been assigned to either 3 (group A) or 6 (group B) IU/m^2/day of recombinant GH (Norditropin$^{®}$). After two years of treatment an interim analysis was performed on a total of 62 patients (34 in group A/28 in group B). A significant increase in height and height velocity was observed over the two years treatment period, which was dose-dependent. The mean (SD) increase in height SDS for chronological age (CA) was +1.3 for group A and +1.7 for group B. The mean increase in height velocity SDS for CA was +4.0 and +5.1 for groups A and B, respectively. An increase in bone maturation was found for each group during two years of GH therapy, which was not dose-dependent. However, a significant improvement in predicted FH was present after two years of GH treatment, suggesting that the increase in height surpassed the advanced bone maturation. In a subgroup of 30 children who had completed 3 years of GH treatment, bone maturation decreased over the third year of treatment, increasing the possibility of an improvement in FH. We stress the fact that data on bone age and height prediction have to be handled with caution since the natural pattern of growth and development may be different in children with IUGR. Plasma levels of IGF-I and IGFBP-3 at the start of the study were significantly reduced compared to healthy controls for both groups. GH treatment induced a significant increase in IGF-I levels and normalization of IGFPB-3 levels. Preliminary analysis showed no correlations between the growth response to GH therapy and pretreatment parameters of GH secretory status. However, the change in IGF-I SDS for CA and in IGFBP-3 SDS over two years of GH therapy was significantly correlated with the change in

$HSDS_{CA}$ over two years of GH treatment (r=0.46 and r=037, respectively; P-values<0.02). Glucose tolerance was maintained at the expense of a significant rise in fasting insulin concentrations and a significant increase in total insulin production after oral glucose stimulation for both groups. In conclusion, GH therapy in children with short stature after IUGR appears to be efficacious in increasing height and height velocity. In addition, a significant improvement in predicted FH was achieved without serious adverse effects, despite advanced bone maturation. However, final results of this ongoing study are required to draw definite conclusions.

INTRODUCTION

Short stature after intrauterine growth retardation (IUGR) is a well known phenomenon. Although postnatal catch-up growth occurs in most of the IUGR newborns, about 15% of these children fail to show catch-up growth (1,2). They present with a height deficit during childhood that may result in short adult stature (3,4). The etiology of IUGR includes maternal factors, such as toxaemia and smoking, and fetal and placental factors. In many cases no underlying condition can be determined. The mechanism of the stunted growth in IUGR children is poorly understood. However, it has been shown previously by us (5) and others (6-8) that disturbances in the GH/IGF axis may account for some of the growth retardation: up to 60 percent of the IUGR children have GH-secretory abnormalities and/or reduced levels of Insulin-like growth factors (IGFs). GH treatment in short children after IUGR has been explored from the early 1970s (9,10). Initial data were disappointing probably due to the low dose and frequency of GH administration. More recent studies have indicated that daily administration of recombinant human GH therapy in varying dosages accelerates growth significantly in children with short stature after IUGR (6,11-14). In order to evaluate whether GH treatment will improve linear growth and final adult height, we set up a randomized, double-blind, dose-response multicenter trial in 79 prepubertal children with short stature

secondary to IUGR. We now report on the efficacy and safety of two doses of GH (3 versus 6 IU/m^2/day) after two years of treatment in 62 patients. In addition, we describe IGF-I and IGFBP-3 plasma levels in relation to GH therapy.

PATIENTS AND METHODS

Patients

The study population comprises 79 prepubertal IUGR children (52 boys, 27 girls). IUGR was diagnosed when length at birth was 2 SD or more below the mean for gestational age according to the standards of Usher and McLean (15). In addition, the following inclusion criteria were used: 1) chronological age (CA) of at least 3.00 years and less than 11.00 years in boys and 9.00 years in girls, 2) growth retardation determined by a height SDS for CA ($HSDS_{CA}$) < -1.88 (that is <P3 for CA according to Dutch references (16)), 3) height velocity (HV) for CA under the 50th percentile for CA (17), 4) prepuberty defined as Tanner stage I or a testicular volume < 4ml (18), 5) uncomplicated neonatal period, that is without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without complicated sepsis neonatorum and without long-term complications of respiratory ventilation such as bronchopulmonary dysplasia or pneumothorax. Excluded from the study were children with syndromes and chromosomal disorders, patients with other organic causes for growth retardation such as severe chronic illness and chondrodysplasia, and patients who had received any previous hormonal treatment; patients with Silver-Russell syndrome (SRS), however, were included in this study.

Study procedure

Four centers in the western part of The Netherlands participated in the study. The study was approved by the ethics committee of each participating center. At preinclusion written informed consent was obtained from the parents.

Upon inclusion, patients were stratified according to age and plasma 24-hours GH profile and then randomly and blindly assigned to either 3 or 6 IU/m²/day (≈0.1 and 0.2 IU/kg/day) of recombinant human GH (Norditropin®, Novo Nordisk A/S), administered once daily by subcutaneous injection. Patients were examined at enrollment and subsequently every 3 months at the four participating centers and always by one and the same investigator (WdW).

Height was measured with a Harpenden stadiometer by the same investigator (WdW). The height reported at each visit was the mean of four successive measurements performed by the same observer using the same stadiometer.

Bone age (BA) was determined at the start of the study and subsequently every 6 months, using the TW2-method (19). All bone ages (TW-RUS) were rated by one and the same pediatric radiologist. Final height (FH) prediction was performed by the method developed by Tanner et al (19).

Blood and urine samples were taken at each visit for determination of complete blood cell count, serum electrolytes, creatinine, alkaline phosphatase, ALAT, ASAT, calcium, phosphate, and urine stick analysis. Thyroid function (TSH, T4) and Hemoglobin-A_{1c} (HbA_{1c}) were measured at start and at 6 monthly intervals.

At start of the study and at 12 months a standard oral glucose tolerance test (oGTT) was performed after an overnight fast. A high carbohydrate intake had been given for 3 days prior to testing. Glucose (1.75 g/kg with a maximum of 75 g) was administered orally. Blood samples were taken at -15, 0, 15, 30, 60, 90 and 120 minutes for glucose determination and at 0, 30, 60 minutes for insulin determination.

Prior to treatment a standard arginine tolerance test (ATT) was performed after an overnight fast. Arginine (0.5 g/kg) was infused over 30 minutes. Blood samples were taken at -15, 0, 15, 30, 45, 60, 90 and 120 minutes for plasma GH determination.

Before treatment 24-hours GH profiles were performed in the first 40 patients who enrolled the study, as described previously (5).

Additional blood samples were taken at the start of the study and at 6, 12 and 24 months for determination of IGF-I and IGFBP-3. After centrifugation, serum samples were frozen (-20°C) until assayed.

Plasma GH was determined by a double antibody RIA, as described previously (20). Concentrations of plasma IGF-I were determined by a specific radioimmunoassay (RIA) after acid chromatography (20). IGFBP-3 was isolated from human plasma in principle according to the method developed by Martin and Baxter (21). IGFBP-3 was determined by a specific RIA using an polyclonal antiserum derived from New Zealand White rabbits. The assay had a sensitivity of 0.5 ng/ml with an intra-assay variation of 6.9% at 2.52 mg/l and 12.9% at 0.83 mg/l. The interassay variation was 10.8% at 2.88 mg/l and 9.9% at 1.67 mg/l. Since levels of both IGF-I and IGFBP-3 are dependent on age and sex, values were transformed to SD scores for CA. For IGF-I reference values were used based on 600 samples from a healthy Dutch population, as described previously (20). Reference data for IGFBP-3 were available from a healthy Dutch population of 286 children aged 0 - 14 years, provided by the laboratory. Serum glucose was measured in automatic analyzers using glucose oxidase, with interassay and intraassay variability < 10%. Insulin was measured by RIA with interassay variability < 12%. HbA_{1c} was determined by HPLC-method (high pressure liquid chromatography) using a Diamat (Biorad, USA).

Methods of expression of growth parameters

Height was expressed as SD score for CA ($HSDS_{CA}$) according to Dutch references (16). Pretreatment HV in cm per year was calculated by fitting a quadratic linear model to the pre-treatment height data collected within four years before start of treatment; HV was calculated by the differential from the fitted model. During treatment, growth was assumed to be linear and HV in cm per year was calculated by piecewise and continuous linear regression for each succesive 6 month period during the first year and then for each successive 12 month period. HV was also expressed in SDS for CA ($HVSDS_{CA}$) using height velocity references

derived from the childhood phase of the Infant-Childhood-Puberty model and adapted for wide age ranges (17). Bone maturation was expressed as the ratio of increase in BA to increase in CA ($\Delta BA/\Delta CA$). The target height (TH) was calculated according to the formula: (length father + length mother + 12)/2 + 3 for boys and (length father + length mother - 12)/2 + 3 for girls, in which 3 cm represents the secular trend (22). THSDS then was calculated using Dutch references of mean and SD of adult men and women (16).

Interim analysis

An interim analysis was planned for a total of at least 60 children having completed the full treatment period of two years. Sample size calculation had been based on previously reported growth responses of growth hormone deficient (GHD) children (23). Sixty children randomized into two groups of 30 to each dosage and a treatment effect within each dose group equivalent to a change in the average height velocity of 1.1 cm/year would result in a statistical significance with a probability of 80% (equivalent to a risk of a type II error of 20%). The difference in height velocity obtained between the dose groups should be in the order of 1.9 cm/year to make statistical significance (with a risk of a type II error of 20%) based on the above number of patients. The estimate of the sample size was based on a within group and a between group comparison of the change in height velocity by a two-sided t=test for paired and unpaired design, respectively and a significance level of 5% (equivalent to a risk of a type I error of 5%). In total, 62 patients were included in the interim analysis.

Statistics

Differences between groups were tested using unpaired t-tests or Wilcoxon's two sample test, as appropriate. The between group comparisons over time were assessed by repeated measures analysis of variance. Within treatment group comparisons were made by paired t-tests or Wilcoxon matched-pairs signed-ranks tests, as appropriate. Correlations between variables were tested using Spearman's

correlation test. The ratio $\Delta BA/\Delta CA$ was calculated and compared within treatment to see if the increase was significantly different to unity indicating acceleration in bone maturation.

During the two years treatment period 10 patients (7 girls/3 boys) entered into puberty. Due to a discrepancy between vial preparation and mode of administration, the first 19 children (9 boys, 10 girls) who enrolled in the study actually received half of the assigned GH dosage in the first three months of treatment. Subgroup analysis was therefore performed excluding either one or both of these groups of patients.

Since the study remains double-blinded, statistical analysis was performed by an independent statistician (N.Baker, Pharmaco LSR Ltd, England) and therefore data are only expressed as mean (SD).

RESULTS

Table 1 shows the baseline clinical data of all 62 patients (39 boys/ 23 girls). No significant differences were found between group A and B in baseline data, except for mother's height; the children from group A had shorter mothers compared to group B ($P<0.05$). Parental height was significantly shorter compared to the Dutch population ($P<0.05$) Consequently the target height of the patients was significantly shorter compared to the normal population. One patient dropped out after the first year of treatment due to lack of motivation despite marked growth acceleration. The subcutaneous injections were well tolerated.

Growth

Table 2 and figures 1 and 2 summarize the effect of 3 IU (group A) versus 6 IU (group B) of GH/m^2/day on $HSDS_{CA}$, HV and $HVSDS_{CA}$ for all children. We found a significant effect of both GH doses on $HSDS_{CA}$ and $HVSDS_{CA}$ compared to pretreatment data (P-values<0.001). This effect sustained over the two years treatment period. In addition, the change in $HSDS_{CA}$ after 2 years of treatment was

significantly different between group A and B (P=0.001). The change in $HVSDS_{CA}$

Table 1 Baseline clinical data for 62 IUGR patients. Data expressed as mean (SD).

	GH dosage groups	
	A (n=34)	B (n=28)
Male/Female	24/10	15/13
Age (yrs)	7.43 (2.30)	6.97 (2.40)
BA (yrs)	6.9 (2.8)	6.5 (3.2)
$HSDS_{CA}$	-2.79 (0.66)	-2.94 (0.66)
Prestudy HV (cm)	5.9 (1.2)	5.9 (1.6)
Prestudy $HVSDS_{CA}$	0.07 (1.19)	-0.17 (1.43)
Birth weight (g)	2031 (701)	1826 (745)
Birth length (cm)	42.6 (4.6)	40.9 (5.6)
Father's height (cm)	173.4 (7.4)	176.1 (6.7)
Father's height SDS	-1.27 (1.12)[a]	-0.88 (1.01)[a]
Mother's height (cm)	158.3 (7.7)	162.5 (7.4)*
Mother's height SDS	-1.62 (1.24)[a]	-0.43 (0.89)*[a]
Target height SDS	-1.04 (0.87)[a]	-0.43 (0.89)[a]*

* significantly different compared to group A (P<0.05)
[a] significantly different from zero (P<0.05)
BA = bone age
$HSDS_{CA}$ = height standard deviation score for chronological age
HV = height velocity
$HVSDS_{CA}$ = height velocity standard deviation score for chronological age
SDS = standard deviation score

over the first 6 months and between 6 and 12 months was significantly different between group A and B (P=0.001, and P=0.011, respectively). However, no significant difference between both groups was found for the change in $HVSDS_{CA}$ over the first year of treatment nor over the second year of treatment.

Table 2. The effect of 3 (group A) versus 6 (group B) IU/m²/day of GH on $HSDS_{CA}$, HV and $HVSDS_{CA}$ in 62 IUGR patients. Data expressed as mean (SD).

		GH dosage groups	
		A	B
$HSDS_{CA}$	pretreatment	-2.79 (0.66)	-2.94 (0.66)
	6 months	-2.34 (0.59)	-2.26 (0.72)
	12 months	-1.95 (0.60)	-1.77 (0.82)
	18 months	-1.67 (0.59)	-1.46 (0.88)
	24 months	-1.47 (0.64)	-1.21 (0.98)
HV (cm/yr)	pretreatment	5.9 (1.2)	5.9 (1.6)
	0 - 6 months	7.9 (1.8)	10.2 (2.4)
	6 - 12 months	10.1 (1.2)	11.1 (1.8)
	12 - 24 months	8.0 (1.1)	9.0 (1.3)
$HVSDS_{CA}$	pretreatment	0.07 (1.19)	-0.17 (1.43)
	0 - 6 months	2.94 (2.44)	5.56 (2.66)
	6 - 12 months	6.11 (1.82)	7.00 (2.54)
	12 - 24 months	4.03 (1.95)	4.95 (2.06)

$HSDS_{CA}$ = Height standard deviation score for chronological age
HV = Height velocity
$HVSDS_{CA}$ = Height velocity standard deviation score for chronological age

Subgroup analysis revealed no change in outcome with respect to the differences between group A and B in the change in $HSDS_{CA}$ or $HVSDS_{CA}$ over the 2 years of treatment. A significant effect of underdosing was only present during the first 3 months of treatment and affected group B more than group A. Subgroup

analysis excluding the underdosed children revealed that a dosage effect was only present on $HVSDS_{CA}$ over the first 6 months of therapy. In addition, subgroup analysis on the prepubertal children only showed a dosage effect on $HVSDS_{CA}$ over the first year of GH treatment (P=0.045).

Figure 1. The effect of 3 (group A) versus 6 (group B) IU/m²/day of GH on height SDS for chronological age in 62 IUGR patients. Data expressed as mean (SD).

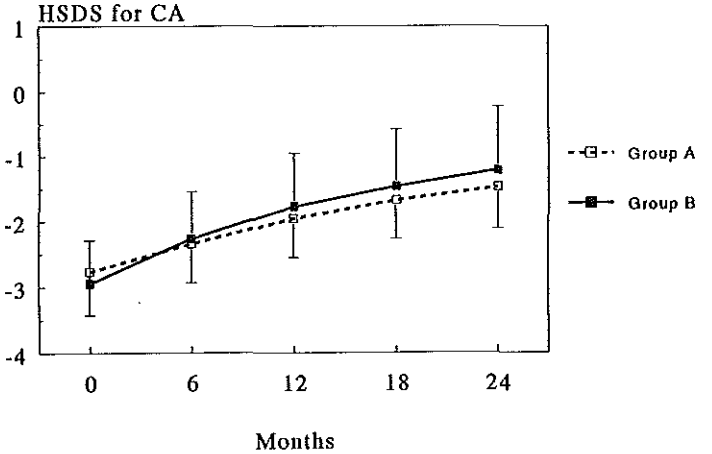
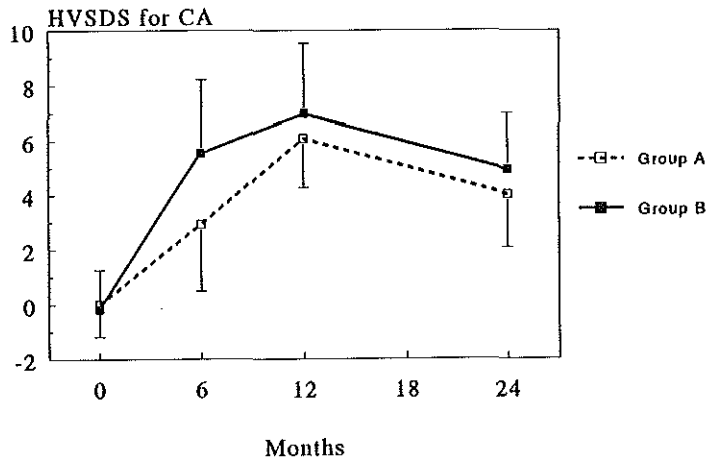


Figure 2. The effect of 3 (group A) versus 6 (group B) IU/m²/day of GH on height velocity SDS for chronological age in 62 IUGR patients. Data expressed as mean (SD).



Bone maturation and height prediction

Table 3 lists the effect of GH treatment on bone maturation. The ΔBA/ΔCA ratios calculated over the first and second year of treatment for both GH doses were all significantly different from 1 (P-values < 0.001), indicating acceleration of bone maturation. No significant difference in bone maturation was found between groups A and B.

Table 3. The effect of 3 (group A) versus 6 (group B) IU/m²/day of GH on bone maturation in 62 IUGR patients. Data expressed as mean (SD).

Bone maturation (ΔBA/ΔCA)	GH dosage groups A	B
All patients		
first year	1.8 (0.9)	1.7 (1.0)
second year	2.2 (0.9)	2.3 (0.9)
Subgroup (n=30)[a]		
third year	*0.6 (0.9)*	

[a] see text

At the time of the interim analysis, BA data of three years of GH treatment were available for 30 children. Since BA showed an acceleration during the first two years of GH treatment, we also calculated the ΔBA/ΔCA ratio over the third year for these 30 children. The ΔBA/ΔCA ratio of this subgroup of children was 0.6 (0.9), being significantly different from 1.

Additionally, in order to look at the effect of GH treatment on FH, FH predictions were performed according to the prediction equations developed by Tanner et al (16). Since these equations are age-limited, complete longitudinal data

of the 2 years treatment period were available in only 48 children, 26 boys and 22 girls. The FH predictions per yearly interval for both boys and girls are given in table 4. Initially, we had planned no formal analysis on FH prediction. Therefore, in this second step analysis, no indepth analyses such as search for dosage effects were performed. FH prediction after 2 years of GH treatment was significantly increased compared to pretreatment predictions for boys as well as for girls (P-values<0.001). The increment of the FH prediction over the two years treatment period was 4.9 (4.0) cm for boys and 5.4 (2.8) cm for girls, being not significantly different between the sexes. At start of therapy, there was a significant difference between the FH prediction and the TH for both boys and girls: the FH prediction was 14.3 (5.4) cm and 10.2 (6.2) cm below the TH for boys and girls, respectively (P-values<0.001), which was also statistically significant between the sexes (P<0.05).

Table 4. The longitudinal effect of 3 (group A) versus 6 (group B) IU/m²/day of GH on final height prediction in 48 IUGR patients[a]. Data expressed as mean (SD).

		Final height prediction			ΔFH 0-2 yr
		start	first yr	second yr	
Boys	n=26	161.0 (3.5)	165.3 (3.9)	165.9 (5.0)	4.9 (4.0)
Girls	n=22	155.5 (4.6)	159.2 (5.5)	160.9 (5.5)	5.4 (2.8)

[a] = For 14 children final height predictions were not available at time of start of GH treatment due to the age-limited equations (19).

ΔFH 0-2 yr = The change in final height prediction over the two years of GH treatment.

GH/IGF axis

At start of the study the mean maximal plasma GH response during ATT was 20.5 (12.2) mU/l in group A and 28.9 (11.1) mU/l in group B, which was significantly different between the groups ($P<0.01$). In a subgroup of 40 children, data of 24 hour GH profiles had been collected prior to GH treatment as described previously (5). We found no significant correlations between parameters of GH-secretion, as determined by 24-hour GH profiles (mean GH, number of peaks above 20 mU/l, peak amplitude and number of peaks) or by pharmacological testing (maximal GH response during ATT), and the change in $HSDS_{CA}$ over two years of treatment.

Mean levels of IGF-I and IGFBP-3 at start of the study, expressed as SDS for CA, were significantly different from zero for both GH groups, indicating reduced plasma concentrations (table 5) (P-values<0.05). During GH treatment, these plasma levels increased significantly during the first year in both groups (P-values < 0.001) and remained so during the second year of treatment. For IGF-I SDS_{CA} but not for IGFBP-3 SDS_{CA}, a significant difference was observed between groups A and B for all time points ($P<0.001$), showing the highest levels in group B. Figure 3 visualizes the change in IGF-I SDS_{CA} and IGFBP-3 SDS_{CA} levels during the 2 yrs of GH treatment for both groups.

Preliminary analysis showed no correlation between the change in $HSDS_{CA}$ over the first or second year of GH treatment and baseline levels of IGF-I and IGFBP-3, expressed as SD scores. However, the change in IGF-I SDS over one year and over two years of GH therapy correlated significantly with the change in $HSDS_{CA}$ over two years of treatment ($r=0.46$, $P=0.003$ and $r=0.49$, $P=0.001$, respectively). Moreover, the change in IGFBP-3 over two years of GH treatment but not over the first year of treatment correlated significantly with the change in $HSDS_{CA}$ over 2 years ($r=0.37$, $P<0.02$ and $r=0.20$, $P>0.10$, respectively).

Carbohydrate metabolism

Table 5 shows plasma glucose and insulin levels during oGTT. Fasting

Figure 3. The effect of 3 (group A) versus 6 (group B) IU/m²/day of GH on IGF-I SDS and IGFPB-3 SDS levels in 62 IUGR patients.

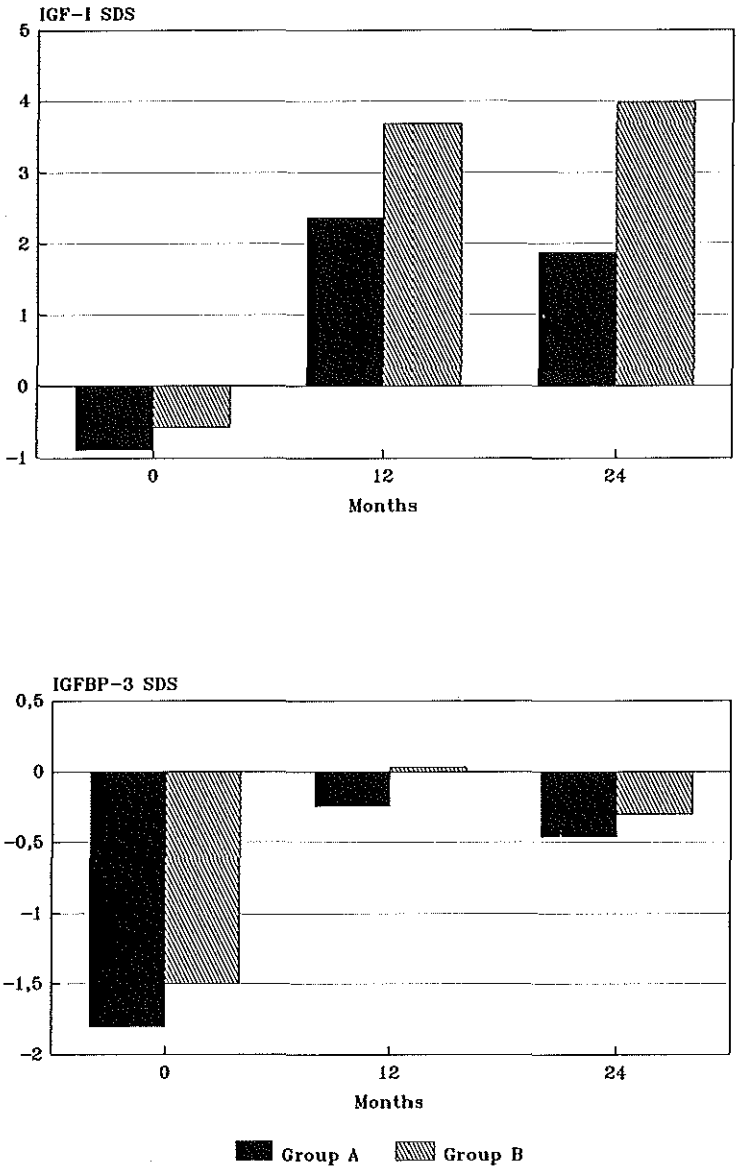


Table 5. The effect of 3 IU (group A) versus 6 IU (group B) of GH/m²/day on mean plasma levels of IGF-I and IGFBP-3, and mean plasma glucose and insulin levels as determined by oGTT in 62 IUGR patients.

		GH dosage group	
		A	B
IGF-I SDS	start	-0.88	-0.57
	1 year	2.36	3.68
	2 years	1.87	3.98
IGFBP-3 SDS	start	-1.80	-1.50
	1 year	-0.24	0.03
	2 years	-0.46	-0.30
Fasting glucose (mmol/l)	start	4.5	4.7
	1 year	4.9	4.9
Fasting insulin (mUl/l)	start	6.4	4.9
	1 year	7.6	8.4
AUC glucose (mmol/l)	start	786	744
	1 year	801	769
AUC insulin (mU/l)	start	1433	1161
	1 year	2101	2634

SDS = standard deviation score
AUC = calculated area under the curve

glucose levels and the calculated area under the curve (AUC) for glucose were not significantly different after 1 year compared to prestudy values and not significantly different between the GH dosage groups. At the start of the study, group A had

significantly higher levels of fasting insulin than group B ($P<0.05$). Fasting insulin levels increased significantly after 1 year of GH treatment for both groups ($P<0.01$). In addition, the calculated AUC for insulin increased significantly for both groups ($P<0.001$), but was not significantly different between the groups.

Mean HbA_{1c} levels were 5.1 (0.3) and 5.0 (0.4) at start of study for group A and B, respectively. GH therapy induced no significant change in mean HbA_{1c} levels in both groups.

Other safety parameters

Throughout the study there were slight, statistically significant but clinically non-significant, time-related changes in blood chemistry and thyroid function. However, all values stayed well within the normal limits, irrespective of GH dosage. Urinalysis revealed no abnormalities during the study in either study group.

DISCUSSION

The present study shows that 2 years of therapy with recombinant GH gives a significant improvement of growth in children with persistent short stature after IUGR. We demonstrated a significant effect of both GH doses (3 (group A) and 6 (group B) IU/m^2/day) on linear growth, expressed as the mean change in $HSDS_{CA}$ and $HVSDS_{CA}$ over 2 years. In addition, a clear dose-response effect was present with the highest dose showing a greater change in $HSDS_{CA}$. In our study, the mean change in $HSDS_{CA}$ was +1.3 and +1.7, whereas the mean change in $HVSDS_{CA}$ was +4.0 and +5.1 for group A and B, respectively. A significant, dose-dependent improvement of linear growth during GH therapy has been described in previous studies (6,8,11-14). However, these studies showed lower growth responses compared to our study. Chatelain et al (13) reported a mean change in $HVSDS_{CA}$ after two years treatment of +1.8 for the highest dose (1.2 IU/kg/week, *i.e.* approximately 5 IU/m^2/day) and Chaussain et al (14) described a change in $HSDS_{CA}$

of +1.0 and +1.4 after two years of treatment using GH doses of 0.7 and 1.4 IU/kg/week, respectively. These observed differences in growth response to GH treatment are likely to be related to differences in patient selection. For instance in our sample, no distinction was made for differences in GH response during stimulation testing, while others (11-14) have included only children with adequate GH responses. The reason for us not to exclude children with suspected GH insufficiency was that in a previous study we did not find a clear relation between GH secretion status and spontaneous growth in these children (5). Thus, as the complete pathophysiological mechanism is unclear, we wanted to study whether there is a relation between growth response to GH treatment and parameters of GH secretion and included all patients regardless of their GH secretion, but after stratification for parameters of GH secretion. Another issue contributing to the differences in growth response is, that children with short stature secondary to IUGR probably represent multiple aetiologies; in some way this may be responsible for a heterogeneous growth response.

In our study, the most remarkable finding was the acceleration in bone maturation during the 2 years of GH treatment, which appeared to be not dose dependent. In the recent studies data on bone maturation are not consistent. Stanhope et al (11,12), studying 24 children with short stature after IUGR, described that height for BA SDS did not increase after 3 and 4 years of treatment with 30 IU/m^2/week. This implies the presence of an advance in epiphyseal maturation, since the increase in HVSDS sustained during treatment. Unfortunately, exact data on bone maturation were not given. In contrast, the combined Belgian/French study (13) treating 95 children with either 0.4 or 1.2 IU/kg/week (*i.e.* approximately 2 and 5 IU/m^2/day, respectively), reported ratios of ΔBA/ΔCA varying from 1.1 to 1.6, which were also not significantly different between both GH dosage groups. In another French study (14) in which 130 children received either 0.7 or 1.4 IU/kg/week (approximately 3 and 6 IU/m^2/day, respectively) or served as controls, bone maturation over two years of GH treatment did not exceed 1.2 years per year in either dosage group. The observed differences in bone maturation are

partly due to differences in bone age assessment: some investigators (13,14) have used bone age determinations according to Greulich and Pyle (24), while others (11,12) and we have used the method of Tanner et al (19). Since advanced progression in bone maturation may attenuate the beneficial effects on final height we looked at the ΔBA/ΔCA ratio over the third year of treatment of a subgroup of children who had completed 3 years of GH treatment. These data showed that a decrease in progression of bone maturation is to be expected, increasing the possibility of an improvement in final height. It should be stressed, however, that the interpretation of bone maturation is hampered by the clinical impression that spontaneous epiphyseal maturation is inappropriately advanced between the age of 6-10 years in many untreated IUGR children with or without dysmorphic features and that precocious or early puberty is seen more frequently than in healthy children (3,25-28). Therefore, one should be cautious to place too much emphasis on bone age data, as also mentioned by others (6,11-13).

Despite the observed progression in bone maturation, a significant improvement in FH prediction was observed during the treatment period. This suggests that the increase in height surpasses the increase in bone maturation. However, as with bone age determination, one should be extremely cautious to rely completely on height prediction. Height prediction in children with growth disorders may overestimate adult final height (29,30). Only long-term studies with the attainment of adult final height will enable definite conclusions on the benefit of GH treatment in children with short stature after IUGR. Our study is an ongoing, double-blinded study on the effect of two doses of GH on linear growth and FH. Therefore, final results have to be awaited.

We have shown previously, that a substantial part of the children with short stature after IUGR have biochemical signs of GH insufficiency (5). This is confirmed by this study on a larger group of IUGR children: we found significantly reduced levels of IGF-I and IGFBP-3 compared to healthy children. Since GH is one of the most important regulatory factors for IGF-I and IGFBP-3, our findings support the hypothesis that persistent defects in the GH/IGF axis are present in children with

short stature after IUGR. The underlying mechanism, however, remains to be established. It is speculative that the disturbances in GH secretion observed in children with IUGR reflect alterations in brain structure and function. In utero, the brain undergoes a period of impressive growth and development (31,32). During this period, it may be vulnerable to nutritional disturbances. Indeed, animal studies have shown evidence that intrauterine undernutrition causes alterations in brain structure and function and affects the postnatal growth programme (33). Other lines of evidence are derived from clinical studies on children with IUGR showing an association between IUGR and an increased prevalence of mental handicap, lower intelligence, neurological deficits, poor academic performance and behavior problems (34-39). However, several children showed no catch-up growth in the absence of distinct GH/IGF axis abnormalities. Therefore, one has to speculate about other factors beside the GH/IGF axis responsible for the stunted growth such as hypothalamic appetite center disturbances (33), reduced cell number (40) or abnormalities at the level of the GH receptor or secondary messenger systems.

The growth response to GH therapy varies considerably. In a preliminary analysis, we found no significant correlations between *pretreatment* parameters of GH secretory status and the growth response to GH therapy. This is in contrast to the study of Albertsson-Wikland (6), who observed an inverse relationship between the amount of GH secreted over 24 hours, as determined by 24 hour GH profiles, and the increase in HVSDS. However, evaluating the relation between the growth response to GH therapy and biochemical changes *during* therapy we did find significant correlations. This implies, that it might be possible to predict the individual growth response to GH therapy after a limited period of GH treatment. In order to predict growth response to GH therapy, Ranke and coworkers (41) performed a regression analysis on data from 135 children with IUGR using various auxological predictor variables. It appeared that the GH dosage applied had the greatest influence on the growth response to GH therapy during the first year of treatment. However, only 23% of the variability of the growth response could be predicted from these variables and the GH dose accounted for most of the

explained variability (13%). In future, more indepth analyses including auxological and biochemical parameters are needed to investigate to what extent the growth response to GH treatment can be predicted in children with IUGR.

Concern has been expressed, that children treated with pharmacological doses of GH may develop carbohydrate intolerance and hyperinsulinaemia similar to those seen in acromegaly (42,43). In our study, glucose tolerance was maintained at the expense of a rise in fasting insulin concentrations and an increase in total insulin production after oral glucose stimulation. These findings are in concert with previous findings in short normal children treated with GH (44-46). It is speculated that the hyperinsulinemia may be a direct trophic and/or insulinotropic effect of GH on beta-cells (44), rather than a secondary consequence of insulin resistance. It cannot be excluded however, that insulin resistance may develop with longer duration of GH treatment leading to glucose intolerance or diabetes. It is therefore recommended to monitor carbohydrate metabolism during and after the course of GH treatment in these children, especially since it has been shown that IUGR *per se* is associated with insulin resistance and impaired glucose tolerance at adult life (47-49).

In summary, treatment with recombinant GH in prepubertal children with short stature after IUGR for two years has resulted in a dose-dependent increase in growth. However, an increase in bone maturation was observed as well, although dose-independent. Nevertheless, a significant improvement in FH prediction was present, suggesting that the increase in height surpassed the increased bone maturation. It should be stressed, however, that data on bone age and height prediction have to be handled with caution since the natural pattern of growth and development may be different in children with IUGR. Preliminary analysis showed no correlations between pretreatment parameters of GH secretory status and the growth response to GH treatment. However, significant correlations were observed between changes in biochemical parameters during GH therapy and the growth response after two years of GH therapy. Additional analyses are required to study the possibility of predicting the growth response to GH therapy in IUGR children.

GH treatment induced no serious adverse effects and glucose tolerance was not affected. However, GH therapy induced a significant increase in fasting insulin and total insulin levels after glucose loading. Monitoring of carbohydrate metabolism is therefore recommended during and after the course of GH treatment. Final height results of this ongoing, double-blind study are required before definite conclusions can be drawn.

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REFERENCES

1. Albertsson-Wikland K, Karlberg J: Natural growth in children born small for gestational age with and without catch-up growth. Acta Paediatr Scand 1994;399(Suppl):64-70
2. Hokken-Koelega ACS, de Ridder MAJ, van Lemmen RJ, den Hartog H, de Muinck Keizer-Schrama SMPF, Drop SLS: Children born small for gestational age: do they catch-up? Ped.Res. 1995;38:267-271
3. Job JC, Rolland A. Histoire naturelle des retards de croissance à début intra-utérin. Arch Fr Pediatr 1986;43:301-306
4. Chaussain JL, Colle M, Ducret JP: Adult height in children with prepubertal short stature secondary to intra uterine growth retardation. Acta Paediatr Scand 1994;399(Suppl):72-73
5. De Waal WJ, Hokken-Koelega ACS, Stijnen Th, de Muinck Keizer-Schrama, Drop SLS and the Dutch Working Group on Growth Hormone: Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. Clin Endocrinol 1994;41:621-630
6. Albertsson-Wikland K: Growth hormone secretion and growth hormone treatment in children with intrauterine growth retardation. Acta Paediatr Scand 1989;349(Suppl):35-41
7. Rochiccioli P, Tauber M, Moisan V, Pinkowski C: Investigation of growth hormone secretion in patients with intrauterine growth retardation. Acta Paediatr Scand 1989;349(Suppl):42-46
8. Stanhope R, Ackland F, Hamill G, Clayton J, Jones J, Preece MA: Physiological growth hormone secretion and response to growth hormone treatment in children with short stature and intra-uterine growth retardation. Acta Paediatr Scand 1989;349(Suppl):47-52
9. Tanner JM, Whitehouse RH, Hughes PCR, Vince FP: Effect of human growth hormone treatment for 1 to 7 years on the growth of 100 children with growth hormone deficiency, low birth weight, inherited smallness, Turner's syndrome, and other complaints. Arch Dis Child 1971;46:745-782
10. Grunt JA, Enriquez AR, Daughaday WH, Budd S: Acute and long-term responses to hGH in children with idiopathic small-for-dates dwarfism. J Clin Endocrinol Metab 1972;35:157-168
11. Stanhope R, Preece MA, Hamill G: Does growth hormone treatment improve final height attainment of children with intrauterine growth retardation? Arch Dis Child 1991;66:1180-1183
12. Albanese A, Stanhope R: Growth and metabolic data following growth hormone treatment of children with intrauterine growth retardation. Horm Res 1993;39:8-12
13. Chatelain P, Job JC, Blanchard J, Ducret JP, Olivier M, Sagnard L, Vanderschueren-Lodeweyckx M on behalf of the Belgian and French pediatric clinics and Sanofi-Choay (France): Dose-dependent catch-up growth after 2 years of growth hormone treatment in intrauterine growth retarded children. J Clin Endocrinol Metab 1994;1454-1460
14. Chaussain JL, Colle M, Landier F: Effects of growth hormone therapy in prepubertal children with short stature secondary to intrauterine growth retardation. Acta Paediatr Scand 1994;399(Suppl):74-75

15. Usher R, MacLean F: Intrauterine growth of liveborn caucasian infants at sea level: Standard obtained from measurements of infants born between 25 and 44 weeks of gestation. J Pediatr 1969;74:901-910
16. Roede MJ, Van Wieringen JC: Growth diagrams 1980, Netherlands. Rhird nation-wide survey. T Soc Gezondheidsz 1985:62(Suppl):1-34
17. Rikken B, Wit JM: Prepubertal height velocity references over a wide age range. Arch Dis Child 1992;67:1277-1280
18. Tanner JM, Whitehouse R: Longitudinal standards for height, weight-height, height velocity and stages of puberty. Arch Dis Child 1976:51:170-179
19. Tanner JM, Whitehouse RH, Cameron N, Marshall WA, Healy MJR, Goldstein H: Assessment of skeletal maturity and prediction of adult height (TW2-method). Academic Press, London, 1983
20. Hokken-Koelega ACS, Hackeng WHL, Stijnen Th, Wit JM, de Muinck Keizer-Schrama SMPF, Drop SLS: Twenty-four-hour growth hormone (GH) profiles, urinary GH excretion, and plasma insulin-like growth factor-I and -II levels in prepubertal children with chronic renal insufficiency and severe growth retardation. J Clin Endocrinol Metab 1990;71:688-695
21. Martin JL, Baxter RC: Insulin-like growth factor-binding protein from human plasma. Purification and characterization. J Biol Chem 1986;261:8754-8760
22. Van den Brande JL, Degenhart HJ: Endocrinologie en groeistoornissen, In: Van de Brande JL, Van Gelderen HH, Monnens LAH (eds). Kindergeneeskunde. Wetenschappelijke Uitgeverij Bungen, Utrecht, 1993, p179-219
23. Job JC: Results of long-term growth hormone replacement therapy in children: when and how to treat. Horm Res 1990;33(Suppl.4):69-76
24. Greulich WW, Pyle SI: Radiographic atlas of skeletal development of the hand and wrist. Stanford University Press, Stanford, 1959
25. Tanner JM, Lejarraga H, Cameron N: The natural history of the Silver-Russell syndrome: a longitudinal study of thirty-nine cases. Pediatr Res 1975;9:611-623
26. Davies PSW, Valley R, Preece MA: Adolescent growth and pubertal progression in the Silver-Russell syndrome. Arch Dis Child 1988;63:130-135
27. Wollmann HA, Ranke MB, Kirchner T, Preece M: Silver Russell syndrome: spontaneous growth and symptoms in 386 patients. Horm Res 1994;41:73
28. Arisaka O, Ariska M, Kiyokama N, Shimizu T, Nakayama Y, Yabuta K: Intrauterine growth retardation and early adolescent growth spurt in two sisters. Clin Ped 1986;25:559-561
29. Zachmann M, Sobradillo B, Frank M, Frisch H, Prader A: Bayley-Pinneau, Roche-Wainer-Thissen, and Tanner height predictions in normal children and in patients with various pathologic conditions. Pediatrics 1978;93:749-755
30. Chaussain JL, Ducret JF, Job JC: Actual versus predicted final height in patients with intrauterine growth retardation. Pediatr Res 1993;5(Suppl):S38
31. Dobbing J, Sands J: Timing of neuroblast multiplication in developing human brain. Nature 1970;226:639-640
32. Dobbing J, Sands J: Comparative aspects of the brain growth spurt. Early Hum Dev 1979;3:79-83

33. Davies DP: Growth of 'small-for-dates' babies. Review. Early Hum Dev 1981;5:95-105
34. Fitzhardinge PM, Steven EM: The small for dates infant II: neurological and intellectual sequelae. Pediatrics 1976;50:30-57
35. Harvey D, Prince J, Bunton J, Parkinson C, Campbell S: Abilities of children who were small-for-gestational age babies. Pediatrics 1982;69:269-300
36. Westwood M, Kramer MS, Munz D, Lovett JM, Watters GV: Growth and development of full-term nonasphyxiated small-for-gestational-age newborns: follow-up through adolescence. Pediatrics 1983;71:376-382
37. Henrichsen L, Skinhoj K, Andersen GE: Delayed growth and reduced intelligence in 9-17 year old intrauterine growth retarded children compared with their monozygous co-twins. Acta Paediatr Scand 1986;75:31-35
38. Matilainen R, Heinonen K, Siren-Tiusanen H: Effect of intrauterine growth retardation (IUGR) on the psychological performance of preterm children at preschool age. J Child Psychol Psychiat 1988;29:601-609
39. Van der Reijden-Lakeman EA: Growing pains? Psychological evaluation of short stature in intrauterine growth retarded children, before and after two years of GH treatment. Thesis, Rotterdam, 1996
40. Winick M: Malnutrition and brain development. J Pediatr 1969;74:667-679
41. Ranke MB, Guilbaud O, Lindberg A, Cole T: Prediction of the grwoth response in children with various growth disorders treated with growth hormone: analyses of data from the Kabi Pharmacia International Growth Study. Acta Paediatr 1993;391(Suppl):82-88
42. Davidson MB: Effect of growth hormone on carbohydrate and lipid metabolism. Endocrine Reviews 1987;8:115-131
43. Gustafsson J: Possible metabolic side effects of high dose growth hormone treatment. Acta Paediatr Scand 1989; 362(Suppl):50-55
44. Walker J, Chaussain JL, Bourgnères PF: Growth hormone treatment of children with short stature increases insulin secretion but does not impair glucose disposal. J Clin Endocrinol Metab 1989;69:253-258
45. Hindmarsh PC, Pringle PJ, Di Silvo L, Brook CGD: Effects of 3 years of growth hormone therapy in short normal children. Acta Paediatr Scand 1990:366(Suppl):6-12
46. McCaughey ES, Mulligan J, Voss LD, Betts PR: Growth and metabolic consequences of growth hormone treatment in prepubertal short normal children. Arch Dis Child 1994;71:201-206
47. Phipps K, Barker DJP, Hales CN, Fall CH, Osmond C, Clark PM: Fetal growth and impaired glucose tolerance in men and women. Diabetologia 1993:36:225-228
48. Phillips DIW, Barker DJP, Hales CN, Hirst S, Osmond C: Thinness at birth and insulin resistance in adult life. Diabetologia 1994;37:150-154
49. Law CM, Gordon GS, Shiell AW, Barker DJP, Hales CN: Thinnes at birth and glucose tolerance in seven year old children. Diabetic Med 1995;12:24-29

CHAPTER 11

General Discussion and Conclusions.

INTRODUCTION

Children growing at the extremes of height may experience psychological, social, practical and/or physical problems. Hormonal treatment intervening in the process of growth may be employed to improve height prognosis. This thesis describes various clinical studies undertaken to obtain more insight into the possibilities and limitations of therapeutic intervention in children presenting with extremes of growth: constitutionally tall stature (CTS) and short stature after intrauterine growth retardation (IUGR). The major question addressed in these studies was: is it possible to influence childhood growth by means of hormonal treatment in such a way that it is beneficial to children with extremes of growth?

EXTREMES OF GROWTH

Constitutionally tall stature

Constitutionally tall stature is a normal variant of childhood growth. Genetic and familial factors play an important role in etiology and pathogenesis.
Endocrinological studies indicate that tall stature is due, at least partly, to increased GH secretion. However, other mechanisms might play a role as well. The concept of treating tall adolescents to reduce final adult height developed from the observations in children with precocious puberty. In these children, premature secretion of sex hormones induces a progression in bone maturation, which leads to early epiphyseal closure and limitation of linear growth. Therefore, sex steroids have been used for final height reduction since the 1950s (1).

Accuracy of height prediction and effect of treatment

The main question was: Is treatment with pharmacological doses of sex steroids effective in order to reduce final adult height in children with CTS without

long-term side effects?

Our study gives a more differentiated answer to this question than many other reports thus far (1-25). The generally accepted opinion is, that sex hormone treatment is effective in reducing final height, although claimed results may vary (1-25). This variability is mainly due to differences in study design, therapeutic regimen, inclusion criteria (such as age and bone age at initiation of treatment) and definition of adult final height (15,17). The main problem in assessing the effect of sex hormone treatment is that the effect of treatment depends on the method used to predict final adult height. A critical appraisal of the quality of the various prediction methods is required especially since most prediction methods are based on growth data derived from normal growing children. The reliability of height prediction methods in CTS has been rarely studied in large groups of untreated tall children (26-29). Therefore, our studies on the reliability of nine different prediction methods in large groups of untreated tall children (55 boys and 88 girls) are clinically relevant.

We demonstrated that height prediction in tall girls is rather accurate: most prediction methods showed a mean error below 1 cm with mean absolute errors varying from +1.9 to +3.7 cm. In contrast, in tall boys height prediction was found to be rather inaccurate with mean errors ranging from -5.3 to +2.8 cm and mean absolute errors varying from +2.3 to +5.3 cm for the various prediction methods. An important finding was that at the time of referral the control groups (girls and boys) were significantly different to children who had received sex hormone treatment. They were shorter, had smaller parents, had a more advanced bone age and, consequently, had smaller final height predictions. This implies that if one wants to establish the effect of sex hormone treatment, one cannot simply correct the calculated effect of treatment for the error of prediction derived from these tall control groups. However, this has been done in most previously reported studies. We used a more direct and conceptually simple approach using multiple linear regression. Basically in this approach, the treatment effect on final height can be calculated while adjusting for differences in age, bone age and height between

treated and untreated children. In this way, the mean adjusted effect for the various prediction methods varied from -1.7 to +0.7 cm in boys and from +1.1 to +2.4 cm in girls. These outcomes are clearly less than reported previously (1-25) and seriously question the applicability of high doses of sex hormones in children with CTS to limit final adult height; this applies particularly to boys. It should be noted however, that there was a marked variation of the individual height reducing effects. For example, in boys a range in effect was found from -4.6 to +15.8 cm while in girls the range in effect varied from -2.6 to +6.2 cm. In fact, we clearly showed that the height reduction was dependent on the bone age at the start of treatment: height reduction was more pronounced when treatment was started at a younger bone age. This bone age-related effect is in concert with previous studies (5,7,13,18,19,21,23,24).

Surprisingly, when treatment in boys had been started at a relatively 'old' bone age *i.e.* bone age > 14.0 to 15.5 years depending on the method used, a growth induction was observed instead of a growth reduction. This finding has not been reported before and contributes to a limitation of androgen use in boys with CTS. We speculate that this might be due to the presence of a sex-specific and age-dependent responsiveness of cartilage and bone cells to sex steroids, as shown *in vitro* (30-33).

Another important issue that caused a significant reduction in the height limiting effect of sex steroid treatment, was the observation of a marked additional post-treatment growth after cessation of therapy. It appeared that this post-treatment growth was mainly due to the fact that treatment had been stopped before complete closure of the epiphyses. Therefore, continuation of treatment until complete closure of the growth plates has occurred will contribute to a more favourable effect in height reduction. According to the method of Greulich and Pyle, this implies that treatment has to be continued preferably until a bone age 18.0 years in boys and 16.5 years in girls.

Long-term effects

At a mean follow-up period of 10 years, we found no evidence that administration of pharmacological doses of sex steroids has long-term side effects. Although sex hormone treatment induced various side effects during treatment, these tended to be mild and occasionally led to cessation of treatment. This is in concert with previous studies (4,5,23,33-35). We obtained information about 127 pregnancies and found no difference in outcome between treated and untreated tall subjects. In addition, sperm quality in men was not affected. However, previously treated tall men had significantly higher levels of plasma FSH. The meaning of this latter finding remains to be established. We speculate that the higher levels of FSH may reflect intratesticular changes due to androgen treatment received in a period of testicular maturation. These increased FSH levels may compensate for partially disturbed germinal function in order to maintain normal sperm quality. On the other hand, the difference in gonadotropin levels may also reflect a change in responsiveness at the hypothalamic-pituitary level. We intend to study sperm quality and plasma FSH levels in these men with increasing age in order to see whether sperm quality will be maintained. In women no case of malignancy was reported. However, the considerable amount of data relating long-term oral contraceptive use to breast cancer cannot be ignored (36-41). Although the potential hazard should not be exaggerated, it might raise the need for long-term follow-up in these girls.

Psychosocial aspects

One may argue about the effectiveness and applicability of sex hormone treatment in the management of tall stature. It is certain however, that some children with CTS experience psychosocial problems related to their tallness. In our study, many tall adults recalled to be subject to hurtful remarks and jokes about their height; they were frequently teased during childhood. Probably, this plays a major role in the decision in favour of hormone treatment. As adults, previously treated men and women showed no major psychological maladjustment compared

to tall controls. Since our study was a retrospective one, it remains speculative, however, whether treatment with sex hormones did have a beneficial effects on psychosocial functioning. Only properly designed prospective studies will enlighten this important issue. Therefore, inventarisation of psychosocial problems prior to possible treatment is urgently needed.

Conclusions and recommendations

Our studies led us to make the following conclusions and recommendations:

1. Whenever a child presents with constitutionally tall stature, a reliable height prediction is of utmost importance. Extrapolation of the height SDS for bone age according to Greulich and Pyle has been shown to be an easy clinical tool to make a rather accurate height prognosis in tall children. Otherwise, the regression equations based on a reference group of untreated tall children can be used, as described in chapter 7.
2. Dependent on the height prognosis, sex steroid treatment for final height reduction may be considered. However, the expected effect of such a treatment is significantly dependent on the bone age at the time treatment may be started. As outlined in chapters 3 and 4, the equations in table 5 can be used to estimate the expected effect of treatment according to various prediction methods and methods of bone age assessment.
3. If treatment is given, it should be continued until complete closure of the epiphyses has occurred. According to the method of Greulich and Pyle, this implies that treatment has to be continued preferably until a bone age 18.0 years in boys and 16.5 years in girls.
4. In boys, we do not favor the start of sex hormone treatment at a bone age beyond 14 years according to Greulich and Pyle and beyond 15 years according to Tanner-Whitehouse. It might increase final adult height rather than limit it.
5. Reassurance can be given to patients and parents about the fact that to date no evidence of long-term side effects have been demonstrated.

However, the presence of elevated FSH levels with normal sperm quality in previously treated tall men should be discussed.

Considerations

In order to increase the height reducing effect of hormonal treatment, other ways of intervention may be explored. Recent approaches have focussed on suppression of GH secretion in children with CTS using somatostatin analogues (42-44). These preliminary results are promising. However, final results remain to be established and the occurrence of adverse effects has to be considered. It may be worthwile to combine these two modes of height intervening strategies: reduction of GH secretion by means of somatostatin analogues and progression of bone maturation via high doses of sex steroids. Hindmarsh et al (44) demonstrated a shift of the growth curve to the right by administration of octreotide (a somatostatin analogue) to prepubertal children with CTS. Therefore it might be considered to treat tall children with octreotide during prepuberty, whereafter sex steroid therapy can be applied or given as adjuvant. Future studies are required to investigate the effect and safety of these theoretical modalities on height reduction in children with tall stature.

Short stature after IUGR

The clinical importance of IUGR is apparent. The association between the degree of IUGR and perinatal morbidity and mortality and the greater risk of asphyxia-induced encephalopathy with consequent risks of neurological dysfunction have been well established. In addition, infants born with IUGR may have lifelong consequences. Barker and associates have presented considerable evidence linking abnormal fetal and/or placental growth to development of cardiovascular diseases (reviewed in reference: 45). Furthermore, an association has been found between IUGR and both insulin resistance and impaired glucose intolerance in adulthood (45,46). Finally, persistent growth failure may be present

(47-52). A recent study showed that children born small-for-gestational age with birth length less than -2 SD below the mean have a 7-fold higher risk for short stature in adulthood compared to non-SGA children (53). In fact, at 18 yrs of age 22% of the total short population were found to be short at birth (<-2SD)(53). The studies presented in the second part of this thesis mainly adressed the problem of persistent growth failure. The main question was whether treatment with recombinant GH would improve growth and ultimately final height in children with short stature after IUGR.

Pathophysiology

Although GH therapy is explored in many short children without any sign of classical GH deficiency such as Turner syndrome (54), supportive evidence for GH intervening therapy in children with short stature after IUGR came from endocrinological studies showing GH secretion disturbances in many of these children (55-57). These studies comprised small groups of children and did not address IGFs or urinary GH excretion. We studied the GH/IGF axis in a group of 40 prepubertal IUGR children and demonstrated that 50-60% of these children have signs of GH insufficiency. The etiology of this remains to be established. Most of our children experienced growth retardation in early pregnancy (symmetric IUGR). It is possible that the factors that induce the pathophysiological state that causes IUGR, may also affect organ development. Especially the brain seems to be vulnerable to disturbances in its nutritional supply, since important brain growth and development occurs in utero (58,59). Indeed, animal studies give arguments supportive to the hypothesis of neuropsychological dysfunction due to IUGR (60). Other lines of evidence are derived from clinical studies in children with IUGR showing an association between IUGR and an increased incidence of mental handicap, lower intelligence, neurological deficits, poor academic performance and behavior problems (61-65). In the parallel study on psychological aspects, IUGR children scored lower in intelligence tests and showed more disturbances in attention and concentration, indicating a possible underlying neurophysiological

deficit (66). It is speculative that the GH secretion disturbances observed in children with IUGR reflect alterations in brain structure and function. Since the high percentage of GH secretory abnormalities might be attributable to GH pulse amplitude disturbances, this dysfunction could be at the hypothalamic level. However, there were several children with no distinct GH/IGF axis abnormalities who still failed to show catch-up growth. Therefore other mechanisms may be operative as well.

An alternative hypothesis explaining why postnatal growth may be disturbed due to prenatal factors concerns an alteration in the nuclei and tracts of the hypothalamic appetite center (67). In the human fetus, the hypothalamic appetite center develops from the fourth to seventh months in fetal life. Intrauterine undernutrition may affect this developing process. Indeed, in many children with IUGR and short stature feeding problems seem to be present. In animal models, temporary early life dietary protein restriction leads to lack of catch-up growth and produces long-term alterations in GH and insulin secretion which may underlie the impairment in subsequent physical growth (68). To our knowledge, there are no studies evaluating the consequences of possible malnutrition on growth during childhood in children with IUGR. Finally, malnutrition in early intrauterine life may interfere with the rate of cell division, leaving the fetus with fewer cells and thus limiting the potential for catch-up growth as suggested by Winick (69).

Children with short stature after IUGR should be distinguished from children with idiopathic or normal short stature. Short stature after IUGR seems to be a distinct entity. This is illustrated by the fact that the intrauterine environment seems to influence postnatal growth in such way that it overrules the genetic potential of the child. In our study, the predicted adult height at start of GH treatment was more than 10 cm below the target height. Usually in idiopathic short stature, no such discrepancy has been found (70). In addition, evidence for GH/IGF disturbances have been described in many patients with IUGR shortly after birth and during childhood (55-57,71-73). By definition, children with idiopathic short stature show no signs of GH insufficiency. However, as in idiopathic short stature, children with

IUGR represent a heterogeneous group. Efforts should be made to categorize those patients who respond to hormonal therapy from those who are non-responders.

Effect of GH treatment

Although the pathophysiological mechanism is not quite clear, it seems justified to start clinical trials to investigate whether treatment with recombinant GH might be beneficial for IUGR children. Therefore, we set up a randomized, double-blind, dose dependent, multicenter study to investigate the effect of GH therapy on linear growth, bone maturation, pubertal development and final height. After two years of treatment, we demonstrated a significant dose-dependent effect of GH therapy on growth acceleration, expressed as the change in $HSDS_{CA}$. However, an increase in bone maturation was also observed, which was dose-independent. The bone maturation slowed down during the third year of treatment. Despite the progression in skeletal maturity, a significant improvement in height prognosis was found during the two years treatment period. Nevertheless, we like to stress that data on bone maturation and height prediction should be interpreted with caution. It is our clinical impression, supported by thrifty literature data (74-78) that spontaneous epiphyseal maturation is inappropriately advanced between the age of 6-10 years in many untreated IUGR children with or without dysmorphic features and that early puberty may occur more frequently than in healthy children. This latter phenomenon might also reflect alterations in brain structure and function as discussed above. It is obvious though, that the possible acceleration of bone maturation by GH will negatively influence the positive effects of GH therapy on final height. Ultimately, only long-term studies with the attainment of final adult height will enable definite conclusions. Therefore, the present study will be continued until all children have attained their final adult height.

In our study, glucose tolerance was maintained at the expense of a rise in fasting insulin concentrations and an increase in total insulin production after oral glucose stimulation. These findings are in concert with previous findings in short

normal children treated with GH (79-81). It is speculated that the hyperinsulinemia may be a direct trophic and/or insulinotropic effect of GH on beta-cells, rather than a secondary consequence of insulin resistance (79). It cannot be excluded however, that insulin resistance may develop with prolonged duration of GH treatment leading to glucose intolerance or diabetes. Since it has been shown that IUGR *per se* is associated with an increased prevalence of insulin resistance and impaired glucose tolerance in adulthood (45,46,82,83), monitoring of carbohydrate metabolism during and after the course of GH therapy is recommended.

The effect of GH therapy on growth rate is known to vary considerably. Therefore, we have searched for factors which might influence the growth response. Obviously this would have clinical implications, as prediction of the growth response to GH treatment is very useful in the management of short stature. Unfortunately, we could not identify a significant marker *prior to* treatment predicting the growth response to GH therapy in children with short stature after IUGR. For instance, no correlations were found between pretreatment parameters of GH secretory status and either growth or growth response to GH therapy. Albertsson-Wikland reported an inverse relationship between the amount of spontaneous GH secretion and the 1-year growth response to GH therapy in children with IUGR (55). However, preliminary analysis revealed that biochemical changes *during* therapy did correlate with the growth response to GH therapy. For instance, the change in IGF-I SDS and IGFBP-3 SDS over the first two years of treatment was positively correlated with the change in $HSDS_{CA}$ over the same period ($r=0.49$ and $r=0.37$, respectively). This implies, that it might be possible to predict the individual growth response to GH therapy after a try-out treatment with GH during a limited period. Ranke et al (84) demonstrated that the GH dosage applied had the greatest influence on the growth response to GH therapy during the first year of treatment in 135 children with IUGR. In their study, only auxological parameters had been included, which could explain only 23% of the total variability in growth response. More indepth analyses are needed including auxological and biochemical parameters to investigate to what extent the growth response to GH treatment can be predicted in children with IUGR.

Conclusions and recommendations

From our studies the following conclusions and recommendations can be made:

1. In 50-60 percent of the children presenting with short stature after IUGR, signs of GH insufficiency are present. Although the complete pathophysiological mechanism has to be established, it seems justified to study the effect of GH therapy in these children.
2. GH treatment induces a significant increase in height and height velocity, which is dose-dependent. However, bone maturation is also increased, independent of the GH dose. This latter finding has to be interpreted with caution, since it may be influenced by the natural history of growth and bone age development of these children. Nevertheless, final adult height prognosis appeared to be significantly improved after two years of GH treatment.
3. To date, it is not possible to predict the growth response to GH treatment in IUGR children adequately. More indepth analyses are needed to evaluate the possibility of growth response prediction.
4. No major adverse effects of GH treatment are demonstrated. However, fasting insulin levels and total insulin production after glucose loading are significantly increased after 1 year of GH treatment. Therefore, it is recommended to monitor carbohydrate metabolism during and after GH treatment.
5. Before GH has proven to be effective, treatment of children with short stature after IUGR with GH should be limited to investigational settings.

Considerations

To evaluate the ultimate effect of GH treatment on final adult height in children with short stature after IUGR, studies including auxological measurements and bone age determinations up to final height in a large group of untreated IUGR children will be of a great value. Establishment of the natural pattern of growth and development can be used as a reference for GH intervention studies.

REFERENCES

1. Goldzieher M: Treatment of excessive growth in the adolescent female. J Clin Endocrinol 1956;16:249-252
2. Schoevaart CE, Drop SLS, Otten BJ, Slijper FME, Degenhart HJ: Growth analysis up to final height and psychosocial adjustment of treated and untreated patients with precocious puberty. Horm Res 1990;34:197-203
3. Brauner R, Adan L, Malandry F, Zantleifer D: Adult height in girls with idiopathic true precocious puberty. J Clin Endocrinol Metab 1994;79:415-420
4. Zachmann M, Ferrandez A, Mürset G, Prader A: Estrogen treatment of excessively tall girls. Helv Paediat Acta 1975;30:11-30
5. Wettenhall HNB, Cahill C, Roche AF: Tall girls: A survey of 15 years of management and treatment. J Pediatr 1975;86:602-610
6. Von Puttkamer K, Bierich JR, Brugger F, Hirche W, Schönberg D: Östrogentherapie bei Mädchen mit konstitu-tionellem Hochwuchs. Dtsch Med Wochenschrft 1977;102:983-988
7. Kuhn N, Blunck W, Stahnke N, Wiebel J, Willig RP: Estrogen treatment in tall girls. Acta Paediatr Scand 1977; 66:161-167
8. Colle ML, Alperin H, Greenblatt RB: The tall girl. Prediction of mature height and management. Arch Dis Child 1977;52:118-120
9. Bierich JR: Estrogen treatment of girls with constitutional tall stature. Pediatrics 1978;62:1196-1201
10. Reeser HM, Heremans GFP, van Gelderen HH: Reduction of adult height in tall girls. Eur J Pediatr 1979;132:37-41
11. Willig RP, Christiansen D, Kuhn N, Schaefer E, Stahnke N: Voraussetzungen und Ergebnisse der Östrogenbehandlung extrem großer Mädchen. Monatsschr Kinderheilkd 1980;128:787-788
12. Andersen H, Jacobsen BB, Kastrup KW, Krabbe S, Peitersen B, Petersen KE, Thamdrup E, Wichmann R: Treatment of girls with excessive height prediction. Acta Paediatr Scand 1980;69:293-297
13. John G, Schellong G: Östrogentherapie hochwüchsiger Mädchen. Monatsschr Kinderheilkd 1980;128:545-550
14. Stöver B, Kollmann F: Ergebnisse der Behandlung hochwüchsiger Mädchen mit conjugierten Östrogenen. Monatsschr Kindrheilkd 1982;130:36-40
15. Sorgo W, Scholler K, Heinze F, Heinze E, Teller WM: Critical analysis of height reduction in oestrogen-treated tall girls. Eur J Pediatr 1984;142:206-265
16. Schambach H, Nitschke U: Die Behandlung des konstitutionellen Hochwuchses bei Mädchen mit physiologischen Östrogendosen in der Präpubertät. Monatsschr Kinderheilkd 1985;133:32-37
17. Bartsch O, Weschke B, Weber B: Oestrogen treatment of constitutionally tall girls with 0.1 mg/day ethinyl oestradiol. Eur J Pediatr 1988;147:59-63
18. Grüters A, Heidemann P, Schlüter H, Stubbe P, Weber B, Helge H: Effect of different oestrogen doses on final height reduction in girls with constitutional tall stature. Eur J Ped 1989;149:11-13

19. Ignatius A, Lenko HL, Perheentupa J: Oestrogen treatment of tall girls: effect decreases with age. Acta Paediatr Scand 1991;80:712-717
20. Svan H, Ritzén EM, Hall K, Johansson L: Oestrogen treatment of tall girls: dose dependency of effects on subsequent growth and IGF-I levels in blood. Acta Paediatr Scand 1991;80:328-332
21. Normann EK, Trygstad O, Larsen S, Dahl-Jorgensen K: Height reduction in 539 tall girls treated with three different dosages of ethinyloestradiol. Arch Dis Child 1991;66:1271-1278
22. Joss EE, Zeuner J, Zurbrügg RP, Mullis PE: Impact of different doses of ethinyl oestradiol on reduction of final height in constitutionally tall girls. Eur J Pediatr 1994;153:797-801
23. Zachmann M, Ferrandez A, Mürset G, Gnehm HE, Prader A: Testosterone treatment of excessively tall boys. J Pediatr 1976;88:116-123
24. Brämswig JH, Schellong G, Borger HJ, Breu H: Testosteron-Therapie hochwüchsiger Jungen. Ergebnisse bei 25 Jungen. Dtsch Med Wschr 1981;106:1656-1661
25. Brämswig JH, von Lengerke HJ, Schmidt H, Schellong G: The results of short-term (6 months) high dose-testosterone treatment on bone age and adult height in boys of excessively tall stature. Eur J Pediatr 1988; 148:104-106
26. Zachmann M, Sobradillo B, Frank M, Frisch H, Prader A: Bayley-Pinneau, Roche-Wainer-Thissen, and Tanner height predictions in normal children and in patients with various pathologic conditions. Pediatrics 1978;93:749-755
27. Willig RP, Christiansen D, Kuhn N, Schaefer E, Stahnke N: Vorraussetzungen und ergebnisse der Östrogenbehandlung extrem großer Mädchen. Monatschr Kinderheilkd 1980;128:787-788
28. Brämswig JH, Hermeling W, von Petrykowski W, Schellong G: Comparison of height predictions with final adult height in boys with constitutional tall stature. In: Borms J, Hausple R, Sand A, Suzanne C, Hebbelinck M (eds). Human growth and development. Plenum Press, New York, 1984; pg.423-429
29. Joss EE, Temperli R, Mullis PE: Adult height in constitutionally tall stature: accuracy of five different height prediction methods. Arch Dis Child 1992;67:1357-1362
30. Blanchard O, Tsagris L, Rappaport R, Duval-Beaupere G, Corvol M: Age-dependent responsiveness of rabbit and human cartilage cells to sex steroids in vitro. J Steroid Biochem Mol Biol 1991;40:711-716
31. Nasatzky E, Schwarz Z, Boyan BD, Soskolne WA, Ornoy A: Sex-dependent effects of 17ß estradiol on chondrocyte differentiation in culture J Cell Physiol 1993;154:359-362
32. Schwarz Z, Nasatzky E, Ornoy A, Brooks BP, Soskolne WA, Boyan BD: Gender specific, maturation-dependent effects of testosterone on chondrocytes in culture. Endocrinology 1994;134:1640-1647
33. Hanker JP, Schellong G, Schneider HPG: The functional state of the hypothalamo-pituitary axis after high dose oestrogen therapy in excessively tall girls. Acta Endocrinol 1979;91:19-29
34. Brämswig JH, Nieschlag E, Schellong G: Pituitary-gonadal function in boys after high dose testosterone treatment for excessively tall stature. Acta Endocrinol 1984;107:97-103
35. Trygstad O: Oestrogen treatment of adolescent tall girls; short term side effects. Acta Endocrinol 1986(Suppl) 279:170-173

36. Olsson H, Möller TR, Ranstam J: Early oral contraceptive use and breast cancer among premenopausal women: final report from a study in southern Sweden. J Natl Cancer Inst 1989;81:1000-1004
37. Schlesselman JJ: Cancer of the breast and reproductive tract in relation to use of oral contraceptives. Contraception 1989;40:1-38
38. Rushton L, Jones DR: Oral contraceptive use and breast cancer risk: a meta-analysis of variations with age at diagnosis, parity and total duration of oral contraceptive use. Br J Obstet Gynecol 1992;99:239-246
39. Grimes DA: The safety of oral contraceptives: epidemiologic insights from the first 30 years. Am J Obstet Gynecol 1992;166:1950-1954
40. Kaunitz AM. Oral contraceptives and gynecologic cancer: an update for the 1990s. Am J Obstet Gynecol 1992; 167:1171-1176
41. Malone KE, Dailing JR, Weiss NS: Oral contraceptives in relation to breast cancer. Epidemiol Rev 1993;15:80-97
42. Tauber MT, Tauber JP, Vigoni F, Harris AG, Rochiccioli P: Effect of long-acting somatostatin analogue SMS 201-995 on growth rate and reduction of predicted adult height in ten tall adolescents. Acta Paediatr Scand 1990; 79:176-181
43. Hindmarsh PC, Pringle PJ, Di Silvo L, Brook CGD: A preliminary report on the role of somatostatin analogue (SMS 201-995) in the management of children with tall stature. Clin Endocrinol 1990;32:83-91
44. Hindmarsh PC, Pringle PJ, Stanhope R, Brook CGD: The effect of continuous infusion of a somatostatin analogue (octreotide) for two years on growth hormone secretion and height prediction in tall children. Clin Endocrinol 1995;42:509-515
45 Barker DJP, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS: Fetal nutrition and cardiovascular disease in adult life. Review article. Lancet 1993;341:938-941
46. Phipps K, Barker DJP, Hales CN, Fall CHD, Osmond C, Clark PMS: Fetal growth and impaired glucose intolerance in men and women. Diabetologia 1993;36:225-228
47. Westwood M, Kramer MS, Munz D, Lovett JM, Watters GV: Growth and development of full-term nonasphyxiated small-for-gestational-age newborns: follow-up through adolescence. Pediatrics 1983;71:376-382
48. Job JC, Rolland A: Histoire naturelle des retards de croissance à debut intra-utérin. Arch Fr Pediatr 1986;43:301-306
49. Paz I, Seidman DS, Danon YL, Laor A, Stevenson DK, Gale R: Are children born for gestational age at increased risk for short stature? Am J Dis Child 1993;147:337-339
50. Chaussain JL, Colle M, Ducret JP: Adult height in children with prepubertal short stature secondary to intrauterine growth retardation. Acta Paediatr Scand 1994;399(Suppl):72-73
51. Tanner JM, Lejarraga H, Cameron N: The natural history of the Silver-Russell syndrome: A longitudinal study of thirty-nine cases. Pediatr Res 1975;9:611-623
52. Davies PS, Valley R, Preece MA: Adolescent growth and pubertal progression in the Silver-Russell syndrome. Arch Dis Child 1988;63:130-135
53. Karlberg J, Albertsson-Wikland K: Growth in full-term small-for-gestational-age infants: from birth to final height. Pediatr Res 1995;38:733-739
54. Hindmarsh PC, Bridges NA, Brook CGD: Wider indications for treatment with biosynthetic human growth hormone in children. Review. Clin Endocrinol 1991;34:417-427

55 Albertsson-Wikland K: Growth hormone secretion and growth hormone treatment in children with intrauterine growth retardation. Acta Paediatr Scand 1989;349(Suppl):35-41

56 Rochiccioli P, Tauber M, Moisan V, Pinkowski C: Investigation of growth hormone secretion in patients with intrauterine growth retardation. Acta Paediatr Scand 1989;349(Suppl):42-46

57 Stanhope R, Ackland F, Hamill G, Clayton J, Jones J, Preece MA: Physiological growth hormone secretion and response to growth hormone treatment in children with short stature and intra-uterine growth retardation. Acta Paediatr Scand 1989;349(Suppl):47-52

58 Dobbing J, Sands J: Timing of neuroblast multiplication in developing human brain. Nature 1970;226:639-640

59 Dobbing J, Sands J: Comparative aspects of the brain growth spurt. Early Hum Dev 1979;3:79-83

60. Thomas YM, Bedi KS, Davies RA, Dobbing J: A stereological analysis of the neuronal and synaptic content of the frontal and cerebellar cortex of weaning rats undernourished from birth. Early Hum Dev 1979;3:109-126

61. Fitzhardinge PM, Steven EM: The small for dates infant II: neurological and intellectual sequelae. Pediatrics 1976;50:30-57

62. Harvey D, Prince J, Bunton J, Parkinson C, Campbell S: Abilities of children who were small-for-gestational age babies. Pediatrics 1982;69:269-300

63. Westwood M, Kramer MS, Munz D, Lovett JM, Watters GV: Growth and development of full-term nonasphyxiated small-for-gestational-age newborns: follow-up through adolescence. Pediatrics 1983;71:376-382

64. Henrichsen L, Skinhoj K, Andersen GE: Delayed growth and reduced intelligence in 9-17 year old intrauterine growth retarded children compared with their monozygous co-twins. Acta Paediatr Scand 1986;75:31-35

65. Matilainen R, Heinonen K, Siren-Tiusanen H: Effect of intrauterine growth retardation (IUGR) on the psychological performance of preterm children at preschool age. J Child Psychol Psychiat 1988;29:601-609

66. Van der Reijden-Lakeman EA: Growing pains? Psychological evaluation of short stature in intrauterine growth retarded children, before and after two years of GH treatment. Thesis, Rotterdam, 1996

67. Davies DP: Growth of small-for-dates babies. Review article. Early Hum Dev 1981;5:95-105

68. Harel Z, Tannenbaum GS: Long-term alterations in growth hormone and insulin secretion after temporary dietary protein restriction in early life in the rat. Pediatr Res 1995;38:747-753

69. Winick M: Malnutrition and brain development. J Pediatr 1969;74:667-679

70. Wit JM, Rietveld DHF, Drop SLS, Oostdijk W, Gons M, Otten BJ, Delemarre-Van de Waal HA, Reeser M, Waelkens JJ, Bot A (Dutch Growth Hormone Working Group): A controlled trial of methionyl growth hromone therapy in prepubertal children with short stature, subnormal growth rate and normal growth hormone response to secretagogues. Acta Paediatr Scand 1989;78:426-435

71. Lasarre C, Hardouin S, Daffos F, Forestier F, Frankenne F, Binoux M: Serum insulin-like growth factors and insulin-like growth factor binding proteins in the human fetus. Relationship with growth in normal subjects and in subjects with intrauterine growth retardation. Pediatr Res 1991;29:219-225

72. Straus DS, Ooi GT, Orlowski CC, Rechler MW: Expression of the genes for insulin-like growth factor-I (IGF-I), IGF-II, and IGF-Binding Proteins-1 and -2 in fetal rat under conditions of intrauterine growth retardation caused by maternal fasting. Endocrinology 1991;128:518-525
73. Unterman TG, Simmons RA, Glick RP, Ogata ES: Circulating levels of insulin, insulin-like growth factor-I (IGF-I), IGF-II and IGF-Binding Proteins in the small for gestational age fetal rat. Endocrinology 1993;132:327-337
74. Job JC, Rolland A: Histoire naturelle des retards de croissance à debut intra-utérin. Arch Fr Pediatr 1986;43:301-306
75. Tanner JM, Lejarraga H, Cameron N: The natural history of the Silver-Russell syndrome: A longitudinal study of thirty-nine cases. Pediatr Res 1975;9:611-623
76. Davies PS, Valley R, Preece MA: Adolescent growth and pubertal progression in the Silver-Russell syndrome. Arch Dis Child 1988;63:130-135
77. Wollmann HA, Ranke MB, Kirchner T, Preece M: Silver Russell syndrome: spontaneous growth and symtoms in 386 patients. Horm Res 1994;41:73
78. Arisaka O, Arisaka M, Kiyokama N, Shimizu T, Nakayama Y, Yabuta K: Intrauterine growth retardation and early adolescent growth spurt in two sisters. Clin Pediatr 1986;25:559-561
79. Walker J, Chaussain JL, Bourgnères PF: Growth hormone treatment of children with short stature increases insulin secretion but does not impair glucose disposal. J Clin Endocrinol Metab 1989;69:253-258
80. Hindmarsh PC, Pringle PJ, Di Silvo L, Brook CGD: Effects of 3 years of growth hormone therapy in short normal children. Acta Paediatr Scand 1990:366(Suppl):6-12
81. McCaughey ES, Mulligan J, Voss LD, Betts PR: Growth and metabolic consequences of growth hormone treatment in prepubertal short normal children. Arch Dis Child 1994;71:201-206
82. Phillips DIW, Barker DJP, Hales CN, Hirst S, Osmond C: Thinness at birth and insulin resistance in adult life. Diabetologia 1994;37:150-154
83. Law CM, Gordon GS, Shiell AW, Barker DJP, Hales CN: Thinnes at birth and glucose tolerance in seven year old children. Diabetic Med 1995;12:24-29
84. Ranke MB, Guilbaud O, Lindberg A, Cole T: Prediction of the growth response in children with various growth disorders treated with growth hormone: analyses of data from the Kabi Pharmacia International Growth Study. Acta Paediatr 1993;391(Suppl):82-88

CHAPTER 12

Summary/Samenvatting

SUMMARY

In this thesis various clinical studies are described on children growing at the extremes of height. Several aspects of growth intervening therapy in the management of children growing at the upper extreme of the growth curve, children with constitutionally tall stature (CTS), and of those growing at the lower extreme of the growth curve, children with short stature after intrauterine growth retardation (IUGR), are studied.

Chapter 1 gives an overview of the hormonal regulation of growth and focuses on growth hormone (GH) as the most important endocrine factor responsible for growth during childhood. From this it is clear that there are several possibilities to interfere in the hormonal regulation in order to influence growth and extremes of growth. Growth reduction is striven for in children with CTS and this might be achieved by administration of pharmacological doses of sex hormones. It is expected that, although sex hormones increase GH secretion and thus stimulate growth, the acceleration of bone maturation caused by these hormones will surpass the growth induction and ultimately will result in a decrease of the expected adult final height. To study the effect of such treatment and to investigate possible adverse effects, a large group of children with CTS, part of whom had received high doses of sex steroids in order to reduce their ultimate height, were contacted by mail and recalled to our hospital. In total, 209 men and 326 women responded to our call and most of them participated in one or more of our studies. The results of these studies are discussed in ***chapters 2 to 8.*** In children with short stature after IUGR, promotion of growth is the purpose of clinical intervention. The unlimited availability of recombinant GH has allowed the possibility to investigate whether GH administration is beneficial to these children. To study the effect of GH on linear growth, bone maturation, pubertal development and adult final height in children with short stature after IUGR a multicenter, randomized, double-blind, dose response study was set up. The study population comprises a total of 79 patients. They had been allocated to a GH dosage of either 3 or 6 IU/m^2/day and are controlled at 3 monthly intervals in one of the four participating centres (Sophia

Children's Hospital, Rotterdam; Wilhelmina Children's Hospital, Utrecht; Academic Hospital of Free University, Amsterdam; Juliana Children's Hospital, The Hague). The results of this study are described in ***chapters 9 and 10.***

Chapter 2 describes the reliability of a recently developed computer-aided skeletal scoring system (CASAS) and the applicability of this system to children with CTS. CASAS estimates Tanner-Whitehouse (TW) skeletal maturity scores automatically, although manual insertion is possible. It rates each of the 13 bones in the TW-RUS classification system (13 bones). A shortened model, using a 6 bone subset is also available. We compared manual ratings and CASAS ratings in 151 radiographs from healthy children and 421 radiographs from 362 children with CTS. Some of the mean differences between the methods were statistically significant, however, since these mean differences were less than 0.4 bone age 'year', they are clinically not significant. In addition, the range of the differences between the methods was considerable, but the combined within and between components of variance (0.7%) was in the same order of magnitude as reported for manual readings. Manual insertions were performed in up to 8%. This study indicates that CASAS is quite reliable in healthy children and applicable in children with CTS. It should be noted though, that this system is not yet completely independent of its user. Therefore, whenever manual substitution is used, the limits for the acceptance of disagreement should be reported.

In *chapter 3*, the accuracy of nine different height prediction methods is evaluated in 55 untreated tall boys and the effect of androgen therapy is evaluated in 60 previously treated tall boys. For this purpose we compared the predicted adult height with the attained height measured at a mean age of 25.0 years. In controls, we found a large variability for the errors of prediction of the various prediction methods, demonstrating that height prognosis in boys with CTS is rather inaccurate. The most reliable prediction was performed by extrapolation of the height SDS for bone age according to Greulich and Pyle. In this study, boys who had ultimately chosen for hormonal treatment were significantly different from those who had not chosen for treatment in several aspects. Therefore, the effect of androgen therapy was evaluated, while adjusting the effect of treatment for diffe-

rences between treated and untreated subjects in age, bone age and height prediction by means of multiple regression analysis. The mean adjusted effect of treatment according to the various prediction methods varied from -1.7 to +0.7 cm, meaning that the height reducing effect of high doses of androgens in tall boys is limited. However, we observed a significant treatment effect when treatment was started at bone ages less than 14 to 15 years. At older bone ages, treatment was found to be contraindicated, since androgen administration had caused extra growth instead of growth inhibition. Finally, we demonstrated a significant mean additional growth of 2.4 cm after cessation of androgen treatment due to the fact that treatment had been stopped before complete closure of the epiphyses.

In ***chapter 4***, the accuracy of nine different height prediction methods is evaluated in 88 untreated tall girls and the effect of oestrogen therapy is evaluated in 159 previously treated tall girls. As in boys, the accuracy of the various prediction methods was evaluated by comparing the predicted adult height with the attained height measured at a mean age of 25.3 years. In tall girls, six of the nine methods applied showed a systematic error within 1 cm of the adult final height, indicating that height prediction is rather accurate and clinically acceptable. Again, the effect of oestrogen therapy was evaluated, while adjusting for differences between treated and untreated subjects in age, bone age and height prediction by means of multiple regression analyses. We found a significant mean height reducing effect of oestrogen treatment varying from +1.1 to +2.4 cm according to the various prediction methods. This effect was more pronounced when treatment had been started at a younger bone age. Serious additional growth of 2.7 cm after cessation of oestrogen treatment was observed which could only be partly explained by incomplete closure of the epiphyses; additional spinal growth may also account for part of the post-treatment growth.

Chapter 5 describes possible long-term side effects of high doses of sex steroids in the management of CTS with special attention to hypothalamic-pituitary-gonadal function. Two hundred and forty-four tall men and women were interviewed in a standardized way at a mean follow-up of 10 years after cessation of sex hormone treatment. Hundred and fifty-five untreated tall men and women

served as controls. Almost 80% percent of the previously treated men and women indicated to have been troubled by one or more side effects during treatment, however, most side effects were mild. The incidence of side effects is probably dose-dependent. Post-treatment amenorrhoea of longer than 6 months was found in 5% of the women. No differences in menstrual cycle characteristics were found between treated and untreated subjects. Information about a total of 127 pregnancies was obtained and revealed no evidence that pharmacological doses of sex hormones have long-term effects on reproductive function. However, this period is still too short to draw definite conclusions.

Chapter 6 evaluates whether treatment with high doses of androgens in tall boys during puberty may influence testicular function in adulthood. We studied sperm quality, testis volume and plasma hormone levels in 43 previously androgen treated tall men and 30 non-treated tall men. It appeared that sperm quality, testis volume and plasma testosterone levels were comparable between treated and untreated tall men. Mean sperm concentration was about 66 x 10^6/ml, which is comparable to that in the normal population nowadays. However, treated men had significantly higher plasma levels of FSH compared with controls (3.3 IU/l versus 2.1 IU/l). The meaning of this finding remains to be established. As a coincidence, we found a high prevalence of left-sided varicocele in the treated and untreated tall men (about 40%).

Chapter 7 describes the development of a new model to predict final height in constitutionally tall children. Standard multiple regression techniques were used to develop prediction equations for final height based on growth data derived from a sample of 143 untreated tall children. Separate regression models for boys and girls were obtained. To test the predictive capability of these newly derived prediction models, a second group of 32 untreated tall children was used. Final height was measured and compared to the predicted final height. The regression models showed satisfying accuracy in height prognosis and, when compared to the currently available prediction methods, were found to be quite promising. However, its additional clinical usefulness has to be ascertained in larger groups of tall children.

In ***chapter 8*** psychosocial aspects of constitutionally tall stature are described. Psychosocial information was obtained via five different questionnaires sent to 145 tall men and 280 tall women, part of whom had received hormonal treatment during puberty. Special areas of interest concerned self-esteem, social anxiety and assertiveness, general psychological well-being and body perception. We observed no major psychological maladjustment in previously treated tall children compared to tall controls. Previously treated tall men scored slightly higher on self-esteem and lower on social anxiety scales than the norms. It is speculative whether the administration of high doses of sex steroids may have contributed to these favourable outcomes. Other psychological mechanisms may have played a role as well. In retrospect, previously treated tall men and women experienced more problems related to their height than tall controls, especially during childhood. This may have played a role for the choice in favour of height reducing therapy.

Chapters 9 and 10 concern studies in children with short stature after IUGR.

Chapter 9 describes the possibility that disturbances in the GH/IGF axis may play a role in the mechanism underlying the failure to catch-up growth of these children. We studied GH secretion by physiological testing (24 hour plasma GH profiles) and by pharmacological testing (arginine provocation test). In addition, plasma IGF-I and IGF-II levels were measured and GH urinary excretion was determined. We demonstrated that 50-60% of children with short stature after IUGR have 24-hour plasma GH profile abnormalities and/or subnormal responses to arginine provocation, while mean IGF-I and -II levels are significantly reduced, indicating GH insufficiency. Compared to controls, urinary GH excretion was lower in boys, but not in girls. Surprisingly, we found no clear relation between GH secretory status and growth. These findings did not enable us to elucidate the complete pathophysiological mechanism in IUGR. A disturbance in the GH/IGF axis is only in part responsible for the failure to catch-up growth; several children had no distinct GH/IGF axis abnormalities, but still lacked postnatal catch-up growth.

Chapter 10 describes the two-years results of an ongoing clinical multicenter trial on the effect of two doses of GH on linear growth, bone maturation, pubertal development and final height of 79 children with short stature after IUGR. We

demonstrated a significant effect of both GH doses (3 and 6 IU/m²/day) on growth acceleration, expressed as the change in $HSDS_{CA}$ and $HVSDS_{CA}$, after two years of treatment. In addition a clear dose-response effect was present with the highest dose showing a greater change in $HSDS_{CA}$ (+1.3 and +1.7 with 3 and 6 IU/m²/day, respectively). An impressive acceleration in bone maturation during the 2 years of GH treatment was observed, which appeared to be not dose dependent. Despite this progression in skeletal maturity, a significant improvement in height prognosis was found during the treatment period. However, data on bone age maturation and height prediction should be interpreted with much caution, since spontaneous epiphyseal maturation is inappropriately advanced in untreated IUGR children and height prediction may overestimate adult final height. IGF-I and IGFBP-3 levels were low in children with IUGR. GH treatment induced normalization of IGFBP-3 levels and a marked increase of IGF-I levels. Preliminary analysis showed no correlations between pretreatment parameters of GH secretory status and the growth response to GH treatment. However, significant correlations were observed between changes in biochemical parameters during GH therapy and the growth response after two years of GH therapy. Additional analyses are required to study the possibility of predicting the growth response to GH therapy in IUGR children. In our study, glucose tolerance was maintained at the expense of a rise in fasting insulin concentrations and an increase in total insulin production after oral glucose stimulation. In conclusion, GH treatment seems beneficial to children with short stature after IUGR in promoting growth without serious adverse effects. However, only long-term follow-up studies such as this with the attainment of final height will enable definite conclusions. Monitoring of adverse effects on for instance carbohydrate metabolism is recommended.

Finally, ***chapter 11*** discusses the results of our studies and their implications for the management of children growing at the extremes of height. In addition, recommendations are made and suggestions for future research are given.

SAMENVATTING

In dit proefschrift worden verschillende klinische studies beschreven welke betrekking hebben op kinderen met extreme lengtegroei. Diverse aspecten van groei-beïnvloedende therapie worden bestudeerd. Dit alles in het kader van de behandeling van kinderen met extreme lengtegroei, dat wil zeggen kinderen met een lengtegroei die aan de bovenzijde van de groeicurves verloopt, constitutioneel lange gestalte (CLG), en kinderen die zich presenteren met een lengtegroei aan de onderzijde van de groeicurves, kleine gestalte na intrauteriene groeivertraging (IUGV).

In *hoofdstuk 1* wordt een overzicht gegeven van de hormonale regulatie van de normale groei. Hierbij wordt voornamelijk stilgestaan bij groeihormoon (GH) als de meest belangrijke endocrinologische factor die verantwoordelijk is voor de groei op de kinderleeftijd. Vanuit dit overzicht wordt het duidelijk dat er verschillende mogelijkheden bestaan om in te grijpen in de hormonale regulatie om op die manier de groei van kinderen met extreme lengtegroei te beïnvloeden. Bij kinderen met CLG wordt getracht een lengtereductie te bewerkstelligen. Dit kan worden bereikt door het toedienen van geslachtshormonen in farmacologische doseringen. De ratio hierachter is, dat hoewel geslachtshormonen de GH secretie en dus ook de groei stimuleren, ze tevens een versnelling van de botrijping veroorzaken. Hierdoor vindt een snellere sluiting van de epiphysairschijven plaats, zodat de groei eerder ophoudt en er uiteindelijk een lengte reductie optreedt. Teneinde het effect van een dergelijke behandeling te bestuderen en na te gaan of er mogelijke bijwerkingen bestaan werd een grote groep kinderen met CLG gevraagd mee te werken aan een uitgebreid na-onderzoek. Deze groep, inmiddels jong volwassenen, was ooit in ons ziekenhuis geweest in verband met hun lange gestalte en een aantal van hen is destijds behandeld met geslachtshormonen. In totaal reageerden 209 mannen en 326 vrouwen op onze oproep en de meesten waren bereid aan ons onderzoek mee te werken. De resultaten van dit follow-up onderzoek worden beschreven in de *hoofdstukken 2 tot en met 8*. Bij kinderen met een kleine gestalte na IUGV wordt groeibevordering nagestreefd. Door de komst

van recombinant GH lijkt het mogelijk onbeperkt GH voor te schrijven. Onderzoek zal echter moeten aantonen of GH therapie ook bij deze groep kinderen zinvol is. Teneinde het effect van GH behandeling op kinderen met een kleine gestalte na IUGV te bestuderen werd een multicenter, gerandomiseerd, dubbel-blind, dosis-respons onderzoek opgezet. In totaal doen 79 prepubertaire kinderen aan dit onderzoek mee. Zij kregen een GH dosering toegewezen van 3 of 6 IU/m^2/dag en worden vervolgens om de drie maanden gecontroleerd in één van de vier deelnemende centra. De deelnemende centra zijn: het Sophia Kinderziekenhuis te Rotterdam, het Wilhelmina Kinderziekenhuis te Utrecht, het Academisch Ziekenhuis van de Vrije Universiteit te Amsterdam en het Juliana Kinderziekenhuis te Den Haag. De resultaten van dit lopende onderzoek worden beschreven in de *hoofstukken 9 en 10.*

Hoofdstuk 2 beschrijft de betrouwbaarheid van een recent ontwikkeld computer systeem voor het automatisch beoordelen van botleeftijden (CASAS). Daarnaast wordt de toepasbaarheid van dit nieuwe systeem geëvalueerd bij kinderen met een CLG. CASAS beoordeelt automatisch de skeletmaturatie scores zoals deze zijn ontwikkeld door Tanner en Whitehouse en medewerkers. Er bestaat echter wel de mogelijkheid tot handmatige correctie. De computer scoort elk van de 13 handbeenderen van het TW-RUS classificatiesysteem. Een verkorte versie is ook beschikbaar; deze maakt slechts gebruik van een zestal handbeenderen. Wij vergeleken handmatige scores met scores van CASAS van 151 handfoto's van een groep gezonde kinderen en van 421 handfoto's van 362 kinderen met CLG. Enkele van de gemiddelde verschillen tussen de methoden waren statistisch significant. Echter, aangezien deze verschillen minder waren dan 0.4 'jaar' in botleeftijd, is dit klinisch niet belangrijk. De range van de verschillen tussen de methoden was aanzienlijk, maar de gecombineerde inter- en intra variantie (0.7%) voor CASAS was vergelijkbaar met hetgeen beschreven is voor handmatige scores. Gebruikmakend van het computersysteem vond handmatige correctie plaats in maximaal 8% van de gevallen. Deze studie laat zien dat CASAS betrouwbaar is bij gezonde kinderen en toepasbaar bij kinderen met CLG. Wel dient te worden opgemerkt dat het computersysteem nog niet volledig onafhankelijk is van de

gebruiker. Derhalve dienen bij het toepassen van handmatige correcties de grenzen van het accepteren van verschillen tussen computer en gebruiker te worden vermeld.

In *hoofdstuk 3* wordt de nauwkeurigheid van negen verschillende voorspellingsmethoden geëvalueerd bij 55 onbehandelde lange jongens. Daarnaast wordt het effect van androgeenbehandeling op de eindlengte bestudeerd bij 60 behandelde lange jongens. We vergeleken de voorspelde eindlengte met de werkelijke, gemeten eindlengte op een gemiddelde leeftijd van 25.0 jaar. In de onbehandelde groep vonden we een grote variabiliteit aan predictiefouten voor de verschillende voorspellingsmethoden. Dit toont aan dat de lengtevoorspelling bij jongens met CLG vrij onnauwkeurig is. De meest betrouwbare voorspelling wordt verkregen door de lengte uit te drukken in standaard deviatie score volgens de botleeftijd (bepaald met de Greulich en Pyle methode) en vervolgens te extrapoleren tot volwassen eindlengte. Het bleek, dat de jongens die uiteindelijk niet hadden gekozen voor hormoonbehandeling, al voor de behandeling op verschillende punten significant verschilden van de jongens die wel waren behandeld. Teneinde nu het effect van de hormoonbehandeling te berekenen werd daarom rekening gehouden met de bestaande verschillen in leeftijd, botleeftijd en lengteprognose tussen behandelde en onbehandelde jongens. Hiertoe werd gebruik gemaakt van multipele regressie analysen. Het gemiddelde, gecorrigeerde effect van behandeling bepaald voor de diverse voorspellingsmethoden varieerde van -1.7 cm tot +0.7 cm. Dit houdt in dat hoge dosering androgenen in lange jongens slechts een beperkte lengtereductie bewerkstelligt. Echter, wanneer de behandeling was gestart op een botleeftijd van jonger dan 14 tot 15 'jaar', werd er een significant effect van de behandeling waargenomen. Wanneer de behandeling was gestart op een relatief 'oude' botleeftijd, had de androgeen toediening juist extra groei veroorzaakt in plaats van groeiremming. Het op 'oudere' botleeftijd starten van androgeenbehandeling is daarom gecontraindiceerd. Tenslotte toonden we aan dat er een aanzienlijk restgroei van gemiddeld 2.4 cm had plaatsgevonden nadat de groeiremmingsbehandeling was gestopt. Dit komt doordat de behandeling werd gestaakt voordat volledige sluiting van de epiphysairschijven had

plaatsgevonden.

In *hoofdstuk 4* wordt de nauwkeurigheid van negen verschillende voorspellingsmethoden geëvalueerd bij 88 onbehandelde lange meisjes. Daarnaast wordt het effect van oestrogeenbehandeling op de eindlengte bestudeerd bij 159 behandelde meisjes. Net als bij de jongens werd de nauwkeurigheid van de diverse voorspellingsmethoden vastgesteld door de voorspelde eindlengte te vergelijken met de werkelijke eindlengte, gemeten op een gemiddelde leeftijd van 25.3 jaar. Bij lange meisjes toonden 6 van de 9 geteste voorspellingmethoden een systematische voorspellingsfout van nog geen centimeter. Dit impliceert dat de voorspelling van de eindlengte bij meisjes met CLG vrij nauwkeurig is en klinisch acceptabel. Ook bij hen werd het groeiremmende effect van de geslachtshormonen geanalyseerd door middel van multipele regressie analysen. Hiermede wordt gecorrigeerd voor de gevonden verschillen in leeftijd, botleeftijd en eindlengteprognose tussen behandelde en onbehandelde meisjes. We vonden dat oestrogeenbehandeling een gecorrigeerd groeiremmend effect induceerde van gemiddeld +1.1 to +2.4 cm afhankelijk van de gebruikte voorspellingsmethode. Het groeiremmende effect was meer uitgesproken naarmate de behandeling op 'jongere' botleeftijd was gestart. Nadat de hormoonbehandeling was gestaakt, had een belangrijke restgroei plaatsgevonden van gemiddeld 2.7 cm. Dit kan niet alleen worden verklaard door het feit dat de behandeling was gestopt voordat complete sluiting van de epiphysairschijven had opgetreden; een gedeelte van de restgroei wordt mogelijk ook veroorzaakt door additionele spinale groei.

Hoofdstuk 5 beschrijft de mogelijke lange termijn effecten van de behandeling met hoge dosering geslachtshormonen bij kinderen met CLG. Speciale aandacht gaat daarbij uit naar het functioneren van het hypothalame-hypofysaire-gonadale systeem. 244 Lange mannen en vrouwen werden op gestandaardiseerde wijze geïnterviewd na een gemiddelde follow-up periode van 10 jaar na het staken van een behandeling met geslachtshormonen. 155 Onbehandelde lange mannen en vrouwen dienden als controlegroep. Bijna 80% van de mannen en vrouwen die behandeld waren, gaven aan last te hebben gehad van 1 of meer bijwerkingen gedurende de hormoonbehandeling. De incidentie van

bijwerkingen is daarbij waarschijnlijk dosis afhankelijk. Een amenorrhoe van langer dan 6 maanden na het staken van de behandeling trad op bij 5% van de vrouwen. Er werd geen verschil gezien in menstruatiepatroon tussen behandelde en onbehandelde vrouwen. In totaal werd informatie verkregen van 127 zwangerschappen. Hieruit kwamen geen aanwijzingen naar voren dat het toedienen van geslachtshormonen in farmacologische dosering lange termijn effecten heeft op de vruchtbaarheid. Echter, deze follow-up periode is nog steeds te kort om definitieve conclusies te kunnen trekken.

Hoofdstuk 6 evalueert de vraag of behandeling met hoge dosering androgenen gedurende de puberteit de testiculaire functie beïnvloedt op de volwassen leeftijd bij jongens met CLG. Hiertoe werden de spermakwaliteit, het testikelvolume en plasmawaarden van verschillende hormonen bestudeerd van 43 behandelde en 30 onbehandelde lange mannen. Het bleek dat de spermakwaliteit, het testikelvolume en de testosteronspiegels in het plasma niet significant verschilden tussen de behandelde en de onbehandelde groep. De gemiddelde sperma concentratie bedroeg 66 x 10^6/ml en dat is vergelijkbaar met die van de normale populatie van deze tijd. Opvallend was, dat behandelde mannen in vergelijking met de controlegroep significant hogere plasmawaarden hadden van het follikel-stimulerend hormoon (FSH), 3.3 IU/l versus 2.1 IU/l. De betekenis van deze bevinding dient nog te worden bezien. Als toevalsbevinding werd geconstateerd dat er bij lange mannen (behandeld en onbehandeld) sprake is van een hoge prevalentie van een links-zijdige varicocele (40%) .

Hoofdstuk 7 beschrijft de ontwikkeling van een nieuw model om de eindlengte te voorspellen bij kinderen met CLG. Met behulp van standaard multipele regressie methoden werden formules ontwikkeld om de eindlengte te voorspellen. Deze formules zijn gebaseerd op groeigegevens van een groep van 143 onbehandelde lange kinderen. Voor meisjes en jongens werden aparte regressiemodellen berekend. Teneinde de waarde van deze nieuwe voorspellingsmethoden te toetsen werd gebruik gemaakt van een tweede groep van 32 onbehandelde lange kinderen. Bij deze groep werd de eindlengte gemeten en vergeleken met de voorspelde eindlengte. De regressiemodellen lieten een

goede nauwkeurigheid zien in het voorspellen van de eindlengte en lijken veelbelovend in vergelijking met bestaande voorspellingsmethoden. Echter, de nadere klinische toepasbaarheid van de nieuw ontwikkelde methoden dient nog nader bekeken te worden bij grotere groepen lange kinderen.

In *hoofdstuk 8* worden de psychosociale aspecten van een constitutioneel lange gestalte beschreven. Door middel van een 5-tal vragenlijsten werd psychosociale informatie verkregen van 145 mannen en 280 vrouwen met CLG; een deel van hen had een hormoonbehandeling ondergaan gedurende de puberteit. Speciale interessegebieden bij dit onderzoek betroffen aspecten van zelf-respect, sociale angst en assertiviteit, algemeen psychologisch welbevinden en lichaamsperceptie. Vergeleken met de controlegroep vonden we geen aanwijzingen voor de aanwezigheid van ernstige psychologische problemen bij behandelde lange kinderen op de volwassen leeftijd. Behandelde lange mannen scoorden wat hoger met betrekking tot zelf-respect en hadden lagere scores voor sociale angst dan de norm. In hoeverre de toediening van hoge dosering geslachtshormonen heeft bijgedragen tot deze gunstige resultaten is een kwestie van speculatie. Andere psychologische mechanismen kunnen eveneens een rol hebben gespeeld. Retrospectief gesteld ondervonden behandelde mannen en vrouwen meer problemen van hun lengte dan onbehandelde lange controles, met name gedurende hun jeugd. Dit kan een belangrijke rol hebben gespeeld bij de keus voor een groeiremmingsbehandeling.

De *hoofdstukken 9 en 10* hebben betrekking op klinische studies bij kinderen met een kleine gestalte na IUGV.

Hoofdstuk 9 beschrijft de mogelijkheid dat verstoringen in de GH/IGF as een rol spelen bij het onderliggend mechanisme dat veranwoordelijk is voor het uitblijven van inhaalgroei groei bij deze kinderen. Wij bestudeerden de GH secretie door middel van fysiologische testen (24-uurs GH profielen) en farmacologische testen (arginine stimulatie test). Daarnaast werden plasma waarden van IGF-I en IGF-II gemeten en werd de uitscheiding van GH in de urine bepaald. We toonden aan dat bij 50-60% van de kinderen met een kleine gestalte na IUGV er sprake is van 24-uurs GH profiel stoornissen en/of een subnormale oploop van GH na

arginine provocatie. Tevens zijn de gemiddelde waarden van IGF-I en IGF-II in het bloed verlaagd. Dit alles wijst op GH insufficientie. In vergelijking met een controlegroep bleek de uitscheiding van GH in de urine verlaagd te zijn bij jongens maar niet bij meisjes. Tot onze verrassing vonden we geen duidelijke relatie tussen de GH secretie en de groei. Met de resultaten van deze studie kon het gehele pathofysiologische mechanisme bij IUGV niet worden opgehelderd. Het blijkt dat stoornissen in de GH/IGF as slechts ten dele verantwoordelijk zijn voor het uitblijven van inhaalgroei; verschillende kinderen hadden geen duidelijke stoornis in hun GH/IGF as maar vertoonden toch geen inhaalgroei na de geboorte.

Hoofdstuk 10 beschrijft de twee-jaars resultaten van een klinisch multicenter onderzoek naar het effect van twee doseringen GH op de lengtegroei, de botrijping, de puberteitsontwikkeling en de eindlengte van 79 prepubertaire kinderen met een kleine gestalte na IUGV. Wij toonden aan dat beide GH doseringen (3 en 6 IU/m^2/dag) na twee jaar een significante groeiversnelling, uitgedrukt als de verandering in $HSDS_{CA}$ en $HVSDS_{CA}$, teweegbrachten. Daarnaast was er een duidelijk dosis-respons effect waarneembaar waarbij de hogere dosering een grotere verandering in $HSDS_{CA}$ liet zien (+1.3 en +1.7 met respectievelijk 3 en 6 IU/m^2/dag). Gedurende de twee jaar van GH behandeling werd een versnelling in botrijping waargenomen, welke niet dosis-afhankelijk bleek te zijn. Ondanks deze progressie in botrijping werd er een significante toename gezien van de lengteprognose gedurende de behandelingsperiode. Echter, de gegevens met betrekking tot de botrijping en de lengteprognose dienen met de nodige voorzichtigheid te worden bekeken, aangezien ook de spontane botrijping bij onbehandelde IUGV kinderen onevenredig kan toenemen en de lengtevoorspellingen de werkelijke eindlengte kunnen overschatten. De waarden van IGF-I en IGFBP-3 waren laag bij kinderen met IUGV. Tijdens GH behandeling normaliseerden de IGFBP-3 waarden en namen de IGF-I waarden aanzienlijk toe. In een voorlopige analyse vonden we geen correlatie tussen de uitgangswaarden van de diverse GH secretie parameters en de groeirespons tijdens GH behandeling. Wel bleek dat er significante correlaties bestonden tussen veranderingen in biochemische parameters tijdens GH therapie en de GH geïnduceerde

groeirespons na twee jaar behandeling. Aanvullende analyses zullen nodig zijn om te bestuderen of het mogelijk is de groeirespons door GH therapie te voorspellen bij kinderen met IUGV. In onze studie werd de glucose toierantie behouden ten koste van een stijging van de nuchtere insuline concentratie en een toename van de totale insuline produktie na een orale glucose belasting. Concluderend kunnen we stellen dat GH behandeling zinvol lijkt bij kinderen met een kleine gestalte na IUGV teneinde de groei te bevorderen zonder dat daarbij ernstige bijwerkingen optreden. Echter, definitieve conclusies kunnen alleen worden getrokken aan de hand van lange termijn studies zoals deze waarbij een en ander tot en met eindlengte wordt vervolgd. Het verdient daarbij aanbeveling de nevenwerkingen van GH op bijvoorbeeld het koolhydraat-metabolisme te blijven vervolgen.

Tenslotte wordt in *hoofdstuk 10* de resultaten van onze studies besproken en de implicaties ervan voor het beleid bij kinderen met extreme lengtegroei. Daarnaast worden aanbevelingen gedaan en worden suggesties gegeven voor verder toekomstig onderzoek.

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CURRICULUM VITAE

Wouter de Waal was born in Egmond aan Zee, The Netherlands, on February 13th, 1965. He passed his secondary school exam (VWO) in 1983 at the Christelijk Lyceum in Veenendaal. In 1983, he started his medical training at the Medical Faculty of State University Utrecht. From September to December 1988, during his study, he performed a literature research on 'CARA and Sports in children' by order of The Foundation "The Sick Child in Exercise", Utrecht (head: Drs. F.J.G. Backx). In March 1990, he completed a clinical clerkship at the Department of Child and Adolescent Psychiatry (supervisor: Drs. D. van Strien) of the Academic Hospital Utrecht. In May 1990 he obtained his Medical Degree.

From September 1990 to April 1995 he was a research fellow (Assistent In Onderzoek (AIO)) at the Division of Endocrinology (head: Prof.Dr. S.L.S. Drop) of the Sophia Children's Hospital, Rotterdam (head: Prof.Dr. H.K.A. Visser). His research project included several studies on the influencing of extremes of growth. During this period he was a member of the Dutch Working Group on Growth Hormone of the Dutch Growth Foundation. In May 1992, during his research fellowship, he passed his Oxford Examination in English as a Foreign Language (higher level). He attended the Erasmus Autumn School on Endocrinology of Erasmus University Medical School, Rotterdam, in October 1992. In October 1993, he passed his exam after the course on Classical Statistical Methods for Data Analysis of The Netherlands Institute for Health Sciences of Erasmus University Medical School, Rotterdam.

In April 1995 he started his specialist training in Pediatrics at the Sophia Children's Hospital, Rotterdam (head: Prof.Dr. H.K.A. Visser/Prof.Dr. H.J. Neijens).

In his spare time, he is an amateur ornithologist, a fervent chess player and he likes ice-skating and gymnastics. In June 1992, during his research period, he performed three parachute jumps in Texel, The Netherlands, with success. Since 1987 he has been married to Laura Pakvis, oncology nurse, and they have one son: Onne.