GROWTH HORMONE TREATMENT MODALITIES IN GIRLS WITH TURNER SYNDROME

GROEIHORMOON BEHANDELINGS MODALITEITEN BIJ MEISJES MET HET SYNDROOM VAN TURNER

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CONTENTS

LIST OF ABBREVIATIONS

apo	apolipoprotein (A1 or B)
ATT	arginine tolerance test
$AUC_{(ab)}$	area under the curve (above baseline)
BA	bone age
BIAC	biacromial diameter
BIIL	biiliacal diameter
BID	twice daily
BMI(SDS)	body mass index (standard deviation score)
B&P	Bayley and Pinneau final height prediction method
BP	blood pressure
CA	chronological age
CASAS	computer aided skeletal age scoring system
6b/13b	6 or 13 bones rating models of CASAS
cSDS	corrected SDS final height prediction method
DR	dose response
DSD	Dutch-Swedish-Danish Turner reference population
ecg	electrocardiogram
echo	echocardiogram
(e)d	(end) diastolic
(e)s	(end) systolic
EE	ethinyl estradiol
ESD	standard deviation of the mean error
FH	final height
FR	frequency response
GH(D)	growth hormone (deficiency)
GHBP	growth hormone binding protein
G&P	Greulich and Pyle method for BA determinations
HbA_{IC}	hemoglobin A_{IC}
HDL	high-density lipoprotein cholesterol
H(V)	height (velocity)
H(V)SDS	height (velocity) standard deviation score
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IPH	index of potential height
IVS	intraventricular septum
LDL	low-density lipoprotein cholesterol

LV(act)	left ventricular
LVID	LV internal diameter
mPAH	modified projected adult height
mIPH	modified index of potential height
PAH	projected adult height
MPH	midparental height
MS	maturity score according to TW2 method
OD	once daily
OGTT	oral glucose tolerance test
PTS	Turner specific final height prediction method
PW	posterior wall
RUS	radius, ulna, and short bone score (TW2 method)
RWT	relative wall thickness
$\int$-value	shape value for two measurements
$SD(S)_{CA}$	standard deviation (score) for chronological age
SE	standard error
SF	shortening fraction
SH	sitting height
TC	total cholesterol
TG	triglycerides
TS	Turner syndrome
TW:	Tanner-Whitehouse Mark 2 adult height prediction
uCp	urinary C-peptide
uGH	urinary growth hormone excretion
z-score	standard deviation score

Chapter 1

General introduction:

Clinical findings, endocrine function and treatment in Turner syndrome

CLINICAL FINDINGS, ENDOCRINE FUNCTION AND TREATMENT IN TURNER SYNDROME

NAMING A SYNDROME

The November 1938 issue of *Endocrinology* published a paper by the American physician Henry Turner which described seven females exhibiting certain physical features including short stature, sexual infantilism, webbing of the neck, low posterior hairline, and increased carrying angle of the elbow [1]. This syndrome now bears his name, although the Munich paediatrician Otto Ullrich had already described patients with similar physical characteristics in 1930 [2]. For this reason, German studies refer to this syndrome as Ullrich-Turner syndrome.

EVOLUTION IN GENETICS

The etiology of this syndrome remained unclear until Wilkins and Fleischmann in 1944 established the presence of underdeveloped ovaries [3], which in 1955 was referred to by Grumbach *et al.* [4] as 'gonadal dysgenesis', a term often used for the disease itself. Further clarification of the etiology of this disorder came after the development of techniques for demonstrating human chromosomes. Ford *et al.* [5] reported in 1959 a missing X chromosome in TS. Before the more advanced and accurate chromosome analyses could be performed, the absence of the 'Barr body' in mucous membranes or of the 'drumsticks' in granulocytes were sufficient for the diagnosis. More general, the staining of material in an area within the cell nucleus in the presence of two X chromosomes was referred to as 'sex chromatin'. Later, it was shown that not all body cells had to have a missing X chromosome to explain the typical features of the syndrome. Usually leucocytes from a simple blood collection are used but the investigation can also be carried out on cells from other tissues, e.g. fibroblasts. The findings from various tissues do not always show complete agreement. Thus, the diagnosis 'Turner syndrome' is based on the total or partial absence of one of the two X chromosomes in at least one tissue of the body [6]. Above indicates that a range of karyotypes is associated with the syndrome.

ALTERATIONS IN GENETIC MATERIAL

An entire X chromosome can be lost due to non-disjunction during either of the two meiotic divisions of gametogenesis or during the first mitotic divisions of the zygote. These all result in numerical abnormalities, 45,X (60% of the cases).

Table 1. Frequency distribution groups for physical features and aberrations of internal organs in girls with Turner syndrome (adapted from M.B. Ranke [6]).

Present in 80-100% of the cases:	
Growth	Small for dates at birth
	Growth retardation after birth
Ovaries	Gonadal dysgenesis
Present in 60-79% of the cases:	
Mouth and jaw	High arched palate
	Small or backward rotated lower jaw
	Defective dental development
Neck	Short, thick ('webbed') neck
	Low neck hair line
	Pterygium colli
Chest	Scutiform thorax (apparant wide nipples)
	Inverted nipples
Skin (appendages)	Lymphedema of hands and feet
	Increased number of pigmented naevi
	Increased body hair growth
	Dysplasia of finger-nail and toe-nail
	Increased skin ridge patterns
	Alopecia
	Vitiligo
Present in 40-59% of the cases:	
Skeleton	Wide angle of arm (cubitus valgus)
	Short metacarpal bones (e.g. 4th)
	Spongiose bone structure
	Scoliosis
Heart and vessels	Stenosis of aortic isthmus
	Bicuspid aortic valve
	Aortic dilatation/aneurysm
Kidneys	Renal malformation (e.g. horseshoe)
	Renal aplasia
	Changes in renal pelvis and ureters
	Vessel abnormalities
Ears	Deformed auricles
	Otitis media
	Impaired hearing
Present in 20-39% of the cases:	
Eyes	Ptosis
	Epicanthus
	Myopia
	Strabismus
	Nystagmus

Structural abnormalities with partial loss of X chromosomal material may be due to transverse meiotic division of the chromosome around the centromere leading to isochromosomes i(Xp) or i(Xq). In patients with an i(Xp) karyotype, gonadal dysgenesis seems to be less severe and stature is often normal. Furthermore, the long or short arm of the X chromosome may be missing (in part), denotation 'del' or '-', but also small fragments can be lost. Finally, an X chromosome may have formed a ring ('r') with the loss of chromosomal material at the point of fusion.

Loss of (a part of) an X chromosome during later cell divisions in the zygote results in mosaics with both normal and reduced chromosome counts. If mosaicism occurs only in certain organs the diagnosis can be missed. This is particularly a drawback if the Barr-body analysis is used for the diagnosis. The last decades have seen a rapid development in knowledge in the field of genetics. It has even been suggested that current PCR-probe techniques will show that all 45,X karyotypes are in fact mosaics [7]. Features of TS are also exhibited in mosaicism with the presence of Y chromosomal material. However, if testicular tissue is present the patient is referred to as having 'mixed gonadal dysgenesis syndrome'.

Mapping of the chromosomes is currently an important field of investigation. Most features of TS have not yet been mapped to precise regions of the X chromosome. The parental origin of the abnormal X chromosome is also uncertain. Evidence suggests that the i(Xq) chromosome and the missing X chromosome in 45,X are paternal [6,8-11]. Many other questions remain yet to be unanswered. The most intriguing one may be 'Why does (partial) loss of an X chromsome result in TS phenotype?'

CHARACTERISTIC CLINICAL FEATURES

Turner syndrome is relatively common, with an estimated incidence of 1:2500 female births [6]. The X chromosomes carry genetic information that may have a direct effect on the clinical features of girls with TS. However, regulatory interactions with the autosomes are also probable since the number and degree of abnormalities show wide variation. Nevertheless, the most marked clinical characteristics are seen in cases with a complete loss of an X chromosome, the 45,X karyotype [6]. The changes in external appearance and aberrations of internal organs in TS are listed as frequency ranges in Table 1 according to Ranke [6]. TS is associated with a wide variety of clinical characteristics. Although the severity of these features also varies, each one can present a medical or psychosocial problem to the girl and/or their custodians. For the latter, acceptance of the diagnosis is important in order to give appropriate guidance the girl.

Table 1 indicates that girls with TS almost invariably present with short stature and gonadal dysgenesis. The clinical features have extensively been described by others [12-14]. Since the scope of the studies in this thesis is related to growth-promoting therapies, only the natural growth pattern and some aspects of gonadal dysgenesis are described here. In addition, other hormonal changes

and possible influences of GH and estrogen treatment, disturbances in carbohydrate and lipid metabolism, and cardiac abnormalities are discussed.

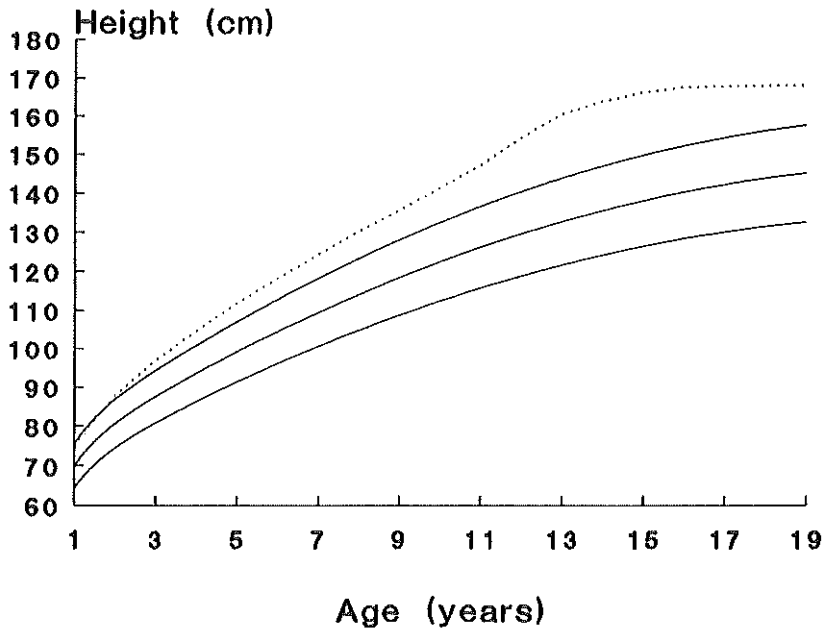

Figure 1. Dutch-Swedish-Danish Turner references (mean +/- 2 SD) for standing height, adapted from Karlberg *et al.* [25], and the 50th percentile for healthy Dutch girls (.....).

NATURAL GROWTH PATTERN

Although smaller cohorts have been described [15], Ranke *et al.* [16,17] documented the pattern of natural growth in a large number of women with TS. Thereafter, reports on many other Turner populations worldwide, including a large Dutch Turner population by Rongen-Westerlaken *et al.* [18], have confirmed this first description [19-24]. Based on data from a large multinational study, a Dutch-Swedish-Danish growth standard for TS has also been compiled [25], see Figure 1.

Four distinct phases can be recognized in the pattern of growth in TS [26]:

1. Intrauterine growth is retarded at some point in time; newborns at term are smaller than average girls, body length by about 3 cm and body weight by about 500 grams.
2. Postnatal growth rate appears to be in the normal female range during the first 2 to 3 years of life.
3. Thereafter, height velocity shows a gradual decrease compared with healthy girls.

4. Due to the non-functional ovaries there is a lack of the pubertal growth spurt evident from the age of 10 years onwards. In fact, growth is prolonged at a slow rate. The delayed epipyseal fusion allows a number of patients to continue growing until their early twenties.

Bone maturation of girls with TS shows similarities with the development of height [16,17,27]: a progressive retardation until about the third year of life, a normal rate of bone maturation until an age of about 12 years, followed by a progressive retardation due to the relative absence of sex steroids compared with their healthy peers.

The mean final height of women with TS in Northwestern Europe is about 147 cm [17,19,25], some 20 cm shorter compared with the normal female population. A relationship with the adult length of the mother has been found [28]. The differences in reported FH may be due to the relatively small numbers of cases, selection bias, or differences in karyotypes, malformations, and/or social backgrounds. Convincing evidence that patients with a 45,X karyotype differ from those with another chromosomal pattern has not been documented [6]. The comparable standard deviations of the mean FH in TS and in the normal (female) population suggests that growth regulation follows similar principles [6].

Rongen-Westerlaken *et al.* [18,29] described the relationship between height and sitting height in an extensive population of women with TS. Disproportionately short stature with short lower extremities and a normal trunk was observed in young girls. While the development of the legs in this cross-sectional study was similar to that in healthy controls, the trunk became relatively shorter with increasing age. At the age of 15-17 years the ratio of sitting height to subischial leg length was even within the range for normal females. In adult women with TS these mean body dimensions were at the limit of normality because of a more pronounced defect of leg growth. Alike controls, growth of the legs appeared to cease at an earlier age than truncal growth.

Gerver *et al.* [30] showed that mean values of a number of measurements, including height, sitting height, arm span, hand, tibia, and foot, were significantly smaller in TS compared with those for normal age-specific females. However, large numbers of girls with abnormal shape values were only found if the measurements of the foot, shoulders or hips were related to the height of the girls.

TURNER SPECIFIC REFERENCE VALUES

For diagnostic purposes, but also when judging the (short-term) effects of growth promoting therapies, the necessity of Turner specific reference populations and even the need to distinguish cultural/regional differences, is clear. As pointed out above, the data described by Karlberg *et al.* include a large cohort of Dutch women with TS [25]. Based on the age of a Turner girl, tables and mathematical equations are given for height and height velocity. Turner specific data for other auxological measurements and bone maturation, as well as for final height prediction, were not available prior to the studies described in this thesis. In order to evaluate whether or not growth during growth-promoting therapy is safe and effective, Turner specific datasets had to be developed.

OVARIAN DYSFUNCTION

During the first three months in utero, apparently, the ovaries develop normally [31], thereafter, oocytes are rapidly lost. By the end of the first year of life this process is completed. Only in a very limited number of cases the loss of germ cells may be more gradual, ovarian function may be sufficient to induce puberty [32], and even pregnancies have been reported [33].

The ovarian dysfunction is also apparent from an insufficient estrogen secretion at the pubertal age. Therefore, a normal growth and development of the uterus, vagina, breasts, and female pubic and axillary hair distribution fails to occur [6].

The intact hormonal feed-back mechanism is the reason for high levels of pituitary hormones which control ovarian function, i.e. follicle stimulating hormone and luteinizing hormone. Although between 3 and 7 years of age basal blood levels of LH and FSH are normal, in the first years of life and from the age of about 8 years onwards the secretion of these gonadotrophic hormones is increased [6,34]. With induction of puberty through exogenous estrogen replacement the increased gonadotrophic levels can be reduced to normal.

The age at which puberty should be induced in relation to optimal growth has been a matter of debate. The main reason is the increase in bone maturation due to sex steroids [35]. It has been proposed that before the introduction of estrogens, even at dosages as low as 0.05 microgram per kilogram, the girl should have reached an acceptable length in relation to her bone age. In the Netherlands it is advocated to administer synthetic ethinyl estradiol or, preferably, the natural estrogen 17-ß-oestradiol, from the age of 12 years onwards. However, individual 'needs' should be taken into account, in particular with regards to psychological aspects. For the adult woman with TS in vitro fertilization using oocyte donation can be offered. This procedure has successfully been performed [36,37], also in the Netherlands.

SOMATOTROPIN-INSULIN-LIKE GROWTH FACTOR AXIS

In healthy individuals secretion of human growth hormone follows a pulsatile pattern [38]. The amplitude and frequency of GH secretory pulses are regulated by a variety of physiological factors through the hypothalamus and by direct influence of hormones and metabolites on the GH secretory cells in the anterior hypopituitary.

The pulsatile release of GH is controlled by a complex neuro-endocrine system in which two antagonistic neurohormones from the hypothalamus play an important role. Initiation of the GH pulses requires stimulatory activity of growth hormone releasing hormone, whereas the amplitude of GH pulses is modulated by the levels of somatotropin release inhibiting hormone (or somatostatin), which basically has inhibitory activity [39,40].

Among the physiological factors stimulating GH secretion [38,41] are gonadal steroids, fasting, sleep, exercise, stress, and metabolic substrates as arginine and hypoglycaemia. The latter two are also used as provocative stimuli to test GH secretion in children.

Plasma GH is bound to two binding proteins. The major one is identical to the extracellular portion of the GH receptor and has high affinity for the 22 kDa (predominant) form of GH [42]. GHBP plasma levels are stable throughout the day in a given individual and the influence of GH on GHBP regulation is not thought to be of great importance. In turn, GHBP prolongs the half-life of GH. Obesity and the use of oral estrogens are associated with increased GHBP levels.

GH stimulates hepatic production of Insulin-like Growth Factor-I, the most important growth factor, through classical endocrine regulatory mechanisms. However, IGF-I is also generated in numerous other tissues and exerts paracrine and autocrine action [43]. IGF-I has a negative feedback effect in the regulation of GH secretion [44]. It is bound in the circulation to several binding proteins of which IGFBP-3, which is also GH dependent, is the most important [45-48]. These binding proteins prolong the half-life of IGF-I and modulate its bioavailability and action.

LONGITUDINAL BONE GROWTH

GH is thought to have a dual effect on the epiphysial growth plates [49,50]. A direct effect of GH by promoting differentiation of growth plate precursor cells as well as an indirect effect through the stimulation of IGF-I production, both systemically and locally, and by inducing IGF-I responsiveness of the chondrocytes. GH deficient children have a severe, but in general proportionate growth retardation. On the other hand, GH excess in acromegaly is characterized by enlargement of the distal parts of the body, although it may involve all body portions.

Sex steroids also have a dual effect on skeletal growth. Apart from a direct effect on the growth plate and on mineralization [51-53], GH and IGF-I act as mediators [51]. In puberty, sex steroids induce not only rapid growth with predominance toward the extremities, but also an increase in bone maturation with a closure of the epiphyseal growth plates. Body growth in precocious

puberty is increased, but bone maturation even more, resulting in an adult height in the low normal range with the main deficit in the extremities [54]. In contrast, adolescents with hypogonadism tend to have disproportionally long legs. Primary hypogonadism in TS is also accompanied with short stature, in particular of the lower limbs [29,30].

ETIOLOGY OF THE GROWTH DISTURBANCE

The etiology of the growth disturbance in TS is unknown. Evidence suggests that deletion of the distal part of the short arm of the X chromosome results in short stature [55]. Physiological and stimulated plasma GH levels are normal [56-61], IGF-I plasma levels are in the (sub)normal range [46,62,63], and binding protein levels of GH and IGF-I are thought to be normal [46]. TS is associated with generalized short stature and characteristic bony anomalies, e.g. cubitus valgus, short metacarpal bones (e.g. fourth metacarpal), and Madelungs deformity. These specific skeletal defects do not seem to be severe or generalized enough to account for the short stature. A generalized skeletal dysplasia seems also unlikely in view of the more or less proportionate reduction in length and width. Moreover, the structure and biochemical composition of epiphysial cartilage is normal [64]. The growth retardation in TS appears to affect all tissues and organs. Therefore, it has been suggested that girls with TS have a relative end-organ insensitivity to growth factors [34].

There is wide support for the hypothesis that at least a part of the growth disturbance of this syndrome, does not result from hormonal abnormalities.

TS AND GH EFFECTS ON CARBOHYDRATE METABOLISM

The prevalence of carbohydrate intolerance and noninsulin-dependent diabetes mellitus (NIDDM) is common in adult women with TS, with frequencies up to 32.5% [65]. In girls with TS the reported frequency rate of impaired glucose tolerance ranges from 15% [66,67] to 43% [68] and was associated with normal or increased insulin responses. Both insulin resistance and hyperglucagonaemia have been implicated [68]. As in other conditions with insulin resistance, e.g. obesity and NIDDM, this may be due at least in part to decreased glycogen synthesis [69]. Age, karyotype, family history of DM, and estrogen replacement are important factors in aggrevating a pre-existent carbohydrate intolerance. In healthy children body composition and insulin sensitivity have been shown to depend on gender and Tanner stage [70]. Carbohydrate tolerance is defective in young girls with TS but improves at pubertal ages due to the almost complete absence of estrogen-progestagen secretion [71].

In small groups of children with GHD following oral glucose loads glucose levels were normal but insulin concentrations were significantly reduced compared with controls [72,73]. Subsequent GH suppletion therapy for 6 to 12 months resulted in one study in improved insulin responsiveness with near normal plasma glucose levels [72]. In another study impaired glucose tolerance developed [73]. A report by Gertner *et al.* [74] indicated that even at GH dosages up

to 0.9 IU/kg/week for several months in GHD children glucose tolerance did not change. Similar findings have been reported by de Muinck Keizer-Schrama *et al.* [75] at maximum GH dosages of 4 IU/m^2/day after 3 years therapy.

Adults with GH deficiency exhibit, in general, normal fasting levels of glucose and HbA_{1C}, reflecting overall carbohydrate tolerance, even after GH treatment [76]. On the other hand, abnormal glucose tolerance was found to be more frequent in GHD adults compared with controls [77]. GHD increases sensitivity to insulin [78], although this has been disputed [79]. Minor detrimental effects on carbohydrate tolerance along with hyperinsulinaemia has been observed after prolonged GH treatment [80].

Supraphysiologic concentrations of GH in acromegalic patients [81] and in normal [82,83] and diabetic [84] adults showed a decrease in glucose sensitivity to insulin, both in liver and in extrahepatic tissues. This insulin resistance can be explained on the basis of a postreceptor defect [85] and results in impaired ability of insulin to suppress (hepatic) glucose production and to stimulate glucose utilization [83]. Diabetogenic effects only occur if compensatory mechanisms fail, e.g. if insulin secretion is deficient. In addition, (asymptomatic) diabetes mellitus disappeared in most cases if the acromegaly was cured [86].

Results after one or two years of GH therapy have indicated that in TS glucose homeostasis is maintained during GH therapy at the expense of a compensatory increase in insulin response [66,67,87].

TS AND GH EFFECTS ON LIPID METABOLISM

Girls with TS at an adolescent age were shown to have higher TC levels, but similar TG levels compared with age-matched controls, even after adjustment for age and for obesity. These findings are likely to be related to the absent influence of estrogens, possibly mediated by GH [88], which lowers LDL and increases HDL levels [89]. Although one group reported an increase in TG levels after 6 months GH therapy in girls with TS [90], other studies have not observed any significant changes in TC or TG concentrations after one or two years GH administration [66,91].

In GHD adults, GH was shown to have an intrinsic lipolytic activity [92]. This results in a reduction of (centralized) adipose tissue and an increase in lean body mass [93]. Normalization of plasma lipids has been observed, in particular a reduction in TG, and to some extent LDL, and an increase in HDL [94]. Other studies reported reductions in TC and LDL [95].

Hypercholesterolaemia is already present in GHD children [96-99]. Contradictory effects of GH therapy in GHD children have been reported [96-99], but in general reduced or equal TC and TG levels have been found. After 3 years GH therapy de Muinck Keizer-Schrama *et al.* reported [75] normalization of the initially raised TC, LDL and apolipoprotein B concentrations. Even a triple GH dose-increase from 0.3 IU/kg/week to 0.9 IU/kg/week (thrice weekly injections) [74], or an equal division of the total GH dose (20 IU/m^2/week) in a thrice daily regimen [100], had no effect on TG and TC plasma levels. Acromegalic patients often have increased serum TG (but not TC) levels which returned within the normal range after surgery [101].

TS AND GH EFFECTS ON THE CARDIOVASCULAR SYSTEM

TS, in particular the 45,X karyotype, is associated with cardiac anomalies (up to 45%), e.g. aortic coarctation, aortic valve abnormalities, and dilated ascending aorta, see Table 1 [102,103]. Because of the frequent association between congenital heart defects and webbed neck it has been suggested that lymphatic sac obstruction is the initiating event in the sequence leading to heart malformation [104]. The increased lymphatic pressure associated with jugular lymphatic sac obstruction distends the thoracic ducts, which compress the ascending aorta altering intracardial blood flow. The redirected intracardiac blood flow results in coarctation of the aorta and other defects in the spectrum of left heart obstruction.

Animal studies have suggested that GH may increase heart size and enhance DNA synthesis of heart muscle [105]. In humans, GH is related to cardiovascular morbidity and mortality, since life expectancy in acromegaly [106] and in GHD [107] is shortened. Left ventricular hypertrophy is a feature of GH hypersecretion in acromegaly [108]. It is directly correlated with the duration of the disease [109] and usually reverses after treatment [110]. A direct inotropic effect of GH is possible, since short-term GH administration in normal subjects increases heart rate, cardiac output, and myocardial contractility [111].

Adult GHD patients show decreased lean body mass, skeletal muscle volume and oxygen uptake [76]. Long-term follow-up (until 38 months) of GHD adults on GH treatment (2 IU/m^2/day) [112] showed that LV diastolic dimensions increased and seemed to normalize, while heart rate and cardiac output increased to supranormal levels. Two years of GH therapy in non-GHD short children resulted in modest changes in LV dimensions, within normal limits [113]. After one year GH treatment in TS a small increase in diastolic LV internal diameter was found, remaining within the norm [114].

TREATMENT MODALITIES IN TS

ESTROGEN THERAPY

Lack of estrogens is the main endocrine defect in Turner girls resulting in the absence of normal pubertal development. While as many as 10-20% of the patients may show early signs of estrogenization, only 1% menstruate, and even fewer are capable of successful pregnancies. Although the likelihood of having functional ovaries is higher in patients with mosaicism, even occasional patients with a 45,X karyotype have successfully conceived.

Therapy with low doses of estrogen has a dual effect in these girls. The induction of pubertal changes concomitantly with their healthy peers reinforces their psychological well-being. This therapy will also induce an increase in height velocity, but whether this will lead to increased final height has not been demonstrated. As in a normal female population, cyclic progesterone therapy should be added to reduce the risk of endometriosis and adenocarcinoma of the uterus.

Ross *et al.* [115] have pointed out that the mode of action of estrogens on growth is biphasic. Lower doses stimulate growth, while higher doses are inhibitory. Also, a direct effect of estradiol on bone growth and maturation cannot be excluded. This may be mediated partly through osteocalcin via stimulation of osteoblasts. It has been shown that 17-ß-estradiol stimulates the growth of osteoblasts directly [116] and thereby increases the synthesis of osteocalcin.

ANDROGENS AND ANABOLIC STEROIDS

For decennia the growth-promoting effects of androgens have been tested. However, there has always been reluctance to use these agents for promotion of linear growth because of undesirable side effects, e.g. masculinization and acceleration of epiphyseal maturation. One of the anabolic steroids more recently successfully synthesized to dissociate anabolic from androgenic effects is oxandrolone [117,118].

The increase of the growth velocity wanes after the first year of oxandrolone treatment in most of the studies in TS [119-127], as well as in other groups of patients [118]. Again the question arises whether or not final height will improve? Sybert *et al.* [119] found an increment of only 1 cm compared to controls on a relatively high oxandrolone dose of 0.13-0.29 mg/kg body weight/day.

Most other studies show an increase in adult height of about 5 cm on oxandrolone dosages of 0.1 mg/kg/day during treatment periods ranging from 1 to 6 years in girls with TS older than 10 years of age. In some of these studies the relatively low final height of the controls has to be considered.

Although a dose response in respect to growth promotion has never been proven, oxandrolone given at a dosage equal or more than 0.1 mg/kg/day can give mild and not always transient virilizing effects in females, e.g. increase in pubic hair, muscular development, deepening of the voice, acne, facial hair growth, cliteromegaly.

With respect to frequency of dosing, Joss *et al.* [122,123] showed that an intermittent regime leads to rapid fall in height velocity. Their results are in conformity with a study by Urban *et al.* [120]. Joss also evaluated another important aspect, i.e. bone age maturation. In his study bone age advanced inappropiately before the age of 8 years [65]. Also, height velocity correlated negatively with bone age from 8-13 years of age [123]. Other long-term studies [119-121,124,126,127] give conflicting reports about the effects on bone age development. It appears that low-dose oxandrolone does not cause unacceptably rapid acceleration of epiphyseal maturation and does not compromise eventual height attainment [125].

Reports from ongoing studies by Rosenfeld *et al.* [126] and Nilsson *et al.* [127] indicated that a combination of GH and oxandrolone results in a better growth response than treatment with either drug alone. The growth velocity also wanes on the combination regimen. Bone age advance is greater in the combination group than in the group treated with GH alone. Nilsson *et al.* concluded that in girls with TS older than 9 years, the combination resulted in a significant growth acceleration and a significant increase of final height compared with the

projected adult height at start of treatment. A GH alone comparator-group was not included in this study. After the first year of therapy the oxandrolone dose in both studies was halved, because of mild virilizing effects.

Based on the findings mentioned above, in 1992 another multicenter study in Turner girls has been initiated by the Working Group on Growth Hormone. In addition to 4 IU GH/m^2 body surface/day and from the age of 12 years 0.05 mg ethinyl estradiol/kg body weight/day, oxandrolone at dosages of either 0.06 or 0.03 mg/kg body weight/day per os is tested against placebo from the age of 8 years onwards.

GH TREATMENT IN TS

In his report in 1938, Henry Turner already mentioned treatment of his short-statured patients with pituitary growth hormones [1]; at that stage it was unsatisfactory. Reasons could be inadequate dosing, because the exact biological activity of the compounds he used was not then known.

Studies in the early 1970s with small numbers of older girls with TS were also disappointing [128]: a mean gain in height velocity (HV) of 1 cm/year during the first year treatment with 20 IU/wk in 2 i.m. injections. Possibly, the injection frequency in this study was insufficient.

Only after 1985 with the unlimited availability of recombinant DNA manufactured human growth hormone, larger populations of girls with TS were treated in standardized studies. Such studies were also conducted in the Netherlands by the Dutch Working Group on GH.

The first study showed that the increase of the HV in the first year of GH therapy (4 IU/m^2 b.s./day) could not be maintained in the subsequent three years [129]. This 'waning' effect is in conformity with the international results of GH treatment in TS [130]. However, at all stages the HV was higher than in untreated age-matched girls with TS. In GHD patients a similar effect is observed, which can be overcome by a two to threefold increase of the GH dose [74,75]. Furthermore, studies in GHD patients have demonstrated the importance of early diagnosis and therapy for optimal FH. GH treatment prevented further loss of stature but could not compensate for the deficit at diagnosis [131]. After the first years of a second Dutch multicenter study of GH treatment in TS it was noted that younger girls responded better than older girls [129,132,133]. In both Dutch TS studies [18,129] bone maturation was faster with GH therapy than the advance in bone age in untreated TS girls, significantly so in 'younger' girls only.

QUESTIONS

Based on the above observations, the following questions were raised:

- What is the optimal GH dose in girls with TS, 4 IU/m^2 body surface/day or more?
- Should the dose -successively- be raised to overcome the waning effect?
- Should GH treatment be started at an early age, at least before the growth curve begins to deviate from the third percentile of the general population?
- Does GH treatment at a younger age result in an undue bone maturation compared with untreated girls with TS and thus compromise final height or its prediction?

MORE QUESTIONS

The second Dutch multicenter study demonstrated that the growth response with six GH injections per week was better than with three-weekly injections [133]. Studies in healthy subjects [134] as well as in TS have demonstrated that GH peaks several times per day [56,60,61,135-138]. Furthermore, studies in hypophysectomized rats [139] and GHD patients [140,141] suggest that a schedule which mimicks normal pulsatile GH secretion may be more effective than a single daily dose. Therefore, yet another question was raised:

- Is more than one GH injection per day beneficial with respect to the growth response?

AIMS OF THE STUDIES

In order to answer these questions, two growth hormone treatment studies have been designed to optimize growth hormone therapy in TS and to evaluate its safety at higher growth hormone dosages. The results are presented in this thesis.

CHAPTER 1

A. DOSE-RESPONSE EFFECTS OF GH THERAPY

The first trial comprises 68 girls with TS, aged 2 to 11 years, followed for four years. In order to counteract the 'waning' effect of growth hormone treatment on height velocity in TS, we investigated whether:

1. A yearly stepwise increment of the GH dose from 4 to 6 to 8 IU/m^2/day could maintain or augment the initial increase in height velocity and thereby improve predicted FH.
2. Treatment from a young age onwards could improve FH prediction.
3. Different growth hormone dosages result in changes in body proportions.
4. The use of higher GH dosages is safe with respect to bone maturation, carbohydrate and lipid metabolism, and cardiac dimensions.

Furthermore, in a subgroup of girls 24-hour growth hormone profile testing was performed during each growth hormone dose to relate the auxological response with biochemical parameters. In the supplement subgroup of girls who received the highest dose, oral glucose tolerance tests were performed to test safety of carbohydrate metabolism.

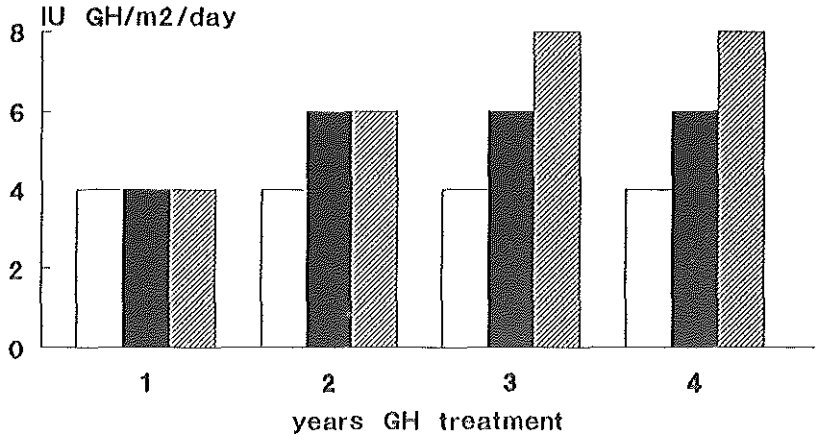

Figure 2. Study design of the 4 year GH dose-response study for groups A (□), B (▓), and C (▨).

B. FREQUENCY EFFECTS OF GH ADMINISTRATION

The second trial comprised 19 girls with TS, aged 11 years or older and with a maximum bone age (RUS score) of 13.5 years, followed for two years. In an attempt to optimize growth hormone treatment by mimicking the normal pulsatile growth hormone secretion more closely,

1. The efficacy and safety of fractionated twice daily versus once daily growth hormone administration (total dose 6 IU/m^2/day) was compared in these girls concurrently receiving low dose ethinyl estradiol.
2. The effect of the two frequencies of growth hormone administration on clinical, auxological (including body proportions), and biochemical parameters was investigated.
3. The safety of two frequencies of growth hormone administration was investigated with respect to bone maturation, carbohydrate and lipid metabolism, and cardiac dimensions.

Moreover, preceeding the two-year follow-up, in a subgroup of 10 girls the growth hormone-insulin-like growth factor axis was studied in detail under GH treatment.

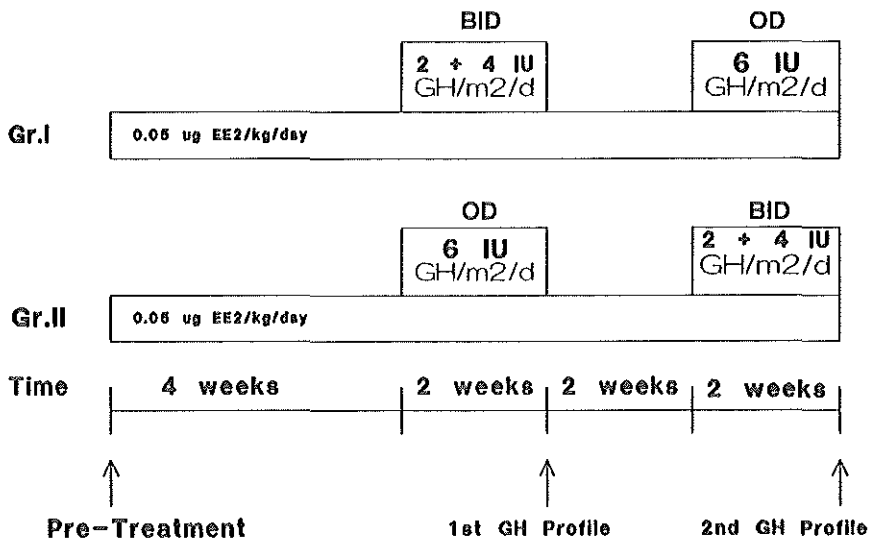

Figure 3. Study design of the 24-hour GH profile study preceeding the GH administration frequency-response study.

CHAPTER 1

OUTLINE OF THE THESIS

Chapters 2 and 3 describe essential background methods for the assessment of treatment effects in girls with TS. First (in **Chapter 2**), a recently developed computerized method for the determination of bone age was validated on healthy children, its applicability in girls with TS was tested, and subsequently radiographs of a large reference population of girls with TS served to develop mixed longitudinal/cross-sectional standard curves for bone maturation. Second (in **Chapter 3**), the accuracy of various existing FH prediction methods was tested on a population of 63 women with TS with 235 measurement points at which height and bone age were known. These data also served to develop a new, Turner-specific FH prediction method, based on regression coefficients for height, chronological age and bone age.

In the subsequent chapters the results of two prospective, open-label, uncontrolled, randomized, parallel designed, multicenter trials in girls with TS are described comparing the efficacy and safety of growth hormone administration. **Chapter 4** discusses auxological aspects and their possible relationships with various growth factors of the four-year growth hormone dose-response study in girls aged 2 to 11 years. **Chapter 5** describes the 24-hour growth hormone profile studies preceeding the two year administration frequency-response study in girls of 11 years or older. The auxological aspects and their possible relationships with various growth factors of the latter study is discussed in **Chapter 6**.

The following chapters describe possible side-effects in both the dose-response and the frequency-response study. First, the effects on body proportions (**Chapter 7**), then the effects on carbohydrate and lipid metabolism, as well as on growth hormone antibody formation, thyroid function, and adverse events reporting (**Chapter 8**) and, finally, the effects on cardiac dimensions (**Chapter 9**) are discussed.

Chapter 10 discusses the significance of the data presented. In addition, conclusions and recommendations are made with respect to growth hormone treatment in TS.

Thereafter, a summary of the thesis in both the English and Dutch language are presented.

Three Appendices have been incorporated in this thesis. **Appendix 1** gives the individual growth curves of all girls in the DR (a) and FR (b) study. The **Appendix 2** compares four different methods to determine body proportions and serves as background information for Chapter 7. **Appendix 3** presents cross-sectional reference tables and curves for various measurements in TS and can also be read in conjunction with Chapter 7.

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Chapter 2

Computer Aided Skeletal Age Scores (CASAS) in healthy children and girls with Turner syndrome

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COMPUTER AIDED SKELETAL AGE SCORES (CASAS) IN HEALTHY CHILDREN AND GIRLS WITH TURNER SYNDROME

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 87 girls with Turner syndrome. In addition, reference curves for bone maturation in TS 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. girls with Turner syndrome (TS). 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 TS as determined by CASAS are presented [4].

POPULATIONS AND METHODS

POPULATIONS

HEALTHY CHILDREN

Eighty-one 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.

TURNER SYNDROME (TS)

Eighty-seven girls with Turner syndrome, aged 2-16 year, participated in this study prior to their enrolment in a drug intervention trial. Furthermore, 818 radiographs of the hand of 314 Dutch girls with TS, born between 1934 and 1992, were gathered from 11 pediatric centres in the Netherlands (see Appendix). The diagnosis was confirmed by chromosome analysis: approximately half of the girls had a 45,X karyotype. Women with conditions known to be related to TS and receiving adequate medical care were included. Three groups were detected: girls without spontaneous menarche or estrogen treatment (A, N=224), girls with spontaneous menarche (B, N=14), and those who received estrogen therapy (C, N=76). About half of Group C received 2.5-5.0 microgram ethinyl estradiol/day, some girls, however, received an ethinyl estradiol dosage up to 50 microgram/day.

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 TS

Radiographs of 87 girls with TS were rated manually and with the 6b model of CASAS; a subgroup of 31 TS girls were rated with the 13b model and compared with the 6b model. Ratings were compared for assessment of agreement.

4. BONE MATURATION CURVES IN TS

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 749 X-rays of girls with TS for analysis with CASAS (6b model). Separate regression equations were estimated for group A and the combined groups B and C in TS.

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.

CHAPTER 2

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 TS reference group was 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 girls with Turner syndrome.

			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
Turner syndrome						
manual vs 13b	F	31	+3.0	-66.5; +72.4	-0.04	-1.36; +1.28
manual vs 6b#	F	87	-20.0*	-88.5; +48.6	-0.43*	-1.71; +0.86
13b vs 6b	F	31	-21.6*	-86.5; +43.3	-0.34*	-1.61; +0.92
manual vs manual	F	31	-16.9*	-75.1; +41.3	-0.27*	-1.13; +0.61

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).

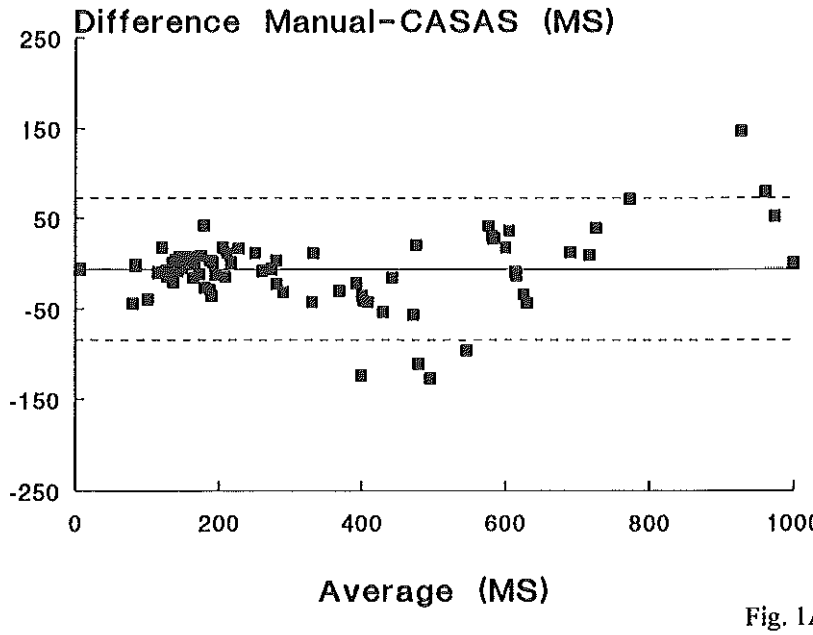

Fig. 1A

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, see next page); mean difference (——), limits of agreement (------).

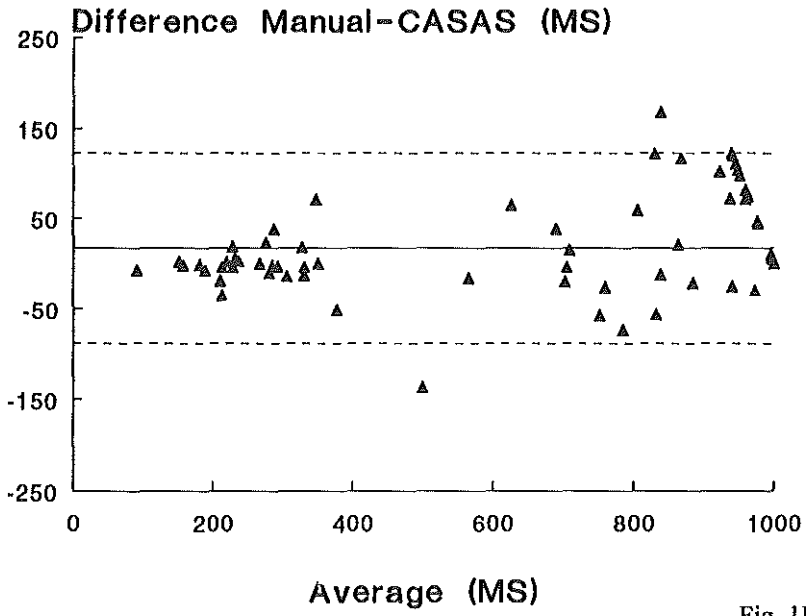

Fig. 1B

3. CASAS IN TS

In girls with TS, four separate comparisons between manual ratings and CASAS were performed (see Table 1). In each comparison the correlation coefficient between the methods was very strong ($r \geq 0.97$; $P<0.01$). On average, the CASAS 6b ratings were significantly higher than the manual ratings ($P<0.0001$), see Figure 2.

The percentage of equal stages on two occasions by a single observer was similar for the manual rating and CASAS 6b; 73% and 72%, respectively. Manually, the proximal phalanges were rated markedly better than the radius and the ulna; 90% and 56%, respectively. Using CASAS 6b there were hardly any differences between the stages of the 6 bones determined at two occasions. The percentage of equal stage ratings between two observers was 73%. The percentage of manual insertions was similar for two observers, about 8%. Most of the 6 bones had manual insertions on both occasions, however the ulna showed a marked inconsistency: in about 20% of instances only on one manual insertion.

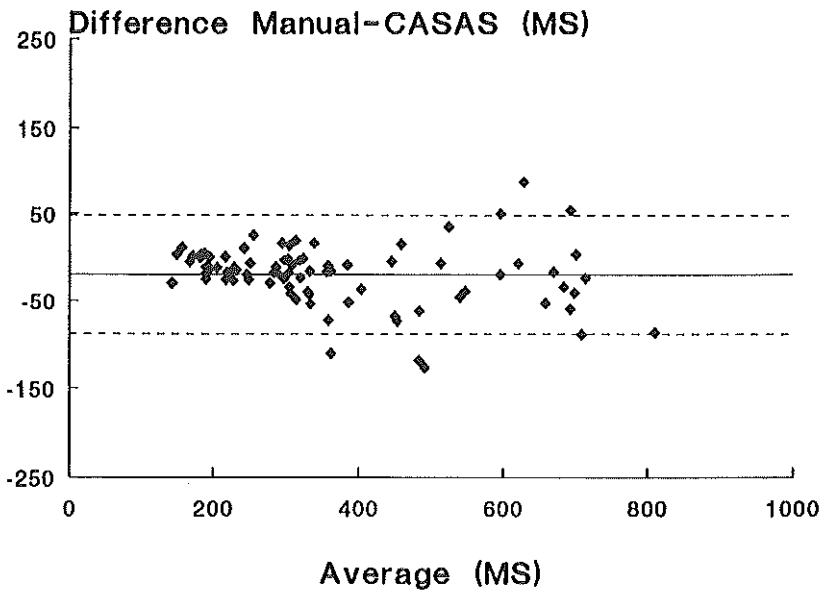

Figure 2. Plot of the difference between manual and CASAS 6 bones ratings against the average of these methods expressed as maturity scores (MS) in girls with Turner syndrome; mean difference (——), limits of agreement (------).

4. BONE MATURATION CURVES IN TS

Figure 3 depicts the CASAS 6b ratings versus CA in TS. A regression analysis of BA (dependent variable) on CA and the presence of estrogens (independent variables) in 749 radiographs of 314 girls with TS yielded the following results:

1. Without estrogens (Group A):

BA (predicted)= $0.522*CA +0.1273*CA^2 -0.01007*CA^3 +0.000212*CA^4$.

2. With estrogens (Groups B and C):

BA (predicted)= $0.538*CA +0.1263*CA^2 -0.01007*CA^3 +0.000219*CA^4$.

The difference in regression lines between groups A and the combined groups B and C was close to the .05 level of significance (likelihood ratio test P=0.0694).

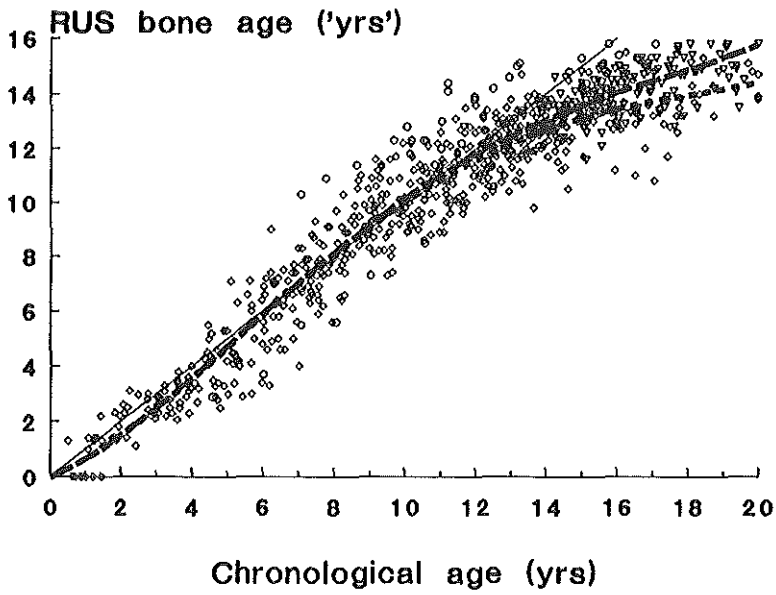

Figure 3. Plot of bone age (BA) determined using CASAS 6 bones versus chronological age (CA) in girls with Turner syndrome. Separate regression lines for Group A (no estrogens, ◊, ▪ ▪ ▪) and the combined Groups B and C (with estrogens, o, ∇, ▬ ▬) are depicted in comparison with the assumed y=x line for BA and CA (——).

The first derivative of BA with respect to CA on these regression equations gives the biological maturation which has passed during a full CA year (dBA/dCA in *'year'*/year):

1. Without estrogens (Group A):

$dBA/dCA = 0.522 + 0.2546*CA - 0.03021*CA^2 + 0.000848*CA^3$.

2. With estrogens (Groups B and C):

$dBA/dCA = 0.538 + 0.2526*CA - 0.03021*CA^2 + 0.000876*CA^3$.

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 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 remains very subjective, in particularly 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 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 has 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 TS

In TS, the mean difference between the manual and the 13b CASAS ratings was close to zero. However, there was a small difference between the manual ratings and CASAS 6b as well as between the duplicate manual ratings. Again, all comparisons - including the duplicate manual assessments - showed fairly large 95% limits of agreement.

The percentage of equal stages on duplicate assessments using the 6b model was lower in these TS girls than in healthy children, but identical to that using the manual rating method in girls with TS. The number of manual insertions was slightly higher (8%) compared with healthy children (5%). In view of the anatomical and structural abnormalities of the hand-wrist bones in these girls this difference seems acceptable.

BONE MATURATION CURVES IN TS

We evaluated the development of bone maturation in TS using CASAS. Our results are comparable with the results described by Ranke *et al.* [10] using the manual TW2 method (400 observations), and by Brook *et al.* [11] and Rochiccioli *et al.* [12] using the G&P method [13]. Latter method is known to have a systematic diminution of BA progression compared with TW2-RUS BA [10].

The BA progression seems to decline until the CA of 3 years. Alternatively the BA is possibly already retarded at birth. It is of interest that in our study population there were 7 girls with BA '0' until the CA of 1.5 years. From the CA of 3 years the rate of BA progression is about 1 *'year'*/ year. Bone maturation reaches its peak (1.15 *'year'*/year) around the CA of 6-7 years. Thereafter, the dBA/dCA declines, resulting in a progressive difference between BA and CA from the CA of 10-11 years, as opposed to 12 years found in the earlier studies [10-12]. At the CA of 20 years, epiphyseal closure was reached in many of the girls with spontaneous menarche and/or estrogen treatment in contrast to those girls without this estrogen influence. Although the girls of

the former group appeared to have a faster progression of BA than the girls without the estrogen influence, the difference between these groups was not significant. The X-ray sampling period spans half a century, however, a time-dependent effect for the bone maturation in girls with TS does not seem to be relevant, because separate regression analyses for women born before and after 1974 (i.e. the median year of birth) revealed similar curves (data not shown).

CONCLUSIONS

In healthy girls and in girls with TS 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 and in girls with TS. 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 girls with TS. The bone maturation curve in TS indicates that from the CA of 9 years the CASAS BA ratings tend to deviate progressively from the theoretical y=x line for BA and CA in healthy children. Estrogen treatment and endogenous estrogens result in a (statistically not significant) faster closure of the epiphyseal growth plates.

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APPENDIX

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Chapter 3

A regression method including chronological and bone age for predicting final height in Turner syndrome, with a comparison of existing methods

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A REGRESSION METHOD INCLUDING CHRONOLOGICAL AND BONE AGE FOR PREDICTING FINAL HEIGHT IN TURNER SYNDROME, WITH A COMPARISON OF EXISTING METHODS

ABSTRACT

A total of 235 measurement points of 57 Dutch women with Turner syndrome (TS), including women with spontaneous menarche and estrogen treatment, served to develop a new Turner specific final height (FH) prediction method (PTS).

Analogous to the Tanner & Whitehouse Mark 2 method (TW) for normal children, smoothed regression coefficients are tabulated for PTS for height (H), chronological age (CA), and bone age (BA), both TW RUS and Greulich and Pyle (GP).

Comparison between all methods on 40 measurement points of 21 Danish TS women showed small mean prediction errors (predicted minus observed FH) and corresponding standard deviation (ESD) of both PTS_{RUS} and PTS_{GP}, in particular at the 'younger' ages. Comparison between existing methods on the Dutch data indicated a tendency to overpredict FH. Before the CA of 9 years the mean prediction errors of the Bayley and Pinneau and TW methods were markedly higher compared with the other methods. Overall, the simplest methods -projected height (PAH) and its modification (mPAH)- were remarkably good at most ages. Although the validity of PTS_{RUS} and PTS_{GP} remains to be tested below the age of 6 years, both gave small mean prediction errors and a high accuracy. FH prediction in TS is important in the consideration of growth promoting therapy or in the evaluation of its effects.

INTRODUCTION

Although reported FH in untreated women with TS may be subject of discussion due to selection bias, sample size or ethnical differences, most studies have confirmed the growth pattern reported by Ranke *et al* [1] with a progressive growth retardation during childhood and an absent pubertal growth spurt. The reported mean FH of Dutch Turner women -- including women with estrogen substitution -- is about 147 cm [2,3].

FH prediction in TS is important in the consideration of growth promoting therapy. Evaluation of treatment effects have to be quantified either using a (historical) control group or by comparing predicted FH at the start of treatment with the attained FH. However, the prediction methods of adult height in these girls, using H, CA, and/or BA, are not very accurate [4-8]. To improve accuracy, Naeraa *et al* [8] suggested that for the evaluation of FH in this growth distorder a method taking BA into account should be combined with a method not taking BA into account.

The present study aimed to design a Turner-specific FH prediction method (PTS) based on regression coefficients of longitudinal growth data of adult women with TS. A comparison is made between a number of existing prediction 63 methods and their combinations. In order to validate PTS, a similar comparison between the various prediction methods is made on data of Danish women with TS.

POPULATION AND METHODS

WOMEN WITH TURNER SYNDROME

Data of 63 Dutch women with TS, born between 1934 and 1973, were available for evaluation. Some women (N=43) had been recruited from an earlier study on spontaneous growth in TS [2], the remainder responded to a call for measurement of their FH (response rate was 95%). Women with conditions known to be related to TS and receiving adequate medical care were included. Women with medication affecting growth were excluded, except for use of estrogens. The diagnosis was made by chromosomal analysis or buccal smears combined with relevant clinical and laboratory findings; 46% had a 45,X karyotype.

Criteria for the achievement of FH were: (1) a follow-up period at least to the age of 20 years, or (2) a height velocity of less than 0.5 cm/year over the previous period of at least one year, or (3) a height velocity of less than 1 cm/year over the previous period of at least two years, and (4) a BA (TW2-RUS, vide infra) of at least 15 'years'. These criteria resulted in exclusion of six women, leaving 57 women with 235 measurement points for analysis (i.e. ages

before reaching FH at which a H measurement and an X-ray were available).

Except for 8 women, all had used estrogens before FH was achieved or a CA of 20 years was reached. Five girls experienced a spontaneous menarche, two of whom never received estrogens.

Height measurements and radiographs of the hand were obtained from hospital files (see Appendix). Sixteen women contributed only one measurement point; the maximum number of data-points per person was ten. All standard deviation scores (SDS: observed minus mean H for age and divided by standard deviation of H for age) were calculated using Dutch-Swedish-Danish TS standards [3]. Midparent height was corrected for the secular trend on the basis of Dutch data [9]: MPH = 1/2*(paternal H + maternal H minus 12 cm) + 3 cm. Table 1 lists the demographic data of 57 women with TS.

Forty measurement points pertaining to 21 Danish women with TS, born between 1956 and 1966, were evaluated. Except for one woman they have been described earlier [8]. Their mean FH was 146.4 (6.3) cm.

SKELETAL AGE DETERMINATIONS

BA was determined by the Tanner-Whitehouse score for the radius, ulna and short bones (RUS)[10] using a recently developed computer-aided image analysis system (CASAS)[11,12]. This system was shown to be applicable in TS. Turner specific equations for the relationship between CA and RUS BA of 314 girls -- with and without endogenous estrogens or estrogen treatment -- have been presented on the basis of 749 X-rays [13]. For the present study, 254 BA determinations from all 63 girls were used. Only one observer operated the computer system. In addition, two investigators (BO and AT) determined simultaneously the BA according to Greulich and Pyle (GP)[14], disregarding the carpals [15].

EXISTING HEIGHT PREDICTION METHODS

1. '*Projected Adult Height*' (PAH). This method assumes that the SDS of FH is equal to the SDS of H for CA ($HSDS_{CA}$) of girls with TS at any moment before FH is reached.

2. '*Modified Projected Adult Height*' (mPAH). Lyon *et al* [16] described a modification to the PAH method, using linear regression analysis on growth data of untreated Turner girls for the relationship between FHSDS and $HSDS_{CA}$, thus FHSDS= a + b*$HSDS_{CA}$. In the present study the Dutch TS data were used to construct a similar equation (N=57).

3. '*Index of Potential Height*' (IPH) [17]. This method assumes that the FHSDS in TS is equal to the HSDS for BA ($HSDS_{BA}$) rather than the HSDS for CA. IPH was calculated using both RUS and GP BA methods, IPH_{RUS} and IPH_{GP}, respectively.

Table 1. Demographic data of 57 women with Turner syndrome with 235 measurement points.

	N	mean	SD
$HSDS_{CA}$ (DSD)	235	0.4	0.94
CA-EEstart (yrs)	49	15.2	1.8
duration EE-XH (yrs)	88	2.0	1.33
CA at FH (yrs)	57	23.4	4.8
FH (cm)	57	147.4	5.6
$FHSDS_{CA}$ (DSD)	57	0.07	0.81
birth H (cm)	21	47.7	2.4
birth W (kg)	46	2.85	0.60
H father (cm)	42	176.0	6.9
H mother (cm)	45	164.3	6.5
TH (cm)	42	167.4	4.8
TH-deficit (cm)	42	-19.9	5.4

N: number of measurement points or women with TS
CA: chronological age
$HSDS_{CA}$ (DSD): height SD-score for CA (Dutch-Swedish-Danish TS references)
$FHSDS_{CA}$ (DSD): final height SD-score for CA (DSD TS references)
CA-EEstart: age at start of estrogen treatment
duration EE-XH: time elapsed between start of estrogen treatment and X-ray date
FH: final height
H: height
W: weight
TH: target height (see Methods-section)
TH-deficit: FH minus TH

4. '*Modified Index of Potential Height*' (mIPH). Lenko *et al* [6,18] developed a modification of IPH in which first CA is expressed as a function of BA in TS and, subsequently, this calculated CA value is used in the PAH prediction method. Again, either the RUS ($mIPH_{RUS}$) or the GP ($mIPH_{GP}$) BA method was applied. A further modification was made by Naeraa *et al* using the mPAH method for the calculated CA instead of the PAH method [8,19].

5. '*Bayley-Pinneau*' (BP) method [20]. In this method the percentage of FH achieved at an observed GP BA is read from a table constructed on the basis of growth data of healthy children and used to calculate FH. Three tables - average, advanced, and retarded - correct for possible differences between CA and BA of more than one year.

6. *'BA corrected SDS'* (cSDS). Price *et al* [21] developed a method in which the BA SDS was subtracted from the $HSDS_{CA}$ using Turner standards developed by Ranke. For each CA class of one year means and SD's of GP skeletal ages were calculated. Since Dutch Turner GP standards were not available, the RUS BA was used [13] in combination with $HSDS_{CA}$, using Dutch-Swedish-Danish TS standards [3].

7. *'Tanner-Whitehouse, Mark 2'* (TW) method [10]. FH is calculated using regression coefficients for each age group, based, as in BP, on healthy children. Although TW provides models with various coefficients, in this study only the pre-menarcheal 3-variate equations have been used, for height, CA, and RUS, respectively.

NEW HEIGHT PREDICTION METHODS

8. *'Turner-specific prediction method'* (PTS). This method was designed to be analogous to the method of TW [10], using the data of Dutch Turner girls. For successive CA classes of one year multiple linear regressions were calculated estimating FH given H, CA, and RUS or GP BA. In each regression analysis measurement points of the 20 different girls whose CA was closest to the integer CA were used. Due to a relative lack of data, analyses were performed from the CA of 6 years. Subsequently, for each predictor variable (H, CA, and BA) the regression coefficients were smoothed using the Kernel smoothing method of S-plus. A FH prediction according to PTS can be calculated as follows:

$$FH\ (\text{in cm}) = (a*H) + (b*CA) + (c*BA) + \text{constant},$$

where a, b, and c are the regression coefficients corresponding to the CA of the girl at prediction listed in Table 2. To investigate the performance of this new method on independent data, PTS_{RUS} and PTS_{GP} were calculated using the Danish Turner data.

STATISTICAL ANALYSIS

For the comparison of the various prediction methods only one measurement point of a Turner girl per CA-class of one year was allowed. The prediction error was calculated as predicted minus observed FH (in cm). The variability in the prediction errors was characterized by the standard deviation of the errors (ESD). Since mean prediction error and ESD depend strongly on age, data were analyzed in 3-year age groups. As well as single FH prediction methods, combinations were studied by taking the average: 1/2*(method 1 + method 2). The Kruskal-Wallis or X^2 test was used to test differences between groups. Correlations were tested with the non-parametric Spearman's rank correlation test. A P-value less than 0.05 was considered significant.

Table 2. Smoothed regression coefficients of PTS_{RUS} and PTS_{GP} for height, chronological age (CA), bone age (RUS and GP, respectively), and the constant (c) per yearly CA-class.

	PTS_{RUS}				PTS_{GP}			
CA (yr)	height (cm)	CA (yr)	RUS (yr)	c (cm)	height (cm)	CA (yr)	GP (yr)	c (cm)
6	1.16	-1.80	-3.25	52.74	1.20	-3.30	-2.07	49.47
7	1.18	-1.54	-3.28	49.18	1.27	-2.84	-2.51	40.63
8	1.18	-1.40	-3.18	46.34	1.32	-2.34	-2.87	32.90
9	1.16	-1.41	-2.84	46.14	1.33	-1.91	-3.01	28.98
10	1.08	-1.26	-2.17	47.35	1.26	-1.49	-2.81	30.85
11	0.97	-0.91	-1.33	48.63	1.12	-1.23	-2.20	39.35
12	0.98	-1.12	-0.87	55.43	1.01	-1.44	-1.75	51.69
13	0.89	-1.70	-1.07	64.51	0.96	-1.75	-1.69	59.04
14	0.94	-1.56	-1.50	58.62	0.99	-1.27	-1.80	49.24
15	1.01	-1.06	-1.82	44.73	1.07	-0.53	-2.12	31.28
16	1.04	-0.78	-1.91	37.90	1.13	-0.13	-2.30	18.60
17	1.01	-0.38	-1.83	33.73	1.09	0.31	-1.91	10.94
18	0.99	-0.09	-1.75	30.58	1.03	0.48	-1.41	8.95
19	0.99	-0.18	-1.66	31.32	1.01	0.17	-1.12	13.60

RESULTS

Table 2 lists the smoothed coefficients for height, CA, and BA of PTS_{RUS} and PTS_{GP} on the Dutch data. The FH prediction for a girl with TS, for example with height 117.1 cm, CA 9.3 years, BA RUS 8.8 years, and BA GP 8.5 years, can be calculated using the coefficients of her CA class, as follows:

PTS_{RUS}: (1.161*117.1) + (-1.408*9.3) + (-2.838*8.8) + 46.14 = 144.0 cm
PTS_{GP}: (1.330*117.1) + (-1.908*9.3) + (-3.013*8.5) + 28.98 = 141.4 cm

For the calculation of the modified PAH, the relationship between FHSDS and $HSDS_{CA}$ was used. FHSDS was estimated on the Dutch data by: -0.2 + 0.836 * $HSDS_{CA}$. For the calculation of the modification of IPH, CA was estimated from BA (N=254) as follows:

CA (RUS)= 1.428 + 0.714 * RUS + 0.0233*RUS^2
CA (GP)= -0.659 + 1.562 * GP - 0.0220*GP^2

Subsequently, these calculated CA values were used in the mPAH prediction method.

The relationship between FHSDS and the fictive '$HSDS_{CA}$', used for the calculation of the modified IPH, was estimated by:

$mIPH_{RUS}$: 0.02 + 0.83 * '$HSDS_{CA}$'
$mIPH_{GP}$: 0.02 + 0.70 * '$HSDS_{CA}$'

Table 3 shows the mean error and the ESD of all prediction methods, including PTS_{RUS} and PTS_{GP}, on the Danish data per age-class of 3 years. Since the mean error and/or ESD of some methods was too high in several age classes compared with other methods, Figures 1A and 1B illustrate the best selected prediction methods. The mean prediction errors of both PTS_{RUS} and PTS_{GP} were small and similar, except for CA 15-18 years (see Figure 1A). The ESD curves of the PTS methods were similar and low in comparison with the other methods, in particular at the 'younger' ages (see Figure 1B).

Table 4 and Figures 2A and 2B represent the mean error and the ESD of the various single-variate prediction methods on the Dutch TS data per age-class of 3 years, respectively. The IPH_{RUS}, IPH_{GP}, and cSDS methods have been left out of the figures, because their mean error and/or ESD was very high in several age-classes compared with other methods

Table 3. Comparison of the mean error (ESD) of all FH prediction methods, including PTS_{RUS} and PTS_{GP}, on Danish Turner data.

	12-15 yr N=13	15-18 yr N=17	≥18 yr N=6
PTS_{RUS}	0.6 (2.9)	-1.8 (2.2)	1.6 (2.0)
PTS_{GP}	-0.5 (3.3)	1.0 (2.1)	0.7 (1.7)
PAH	0.4 (3.3)	0.7 (2.1)	-0.8 (1.4)
mPAH	-0.5 (3.4)	-0.3 (2.3)	-2.0 (2.0)
IPH_{RUS}	6.1 (2.9)	5.9 (2.2)	6.1 (2.2)
IPH_{GP}	10.4 (4.7)	12.1 (2.1)	11.7 (2.7)
$mIPH_{RUS}$	1.0 (3.1)	-0.4 (2.0)	-0.8 (1.6)
$mIPH_{GP}$	0.3 (3.3)	1.4 (2.4)	0.7 (2.2)
BP	2.5 (5.6)	3.4 (2.5)	2.9 (3.6)
TW	2.1 (3.2)	0.3 (2.1)	-1.1 (2.4)
cSDS	9.3 (5.4)	2.4 (3.5)	0.3 (4.8)

N: number of women with TS
mean error: predicted minus observed FH (cm)
ESD: standard deviation of the prediction error (cm)
for abbreviations of the prediction methods see Methods-section

(see Table 4). The mean error and ESD of PTS_{RUS} and PTS_{GP} were (among) the lowest in any age-group.

There was an overall tendency to overpredict FH, indicated by positive mean errors of the age-classes (see Figure 2A). A tendency to underpredict FH was only found in the age class of 9-12 years for mPAH, $mIPH_{RUS}$, and $mIPH_{GP}$. Before the CA of 9 years, the mean prediction errors of the BP and TW methods were markedly higher compared with the other four methods (Figure 2A). Overall, the smallest mean prediction errors were observed using mPAH. The PAH method parallels the mPAH method but showed mean prediction errors about 1-2 cm higher at any CA. The two mIPH methods also showed an evident similarity; using the modification for IPH resulted in lower values than the unmodified method, for both the RUS and GP BA. The cSDS method overpredicted FH, at younger ages more so than at older ages (see Table 4).

The ESD's in Figure 2B show an evident downward trend with CA; from 5 cm around 9 years of age to 1-2 cm for most methods around the age of 20. There was hardly any difference between the six methods from the CA of 9 years onwards. At closer inspection, the BP method showed a lower accuracy compared with the other methods, indicated by higher ESD's between 9 and 18 years of age. The ESD curves of the two mIPH methods were very similar after the age of 9 years. The ESD's of the PAH and mPAH methods were almost identical, with very good accuracy from 9 years onwards.

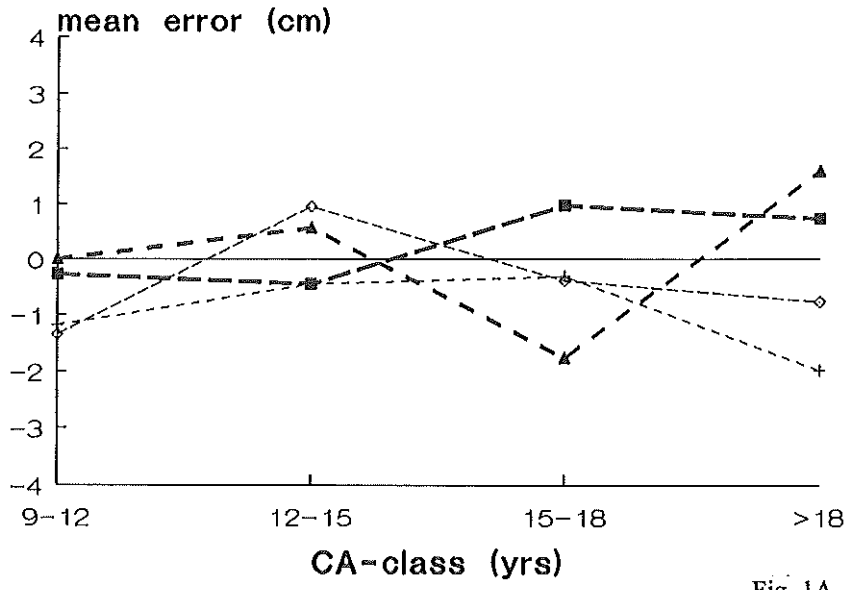

Fig. 1A

Figure 1. Mean error (A) and standard deviation ESD (B) in 3-yearly chronological age classes for PTS_{RUS} (▬ ▲▬), PTS_{GP} (▬ ▓▬), mPAH (– –+–), and $mIPH_{RUS}$(– –◇–) on Danish Turner data.
For abbreviations see Methods-section.

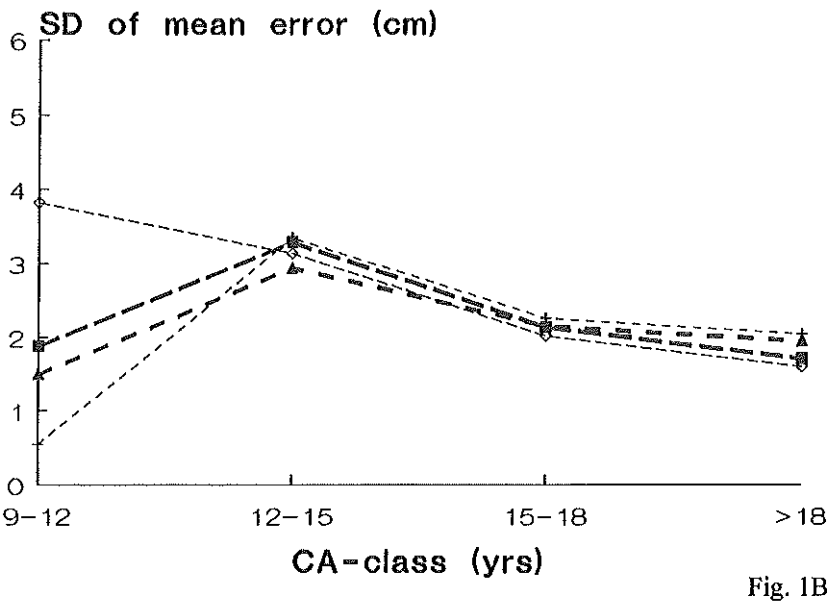

Fig. 1B

Table 4. Mean error (ESD) of all single-variate prediction methods on the Dutch data.

	3-6 yr N=7	6-9 yr N=18	9-12 yr N=36	12-15 yr N=67	15-18 yr N=79	≥ 18 yr N=25
PTS_{RUS}	-0.9 (6.4)	0.4 (4.2)	-0.5 (3.3)	1.0 (2.9)	0.4 (1.6)	1.7 (1.7)
PTS_{GP}	-0.7 (7.5)	0.4 (5.1)	-0.1 (3.1)	0.7 (2.7)	1.2 (1.8)	0.9 (1.4)
PAH	2.9 (7.1)	1.3 (5.7)	-0.4 (3.4)	1.6 (3.1)	2.7 (2.2)	0.7 (1.6)
mPAH	1.2 (7.0)	0.2 (5.6)	-1.8 (3.2)	0.1 (3.1)	1.0 (2.2)	-0.4 (1.9)
IPH_{RUS}	9.0 (4.0)	5.1 (5.0)	1.9 (4.1)	4.9 (3.6)	7.7 (1.9)	8.1 (1.6)
IPH_{GP}	15.8 (4.6)	11.0 (6.4)	8.0 (4.1)	10.7 (3.4)	10.6 (2.6)	9.9 (2.9)
$mIPH_{RUS}$	0.3 (5.1)	1.3 (4.8)	-2.1 (4.1)	-1.0 (3.6)	0.8 (2.1)	1.9 (1.4)
$mIPH_{GP}$	4.0 (4.0)	-0.1 (5.9)	-3.2 (3.9)	-0.6 (3.4)	0.7 (2.5)	2.0 (2.1)
BP		6.1 (5.9)	1.8 (4.7)	2.6 (3.8)	1.8 (2.5)	1.5 (2.0)
TW	10.8 (7.2)	9.2 (4.8)	3.6 (3.9)	1.0 (3.4)	1.5 (2.6)	0.9 (2.7)
cSDS	2.9 (3.5)	3.4 (4.6)	2.0 (5.3)	2.0 (5.2)	1.4 (4.7)	0.8 (4.9)

N: number of women with TS
mean error: predicted minus observed FH (cm)
ESD: standard deviation of the prediction error (cm)
for abbreviations of the prediction methods see Methods-section

Due to the lower GP BA limit of 6 years of the BP prediction method, the number of measurement points in the age-class 6-9 years was reduced for BP to 10.

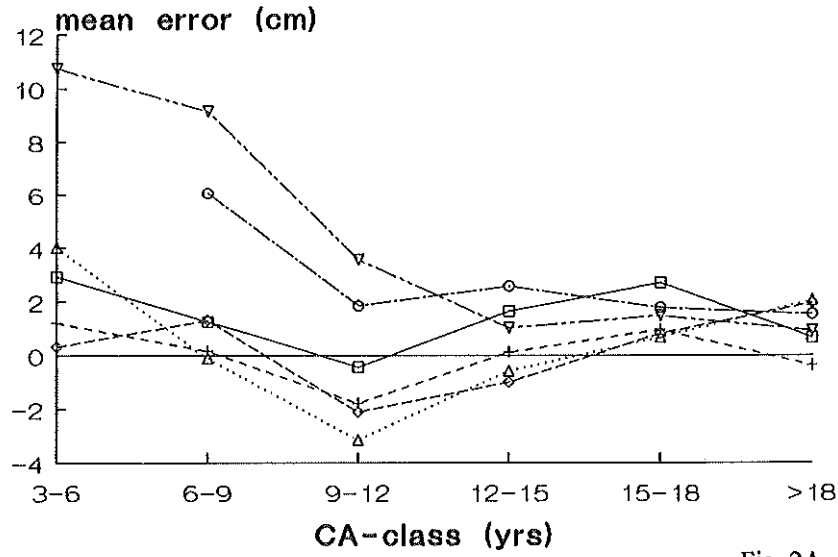

Fig. 2A

Figure 2. Mean error (A) and standard deviation ESD (B) in 3-yearly chronological age classes for various single final height prediction methods on Dutch Turner data; PAH (–□–), mPAH (– –+– –), $mIPH_{RUS}$ (– –◇– –), $mIPH_{GP}$ (···△···), BP (–○–), TW (– ▽–).
For abbreviations see Methods-section.

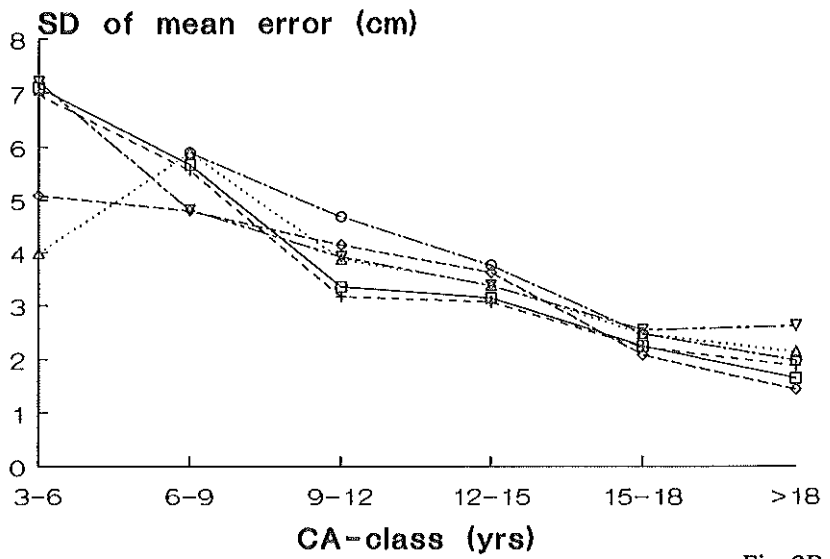

Fig. 2B

Table 5. Mean error (ESD) of all combined prediction methods on Dutch data.

combinations with mPAH	3-6 yr N=7	6-9 yr N=18	9-12 yr N=36	12-15 yr N=67	15-18 yr N=79	≥18 yr N=25
IPH_{RUS}	5.1 (3.9)	2.6 (4.4)	0.0 (3.3)	2.5 (3.1)	4.3 (1.7)	3.9 (1.3)
IPH_{GP}	8.5 (5.4)	5.6 (5.3)	3.1 (3.1)	5.4 (2.7)	5.8 (1.7)	4.7 (1.6)
$mIPH_{RUS}$	0.9 (5.7)	0.7 (4.4)	-2.0 (3.3)	-0.4 (3.1)	0.9 (1.9)	0.8 (1.6)
$mIPH_{GP}$	2.6 (4.0)	0.0 (5.3)	-2.5 (3.2)	-0.2 (3.0)	0.8 (2.1)	0.8 (1.9)
BP		3.8 (5.1)	0.0 (3.2)	1.3 (2.8)	1.4 (1.7)	0.6 (1.4)
TW	6.0 (6.9)	4.7 (5.1)	0.9 (3.2)	0.6 (2.9)	1.2 (1.9)	0.3 (1.6)

N: number of women with TS
mean error: predicted minus observed FH (cm)
ESD: standard deviation of the prediction error (cm)
for abbreviations of the prediction methods see Methods-section

Due to the lower GP BA limit of 6 years for the BP prediction method, the number of measurement points in the age-class 6-9 years was reduced for BP to 10.

Table 5 represents the mean error and the ESD of mPAH combined with the BA dependent IPH_{RUS}, IPH_{GP}, $mIPH_{RUS}$, $mIPH_{GP}$, BP, and TW methods on the Dutch data per age-class of 3 years.

The mPAH combinations resulted in a decrease of the mean prediction error of 0.5-1 cm in most age groups compared with the single-variate prediction methods; the ESD's of the combined methods were about 1 cm lower than the single prediction methods and almost identical after the CA of 9 years. The gain in accuracy of the combinations was most pronounced between 9 and 18 years. When the non-modified PAH was used for the combinations the mean prediction errors were 0.5-1 cm higher in all age groups compared with the mPAH combinations. All mean ESD values were identical (data not shown). The mPAH combinations with $mIPH_{RUS}$ and $mIPH_{GP}$ showed overall the smallest mean prediction errors. From the CA of 9 years mPAH combinations with BP and TW were also very good. The mean errors of combinations of the non-modified IPH methods with mPAH were still not acceptable in most age groups.

The correlation between FH and the MPH was significant (N=42; r=0.45; P=0.003). The mean difference between FH and MPH was 19.9 cm. There was a small, but significant correlation of FH with the year of birth (beta of the slope 0.207; r=0.38; P=0.004). FH was not significantly different between girls with (N=5) and without (N=52) spontaneous menarche (P=0.33). Also, FH was not significantly different between girls without (N=8) and with estrogen exposition (N=49) due to own production or estrogen treatment (P=0.49).

DISCUSSION

We present a new Turner-specific FH prediction method based on regression analysis. Since Danish Turner women are known to have a similar growth pattern and FH compared with the Dutch Turner women [3,19,22], the validity of the coefficients of PTS_{RUS} and PTS_{GP} (Table 2) was tested on Danish Turner women. The mean prediction errors and ESD values of all prediction methods on the Danish data (Table 3) were in good agreement with the Dutch values at 12 years and older (Table 4). As expected, the latter mean errors of the PTS_{RUS} and PTS_{GP} were in all age-groups very small, since the methods were developed on the same dataset. Still, the ESD's were in any age-group (among) the lowest. Figures 1A and 1B (on the Danish data) show that both PTS methods resulted in small mean prediction errors, comparable to mPAH and $mIPH_{RUS}$. The ESD curves of the PTS methods were similar and the accuracy proved to be among the highest, in particular at the 'younger' ages (Figure 1B). Although the youngest CA class given for the PTS methods is 6 years, we had no data available to test the new prediction method at ages below 9 years. Still, at

ages below 6 years the coefficients of the 6-year CA-class might be used.

Since the reported differences in FH between populations are small and the growth pattern as well as BA maturation are similar [2,3,13], it can reasonably be assumed that the PTS methods will be applicable in other populations. In the present study a significant correlation was found between FH and TH, suggesting that when differences between MPH's of various populations become too large, the PTS methods might need systematic correction. Incidentally, earlier findings of a 20 cm deficit between the FH and MPH of Turner women [19,23-25] were confirmed in this study. Whereas other investigators did not not find a secular trend [26], a significant, positive correlation was found between FH and year of birth, confirming a secular trend in the general Dutch female population of +2 cm per decade [9,27], but also in TS of +1.5 cm [28]. If desired, a correction for girls with TS can be performed, based on our data, of 2.1 cm per decade.

In this study FH was not significantly different between women with and without spontaneous menarche, nor between girls with or without estrogen exposition. Although most women in this study received estrogens, the start of treatment occurred at a rather late mean CA of 15.2 years. Estrogen treatment does not lead to a pubertal growth spurt as described for ordinary girls [2]. A minor increase in height velocity without BA acceleration and consequently a temporary improvement in predicted FH is possible in our girls, but should be comparable between the various prediction methods.

Since our historical sample included women with spontaneous puberty as well as with estrogen treatment, the PTS methods should be applicable in any contemporary Turner girl for whom growth promoting treatment in addition to induction of puberty is considered. When growth promoting therapy is applied the effects on growth should thus solely reflect that therapy and should be independent of possible pubertal effects.

OVERPREDICTION

Most single variable prediction methods, except for PTS, showed a general tendency towards overprediction on the Dutch Turner data. The overprediction of BP and TW before the age of 9 years is, as expected, large. Both BP and TW methods were developed from data on healthy children, including prediction of a pubertal growth spurt. The overprediction of FH for BP from the age of 9 years onwards is in agreement with earlier studies [4,5,8]. The ESD's in this study were somewhat lower than previously reported [8]. There are also drawbacks in using the BP method. First, it can only be used after a GP BA of 6 years. Second, the separate tables for girls with a GP BA retardation of more than one year may lead to marked differences in the FH prediction; e.g. when the GP BA retardation changes from just over to just under one year.

Since in 28% of the girls only one measurement point was available, in this study only the premenarcheal 3-variate equations of the TW method

have been used, for height, CA, and RUS, respectively. The postmenarcheal and the higher variate equations improve FH prediction because an allowance is made for the pubertal growth spurt. But, even if untreated Turner girls experienced a pubertal growth spurt [23,29] it is much smaller than that of ordinary girls and does not result in a FH significantly different from Turner girls without spontaneous puberty [2,23,25,30]. The marked decrease in FH overprediction with CA using the TW premenarcheal tables (see Figure 2A) is in good agreement with earlier findings [5,8], and should be kept in mind when a girl is monitored longitudinally. The use of postmenarcheal tables after the age of 14.5 years by Naeraa *et al* resulted in a marked underprediction [8].

Modification of PAH

The modification of the PAH method was first suggested by Lyon *et al* [16]. They found a higher correlation coefficient between H-SDSca and FH-SDSca (r=0.95) than did Naeraa or the present study (r=0.8). In our study, the slope of the regression line between these two variables was not as steep (beta: 0.84) as that reported by Lyon (beta: 1.13). As a result, the range of the mPAH predictions is lower using the modification of PAH from the present study.

The PAH and its modified method show similar patterns, both for mean prediction error and ESD, as was reported earlier [8]. We prefer the use of the modified PAH because the mean error values were smaller at all ages, except for 9-12 years. In fact, the smallest mean prediction errors at most ages were observed using mPAH, with a very good accuracy from the age of 9 years onwards.

Modification of HSDS for BA

Although BA determinations are known to have a considerable measurement error [13], the retardation for BA progresses with CA in a similar manner to that of standing height [1,2,13]. Thus, it was surprising that the IPH methods showed a progressive mean overprediction error at older ages. Apparently, the compensation of the BA retardation made for the lack of a pubertal growth spurt is too large. Applying the modified IPH_{GP} method Joss *et al* [4] gave a mean FH underprediction of 1.2 cm. Although the age distribution of the untreated controls at the moments of FH prediction is unclear, their findings are in agreement with those reported in our 12-15 year age-class and by Naeraa *et al* [8]. However, our ESD values are in conformity with those of Naeraa and higher than the 1.9 cm reported by Joss. In general, mean prediction errors for both mIPH methods in this study were somewhat lower compared with those reported by Naeraa.

CHAPTER 3

COMBINATIONS OF FH PREDICTION METHODS

Naeraa *et al* found that combining prediction methods with and without allowance for BA resulted in a considerable improvement in accuracy (ESD) when compared with the accuracy of single methods [8]. The mPAH method was chosen to be combined with the BA dependent prediction methods, because overall it showed the best results. Compared with the single variate prediction methods, a marked decrease in mean prediction error was only found for those with the highest overprediction. Naeraa *et al* suggested that the improvements in accuracy were most pronounced at ages below 16 years [8]. In agreement, the gain in accuracy in the present study was most pronounced between 9 and 18 years. It must be noted that their 'youngest' girls were aged 9, whereas 6-year-old girls were included in the present study. Only the 6-9 year group did not show a marked gain in accuracy. In this context it should be mentioned, that a gain in accuracy (ESD) of 1 cm results in a reduction of about 2 cm in both the upper and lower 95% confidence limit of the prediction and thus the range of the FH prediction is reduced by 4 cm. The over- or underprediction of a girls' FH can be corrected by the mean prediction error in an age-class. The mPAH combinations with $mIPH_{RUS}$ and $mIPH_{GP}$ showed overall the smallest mean prediction errors. However, below 6 years of age the accuracy of mPAH with the non-modified IPH_{RUS} method is better. From the CA of 9 years mPAH combinations with BP and TW were also very good.

CONCLUSIONS

The new, Turner-specific, FH prediction methods, PTS_{RUS} and PTS_{GP}, both gave small mean prediction errors and a high accuracy. The validity at younger ages remains to be tested. The PTS methods are a valuable addition to other methods in determining FH prediction in girls with TS and in deciding whether or not growth promoting treatment is indicated, or in evaluating its effects.

Of the single-variate FH prediction methods, the smallest mean prediction errors at most ages were observed using the modified PAH, with a good accuracy from the age of 9 years onwards. Averaging mPAH with methods allowing for BA increased the accuracy of the more inaccurate method substantially. Thus, if population specific Turner reference data are available, a number of calculations (with possible errors) can result in a smaller mean prediction error and a higher accuracy. On the other hand, the simplest methods, the mPAH and PAH, were remarkably good at most ages.

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APPENDIX

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Chapter 4

Yearly stepwise increments of the growth hormone dose results in a better growth response after four years in girls with Turner syndrome

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YEARLY STEPWISE INCREMENTS OF THE GROWTH HORMONE DOSE RESULTS IN A BETTER GROWTH RESPONSE AFTER FOUR YEARS IN GIRLS WITH TURNER SYNDROME

ABSTRACT

To optimize growth promoting effect of growth hormone (GH), 65 previously untreated girls with Turner syndrome (TS), chronological age (CA) 2-11 years, were randomized into 3 dosage regimen groups A, B, and C, with a daily r-hGH dose during 4 study years of 4-4-4-4, 4-6-6-6, and 4-6-8-8 IU/m^2 b.s., respectively.

The first GH dosage increase in groups B and C resulted in a significantly higher mean height velocity (HV) compared with constant dose group A. During the third year, when only in group C the dose was raised again, mean HV was significantly higher in groups B and C than in group A, and in group C compared with group B. In year 4 only group C mean HV remained significantly higher than group A. The pattern of change in $HSDS_{CA}$ (Dutch-Swedish-Danish Turner references) was identical, however in year 4 mean $\Delta HSDS_{CA}$ in group B also remained significantly higher than group A. After 4 years GH treatment: [1] the mean $\Delta HSDS_{CA}$ was significantly higher for groups B and C compared with group A, but not significantly different between groups B and C. Although significantly higher compared with estimated values for untreated Dutch girls with TS, [2] bone maturation of the GH treated girls was not significantly different between groups and [3] it was positively related with the degree of bone age (BA) retardation at start of study and negatively with baseline CA. [4] Both the modified Index of Potential Height ($mIPH_{RUS}$) and a recently developed Turner-specific final height (FH) prediction method (PTS_{RUS}), based on regression coefficients for H, CA, and BA, showed significant increases in mean FH prediction, without significant differences between groups. PTS_{RUS} values were markedly higher than the $mIPH_{RUS}$ values.

Dose-dependency could be shown for the area under the curve (AUC) for GH, but $\Delta HSDS_{CA}$ was not linearly related with AUC. Baseline GH binding protein (BP) levels were in 84% of the within the normal age range; the decrease in mean levels after 6 months GH was not significant. Mean IGF-I and IGFBP-3 plasma levels increased significantly, without significant differences between groups. $\Delta HSDS_{CA}$ during GH was dependent on IGF-I plasma levels at baseline and during the study period, beta's -0.002 and 0.0004. Thus, a stepwise GH-dosing approach reduced the 'waning'-effect of the growth response after 4 years treatment without undue bone maturation. FH prediction was not significantly different between treatment groups. Irrespective of the GH dose used, initiation of GH treatment at a younger age is beneficial after 4 years

GH when expressed as actual cm gained or as gain in FH prediction, but not statistically significant when expressed as $\Delta HSDS_{CA}$ over the study period.

INTRODUCTION

Stunted growth is an almost invariable hallmark of girls with Turner syndrome (TS). Although these girls are not clearly GH-deficient (GHD) [1-4], GH therapy results in a marked increase of height velocity (HV) [5-7]. Studies in GHD patients [8-10] and in TS [5-7,11-13] have shown that the growth response to GH treatment is dependent on the dose and frequency of administration. Frasier *et al.* [14] reported in a one year, parallel study in prepubertal GHD children that doubling the GH dose of 30 mU/kg thrice weekly intramuscularly resulted only in a 1.3-fold increase of the first year HV. Also in TS daily GH injections were shown to be more effective than the same weekly dosage in two or three weekly injections [12]. In the past years, the most commonly used GH dosages in TS varied from 2 to 4 IU/m^2 body surface/day subcutaneously [5-7,12,15], where 4 IU/m^2 is equivalent to 0.045 mg/kg. In studies using GH dosages only up to 4 IU/m^2/day the growth rate outweighed the accelerated bone maturation [13,16], and thus, final height (FH) prediction improved more than with lower GH doses [5,7,12,15].

An earlier GH treatment study of girls with TS in the Netherlands [16] showed a doubling of the HV in the first year of treatment with 4 IU GH/m^2/day compared with pretreatment values. However, this increase could not be maintained during the subsequent years of treatment. This so-called 'waning'-effect has also been reported by others [10,17]. In GHD patients, a similar effect is observed, which can be overcome by a two to threefold increase of the GH dose [10,18].

Furthermore, studies in GHD patients have demonstrated the importance of early diagnosis and therapy. GH treatment prevented further loss of stature but could not make up the deficit at diagnosis [19]. The previous Dutch Turner studies confirmed that the growth response in younger girls was better than in older girls [12,13,16].

In contrast to the logarithmic relationship between GH dose, given thrice weekly intramuscularly, and HV in GHD children, the effect of GH on IGF-I plasma levels has been shown linear between 0 and 3 IU GH/m^2/day in GHD adults [20]. In the present study in TS this concept is extended to the 4-8 IU/m^2/day GH range.

In order to optimize GH treatment in TS, we investigated whether [1] a yearly stepwise increment of the GH dose could maintain or augment the initial increase in HV and (thereby) improve FH prediction. In addition, we investigated whether [2] treatment from a young age onwards could improve FH prediction. Moreover, in a subgroup of 12 girls the GH-insulin-like growth factor (IGF)-I axis was studied in detail under GH treatment.

SUBJECTS AND METHODS

STUDY GROUP

Sixty-eight previously untreated girls with TS were enrolled in a 4 year, multicenter GH dose-response study. The diagnosis was confirmed by lymphocyte chromosomal analysis. Clinical data and karyotype of the girls are listed in Table 1. Inclusion criteria were a chronological age (CA) between 2 and 11 years, height below the 50th percentile for Dutch children [21], and a normal thyroid function. Exclusion criteria were: associated endocrine and/or metabolic disorders, growth failure due to other disorders or emotional deprivation, hydrocephalus, previous use of drugs that may interfere with GH therapy, and Tanner puberty stage ≥ B2 [22]. No provision with regard to the baseline GH stimulation tests was made.

STUDY DESIGN

The girls were randomized into three GH dosing groups with stratification according to CA and height standard deviation score ($HSDS_{CA}$):

- **A** (n=23) 4 IU/m^2 body surface (equivalent to 0.045 mg/kg)/day for 4 years,
- **B** (n=23) 4 IU/m^2 in the first year, followed by 6 IU/m^2/day during the second through fourth year,
- **C** (n=22) 4 IU/m^2 in the first year, 6 IU/m^2 in the second year, and 8 IU/m^2/day during the third and fourth year.

Biosynthetic (B)-hGH (Norditropin^R) was given subcutaneously at bedtime by means of a pen injection system (Nordiject^R 24). None of the girls received estrogens during the 4 year study period. Written, informed consent was obtained from the parents or custodians of each child. The study protocol was approved by the Ethics Committee of each participating centre.

GROWTH EVALUATION

Height measurements were determined at baseline and three monthly by one investigator (AT) according to Cameron [23], using a Harpenden stadiometer. Height was expressed as SD-score for CA ($HSDS_{CA}$, $HVSDS_{CA}$) using the Dutch-Swedish-Danish (DSD) Turner data [24], or reference data of normal Dutch girls[21]. The gain in height for untreated girls with TS was estimated from the equations of the DSD Turner data [24], in which the height of an average girl with TS is indicated by her CA.

Table 1. Baseline data for each treatment group.

	Group A	Group B	Group C
Number of girls	22	22	21
CA (yr)	6.1 (2.1)	6.7 (2.4)	6.5 (2.4)
RUS BA (yr)	5.5 (2.2)	6.0 (2.5)	5.8 (2.4)
$HSDS_{CA}$ (RvW)	-2.7 (0.9)	-2.4 (1.0)	-2.6 (1.0)
$HSDS_{CA}$ (DSD)	0.06 (1.03)	0.42 (1.05)	0.18 (1.06)
$HVSDS_{CA}$ (DSD)	0.32 (0.80)	0.08 (0.91)	0.16 (0.71)
$mIPH_{RUS}$ (cm)	147.8 (7.7)	148.0 (4.8)	147.4 (5.5)
PTS_{RUS} (cm)	145.6 (5.9)	147.6 (5.2)	146.3 (5.0)
MPH (cm)	169.4 (5.9)	170.6 (6.0)	169.7 (5.7)
BMI-SDS	0.24 (1.20)	0.27 (1.33)	0.20 (1.29)
Karyotype: 45,X	18 (82%)	21 (96%)	16 (76%)
Karyotype: other	4 (18%)	1 (4%)	5 (24%)
maxGH (ATT) mU/L	23.5 (16.6)	20.4 (15.2)	25.6 (16.4)

Values are given as mean (SD)

CA:	chronological age
RUS BA:	RUS-bone age
SDS:	standard deviation score
H:	height
HV:	height velocity
RvW:	Dutch reference standard for girls
DSD:	Dutch-Swedish-Danish Turner references
$mIPH_{RUS}$:	modified Index of Potential Height
PTS_{RUS}:	Turner-specific final height prediction using RUS bone age
MPH:	midparental height
BMI:	body mass index-SDS
maxGH (ATT):	maximum GH plasma concentration after Arginine stimulation

Midparental height (MPH) was adapted for Dutch reference data [21] with the addition of 3 cm for secular trend: $MPH=1/2*(H_{mother} + H_{father} -12) + 3$ cm. The degree of obesity was expressed as body mass index (BMI) SDS [25]. Bone age (BA) was determined by one investigator (AT) according to Tanner & Whitehouse RUS-score [26]. Bone maturation ($\Delta BA/\Delta CA$) was compared with estimated values from the equations of untreated Dutch Turner girls [27]; in these equations the BA of an average girl with TS is indicated by her CA. FH prediction was estimated using the modified Index of Potential Height ($mIPH_{RUS}$) method [28,29] and a recently developed Turner-specific method (PTS_{RUS}) [29]. Both methods comprise Dutch Turner references. Analoguous to the Tanner and Whitehouse mark 2 FH prediction method for normal children, the PTS method gives smoothed regression coefficients for H, CA, and BA.

BIOCHEMICAL PARAMETERS

At baseline all girls underwent a GH provocation test. Arginine 0.5 g/kg body weight was infused in 30 min. Blood samples were drawn at 15-min intervals from -15 to +60 min and every 30 min during the second hour.

At baseline, six months after initiation of GH, and six months after each GH dosage increase, a 24-hour GH profile was performed in a subgroup of 12 girls of group C. Starting at 8.30 am, blood was withdrawn from an indwelling venous catheter with a heparin lock. Blood was collected every 20 minutes for GH measurement and at the start a single additional sample was obtained for measurement of IGF-I and IGFBP-3. The girls kept normal diets served at hospital mealtimes and kept normal activity and sleeping habits. GH was injected before going to bed. At the above study time points blood was collected from all girls for the determination of IGF-I and IGFBP-3, and at 42 and 48 months only for IGF-I. GH binding protein (GHBP) was determined at baseline and six months after initiation of GH therapy. All blood samples were stored on ice for no more than 3 hours until centrifugation. The plasma samples were frozen (-20° C) until assayed.

HORMONE ASSAYS

The RIA measurements of plasma GH, IGF-I and IGFBP-3 were performed as described previously [30-32]. The 95th percentile for peak pubertal levels in a normal female population for IGF-I and IGFBP-3 are 700 µg/L and 5 mg/L, respectively. Plasma GH binding protein (GHBP) was determined by LIFA [33,34]. All measurements were performed in the same laboratories.

STATISTICAL ANALYSES

Results are expressed as mean (SD), unless indicated otherwise. Differences between groups were tested by Student's *t*-tests or a oneway analysis of variance (followed by the Student-Newman-Keuls test for multiple comparisons between groups at the P=0.05 level). Differences between points in time were tested by paired Student's t-tests. The Kruskal-Wallis test was used to test for differences between stimulated maximum GH levels and Tanner breast-stage groups, the Chi-square test for differences between karyotype groups (45,X and others). Correlations were tested with Pearson's linear correlation coefficient. For this purpose, IGF-I and IGFBP-3 plasma levels were transformed into log-values. To study the relation between growth response variables (the change in $HSDS_{CA}$, HVSDS, bone maturation, or PTS_{RUS}) and growth parameters measured at baseline (CA, BA, BA retardation (=CA-BA), HV, maximal GH peak after stimulation, SD scores for BMI, H, and HV), adjusted for the dose regimen (i.e. group), multiple linear regression (MLR) analyses were used. Statistical procedures were performed using the SPSS/PC+ program version 4.0 (SPSS Inc, 1990). A repeated measures analysis of variance model (adjusted for dose-increment steps and duration of treatment) was used to determine the influence of baseline IGF-I levels on those during GH therapy and of IGF-I levels (at baseline and during GH therapy) on $HSDS_{CA}$

during GH therapy, using BMPD module 5V. A P-value < 0.05 was considered significant.

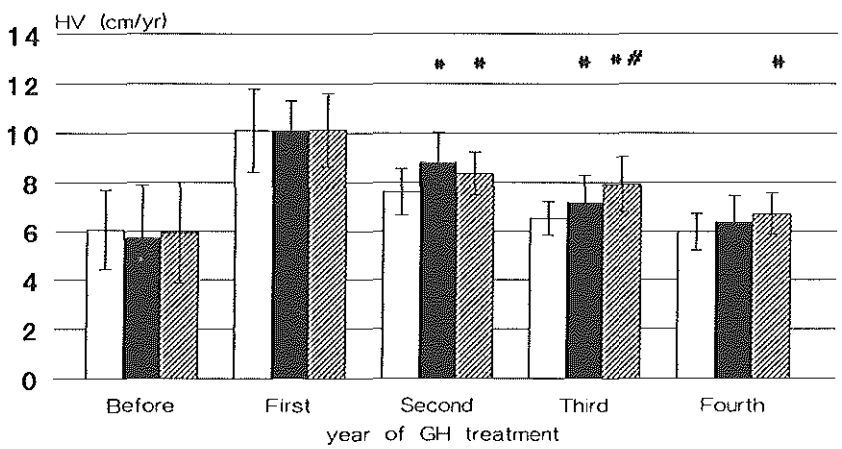
The spontaneous 24-hour GH profiles at baseline were analyzed with the Pulsar program as described previously [30]. The area under the curve (AUC) was determined after the GH injection at 8 p.m. using the trapezoideal rule. The total body clearance was calculated from the injected dose divided by the AUC. The time interval from GH injection to maximum GH levels was recorded as T_{max}. To determine the elimination half-time ($t_{1/2}$) a linear regression analysis was performed on the GH levels starting from one hour after T_{max}.

RESULTS

CLINICAL DATA

In each group only one girl dropped out of the study for the following reasons: non-compliance, alleged increase of muscle mass and decline in school performance, and desire to initiate estrogen therapy before the end of the study period. Eight girls changed during the course of the study from Tanner puberty

Figure 1. Development of mean (SD) HV (cm/yr) for treatment groups A (□), B (▩), and C (▨). Significant differences (P<0.05) compared with group A (*) and with group B (#) are indicated.



stage B1 to B2, at a median age of 13.2 (range 10.9-15.0) years. Their distribution among the treatment groups A, B, and C was 2, 4, and 2 girls, and among karyotypes (45,X and others) 4 and 4 girls, respectively. There were no significant differences between these girls and the girls without signs of endogenous estrogen production with respect to growth response and bone maturation after 4 years GH therapy within each dose group. The number of adverse events was small, all were mild and transient.

GROWTH RESPONSE

Compared with pretreatment, mean HV increased significantly for all three groups from about 6 cm/yr to 10 cm/yr in the first year of GH therapy. Thereafter, a waning of the growth response was observed (Fig. 1). In the second year mean HV in groups B and C on a 50% higher GH dose were significantly higher compared with group A. When subsequently, in group C only, the dose was increased once again, mean HV in groups B and C were both significantly higher than in group A, but in group C also significantly higher compared with group B. In the fourth year of GH treatment only in group C the mean HV remained significantly higher than group A. During the first year of treatment 29% of all girls managed to double their HV.

If the growth response is represented as change in $HVSDS_{CA}$ relative to prestudy values (see Table 1), $\Delta HVSDS_{CA}$ in groups B and C was significantly higher than in group A in the second through fourth year of GH therapy. However, in the third and fourth year ΔHVSDS in group C was not significantly different from group B.

After the first dose-increment for groups B and C both the change in $HSDS_{CA}$ from the first year was significantly higher for the combined groups B and C compared with group A ($P<0.0001$). The second dose-increment resulted in the third year of treatment as well as in the combined third and fourth year in a significantly higher change from year 2 in $HSDS_{CA}$ for group C compared with group B, P-values 0.04 and 0.02. The increase in mean $HSDS_{CA}$ was highest in the first year of GH (>1 SDS), without a difference between groups (Fig. 2). In the subsequent years of treatment, the change in mean $HSDS_{CA}$ showed the same pattern as that of HV. The mean increment in $HSDS_{CA}$ over 4 years was significantly higher for groups B and C compared with group A. However, the gain was not significantly different between groups B and C (Table 2). The change in $HSDS_{CA}$ after 4 years was unrelated to karyotype. When the gain in height was corrected for the estimated gain for untreated girls, the results were similar (Table 2 and Fig. 3). The range of the gain in height over estimated untreated values for all girls was 5.5 to 21.9 cm; for four girls (all Group A) it was below 10 cm.

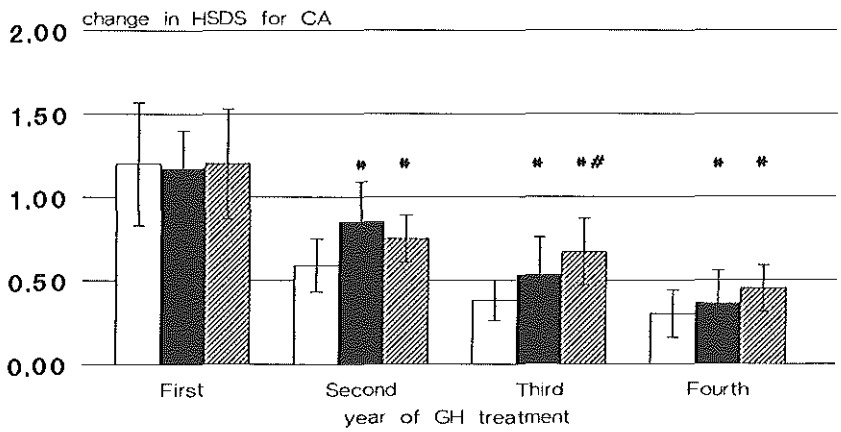


Figure 2. Development of mean (SD) change in $HSDS_{CA}$ for treatment groups A (□), B (▩), and C (▨). Significant differences ($P<0.05$) compared with group A (*) and with group B (#) are indicated.

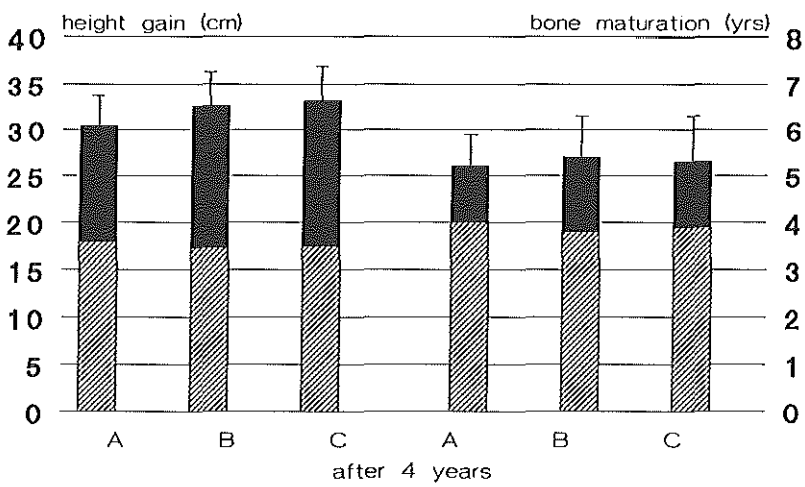


Figure 3. Stacked mean (SD) of treated (▩) over estimated untreated values (▨) in girls with TS after four years GH treatment, both for gain in height (in cm) and for bone maturation (y/4y) in each of the treatment groups A, B, and C.

Table 2. Mean (SD) change during 4 years of GH treatment for every treatment regimen.

	Group A	Group B	Group C	P
$\Delta HSDS_{CA}$ (DSD)	2.46 (0.53)	2.91 (0.54)*	3.07 (0.57)*	0.004
height gain (cm)	12.4 (2.8)	15.3 (3.1)*	15.7 (2.5)*	0.0004
$HVSDS_{CA}$ (DSD) (y4-prestudy)	1.60 (0.97)	2.43 (1.22)*	2.62 (0.99)*	0.007
ΔBA/ΔCA (y/4y) with GH	5.2 (0.7)	5.4 (0.9)	5.3 (1.0)	NS
ΔBA/ΔCA (y/4y) untreated	4.0 (0.5)	3.8 (0.6)	3.9 (0.6)	NS
$\Delta mIPH_{RUS}$ (cm)	4.9 (4.8)	6.6 (3.1)	7.1 (4.5)	NS
ΔPTS_{RUS} (cm)	12.3 (3.8)	14.1 (3.1)	14.7 (3.5)	NS

P: level of significance in a oneway ANOVA
*: significantly different from group A
Δ: change during a period of time for a variable
height gain: gain in cm over estimated untreated values
HV: height velocity
SDS: standard deviation score
DSD: Dutch-Swedish-Danish Turner references
ΔBA/ΔCA: bone maturation (RUS-score): change in BA during 4 years GH treatment; untreated values were estimated using Dutch Turner references
$mIPH_{RUS}$: modified Index of Potential Height
PTS_{RUS}: Turner-specific final height prediction using RUS bone age

BONE MATURATION

The change in RUS BA over the change in CA was not significantly different between groups over 4 years (Table 2), nor during any individual year of treatment. The mean values differed somewhat between the years: for all groups the highest advance was found during the third year and the lowest during the fourth year of GH (data not shown). Compared with estimated values for untreated TS girls bone maturation of the GH treated girls was significantly higher in every year, except for group A in the second and fourth year; for 4 year results see Fig. 3. There was no significant difference in the change in bone maturation over 4 years between the girls with breast-stage B1 and B2 within groups. Bone maturation after four years of treatment was positively related with the degree of BA retardation at start of study (beta: 0.12) and negatively with baseline CA (beta: -0.04).

Table 3. Median (range) values for characteristics of the 24-hour GH profile tests for the 12 girls of Group C at baseline (determined by Pulsar) and 6 months after each dose-increment step (see Methods-section).

	baseline	4 IU/m^2/day	6 IU/m^2/day	8 IU/m^2/day
number of peaks	10 (8;13)			
mean GH (mU/L)	4.3 (1.64;6.78)			
max GH (mU/L)	23.5 (8;42)	63 (27;127)	113 (57;174)*	197 (101;405)*#
AUC (mU/L*24h)	99.1 (39.8;160.3)	341 (160;157)	570 (352;896)*	962 (554;1530)*#
Clearance (ml/min)		420 (224;680)	412 (203;568)	362 (214;566)
$t_{1/2}$ (hrs)		0.5 (-.7;-.2)	0.5 (-.7;-.3)	0.5 (-.9;-.2)
T_{max} (hrs)		2.9 (1.3;4.3)	3.2 (1.3;6.0)	3.2 (1.7;5.3)

* significantly greater compared with 4 IU/m^2/day at P<0.05 level
# significantly greater compared with 6 IU/m^2/day at P<0.05 level

no of peaks:	number of peaks
mean GH:	overall mean GH plasma concentration
max GH:	maximum GH plasma concentration
AUC:	area under the time-concentration curve
clearance:	total body clearance
$t_{1/2}$:	elimination half-time
T_{max}:	time to peak value

FINAL HEIGHT PREDICTION

Mean FH prediction increased significantly for all groups after 4 years GH treatment (Table 2); values with the PTS_{RUS} method were markedly higher compared with the $mIPH_{RUS}$ method. Significant differences between groups for the 4 years change in either FH prediction method were not found, though mean values with both methods in groups B and C were higher than those in group A.

GH MEASUREMENTS

Baseline Arginine-stimulated GH plasma levels ranged from 3-74 mU/L (Table 1). The stimulated GH levels (mU/L) were subdivided in the following level-ranges: <10, ≥10 and <20, and ≥20, with 9, 28, 31 girls, respectively. These numbers were similarly distributed among the three treatment groups. The girls with maximum stimulated GH levels below 20 mU/L did not differ significantly from those with 'normal' stimulated levels (>20 mU/L) in their growth response expressed as the change in $HSDS_{CA}$ after 4 years of GH treatment. Maximum stimulated GH levels were significantly negatively correlated with BMI-SDS at baseline ($r= -0.31$, $P=0.01$). At baseline, the spontaneous and stimulated maximum GH levels in group C were not significantly different and were positively correlated ($r=0.5$, $P=0.05$).

Table 3 includes some of the calculated variables of the spontaneous 24-hour GH profiles of 12 girls of Group C (at baseline). There was no correlation between any of these characteristics and prestudy $HSDS_{CA}$. Furthermore, the characteristics of the 24-hour GH profile tests six months after each dose-increment are shown. There was a significant, dose-dependent increase of the maximum GH level and the AUC. In contrast, the T_{max}, the clearance and the elimination half-life were not significantly different between the three GH doses. The latter is indicated by the parallellism between the curves after the maximum has been reached (Fig. 4).

GH BINDING PROTEIN

Baseline measurements showed no differences between groups (Table 4), the mean (SD) for all girls being 229.4 (127.1) pmol/L. Compared with girls in a normal population [34] 84% of the study group had GHBP levels within the normal age range, in 9 girls the levels were above normal and in only 1 girl it was below normal. Baseline GHBP levels as well as the change after 6 months from baseline were not significantly different between the girls with stimulated GH levels above or below 20 mU/L. GHBP levels after 6 months treatment differed not significantly from baseline.

Table 4. Mean (SD) of IGF-I, IGFBP-3, and GHBP levels for every treatment regimen at baseline, 6 months after initiation of GH therapy and each GH dose-increment, and at 42 and 48 months of GH treatment.

	Gr	baseline	6 months	18 months	30 months	42 months	48 months
IGF-I	A	76.5 (32.7)	213.9 (97.5)	276.6 (91.2)	371.2 (142.8)	393.1 (124.8)	512.7 (143.7)
(mcg/L)	B	104.9 (41.2)#	263.7 (126.4)	363.6 (169.2)	562.3 (227.6)*	525.3 (168.6)*	608.1 (191.6)
	C	85.8 (37.7)	245.1 (100.5)	348.0 (170.7)	501.8 (139.7)*	526.5 (154.5)*	678.6 (179.0)*
IGFBP-3	A	2.54 (0.57)	4.15 (0.79)	4.22 (0.86)	4.08 (0.92)		
(mg/L)	B	2.99 (0.75)	4.71 (1.34)	5.08 (1.26)	4.99 (1.44)		
	C	2.78 (0.55)	4.29 (0.90)	4.58 (0.97)	4.62 (0.80)		
GHBP	A	217.1 (94.0)	201.0 (81.6)				
(pmol/L)	B	256.9 (149.4)	219.8 (95.5)				
	C	224.1 (140.4)	223.4 (164.8)				

\# significantly different from group A and C
* change from baseline significantly different from group A
Gr: Group

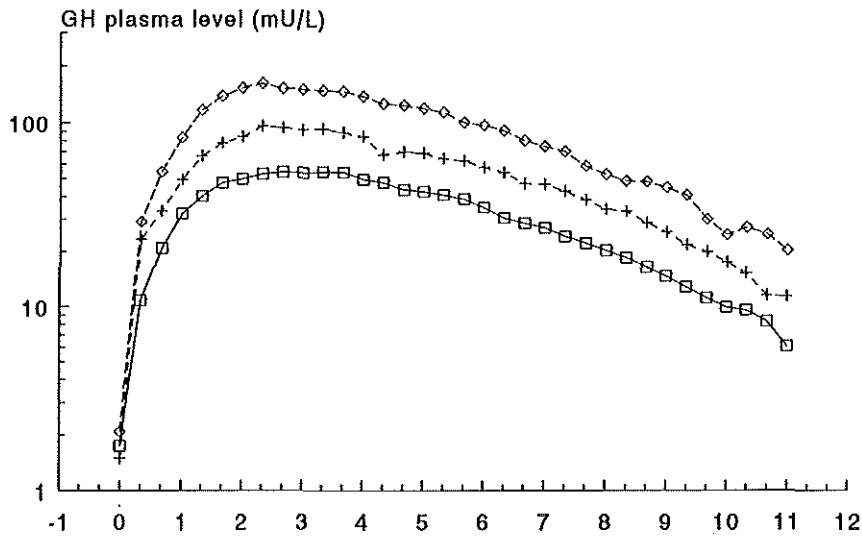


Figure 4. Mean 24-hour GH plasma curves (logarithmic scale) versus time for each of the three GH dosages (4 IU—□—, 6 IU – –+–, and 8 IU – –◇–) employed in a subgroup of 12 girls of Group C, six months after initiation and following each GH dose-increment.

IGF-I AND IGF BINDING PROTEIN-3

At each time-point large interindividual differences existed within groups (Table 4). Mean baseline IGF-I level of group B was higher compared with the other groups. Within groups each point in time was significantly higher than the previous, except for 30 months (all groups) and 42 months (group B). Only after 30 months after start of therapy IGF-I levels (adjusted for baseline levels) for groups B and C became significantly higher compared with group A ($P<0.004$), but at 48 months only group C was still significantly higher than group A ($P=0.008$). The repeated measures model showed that the change in IGF-I levels during GH therapy was dependent on the dose, the duration of treatment and baseline IGF-I level.

At baseline, mean IGFBP-3 levels for group B were higher compared with the other two groups. After adjustment for baseline, IGFBP-3 levels were not significantly different between groups (Table 4). Mean IGFBP-3 levels only increased signicantly after six months of treatment ($P<0.0001$). At the end of study 31% of the girls had plasma IGF-I levels and 35% had IGFBP-3 levels higher than 95th percentile for normal girls at the pubertal peak. There were no differences between treatment groups.

The ratio of IGF-I and IGFBP-3 levels showed an increase over time, but there were no significant differences between groups. Log-values of IGF-I and IGFBP-3 levels both at baseline or their change after 30 months revealed a significant correlation ($r=0.76$, $P<0.0001$ and $r=0.25$, $P=0.04$, respectively).

DETERMINANTS OF GROWTH RESPONSE

MLR analyses showed that there were no significant relationships between [a] $HVSDS_{CA}$ in the fourth year GH (dependent variable) and pretreatment HVSDS; [b] the change in $HSDS_{CA}$ after 4 years GH (dependent variable) and baseline: CA, BA RUS, BA retardation, $HSDS_{CA}$, or Arginine-stimulated maximum GH levels (beta=-0.008; P=0.07). [c] prestudy HV or HVSDS and baseline IGF-I, or IGFBP-3 levels, nor the IGF-I to IGFBP-3 ratio. However, the four-years change in $HSDS_{CA}$ was significant, negatively related to baseline IGF-I and IGFBP-3 levels, and their ratio (beta-values -0.006, -0.32, and -0.015, respectively); even when the baseline IGF-I and IGFBP-3 concentrations were expressed as SDS relative to CA only for the girls with a baseline CA below 10 years (N=64). The change in $HSDS_{CA}$ after 30 or 48 months GH treatment was also significant, positively related to the change in IGF-I, and IGFBP-3 levels after 30 months of GH treatment, but not to their ratio. The repeated measures model with dose-increment steps and duration of treatment as covariates also showed that the change in $HSDS_{CA}$ during GH therapy was dependent on IGF-I plasma levels at baseline and during the study period (beta's -0.002 and 0.0004). The gain in height over estimated untreated values at end of study (dependent variable) was significantly negatively correlated ($P<0.0001$) with age at start of treatment. The change in PTS_{RUS} after four years GH (dependent variable) was significantly negatively related with CA or BA retardation at start of study, as well as with bone maturation during the study period.

Finally, there was no linear relationship between [a] the change in $HSDS_{CA}$ and in the plasma GH AUC at each corresponding point in time; [b] the change in IGF-I or IGFBP-3 plasma levels, or in the IGF-I to IGFBP-3 ratio and the change in AUC at each corresponding point in time; and [c] baseline GHBP levels and baseline CA, $HSDS_{CA}$, HV, stimulated GH levels. Only GHBP levels and BMI-SDS at start of treatment were related (r=0.45, P=0.003).

DISCUSSION

GROWTH RESPONSE

The present study shows that raising the GH dose in subsequent years results in a significant, dose-dependent increase of linear growth expressed as HV or $HSDS_{CA}$. After 4 years of GH treatment only the higher dose groups B and C differed significantly from the constant-dose group A, in terms of gain in cm, and expressed as the change in $HVSDS_{CA}$ (relative to baseline) and in $HSDS_{CA}$; however, after 4 years group C was no longer different from group B. Although during the course of the study bone maturation proceeded significantly faster than that estimated in untreated girls, there was no significant difference between the treatment groups. Bone maturation was negatively related with baseline CA and positively with the degree of BA retardation. The gain in height outweighed the increase in bone maturation, therefore FH prediction improved markedly, the magnitude being dependent on

the method used, but not significantly different between groups. Age, BA RUS, BA retardation, or $HSDS_{CA}$ at start of therapy was not related to the change in $HSDS_{CA}$ over four years in this study group aged 2-11 years. On the other hand, the gain in height over estimated untreated values as well as the change in PTS_{RUS} after 4 years of GH treatment were negatively related to prestudy CA, BA RUS, or the change in bone maturation. A repeated measures model showed that each, yearly change in $HSDS_{CA}$ significantly correlated with IGF-I plasma levels.

DOSE-RESPONSE STUDIES

Dose-response relationships in GH-deficient patients have been described earlier [20]. De Muinck Keizer-Schrama *et al.* [10] reported in GHD children a significantly higher HVSDS and $HSDS_{CA}$ in 17 transfer patients (previously treated with 12 IU GH/m^2/week) on 4 compared with 2 IU GH/m^2/day. Preliminary reports in TS indicated that the increase in HV outweighed the increase in bone maturation and therefore FH prediction was more marked and sustained with higher GH dosages [5,7,15]. However, a comparison with other studies is difficult because of the differences in design and GH dose, entry criteria (e.g. a lower limit for GH provocative testing), age at start of treatment, variables and duration of study reported, and reference populations used.

Takano *et al.* [15] investigated two constant GH dosage regimens in prepubertal girls with TS, 0.5 and 1.0 IU/kg/week (comparable with 2 and 4 IU/m^2/day). Dose-dependency was shown by the significantly higher mean change in HVSDS (Japanese references) during the first four years in the highest dose group, in which the dose was similar to group A in the present study, compared with the lower dose group. In the fourth year of treatment HV in the highest dose-group was no longer significantly higher compared with the lower dose-group. The same phenomenon might also develop in our study, since after a prolonged period on fixed doses, only the HV in group C remained significantly higher compared with group A in year four. Nonetheless, this may already have resulted in a substantial difference in height gain. Since the mean change in HVSDS during the fourth year (see Table 2) was well above 'zero', the girls still exerted catch-up growth. Only 8 girls showed signs of pubertal development at a median age of 13.2 (range 10.9-15.0) years. There were no significant differences between this group and the prepubertal girls with respect to growth response and bone maturation after 4 years GH therapy.

Although FH prediction methods all have their inadequacies, it has been shown in a previous report [29] that the $mIPH_{RUS}$ and PTS_{RUS} methods have the lowest mean error compared with the FH actually reached by girls with TS. Furthermore, FH prediction methods should not be used during growth promoting therapy, since they are based on spontaneous growth. However, since $mIPH_{RUS}$ and PTS_{RUS} both include CA, BA (RUS), and height for the estimation of FH, they reflect the influence of GH on growth as well as bone maturation. In the present study both methods showed significant increases in mean FH prediction after four years of GH therapy, without significant group differences.

Only a trend towards higher values could be observed in the higher dose-groups (B and C) for both the actual and estimated (FH prediction) cm gained.

Chaussain *et al.* [6] performed a study in TS (CA ranged from 5-15 years) with a GH dose of 0.7 IU/kg/week (about 3 IU/m^2/day). If HV after 6, 12, or 24 months had not doubled, this dose was increased by the same amount (to a maximum of 2.1 IU/kg/week). Fourteen of those 24 girls (58%) and 49% of all girls in the present study were unable to double their HV on 4 IU GH/m^2/day after 6 months, and 71% not after one year (data not shown). After 3 years, 12 out of the 22 girls (55%) were on the maximum GH dose. In agreement with the present study, increasing the GH dose did not lead to an acceleration of bone maturation and FH prediction was therefore improved.

GROWTH HORMONE

In general, spontaneous as well as stimulated [3,35,36] GH levels in prepubertal girls with TS have been reported as being near 'normal' [2,4,35,37-39]. Despite differences in the assays used, both spontaneous and stimulated GH plasma levels were comparable with those in prepubertal Dutch TS girls in another study [4]. In the present study the maximum GH levels after Arginine stimulation were similar between groups, but the range was very wide (3 to 74 mU/L). Fifty-four percent of the girls had a maximum level below 20 mU/L, the cut-off point generally accepted to define GH deficiency. Although the mean BMI-SDS was close to zero in these girls (Table 1), obesity could explain the rather low maximal GH levels in these girls [3,40,41], since a negative correlation between stimulated GH levels and baseline BMI-SDS was found. Although representing only a single test, stimulated GH levels were related to the change in $HSDS_{CA}$ after 4 years GH treatment at the 0.07 level of significance. This is in agreement with a report in normal children [42] and in disagreement with a Japanese report in TS [3].

At baseline, a good correlation between spontaneous and stimulated maximum GH levels was observed in a subgroup of 12 girls of group C, in accordance with a previous report [41]. Compared with prepubertal Dutch TS girls in an earlier study [4], and assuming a conversion factor from µg/L to mU/L of two, maximum GH levels were comparable (25.6 vs 27.6 mU/L), mean 24-h GH levels were rather low (4.3 vs ± 6.0 mU/L) and the mean number of peaks was high (10.2 vs 4.5) in the present study. It has been suggested [4] that an elevated spontaneous pulse frequency pattern might be associated with relatively low IGF-I levels and slow baseline growth and that these girls might benefit most from GH treatment. This seems in line with the present study. Furthermore, the change in $HSDS_{CA}$ after 4 years GH treatment was negatively related to baseline IGF-I and IGFBP-3 levels (or their SD-scores for CA), or their ratio.

In agreement with earlier short-term findings in GHD patients [20], a clear-cut increase in maximum GH plasma levels and AUC values was observed during GH treatment with increasing dosages. The elimination half-time, however, was similar between the three GH dosage regimens, suggesting that increasing the GH dose results in a higher bioavailability of GH without

accumulation. In addition, injection of higher dosages (and volume) did not result in a delayed time to maximum plasma GH levels. Nevertheless, in this small subgroup of girls the change over time of the plasma GH AUC was not significantly related to the corresponding growth response expressed as change in $HSDS_{CA}$, nor to the corresponding change in IGF-I or IGFBP-3 levels, or their ratio.

IGF-I AND ITS MAIN BINDING PROTEIN

Except for the 30 months time-point in group B, IGF-I levels showed a progressive, significant increase during the four treatment years, which in part can be explained by the age-dependency of this measurement. At 48 months only group C still had significantly higher IGF-I levels (adjusted for baseline) than group A (P=0.008). In a report after three years in Japanese girls with TS [43] mean IGF-I levels were statistically higher with 1 IU GH/kg/wk than with 0.5 IU/kg/wk. In the present study the change in IGF-I levels after four years GH therapy was dependent on the dose, the duration of treatment and the baseline IGF-I level. Thirty-one percent of the girls had plasma IGF-I levels after four years GH > P95 for normal girls at the pubertal peak, without significant differences between groups. Only two of these girls were younger than 10 years. At a lower GH dose than used in group A in the present study (0.68 IU/kg/wk) Ranke et al [44] reported 15% of the TS girls to have IGF-I plasma levels above the pubertal peak after one year. Moreover, baseline IGFBP-3 plasma levels in that study hardly deviated from the normal range, but after one year of GH therapy more than 20% of the girls had IGFBP-3 levels above the pubertal peak. In the present study, determined in the same laboratory, at baseline only 3 and after 30 months 23 girls (35%) had IGFBP-3 levels greater than 5 mg/L (P95 at peak pubertal level), with a similar distribution between the groups. IGFBP-3 was not determined at 42 and 48 months, but a plateau seemed to have been reached already after 6 months of therapy, despite age-dependency of this binding protein and a further increase of the GH dose.

Baseline log-values of IGF-I versus IGFBP-3 levels showed a significant positive correlation ($r=0.75;P<0.0001$), but also the change of these values after 30 months from baseline was significantly positively correlated ($r=0.25;P=0.01$). This is in line with earlier findings in TS [44,45], and not unexpected, since both proteins are GH dependent. A progressive rise of the IGF-I to IGFBP-3 ratio could be an indicator of the growth response [44]. However, after 30 or 48 months of treatment neither a significant relationship was observed between the change in IGF-I to IGFBP-3 ratio and the change in $HSDS_{CA}$, nor was there a significant difference between groups of the change in this ratio, although a trend was apparent after 30 months: the mean change in IGF-I to IGFBP-3 ratio was 60, 82, and 81, for group A, B, and C. Also, neither IGF-I nor IGFBP-3 levels, nor their ratio were related to the pretreatment HV(SDS). Taken together, there is little evidence to support an explanation of the differences in growth response between the groups by a change in the IGF-I to IGFBP-3 ratio. Nevertheless, a repeated measures model with the dose-increment steps and duration of treatment as covariants, showed that each

change in $HSDS_{CA}$ correlated significantly with IGF-I plasma levels. Thus, free IGF-I might still be a determining factor.

GHBP

In contrast to an earlier study with an older group of girls with TS [32], but in agreement with another report in TS [46], the decrease in GHBP levels after 6 months treatment was not significant from baseline. This might be due to a large interindividual variation. At baseline there were no differences between groups. Most of the girls had GHBP levels within the normal age range, as was shown previously [34]. At baseline a linear relationship between GHBP levels and age or stimulated GH levels at start of treatment in the present group of girls with TS was not observed, in contrast to earlier reports in normal children [34]. In agreement with the latter report, however, GHBP levels at baseline correlated positively with BMI-SDS.

CONCLUSIONS

A stepwise GH-dosing approach reduced the 'waning'-effect of the growth response after 4 years treatment without undue bone maturation. The increase in FH prediction was not significantly different between treatment groups. Irrespective of the GH dose used, initiation of GH treatment at a younger age is beneficial in terms of cm gained either, at end of study or in terms of predicted FH, but not when expressed as the change in $HSDS_{CA}$ over the study period. The lower the baseline IGF-I and IGFBP-3 plasma levels as well as their ratio, and the higher the change in IGF-I and IGFBP-3 plasma levels, the greater is the change in $HSDS_{CA}$. The ultimate proof of the effect of the three GH treatment regimens is FH. Therefore, the present treatment protocol will be extended until FH is reached.

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Chapter 5

Effect of growth hormone administration frequency on 24-hour growth hormone profiles and levels of other growth related parameters in girls with Turner syndrome

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EFFECT OF GROWTH HORMONE ADMINISTRATION FREQUENCY ON 24-HOUR GROWTH HORMONE PROFILES AND LEVELS OF OTHER GROWTH RELATED PARAMETERS IN GIRLS WITH TURNER SYNDROME

ABSTRACT

The optimal dose and frequency of GH administration in Turner syndrome is unknown. There is some evidence that a schedule which mimicks normal pulsatile GH secretion may be more effective than a single daily dose.

Objective: To obtain insight in the influence of the frequency of GH administration on 24-hour GH profiles and levels of other growth related factors in Turner syndrome.

Design: Four weeks after initiation of 0.05 ug/kg/d ethinyl estradiol, we compared twice daily (BID - fractionated dose) with once daily (OD) sc injections of 6 IU GH/m^2/d in a 2-week cross-over design with a 2-week washout interval. Each treatment period was concluded with 24h GH profile tests. Pretreatment plasma/serum levels of GH, IGF-I, binding proteins for GH (GHBP) and IGF-I (IGFBP-3) were used as a basis for comparison of the levels found after either regimen. A one-compartment open model was used for estimation of pharmacokinetic parameters.

Subjects: Ten previously untreated girls with TS aged ≥11 years.

Measurements: plasma levels of GHBP by standardized binding assay; GH, IGF-I, and IGFBP-3 serum/plasma levels by RIA.

Results: There were significantly higher maximum GH levels and a larger area under the curve with OD than with BID GH, while GH clearance was larger with BID The pharmacokinetic values with OD injections were in conformity with values for healthy and GH-deficient children. Pretreatment GHBP levels tended to be high compared with values in healthy prepubertal children. These levels decreased with GH therapy, significantly so with BID GH only. There was a significant increase in levels of IGF-I and IGFBP-3, irrespective of regimen. The IGF-I to IGFBP-3 ratio, a possible indicator of the growth response, rose significantly and comparable with both regimens. There was no consistent diurnal variation with either regimen in GHBP, IGF-I, or IGFBP-3 levels. Four-hourly levels of GH, GHBP, IGF-I, and IGFBP-3 were not correlated.

Conclusions: Although the 24-hour profiles differed during once or twice daily administration of the same total GH dose, the diurnal pattern and mean

levels of factors involved in the biological effects of GH are comparable for both frequency regimens.

INTRODUCTION

The reasons for growth retardation in Turner syndrome (TS) are unknown. Administration of GH results in growth acceleration, but optimal dosage of GH in TS has yet to be established, particularly concerning frequency of administration. Studies in hypo-physectomized rats [1] and GH-deficient (GHD) patients [2,3] suggest that a schedule which mimicks normal pulsatile GH secretion may be more effective than a single daily dose. The biological activity of GH and IGF-I is believed to be modulated by binding proteins (BP), mainly the high affinity BP for GH [4] and IGFBP-3 for IGF-I [5].

To obtain insight in the influence of the frequency of GH administration on these growth related parameters in TS, we investigated the comparative effect of fractionated twice daily (BID) versus once daily (OD) GH administration on GH, GHBP, IGF-I, and IGFBP-3 levels in girls with TS concurrently receiving low-dose ethinyl estradiol.

PATIENTS AND METHODS

STUDY POPULATION

We studied ten previously untreated girls with TS, confirmed by lymphocyte chromosomal analysis. Clinical data for the girls, all Tanner puberty stage B1 [6], are listed in Table 1. Tanner-Whitehouse classification (TW2-RUS) was used for determination of bone age [7]. Height was expressed as SD-score for chronological age ($HSDS_{CA}$) using Dutch reference data [8]. The body mass index for each subject was calculated by equating weight in kg/height in m^2 and SD-scores were determined [9]. After matching for bone age and $HSDS_{CA}$, they were randomly divided into two groups for GH trials. Written, informed consent was obtained from the girls and their parents. The study protocol was approved by the Ethics Committee of each participating center.

STUDY DESIGN

To determine unbiased pretreatment levels of GH, IGF-I, GHBP, and IGFBP-3, all girls underwent blood sampling (at 1100h). Subsequently,

Table 1. Clinical data for 10 girls with TS; Mean values (range).

Chronological age (years)	13.6 (11.3-16.4)
Bone age (TW2-RUS) (years)	12.6 (11.6-13.3)
Height SDS_{CA}	-3.77 (-6.01 to -2.01)
Body mass index SDS	0.63 (-0.84 to 3.28)
Karyotype	45,X (n=3)
	45,X/46,X,iso X(q) (n=7)

they started taking ethinyl estradiol (0.05 ug/kg/d). GH therapy (Norditropin^R, Novo-Nordisk A/S, Denmark) followed four weeks later. The girls were trained to inject GH sc over the quadriceps region by means of pen injection (Nordiject^R) in a daily dose of 6 IU GH/m^2, equalling 0.07 mg/kg/d, rounded off to 0.5 IU. Mimicking the diurnal secretory GH pattern [10] one group received 1/3 of the total dose at 0800h and 2/3 at 2000h for 2 weeks. Following a washout interval of 2 weeks, GH therapy was resumed with OD (total dose) injections at 2000h for 2 weeks. The other group followed the same schedule in reverse. Each GH treatment period concluded with 24h GH profile testing. Starting at 1900h, blood was withdrawn from an indwelling venous catheter with a heparin lock and stored on ice in heparin-coated tubes for no more than 3 hours. A sample of 0.5 ml blood was collected every 20 minutes for GH measurement and an additional sample of 5 ml every 4 hours for measurement of GHBP, IGF-I, and IGFBP-3. After centrifugation, all samples were frozen at -20°C until assayed. Although hospitalized for the duration of the test, the girls kept normal diets served at hospital mealtimes and continued normal activity and sleeping habits.

HORMONE ASSAYS

A double antibody RIA served to measure duplicate samples of GH, while a specific RIA measured IGF-I levels as described previously [11]. Pretreatment levels were compared with mean IGF-I levels of healthy, prepubertal, 8-9 year old Dutch girls tested in the same laboratory (mean ± SD 181 ± 47 ng/ml).

Determination of plasma GHBP was performed by standardized GH-binding assay and correction was made for saturation by high GH levels (>7 ug/L) in 2-4 of the 7 samples during the 24h test-ing period, as previously described [12]. GHBP levels in healthy 8-10 year old children amount to 55-60% of adult levels (mean adult levels ± SD: 13.5% ± 2.3% GH bound/400ul plasma).

The acid-stable subunit of the 140 kD IGFBP-3, was measured in duplicate in unextracted serum by specific RIA [13]. Pretreatment levels were compared with mean IGFBP-3 levels of healthy, prepubertal, 7-9 year-old German girls tested in the same laboratory (log-normal mean ± SD 2.716 ± 1.25 ug/L).

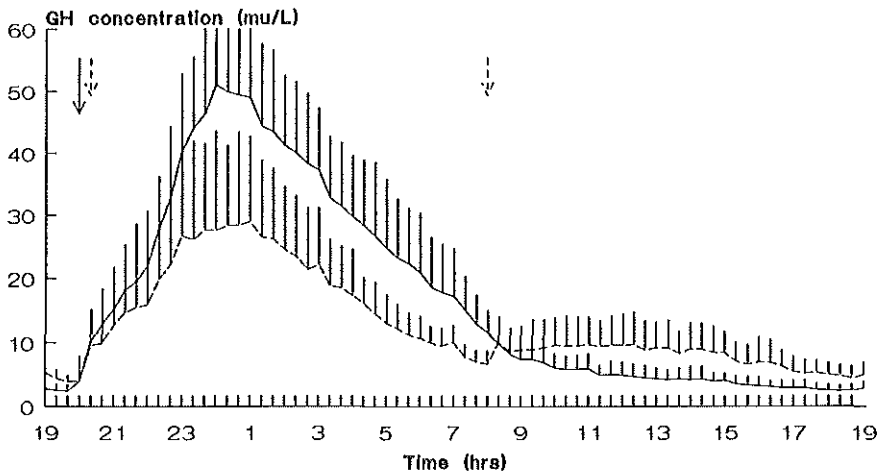
STATISTICAL ANALYSIS

Results were expressed as the mean ± SD. GH concentrations in plasma were plotted in relation to time. Visual inspection of maximum GH concentration and GH levels after 0800h served for comparison of individual 24h GH profiles after either BID or OD GH. The area under the curve for time-concentration (AUC) was determined using the trapezoideal rule. The total body clearance was calculated from the total dose divided by the AUC. The duration of time from GH injection till maximum GH levels was recorded as T_{max}. Plasma concentrations versus time data for GH were fitted using the SAS NLIN computer program package. A one-compartment open model was selected after application of Akaike's information criteria [14]. Allen's equations [15] were used to estimate model-dependent pharmacokinetic parameters: time-lag till GH appeared in blood (T_{lag}) absorption rate constant (K_{abs}), and elimination half-time ($t_{1/2}$).

Statistical analysis was performed by paired Student's t-tests, repeated measures ANOVA with the SPSS/PC+ statistical package, and cluster analysis [16]. Since the distribution of the AUC was skewed, confidence intervals were calculated after log-transformation.

Figure 1. Mean ± SD plasma GH levels during 24 hrs in 10 girls with TS, during OD (——) and BID (– – –).

GH injections: ——> OD 1/1 at 0800h
– –> BID 2/3 at 0800h, 1/3 at 2000h
Blood sampling: ⌊⌋ GH every 20 min.
o o GHBP, IGF-I, and IGFBP-3 every 4h



RESULTS

GROWTH HORMONE PROFILES

No carryover effect of the crossover design was found for any parameter. The comparative results of plasma/serum levels of GHBP, IGF-I, and IGFBP-3 versus pretreatment levels are based on 1100h determination.

Individual curves for plasma GH concentration in relation to time reflected the frequency of GH administration in all but one case. Figure 1 represents the 24h GH profiles depicting mean (SD) GH plasma levels. Table 2 summarizes the pharmacokinetic data for these girls. The AUC appeared to be higher with OD GH than with BID GH, although the difference was not significant (p=0.06). Mean AUC values for the first 12 hours of testing (nighttime) were 1.9 (95% confidence limits 1.19-2.42) times higher with OD GH than with BID GH. Regarding BID GH, the AUC value was 3.1 (95% confidence limits 1.47-3.49) times higher for the first than for the second test-period. The mean maximal GH plasma level was 2.07 (95% confidence limits 1.20-2.94) times higher with OD GH than with BID GH injections.

Table 2. Pharmacokinetic GH parameters of 24h profiles for girls with TS

	n	GH OD	GH BID	p#
Max. value (mU/l)	10	53 ± 20	31 ± 15	0.03
AUC (mU h/l)	10	409 ± 97	305 ± 87	0.06
Cl (ml/min)	10	316 ± 80	430 ± 119	0.04
T_{max} (h)	10	4.3 ± 0.9	4.6 ± 0.9	0.47
T_{lag} (h)	9	0.28 ± 0.1		
K_{abs} (h^{-1})	9	0.31 ± 0.06		
$t_{1/2}$ (h)	9	2.9 ± 0.7		

Data are expressed as the mean ± SD

#:	p-value between frequency regimens
Max.value:	maximum GH plasma concentration
AUC:	area under the time-concentration curve
Cl:	total body clearance
T_{max}:	time to peak value
T_{lag}:	time to GH appearance in blood
K_{abs}:	absorption rate constant
$t_{1/2}$:	elimination half-life

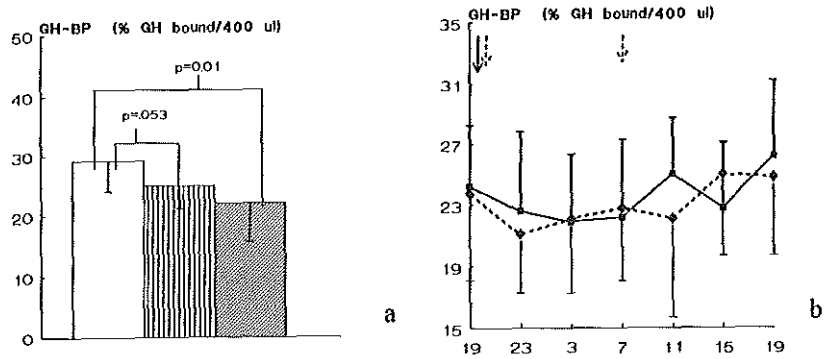


Figure 2. GH-binding protein plasma levels (mean ± SD) before and after 2 weeks GH (6 IU/m^2/day) in 10 girls with TS. [a] Mean 1100h pretreatment (□) and GH (OD ▒ and BID ▒) treatment levels. [b] During 24h profiles (OD —■— and BID - -◆- -).

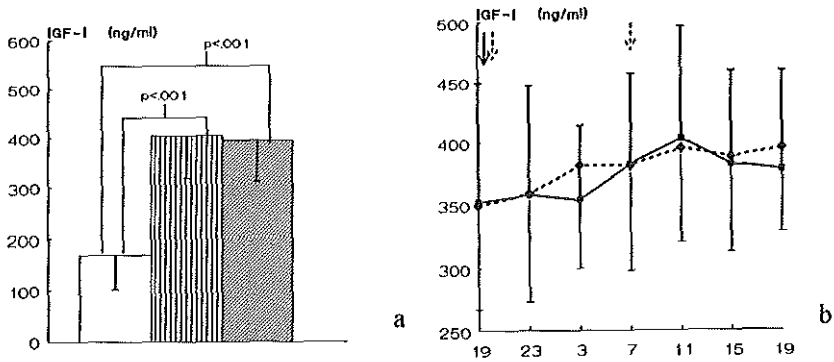


Figure 3. IGF-I plasma levels (mean ± SD) before and after 2 weeks GH (6 IU/m^2/day) in 10 girls with TS. [a] Mean 1100h pretreatment (□) and GH (OD ▒ and BID ▒) treatment levels. [b] During 24h profiles (OD —■— and BID - -◆- -).

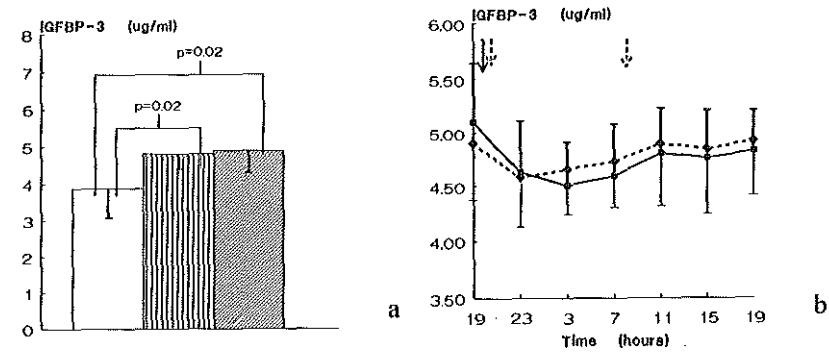


Figure 4. IGF-I-binding protein-3 serum levels (mean ± SD) before and after 2 weeks GH (6 IU/m^2/day) in 10 girls with TS. [a] Mean 1100h pretreatment (□) and GH (OD ▒ and BID ▒) treatment levels. [b] During 24h profiles (OD —■— and BID - -◆- -).

A good curve-fit for plasma GH concentrations in relation to time was found for nine girls on OD GH (Table 2). With the BID regimen there was an acceptable curve-fit for the first (nighttime) GH peak in four cases only, whereby pharmacokinetic values closely resembled those for the OD regimen. No significant correlation between body mass index and T_{lag} was found.

GROWTH HORMONE BINDING PROTEIN

Pretreatment levels were in the high normal range compared with healthy, prepubertal, 8-10 year old children. Average levels of GHBP were lower during GH treatment than before therapy, but the decrease only became significant with BID GH (Fig. 2a, Table 3). The individual curves for GHBP levels showed no consistent diurnal variation. When pooled, cluster analysis showed a small but significant ($p<0.05$) decline in 1100h GHBP levels during BID GH only, while ANOVA showed a significant ($p<0.03$) and similar time-trend with both frequency regimens (Fig. 2b).

Table 3. 1100h GHBP, IGF-I, IGFBP-3 levels, and IGF-I:IGFBP-3 ratio for 10 girls with TS.

	Pretreatment	GH OD	GH BID	p#
GHBP (%GH/400 µl)	29.2 ± 5.2	25.0 ± 3.7	22.1 ± 6.4*	0.30
IGF-I (µg/l)	169 ± 67	404 ± 85*	395 ± 83*	ns
IGFBP-3 (µg/ml)	3.89 ± 0.8	4.81 ± 0.4*	4.90 ± 0.6*	ns
IGF-I:IGFBP-3	44.3 ± 19	83.6 ± 16*	81.0 ± 13*	ns

Data are expressed as the mean ± SD

*: p-value ≤ 0.05 compared with pretreatment levels

#: p-value between regimens

ns: $p \geq 0.05$

GHBP: GH binding protein

IGFBP-3: IGF-I binding protein 3

IGF-I AND ITS BINDING PROTEIN

Pretreatment IGF-I plasma levels were low-normal compared with healthy, prepubertal, 8-9 year old girls and rose significantly with GH therapy, irrespective of frequency regimen (Fig. 3a, Table 3). Neither regimen resulted in a clear circadian pattern in IGF-I plasma levels. While cluster analysis revealed no significant diurnal pattern, a small but significant positive time-trend did emerge for BID GH in the course of the 24h test (ANOVA, $p=0.02$). Nevertheless, there was no significant interaction between the regimens (Fig. 3b).

Mean pretreatment IGFBP-3 serum levels, which were high-normal compared with healthy, prepubertal, 7-9 year old children, also rose significantly with GH therapy, irrespective of frequency regimen (Fig. 4a, Table 3). Many of the individual curves showed minor variations, although most

values were within 95% confidence limits based on the assay error. No clear circadian pattern emerged for these changes and there was no difference between regimens. Pooled, IGFBP-3 levels showed time-related changes throughout the 24h test period (cluster and ANOVA $p<0.05$), without a significant interaction between the regimens (Fig. 4b). The ratio IGF-I to IGFBP-3 almost doubled with GH therapy, without a difference between regimens.

There was no correlation between GH, GHBP, IGF-I, and IGFBP-3 levels at the four-hourly intervals.

DISCUSSION

GROWTH HORMONE PROFILES

Individual curves for GH concentration in relation to time reflected the frequency regimen in all but one case. The reproducibility of the 24h GH curves was not tested, but two 24h GH profiles within 3 days of one and the same girl on the once daily regimen, resulted in quite similar curves. As expected, resulted the total GH dose administered in one bolus in significantly higher maximum GH concentrations. The clearance was significantly higher with twice daily than with once daily GH injections. There was a tendency towards a higher area under the curve when all the growth hormone was given in the evening as compared to the same total dose given twice a day ($p=0.06$). Although not statistically significant, our data seem to support the findings in GHD children, in which GH administered in the evening resulted in a higher maximum concentration and a larger area under the curve than the same dose administered in the morning. This was attributed to a change in sc absorption as a consequence of the higher skin temperature in bed [17].

The time to peak value (T_{max}), reflecting absorption of sc administered GH, is supposed to amount to 3.3 hours for healthy adult males [18] and to vary between 4 and 5 hours for GHD children [19-21]. The results for this TS study population were in the same range, irrespective of regimen. During once daily injections, both absorption rate constant ($0.31 \pm 0.06\ h^{-1}$) and timed appearance of GH in blood (T_{lag}, 0.28 ± 0.10 h) were comparable to values found in GHD patients, $0.45 \pm 0.05\ h^{-1}$ and 0.30 ± 0.10 h [20]. A positive correlation was found between the T_{lag} and body mass index of GHD children, whereby the higher body mass index was attributed to the delay in absorption after sc administration of GH [20]. We found no such correlation in these girls with TS, despite a wide range of the body mass index. Plasma GH levels are also influenced by elimination. A GH elimination half-time of 2.9 hours during once daily injections conforms with healthy adult males [18] and GHD children [20,21]. Plasma GH levels of girls on a single dose regimen decreased to the lower limit of detection during the second 12 hours of testing. This seems somewhat later than in GHD patients, whereby a return to baseline levels was reached within 11-12

hours [19]. The girls on fractionated administration, received a second GH injection before the detection limit had been reached.

An acceptable curve-fit for GH concentration in relation to time could not always be established for twice daily injections. However, in four cases tested, values during the first 12 test hours were almost identical to those for once daily administration. No second peak could be discerned during the twice daily regimen due to the inconsistent pattern of GH plasma concentrations. Assuming that T_{lag}, K_{abs}, and $t_{1/2}$ were not dose-dependent with the range used, a significant difference between frequency regimens seems unlikely.

GROWTH HORMONE BINDING PROTEIN

The bioavailability of GH and IGF-I is modified by their main binding proteins, GHBP and IGFBP-3. While these parameters may have been influenced by the concurrent low-dose ethinyl estradiol therapy in our study, we may assume that such an effect would be identical for both frequency regimens.

In conformity with findings in healthy adults and children [22-24] GHBP plasma levels remained fairly constant throughout the 24h period, irrespective of frequency regimen. No correlation was found between frequency of GH administration and GHBP, such as found for the endogenous secretory GH pattern [24,25]. The mean pretreatment GHBP level was in the high normal range for healthy prepubertal girls, in conformity with another TS study [26]. These levels decreased to mean values for healthy controls with both regimens, but only significantly with the twice daily regimen. This is in contrast with a marked increase during GH treatment found by others in TS [25], GHD [27], and idiopathic short stature [28]. As GHBP corresponds to the extracellular domain of the GH-receptor [29], a possible explanation for our findings might be that TS is associated with a state of relative (intra-cellular) resistance to GH, which might result in compensatory up-regulation of GH-receptor/GHBP. Exogenous GH would then result in down-regulation of receptor and GHBP levels towards the normal range.

IGF-I AND ITS BINDING PROTEIN

In conformity with other TS studies [30-32], the mean pretreatment IGF-I plasma level was in the subnormal range. GH therapy induced a dramatic rise, without significant difference between frequency regimens. The marked improvement of IGF-I levels is comparable with a reported increase of IGF-I levels during GH treatment in TS [32,33]. ANOVA revealed a significant trend in time of the pooled IGF-I levels during twice daily injections only. However, neither regimen showed a clear circadian pattern of the individual curves. These findings are in conformity with observations in GHD patients on once daily (evening) injections with the same total GH dose [34].

Mean pretreatment IGFBP-3 levels of our subjects were in the high-normal range and are in agreement with values in TS found by Ranke et al [33]. In contrast with their findings, however, the rise in IGFBP-3 levels following GH treatment in our subjects was significant, irrespective of GH regimen. Although both ANOVA and cluster analysis revealed minor diurnal variations of the

pooled IGFBP-3 serum levels irrespective of frequency regimen, the individual curves revealed no consistent pattern. This is in agreement with the minute, but significant changes found in both GHD children and healthy controls [35]. Interestingly, no correlation was found between IGF-I and IGFBP-3 levels at any time-point.

Ranke et al observed a marked increase of the ratio IGF-I to IGFBP-3, suggesting that this might be a possible indicator of the growth response [33]. Furthermore, in GHD patients, the IGF-I to IGFBP-3 ratio was higher after frequent pulsatile and constant iv GH delivery than with 2 evening iv GH injections, suggesting that IGF-I generation is dependent on the pattern of GH delivery [35]. Our results do not support this suggestion, because the ratio almost doubled with sc GH therapy, irrespective of frequency regimen.

CONCLUSIONS

GH administration to girls with TS, as with GHD children, results in pharmacokinetic GH values similar to those found in healthy individuals. The pharmacodynamics of once or twice daily sc GH injections do not enable imitation of the secretory pattern of GH, but twice daily GH administration bears a slightly closer resemblance to the physiological state than once daily injections. Although the 24-hour profiles differed during once or twice daily administration of the same total GH dose, the diurnal pattern and mean levels of factors involved in the biological effects of GH are comparable for both frequency regimens. The predictive importance of these parameters will be assessed by comparing the long-term auxological response to GH administration in either frequency.

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Chapter 6

Growth response and levels of growth factors after two years growth hormone treatment are similar for a once and twice daily injection regimen in girls with Turner syndrome

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GROWTH RESPONSE AND LEVELS OF GROWTH FACTORS AFTER TWO YEARS GROWTH HORMONE TREATMENT ARE SIMILAR FOR A ONCE AND TWICE DAILY INJECTION REGIMEN IN GIRLS WITH TURNER SYNDROME

ABSTRACT

Objective: To improve the growth response following GH therapy by trying to mimick the normal pulsatile GH secretion in Turner syndrome (TS) more closely.

Design: In a two year study the effect of fractionated twice daily (BID) versus once daily (OD) s.c. injections of a total GH dose of 6 IU/m^2/day was compared. BID injections were administered as two-thirds at bedtime and one-third in the morning. The subjects concurrently received low dose ethinyl estradiol (0.05 µg/Kg/day, orally).

Subjects: Nineteen girls with TS aged 11 years or over, who were previously engaged in a 10-week GH cross-over study.

Measurements: Height and bone age were evaluated in relation to untreated Turner reference data. Final height (FH) was predicted using the Bayley & Pinneau (BP) method, the modified Index of Potential Height ($mIPH_{RUS}$), and a recently developed Turner-specific method (PTS_{RUS}) based on regression coefficients for H, CA, and BA. Plasma levels of GH, GHBP, IGF-I, and IGFBP-3 were determined by RIA.

Results: After two years treatment the growth response expressed as HV, HVSDS, the change in $HSDS_{CA}$, the gain in height over estimated untreated values and in FH prediction all showed significant improvements. Although mean values tended to be higher with OD injections, significant differences between groups were not found. Bone maturation was similar between groups and compared with untreated estimated values. Independent of treatment group, the change in $HSDS_{CA}$ after two years of GH treatment was related, negatively to the baseline CA and $HSDS_{CA}$, and positively related to BA delay at baseline. After 18 months of GH treatment the significant decrease in GHBP plasma levels observed after 6 months was no longer significant. In contrast, IGF-I and IGFBP-3 plasma levels and the IGF-I to IGFBP-3 ratio increased significantly during 18 months GH therapy. None of these growth related factors showed a difference between groups in their 18 months change. Relevant side-effects were not observed during the first two years of GH treatment.

Conclusions: The present growth data are in conformity with the data of the earlier 24-hour GH profiles. The growth response and plasma levels of growth related factors after two years GH on a total dose of 6 IU/m^2/day in combination with low-dose estrogens was not significantly different between the once daily and the twice daily GH injection regimen.

INTRODUCTION

Growth hormone (GH) is known to improve height velocity (HV) in girls with Turner syndrome (TS). In order to improve the growth response three weekly intramuscular injections have been replaced by a daily subcutaneous regimen [1,2]. However, just as in girls in a normal population [3], spontaneous GH secretion in TS is characterized by a large peak soon after falling asleep and the occurence of several other peaks during the course of a 24-hour period [4-10]. A more frequent injection regimen might thus be beneficial for the growth response, as has also been suggested by studies with hypophysectomized rats [11] and GH-deficient (GHD) patients [12,13].

Recently, it was reported that in TS once daily (OD) and twice daily (BID) GH injections of a total dose of 25 IU/m^2/week did not result in a significantly different growth response or side-effects [14]. However, the follow-up period lasted only one year and an equal division was made for the BID injection dose. An earlier study in girls with TS by our group [15] showed that after two week treatment periods, the GH bioavailablity favoured OD over BID injections of the same total dose. Although the diurnal pattern and mean levels of IGF-I and its main binding protein IGFBP-3 increased comparable for both regimens, GH binding protein (GHBP) levels decreased only significantly with BID injections. In that study and the present one, in an attempt to mimick the normal pulsatile GH secretion more closely, the total BID dose of 6 IU/m^2/day was divided in two-thirds in the evening and one-third in the morning. A total dose of 6 IU GH/m^2/day was chosen in stead of the more commonly used 4 IU/m^2/day since an earlier study has shown [2] that on the latter dose the growth response in a somewhat older subgroup of girls with TS was relatively poor compared with younger girls.

We now report two year follow-up comparing the effect of fractionated BID versus OD GH administration in 19 girls with TS aged 11 years or over concurrently receiving low dose ethinyl estradiol.

SUBJECTS AND METHODS

STUDY GROUP

We studied 19 previously untreated girls with TS, confirmed by lymphocyte chromosomal analysis. Ten girls were previously enrolled in a 10-week cross-over design described earlier [15]. In brief, they started taking ethinyl estradiol (0.05 µg/Kg/day) for 4 weeks before they were randomly divided in OD or BID GH injection groups (vide infra). GH was administered for 2 weeks. Following a washout interval of 2 weeks GH therapy was resumed for a period of 2 weeks in the alternate injection frequency. The additional nine girls followed randomly the same schedule, only without 24-hour GH profile testing. After a second randomization (baseline), all 19 girls entered the present study immediately after completion of the 10-week design.

At start of the cross-over study all girls had Tanner puberty stage B1 [16]) and were aged 11 years or over, and the Tanner & Whitehouse RUS bone age (BA) [17] was less than 13.5 years. Exclusion criteria were: associated endocrine and/or metabolic disorders, growth failure due to other disorders or emotional deprivation, hydrocephalus, previous use of drugs that may interfere with GH therapy.

STUDY DESIGN

After matching for RUS BA and height SD-score for chronological age (CA, $HSDS_{CA}$), the girls were randomly divided into two GH injection frequency groups for continuous GH treatment (baseline). One group (N=9) received 6 IU GH/m^2 body surface once daily (OD), a second group (N=10) received the same total GH dose divided in one-third in the morning and two-thirds at bedtime (BID injections). In addition to r-hGH therapy (Norditropin^R, Novo Nordisk A/S, Denmark) by means of a pen injection system (Nordiject^R 24) the girls received ethinyl estradiol (0.05 µg/Kg/day), once daily, orally. Written, informed consent was obtained from the girls and their parents. The study protocol was approved by the Ethics Committee of each participating centre.

GROWTH EVALUATION

Height measurements were determined at baseline and three monthly by one investigator (AT) according to Cameron [18] using a Harpenden stadiometer and expressed as SD-score for chronological age ($HSDS_{CA}$, $HVSDS_{CA}$) using the Dutch-Swedish-Danish (DSD) Turner reference data [19]. The gain in height for untreated girls with TS was estimated from the equations of the DSD Turner data [19], in which the height of an average girl with TS is indicated by her CA. Midparental height (MPH) was adapted for Dutch reference data [20] with addition of 3 cm for secular trend: $MPH = 1/2*(H_{mother} + H_{father} - 12\ cm) + 3\ cm$.

The degree of obesity was expressed as body mass index (BMI) SD-scores [21]. Pubertal stages according to Tanner [22] were compared with longitudinal British references [23]. BA was determined by one investigator (AT) according to Tanner & Whitehouse RUS-score [17] and to Greulich and Pyle (GP) [24]. Bone maturation (ΔBA/ΔCA) was compared with estimated values from the equations of untreated Dutch Turner girls [25]; in these equations the BA of an average girl with TS is indicated by her CA. FH was predicted using the Bayley & Pinneau (BP) method [26], the modified Index of Potential Height ($mIPH_{RUS}$) method [27,28] and a recently developed Turner-specific method (PTS_{RUS}) based on regression coefficients for H, CA, and BA [28]. The latter two methods comprise Dutch Turner references and are thought to be the methods of choice in a therapeutic intervention study [28].

BIOCHEMICAL PARAMETERS

Prior to any treatment all girls underwent an Arginine provocation test. Arginine 0.5 g/Kg body weight was infused in 30 min. Blood samples were drawn at 15-min intervals from -15 to +60 min and every 30 min during the second hour. Additional blood was taken for the determination of IGF-I, IGFBP-3, and GHBP at the start of the test (pretreatment) and subsequently at 6 and 18 months after the start of the present study. All blood samples were stored on ice for no more than 3 hours until centrifugation. The plasma samples were frozen (-20° C) until assayed 1-3 weeks later (IGF-I) or 3 years later (IGFBP-3 and GHBP).

HORMONE ASSAYS

The RIA measurements of plasma IGF-I and IGFBP-3 were performed as described previously [15,29,30]. The 95th percentile for peak pubertal levels in a normal female population for IGF-I and IGFBP-3 are 700 μg/L and 5 mg/L, respectively. Plasma GHBP was determined by RIA [31,32]. All measurements were performed in the same laboratories.

STATISTICAL ANALYSIS

Results are expressed as median (minimum;maximum), unless indicated otherwise. To test between group differences the Mann-Whitney U or Chi-square test was used and within group differences were tested with the Wilcoxon signed rank test. Correlations were tested with the Pearson's linear correlation coefficient. For each combination of a growth response variable (the change in $HSDS_{CA}$ or bone maturation) and a baseline measurement -CA, BA, BA retardation (=CA-BA), HV, $HSDS_{CA}$, HVSDS, maximal GH peak after stimulation, and GHBP, IGF-I, and IGFBP-3 plasma levels- a multiple linear regression analysis, adjusted for treatment group, was done. A P-value < 0.05 was considered significant.

RESULTS

Table 1 lists the baseline clinical data of the girls. There were no relevant differences between groups for any of the variables. Prior to treatment the mean CA and BA of the girls in BID injection group was slightly older and their mean $HSDS_{CA}$ was about 1 SDS higher compared with those in the OD group. One girl in the BID group was already remarkably tall at start of study, $HSDS_{CA}$ (DSD) 3.54. The following frequency-groups of the maximum GH levels (mU/L) during a pretreatment Arginine-stimulation test were made: <10, ≥10 and <20, and ≥20, with 4, 10, and 5 girls, respectively.

Table 1. Pretreatment clinical data, median (range).

	OD Group (N=9)	BID Group (N=10)
CA (y)	13.3 (11.2; 16.7)	13.8 (11.0; 17.6)
RUS BA (y)	12.2 (10.3; 13.3)	12.7 (11.1; 13.9)
$HSDS_{CA}$ (RvW)	-3.66 (-6.02;-2.11)	-2.70 (-5.16;-1.21)
$HSDS_{CA}$ (DSD)	0.19 (-1.54; 1.56)	1.09 (-0.64; 3.54)
$HVSDS_{CA}$ (DSD)	0.52 (-1.02; 1.92)	0.45 (-1.17; 1.91)
PTS_{RUS} (cm)	148.3 (137.8;155.3)	152.3 (142.4; 163.9)
MPH (cm)	170.2 (163.4;175.4)	173.1 (161.5; 180.3)
BMI-SDS	1.38 (-0.27; 3.62)	0.86 (-0.84; 4.29)
max GH (ATT)	15 (2 ; 27)	13 (3; 34)
Karyotype:		
45,X	6	8
other	3	2

OD:	once daily GH injections; total dose 6 IU/m^2/day
BID:	the total GH dose of 6 IU/m^2/day is divided in one-third in the morning and two-thirds at bedtime
CA:	chronological age
RUS BA:	bone age, RUS-score
$H(V)SDS_{CA}$:	height (velocity) standard deviation score for chronological age
RvW:	healthy Dutch girls references (Roede en van Wieringen)
DSD:	Dutch-Swedish-Danish references for girls with TS
PTS_{RUS}:	Turner-specific FH prediction method (RUS bone) age
MPH:	midparental height
BMI-SDS:	body mass index standard deviation score
max GH (ATT):	maximum GH response during an Arginine tolerance test

Table 2. Growth response, mean (SD), during two years of GH treatment for both frequency regimens.

		OD Group	BID Group
HV (cm/y)	pretreatment	3.7 (1.0)	3.5 (1.1)
	year 1	8.0 (1.4)*	7.6 (2.2)*
	year 2	5.4 (1.3)*+	4.4 (1.7)+
$HVSDS_{CA}$	pretreatment	0.5 (0.9)	0.5 (1.1)
	year 1	5.4 (1.2)*	5.3 (2.0)*
	year 2	3.1 (1.0)*+	2.3 (1.4)*+
$\Delta HSDS_{CA}$	year 1	0.9 (0.2)	0.9 (0.4)
	year 2	0.5 (0.2)+	0.3 (0.2)+
H gain in 2y	untreated	5.2 (1.0)	4.9 (1.1)
(cm)	treated	8.2 (1.9)#	7.2 (3.1)#
ΔBA/ΔCA (y/y)	year 1	0.6 (0.5)	0.6 (0.4)
(y/y)	year 2	0.8 (0.4)+	0.8 (0.4)
(y/2y)	2 years	1.4 (0.8)	1.2 (0.6)
(y/2y)	untreated	1.1 (0.2)	1.1 (0.2)
$\Delta mIPH_{RUS}$ (cm)	year 1	4.1 (2.1)	4.3 (1.5)
	year 2	1.9 (1.8)+	2.1 (1.5)+
ΔBP (cm)	year 1	6.6 (2.0)	5.2 (2.6)
	year 2	3.0 (1.3)+	2.1 (1.7)+
ΔPTS_{RUS} (cm)	year 1	4.6 (1.8)	4.6 (2.3)
	year 2	2.4 (1.8)+	1.2 (1.5)+

*: change from pretreatment different (P<0.05) within group
+: within group difference (P<0.05) between two treatment years
#: within group difference (P<0.05) between untreated (estimated) vs treated girls

OD: once daily GH injections; total dose 6 IU/m^2/d
BID: total GH dose (6 IU/m^2/d) divided: 1/3 in the morning and 2/3 at bedtime
HV: height velocity
$HVSDS_{CA}$: height velocity SD-score for CA (using Dutch-Swedish-Danish standards)
$\Delta HSDS_{CA}$: change in height SD-score for CA (Dutch-Swedish-Danish standards)
H gain: estimated gain in cm after 2 years
ΔBA/ΔCA: change of RUS bone age per chronological year
$\Delta mIPH_{RUS}$: change of modified index of potential height
ΔBP: change in Bayley & Pinneau FH prediction
ΔPTS_{RUS}: change of Turner specific height prediction method

Table 2 shows HV and $HVSDS_{CA}$ during the two study years, the change in $HSDS_{CA}$, the gain in height of the treated groups in relation to estimated untreated values, bone maturation of the treated groups over the estimated untreated values, and for three FH prediction methods, $mIPH_{RUS}$, BP, and PTS_{RUS}. None of the group comparisons were significantly different. However, some minor differences or trends were observed:

The mean HV both in cm/yr and as SD-score was higher during the second year of study in the OD group compared with the BID group. Likewise, the mean change of $HSDS_{CA}$ during the second year and over both years of study was higher in the OD group compared with the BID group. The gain in height was also not significantly different between groups, while each of the means of the treated groups was significantly higher than the estimated value if the girl had not been treated. Three of the 9 girls (33%) in the OD group and 7 of the 10 (70%) girls in the BID group had reached a height ≥150 cm after two years of treatment. The mean change of RUS BA per chronological year (ΔBA/ΔCA) was almost identical between groups, both when treated and (estimated) untreated. Each of the means of the treated groups was not significantly different compared with the mean expected change when not treated. In both groups the bone maturation in the second year was somewhat faster than in the first year of treatment. Bone maturation after two years GH was significantly related, negatively with the age at start of treatment (r=-0.63,

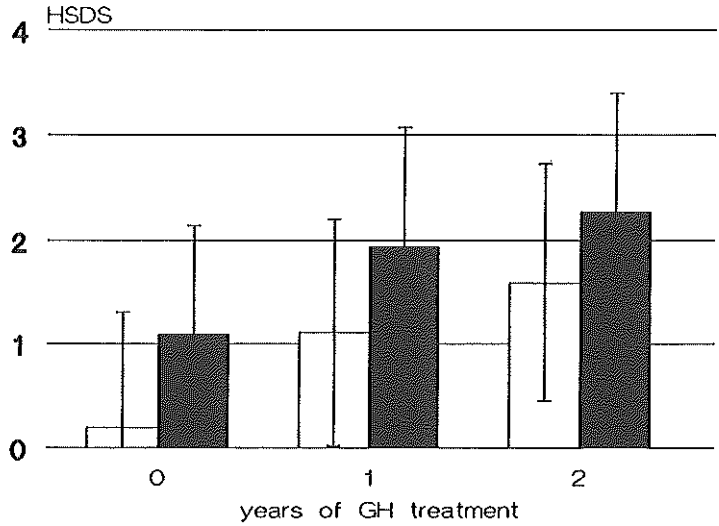

Figure 1. Development of the mean (SD) $HSDS_{CA}$ for the OD group (▭) and the BID group (▬).

P<0.0001) and positively with the degree of BA retardation at start of study (r=0.67, P=0.0003). Except for the BP method, all FH prediction methods showed improvements after two years of treatment in the same order of magnitude, without differences between groups. The mean change in two years of BP and PTS_{RUS} was somewhat higher in the OD group than in the BID group; only $mIPH_{RUS}$ showed the reverse result. The SD of all methods was substantial.

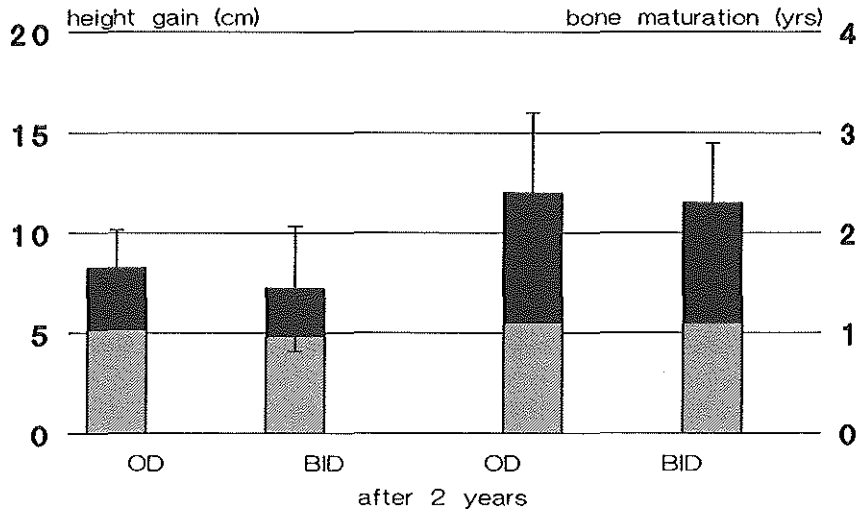

Figure 2. Stacked mean (SD) of treated (▓▓) over estimated untreated (▨▨) values for girls with TS after two years GH treatment, both for gain in height (in cm) and for bone maturation (y/2y) in the OD and BID group.

PUBERTAL DEVELOPMENT

The development of the Tanner breast stages was not significantly different between the OD and the BID group: at end of study the distribution for stages B1 to B5 was 1-4-4-0-0 and 0-5-3-1-1, respectively. Therefore, pooled data is compared with normal British girls with spontaneous puberty [23]. The mean CA at onset of puberty, defined as Tanner breast stage B2, was 15.1 (1.7) years as opposed to 11.2 (1.1) in the reference group. One girl was still prepubertal after 2.25 years of low-dose estrogens. For twelve girls known to have changed during the study period from Tanner breast stage B1 to B2 the median duration of the interval between Tanner stage B2 and B3 was 1.72 (SE 0.27) years. This was significantly slower compared with the 0.93 (SE 0.06) years in the reference group. By the end of the study period 9 girls had reached stage B3 at a mean CA of 15.8 (1.6) years compared with 13.1 (1.2) years in the reference group. One girl experienced her menarche at the age of 15.7 years, 21 months after initiation of estrogen treatment.

BIOCHEMICAL PARAMETERS

Plasma levels of IGF-I, IGFBP-3, and GHBP were not significantly different between groups at any point in time or as change over time, although the change from 6 to 18 months of therapy for IGF-I (P=0.06) and IGFBP-3 (P=0.07) approached the 5% level of significance (Table 3). The change after 6 months from pretreatment in the IGF-I to IGFBP-3 ratio was significantly different between treatment groups (P=0.04).

After the first six months of continuous GH treatment all groups showed a similar pattern: a significant decrease in GHBP levels and a significant increase in IGF-I and IGFBP-3 levels, and in their ratio. After 18 months daily GH therapy the GHBP levels showed an increase compared with the levels after 6 months (P-value for OD 0.08, for BID 0.33); the decrease compared with pretreatment was no longer significant (P=0.09 for OD and BID). From 6 to 18 months of GH treatment increments were measured: for IGF-I levels in both groups (OD: P=0.008), for IGFBP-3 only in the OD group (NS), whereas the BID group showed a significant decrease (P=0.03). After 18 months of GH treatment, IGF-I and IGFBP-3 levels, and their ratio increased significantly (P<0.02), irrespective of treatment group.

Table 3. Median (range) of GHBP, IGF-I, IGFBP-3 plasma levels, and the IGF-I to IGFBP-3 ratio for both treatment regimens at baseline, 6 and 18 months after initiation of GH therapy.

	Gr	Baseline	6 Months	18 Months
GHBP	OD	14.9 (9.2-28.2)	9.2 (6.9-17.2)*	13.2 (8.1-15.1)
(mcg/l)	BID	11.8 (8.8-20.6)	9.8 (6.6-13.6)*	9.6 (6.6-24.3)
IGF-I	OD	140 (63-303)	470 (287- 680)*	696 (357-756)*
(mcg/l)	BID	203 (66-251)	467 (365-1027)*	586 (47-840)*
IGFBP-3	OD	2.9 (1.2-3.9)	3.5 (2.6-5.9)*	4.6 (2.8-5.7)*
(mcg/ml)	BID	3.2 (2.5-4.4)	5.6 (3.7-6.2)*	4.4 (2.5-5.6)*
IGF-I:IGFBP-3	OD	55.5 (31.1-94.6)	113.5 (79.6-183.3)*	149.2 (83.7-207.
	BID	60.8 (44.0-97.7)	96.9 (79.4-273.0)#	137.9 (10.3-245.

Gr: Group
OD: once daily; BID: twice daily injections
*: change from baseline significantly different
#: change from baseline significantly different between groups

RELATIONSHIPS WITH GROWTH RESPONSE

Multiple linear regression analyses of the change in $HSDS_{CA}$ after two years GH treatment (dependent variable), adjusted for treatment group, revealed a negative, significant correlation with both baseline age at start of treatment (beta=-0.15) and $HSDS_{CA}$ (beta=-0.2), whereas pretreatment BA retardation was positively related (beta=0.29). The change in $HSDS_{CA}$ after two years GH was not related to pretreatment HV, HVSDS, maximum stimulated GH levels, plasma GHBP, IGF-I, or IGFBP-3 levels, or IGF-I to IGFBP-3 ratio; nor to the change after the first 6 months of GH treatment in plasma GHBP, IGF-I, or IGFBP-3 levels, or IGF-I to IGFBP-3 ratio.

Both baseline CA and BA retardation were significantly related to bone maturation after two years, beta's -0.11 and +0.23, respectively. Neither the gain in height, nor the gain in FH prediction for each of the three methods were related to the age at start of treatment.

Multiple linear regression analyses using the subgroup of girls (N=10) of the cross-over study showed no significant relationship between the change in $HSDS_{CA}$ after two years (dependent variable) and the AUC for GH or the maximum GH plasma level during the 24h test corresponding to their injection regimen during the present study.

DISCUSSION

GROWTH EVALUATION

A division of the total GH dose in 2/3 in the evening and 1/3 in the morning is not advantageous over the OD GH regimen after two years treatment with respect to the growth response expressed as HV, HVSDS, the change in $HSDS_{CA}$ and the gain in height. These parameters tended towards higher mean values with OD GH compared with BID GH, but significant differences between the treatment groups were not found. In contrast, mean bone maturation during the study period was similar between the groups and compared with untreated estimated values. All FH prediction methods showed improvements, again without group differences. The change in $HSDS_{CA}$ after two years GH treatment was -independent of treatment group- related, negatively to the baseline CA and $HSDS_{CA}$, and positively to BA delay at baseline.

During 24h GH profile tests in ten of the girls [15] it was shown that with the BID regimen, a second GH injection at 8 a.m. in the morning with only 1/3 of the total dose resulted in a detectable increase in GH plasma levels. Nevertheless, the divided dose regimen resulted in a loss of GH bioavailability since the mean AUC was 3.1 times higher for the night-time period compared with the day-time period; also, the mean AUC values for the night-time period were 1.9 times higher with OD than with BID GH. Therefore, the AUC over the 24h period differed at the 6% level of significance detriment of the BID

regimen. The trend towards a higher AUC for GH and $\Delta HSDS_{CA}$ with OD after two years GH treatment in the present study is also in line with a correlation between these two variables in a normal population [3].

Growth in childhood and adolescence has been shown to be mainly GH pulse amplitude modulated with a relatively fixed periodicity [33]. During the cross-over study with ten girls the maximum GH plasma value was significantly higher with OD GH than with BID GH treatment [15]. The growth response in the present study tended also to be in favour of the OD GH regimen. Based on the assumption that equal dosages result in similar peak levels, it could be argued that an equal division of the dose would be more advantageous than a twothird-onethird division used in the present study. However, a study in GHD children showed that the peak serum GH levels and the AUC following a morning GH injection of 2 IU subcutaneously were significantly lower when compared with the same injection in the evening [34]. In a study comparing an OD GH injections (20 IU/m^2/wk) with an equally split thrice daily GH regimen in short children [35] the 'waning'-effect of HVSDS during the second year of treatment was less pronounced with the OD regimen. In a Belgian study in girls with TS, comparing an OD regimen with an equal division of a lower daily GH dose (25 IU/m^2/wk) [14], the change in HV after one year treatment was somewhat, but not significantly lower with OD compared with BID GH injections. In most studies the trends are in favour of undivided doses, but perhaps the treatment groups have been too small and/or the treatment period was too short. Furthermore, the possibility of endogenous GH production in short children and girls with TS cannot be ruled out. Finally, different GH injection regimens may have other long-term effects on carbohydrate and lipid metabolism [36].

GH BINDING PROTEIN

In both frequency groups GHBP plasma levels after 6 months GH treatment were lower compared with pretreatment. This is in line with the two-week cross-over study [15], despite the use of different assays. After 18 months GH treatment mean GHBP levels were still lower than pretreatment but the overall decrease was no longer significant. In contrast, Saggese *et al.* [37] observed an upward trend in GHBP plasma levels, which became significantly different from baseline after one month GH treatment (about 4 IU/m^2/day), but returned to baseline again during the second half of the first year of GH treatment. Carlsson *et al.* [38] found a decrease in GHBP plasma levels when TS girls were treated with GH only (0.1 IU/Kg/day), but when ethinyl estradiol (0.1 mg/Kg/day) was given in addition the GHBP levels showed an upward trend, which became significantly different from baseline after 12 months treatment. In the present study all girls started with estrogens concurrently with GH therapy. These studies indicate that GHBP is influenced by the duration of GH treatment as well as by estrogen treatment, although GHBP levels in untreated girls with TS were not significantly different from controls at a pubertal age [39].

A decrease in GHBP levels could well explain the growth response, since it suggests a higher proportion of unbound GH. In line with an earlier report [37], a linear relationship between GHBP levels and the growth response following

GH treatment, as has been described in GHD children [40], could not be demonstrated in TS.

IGF-I AND IGFBP-3

The increase in plasma IGF-I and IGFBP-3 levels after two weeks GH was not significantly different between the two frequency regimens during the cross-over study [15], nor during the present study at any point in time. In contrast, in a four-week cross-over study with GHD adults the division for BID GH was identical to that in the present study and also compared with an OD GH regimen (3 IU/m^2/day) [36]. In both studies above IGF-I and IGFBP-3 plasma levels showed a remarkably similar pattern over the day with the two injection frequencies. This suggests that the generation of these GH dependent proteins in the two patient groups is independent of the GH injection frequency during a 24 hour period. In the GHD study mean IGF-I plasma levels with BID GH were significantly higher than with OD GH, whereas IGFBP-3 plasma levels were comparable [36]. The authors proposed that the continuous presence of GH was more important for the generation of IGF-I than of IGFBP-3. In TS [15], however, the mean AUC for GH during the day-time period was only slightly greater with BID GH injections than with a OD regimen. Endogenous GH secretion in our girls with TS cannot be excluded, although the day-time plasma GH levels during the OD GH regimen were close to zero and showed no significant pulses during the cross-over study [15].

Ranke *et al.* [41] have suggested a relationship between plasma levels of IGF-I and IGFBP-3, and/or the IGF-I to IGFBP-3 ratio with the growth response following OD GH therapy in TS. In the present study no such relationship was found. The change in $HSDS_{CA}$ after two years GH (in combination with low-dose estrogens) was neither correlated with the pretreatment levels of IGF-I, IGFBP-3, or their ratio, nor to the change in these values during the first 6 months of continuous GH therapy.

BONE MATURATION

In a Belgian frequency response study in girls with TS [14] the mean bone age increment during the first year of treatment was faster (1.3 year) compared with the present study (0.6 year). This is most likely due to the higher number of younger girls in the former study. A negative relationship between $\Delta BA/\Delta CA$ after one year treatment and age at start of treatment was reported in the Belgian study and confirmed in the present study (beta -0.11); the relation between the BA delay and bone maturation after two years of treatment is even stronger, beta +0.23. In both studies bone maturation during GH treatment was not significantly different between the frequency groups. In the present study bone maturation after two years GH treatment was faster than the estimated values should no treatment have been given.

ESTROGEN EFFECT

Despite GH therapy, the progression through puberty on this low estrogen dose (0.05 μg/Kg/day) seemed slower in a subgroup of girls compared with the British reference population as indicated by the mean interval from B2-B3, 1.72 (SE 0.27) vs 0.93 (SE 0.06) year for TS and reference girls, respectively. In the present study progestagens were not (yet) administered. Only one girl experienced menarche.

CONCLUSIONS

The present growth data are in conformity with the data of the earlier 24-hour GH profiles. The growth response after two years GH on a total dose of 6 IU/m^2/day in combination with low-dose estrogens was not significantly different between the once daily and the twice daily GH injection regimens. Only, a tendency in favour of once daily injections could be observed. After 18 months of GH treatment the significant decrease in GHBP plasma levels observed after 6 months was no longer significant. In contrast, IGF-I and IGFBP-3 plasma levels and the IGF-I to IGFBP-3 ratio increased significantly during 18 months GH therapy. None of these growth related factors showed a difference between groups in their change over 18 months. Relevant side-effects were not observed during the first two years of GH treatment. Moreover, bone age maturation was not significantly different between groups, nor from untreated girls with TS. Compliance proved to be equally good between groups, showing that the treatment was well accepted even by the group which injected GH twice daily. All girls agreed to continue GH therapy in their randomized groups until FH.

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Chapter 7

Body proportions in girls with Turner syndrome prior to and during various growth hormone dosing regimens

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BODY PROPORTIONS IN GIRLS WITH TURNER SYNDROME PRIOR TO AND DURING VARIOUS GROWTH HORMONE DOSING REGIMENS

ABSTRACT

In 87 girls with Turner syndrome (TS), aged 2-16 years, concerns about disproportionate growth with long-term growth hormone (GH) treatment were addressed in a 4 year dose-response and in a 2 year administration frequency response study by using *z*-scores and shape (*f*)-values for combinations of measurements. Comparison was made to healthy Dutch girls and to untreated girls with TS.

Although baseline *z*-scores and *f*-values were 'typical' compared with age-matched girls with TS, the vast majority of the girls in the two studies (>85%) had 'atypical' baseline *z*-scores and *f*-values compared with age-matched healthy girls. In general, these values worsened with age. GH treatment resulted in significant improvements of most *z*-scores. The change from baseline to end of study was only significantly dose-dependent for *z*H, *z*Hand, and *z*Foot. During the GH studies *f*-values improved significantly towards the 'typical' range of the normal female reference population, in particular at younger ages and for *f* H&Hand, *f* H&Foot, and *f*H&Biiliacal diameter. A significant dose-dependent improvement was only noted for *f*H&Biiliacal diameter. The change at end of study from baseline in *z*-scores and *f*-values was negatively related with its baseline value and age, only in the FR study the change *z*-scores showed a positive relationship with baseline age.

INTRODUCTION

Impaired growth is usually present in girls with Turner syndrome (TS). Reports on body proportions in these girls are scarce. Most studies on girls with TS only present standards for height (H) and sitting height (SH). Impaired growth of the lower extremities has been reported by a number of investigators [1-5], others found that girls with TS were normally proportioned despite their short stature [6,7]. In one study measurements of body width have been described. In comparison to height a relatively large Biiliacal diameter and Biacromial diameter were observed [4]. But, other parts of the body might also be affected by lack of growth.

In the past decade growth hormone (GH) has been administered in disorders with short stature. One of the concerns of long-term GH treatment is

Table 1. Baseline clinical data of the groups for both studies.

	DR study			FR study	
	Group A	Group B	Group C	OD Group	BID Group
Number of girls	23	23	22	9	10
CA (yr)	6.3 (2.2)	6.8 (2.4)	6.5 (2.3)	13.3 (1.7)	13.8 (1.8)
RUS BA (yr)	5.6 (2.3)	6.2 (2.6)	5.9 (2.4)	12.2 (1.0)	12.7 (0.9)
$HSDS_{CA}$ (DSD)	0.1 (1.0)	0.3 (1.1)	0.1 (1.1)	0.2 (1.1)	1.1 (1.3)
karyotype:					
45,X	19	21	17	6	8
other	4	2	5	3	2

DR: dose-response
FR: administration frequency-response
CA: chronological age
RUS BA: bone age according to Tanner & Whitehouse RUS-score
$HSDS_{CA}$ (DSD): height standard deviation score for CA (according to the Dutch-Swedish-Danish Turner reference population[19])

disproportionate growth of various parts of the body. To our knowledge only Gerver *et al.* [8] described in detail the results of two years GH treatment on body proportions in a previous Dutch Turner study. Apart from SD-scores, the concept of 'shape values' [9,10] was used to describe combinations of measurements.

As in other disorders, the growth response to GH treatment in TS appeared to be dependent on the dose and frequency of administration [11]. In this report we present data on body proportions using (an adapted version of) the shape value to describe possible changes during two GH treatment studies in girls with TS: a four year dose-response study and a two year administration frequency-response study. For a number of combinations of measurements in all girls (age range 2-16 years) comparison is made to healthy Dutch girls [12] and to untreated girls with TS.

SUBJECTS AND METHODS

STUDY GROUPS

Girls with TS were enrolled in two nationwide, multicentre trials. The diagnosis was confirmed by lymphocyte chromosomal analysis. Clinical data for the girls, all prepubertal (Tanner stage B1[13]), are listed in Table 1. The girls were selected to have a height below the 50th centile for Dutch children [14], and a normal thyroid function. Exclusion criteria were: endocrine and metabolic disorders, growth failure due to other disorders or emotional deprivation, hydrocephalus, the (previous) use of drugs for another study or drugs that may interfere with GH therapy, abnormalities in general paediatric- and biochemical screening. Written, informed consent was obtained from the parents. The study protocol was approved by the Ethics Committee of each participating centre. In both studies biosynthetic human GH (Norditropin^R, Novo Nordisk A/S, Denmark) was daily injected subcutaneously by means of a pen injection system (Nordiject^R 24).

DOSE-RESPONSE (DR) STUDY

Sixty-eight girls, aged 2-11 years, participated in a 4 year GH dose-response study. The girls were randomized into 3 GH dosing groups with stratification according to chronological age (CA) and standard deviation score of height for CA ($HSDS_{CA}$):

- **A** (N=23) 4 IU GH/m^2 body surface/day for 4 years,
- **B** (N=23) 4 IU in the first year and during the second through the fourth year 6 IU GH/m^2/day,
- **C** (N=22) 4 IU in the first year, 6 IU in the second year, and during the third and fourth year 8 IU GH/m^2/day.

In each group only one girl dropped out of the study for the following reasons: (a) non-compliance, (b) alleged increase of muscle mass and decline in school performance according to the mother, and (c) desire to initiate estrogen therapy before the end of study. In this study 7 girls were of non-Caucasian origin, 3, 3, and 1, in groups A, B, and C, respectively. For three of the youngest girls (2 in Group A and 1 in Group B) it was impossible to perform the measurements at start of study.

FREQUENCY-RESPONSE (FR) STUDY

Nineteen girls, aged 11-16 years, took part in a two year study on the effect of two administration frequency regimens. After matching for bone age (BA) and $HSDS_{CA}$ age, the girls were randomly divided into two GH injection frequency groups. One group (N=9) received 6 IU GH/m^2 once daily (OD), and a second group (N=10) received the same total GH dose divided in one-third in the morning and two-thirds at bedtime (BID injections). In addition to GH therapy the girls received ethinyl estradiol (0.05 µg/Kg/day orally) to induce puberty.

Immediately prior to the present study, all girls followed the same 10-week cross-over design as described earlier [15]. In brief, the girls started after 4 weeks on ethinyl estradiol with 2 weeks GH in either frequency group. Following a wash-out period of 2 weeks they received GH again, but in the alternate administration frequency. In this study 5 girls were of non-Caucasian origin, 3 in the OD groups and 2 in the BID group.

STUDY PROTOCOL

Prior to continous treatment (baseline) and subsequently every six months all girls were seen at their local hospital. All measurements were performed by one anthropometrist (AT) according to Cameron [16]. H and SH were determined using a Harpenden stadiometer and SH table. The other measurements of the left hand (Hand), and foot (Foot), as well as biacromial (Biac) and biiliacal (Biil) diameter were taken with a Harpenden anthropometer. Bone ages were determined by a single observer (AT) according to the Tanner & Whitehouse RUS-score [17].

SHAPE CHARACTERISTICS

Standard deviation or *z*-scores were calculated using 1105 healthy girls aged 6 weeks to 19 years from the Dutch Oosterwolde study [12]. In addition, a reference population of 187 untreated girls with TS (CA ranging from 2 to 20 years) was measured prior to possible admission in GH treatment studies. Forty-nine girls of this Turner reference population lacked measurements for Hand, Foot, Biac, and Biil. In the original description of the healthy reference population [10] a division in age-classes was made. However, for this study an altered method was used. For each individual patient a number of girls, N, from the reference population closest to her age were used to calculate *z*-score; N=50 for healthy girls and N=20 for untreated Turner girls. A girl was considered atypical for a measurement (m) if $|z_m| > 1.96$.

As a description of shape of a population a multivariate normal distribution was considered for which the age-dependent mean and covariance matrix were computed based on either the healthy girls or on untreated girls with TS. The probability that a specific girl belongs to the given reference population is used as a measure to judge the physical shape of that girl. The joint, multivariate distribution of two standardized variables z_m and $z_{m'}$ at age t is characterized by bivariate normality and correlation coefficient r(m,m'). Since z_m-$z_{m'}$ might be regarded as characteristic for shape, a person is atypical if the shape (f)-value [9,10]:

$$|Z_m-Z_{m'}| / (2-2r_t(m,m'))^{1/2} > 1.96$$

Also, a minimal f-value was computed by varying the age of the girl under observation. This minimum f-value provides the age at which the girl's shape would best fit the reference population. Subsequently, the time difference to the minimal f-value can be determined. Finally four 'Appearance' classes are introduced with reference to the 'typical' range for z-scores or f-values (<1.96):

- A1: measurements typical, however, they form an odd combination for their age (z-scores ≤1.96, f-value >1.96).
- A2: either or both measurements atypical, however, the combination is typical for their age (z-scores >1.96, f-value ≤1.96.
- A3: both measurements as well as their combination is typical for their age (z-scores and f-value ≤1.96).
- A4: either or both measurements and their combination atypical for their age (z-scores and f-value >1.96). For group A4 a subdivision was made:
  a. the combination of measurements is within the 'typical' range for another age, usually younger. The time difference between the age of the girl and the age which fits the combination of measurements best (minimal f-value) is given;
  b. the combination of measurements is not within 'typical' range for any age.

STATISTICAL ANALYSIS

The results are expressed as means (SD), unless indicated otherwise. Differences between groups were tested by the non-parametric, two-sample Kruskal-Wallis test. Differences between points in time were tested by paired Student's t-tests. Correlations were tested with the non-parametric Spearman's rank correlation test. A P-value < 0.05 was considered significant.

Table 2a. *Z*-scores and shape (∫)-values with reference to healthy Dutch girls prior to start and at the end of GH treatment in the dose-response study. P-values are given for significant changes after 4 years between groups.

	Baseline			After 4	yrs		P-value
	Gr. A	Gr. B	Gr. C	Gr. A	Gr. B	Gr. C	
*z*H	-3.1 (1.1)	-2.9 (1.1)	-3.0 (1.1)	-1.4 (1.0)	-1.0 (1.2)	-0.9 (1.1)	0.05
*z*SH	-2.0 (1.1)	-1.7 (1.2)	-2.1 (1.3)	-0.2 (1.2)	0.3 (1.3)	0.1 (1.2)	NS
*z*Hand	-1.9 (1.1)	-1.8 (1.2)	-2.0 (1.0)	-0.5 (1.1)	-0.1 (1.2)	0.0 (1.2)	0.01
*z*Foot	-2.4 (1.0)	-2.0 (1.1)	-2.0 (1.0)	-0.7 (1.1)	0.4 (1.1)	0.4 (1.0)	<.0001
*z*Biac	-1.1 (1.0)	-0.7 (1.1)	-1.1 (0.9)	0.3 (1.1)	1.0 (1.4)	0.6 (1.1)	NS
*z*Biil	-1.4 (0.9)	-0.9 (0.9)	-1.3 (0.8)	0.0 (0.9)	0.9 (1.0)	0.0 (0.9)	NS
∫H&SH	3.5 (0.9)	3.5 (1.1)	3.5 (1.0)	3.0 (0.9)	3.0 (1.0)	2.6 (0.9)	NS
∫H&Hand	3.4 (1.0)	3.2 (1.1)	3.1 (1.1)	2.0 (0.8)	1.8 (1.0)	1.9 (0.7)	NS
∫H&Foot	3.3 (1.0)	3.2 (1.1)	3.2 (1.1)	1.9 (0.8)	2.3 (1.2)	2.3 (0.8)	NS
∫H&Biac	3.6 (1.0)	3.6 (1.0)	3.3 (1.1)	2.5 (0.8)	2.7 (1.0)	2.1 (0.7)	NS
∫H&Biil	3.5 (0.8)	3.6 (1.3)	3.3 (1.1)	2.4 (0.9)	2.7 (1.1)	1.8 (0.8)	0.06
∫Biac&Biil	1.7 (0.8)	1.4 (0.7)	1.7 (0.8)	1.2 (0.6)	1.8 (1.0)	1.3 (0.7)	0.08

RESULTS

Z-SCORES

In general all *z*-scores improved significantly within treatment group. Therefore, emphasis is on differences in improvement (Tables 2a and 2b, and Figures 1a, 1b, 3a, and 3b). Over 4 years of GH therapy in the DR study the change in mean *z*H showed a dose-regimen dependent increase, which was only significantly higher for Group C compared with Group A (P=0.01). During the second year of treatment -after their first increase of the dose- Group B and C showed a similar development, both higher compared with constant dose-group A (P=0.06). During the third year of GH -after yet another dosage increase only in Group C- the increase in mean *z*H for Group C was larger than that of Group B, although not significantly (P=0.09).

Although in the DR study the mean baseline levels of the *z*-scores for SH, hands, and feet were higher than that of *z*H, their development is similar to that of *z*H. In general, the mean baseline *z*-scores for Group B were somewhat higher than in Group A and C. On the other hand, Group

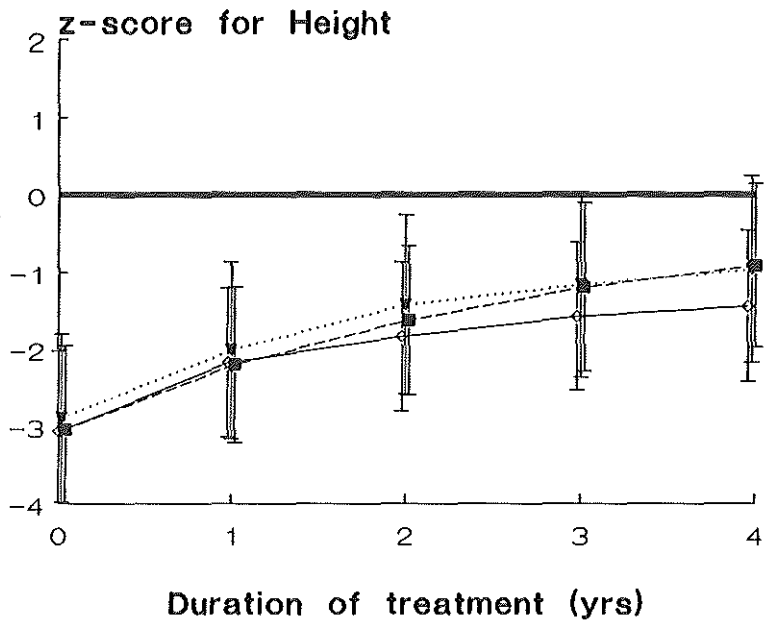

Fig. 1A

Figure 1. Development of the mean (SD) *z*-scores for Height (a) and Sitting Height (b, see next page) for each GH treatment group (A:—◇—; B: ····▼···· ; C: – ■–) in the dose-response study. The horizontal line at *z*=0 represents the mean for age-matched healthy girls.

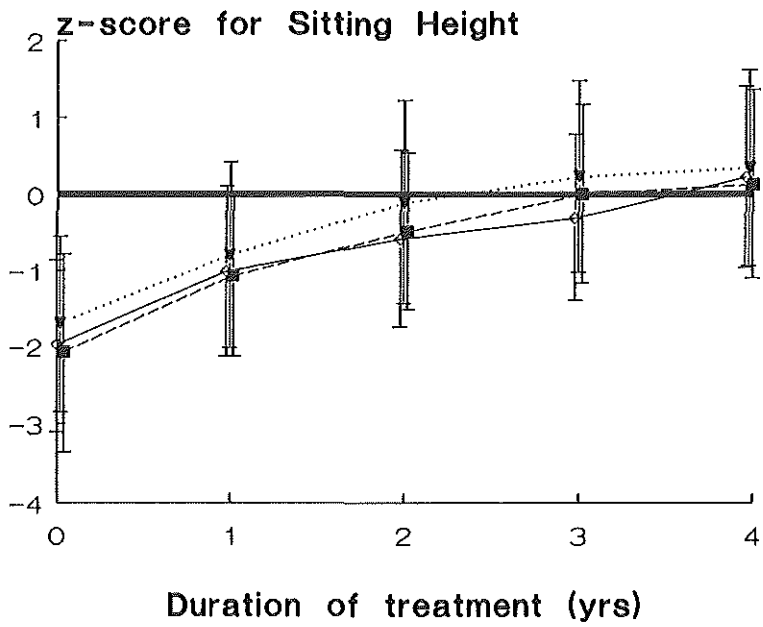

Fig. 1B

C shows the largest change in mean *z*-scores during 4 years treatment, except for *z*Foot. The mean increase of *z*Foot in Group B and C was similar and larger than in Group A. Compared with the other *z*-scores, baseline mean *z*-scores for Biac and Biil were markedly higher (about -1) and their development between groups was also markedly different. The mean *z*-scores for Group B remained during treatment at a higher, parallel level compared with Group A and C. After 4 years GH mean values for *z*Biac were positive in all groups, whereas for *z*Biil only Group B had a mean value markedly higher than zero.

Mean baseline *z*-scores in the FR study were only for *z*H and *z*SH markedly lower than those in the DR study. Also, all OD group means were smaller compared with those of the BID group (NS). The mean change in *z*-scores during the 2 years FR study was similar for H, Biac, and Biil; for H and Biac in the OD group more than in the BID group. For *z*SH and *z*Hand the mean 2 years change for the OD group was less than for the BID group, for *z*Foot this change was significantly larger in the OD group (P=0.05). Within treatment groups, only *z*SH in the OD group did not change significantly.

SHAPE ($\int$)-VALUES

Since the deficit, expressed as mean *z*-score, with reference to healthy Dutch girls was larger for *z*H compared with the other standardized measurements, the mean $\int$-value was likely to be outside the range of

Table 2b. *Z*-scores and shape ($\int$)-values with reference to healthy Dutch girls prior to start and at the end of GH treatment in the frequency-response study. P-values are given for significant changes after 2 years between groups.

	Baseline		After 2 yrs		P-value
	OD Gr.	BID Gr.	OD Gr.	BID Gr.	
*z*H	-4.2	-3.4	-3.3	-2.7	NS
	(1.7)	(1.3)	(1.2)	(1.3)	
*z*SH	-3.1	-2.7	-2.8	2.2	NS
	(1.5)	(1.9)	(1.1)	(1.9)	
*z*Hand	-2.6	-2.1	-1.2	-0.4	NS
	(0.9)	(1.1)	(1.0)	(1.6)	
*z*Foot	-2.4	-1.4	-1.0	-0.5	0.05
	(1.1)	(1.3)	(1.0)	(1.3)	
*z*Biac	-1.5	-1.0	-0.7	-0.5	NS
	(1.1)	(0.9)	(0.8)	(1.2)	
*z*Biil	-1.3	-0.6	-0.6	0.1	NS
	(1.0)	(1.2)	(1.0)	(1.2)	
$\int$H&SH	4.5	3.8	3.4	3.1	NS
	(1.6)	(1.4)	(1.1)	(1.6)	
$\int$H&Hand	4.3	3.6	3.8	3.6	NS
	(1.7)	(1.3)	(1.4)	(1.4)	
$\int$H&Foot	4.3	3.9	3.7	3.3	NS
	(1.6)	(1.1)	(1.0)	(1.2)	
$\int$H&Biac	4.5	3.7	3.5	3.1	NS
	(1.5)	(1.2)	(1.3)	(1.3)	
$\int$H&Biil	4.7	3.9	3.6	3.3	0.05
	(1.4)	(1.1)	(1.0)	(1.2)	
$\int$Biac&Biil	1.9	1.7	1.4	1.4	NS
	(1.1)	(0.6)	(0.8)	(0.8)	

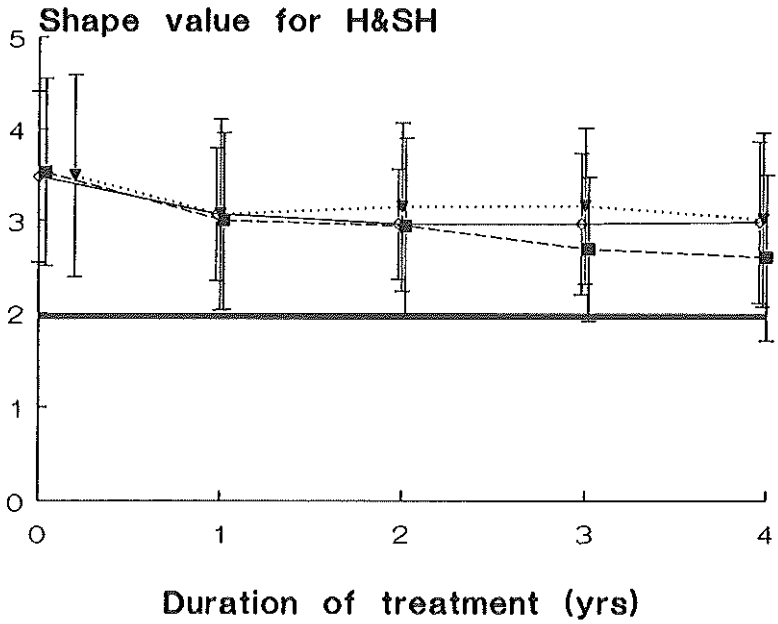

Figure 2. Development of the mean (SD) *f*-value for Height & Sitting height for each GH treatment group (A: —◇—; B: ····▼···· ; C: – ■ –) in the dose-response study. The horizontal line at *f*=1.96 represents the mean for age-matched healthy girls.

'typicality' compared with age-matched healthy girls (*f*>1.96) (Tables 2a and 2b, and Figures 2, 4a, and 4b). Although the mean gain of the various *z*-scores during 4 years in the DR study varied between 1.26 and 2.43 SDS, the mean change in *f*-value during this period was much lower; for *f*H&SH and *f*Biac&Biil the smallest improvements were seen. Note that the mean *f*Biac&Biil of Group B even worsened, but remained within the range for 'typicality'. In both studies most of the *f*-values with relation to height at the end of the study period were significantly lower compared with baseline (within-group comparison), except for *f*H&SH in Group A and B of the DR study, and in the FR study *f*H&Hand (both groups).

In general, the most significant improvement in mean *f*-value was observed during the first year of treatment in the DR study.

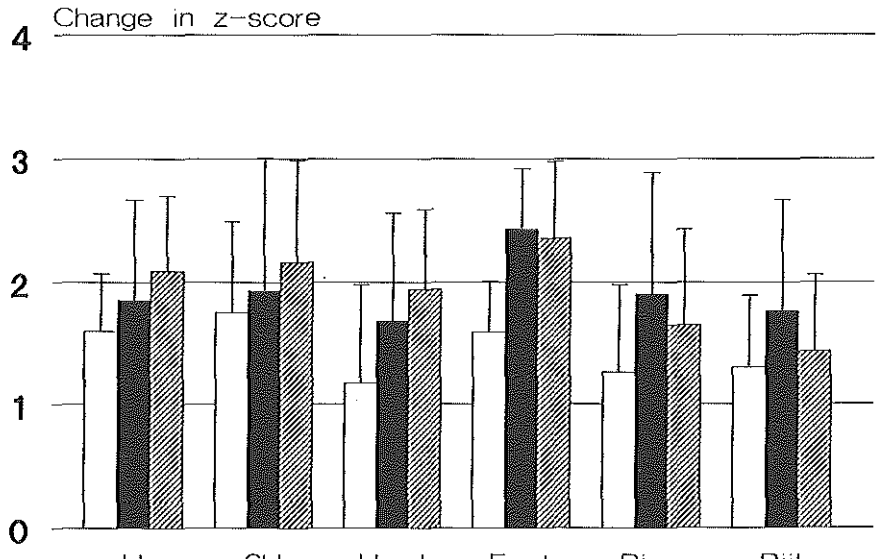

Fig. 3A

Figure 3. Mean (SD) of the change in all *z*-scores (H=height, SH=sitting height, Ha=hand, Fo=foot, Biac=biacromial diameter, and Biil=biiliacal diameter) for each GH treatment group (a) in the 4 years dose-response study (A: ▭; B: ▬; C: ▨) and (b) in the 2 years frequency-response study (OD=once daily: ▭; BID=twice daily injections: ▬).

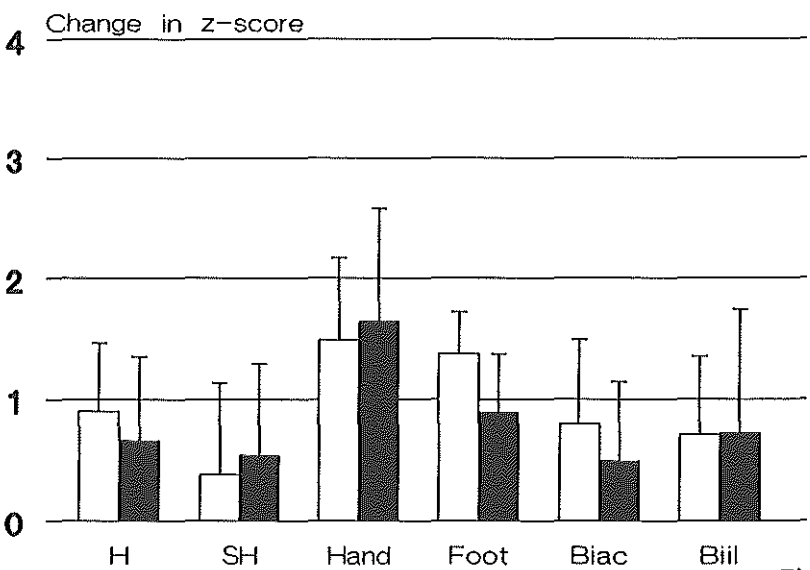

Fig. 3B

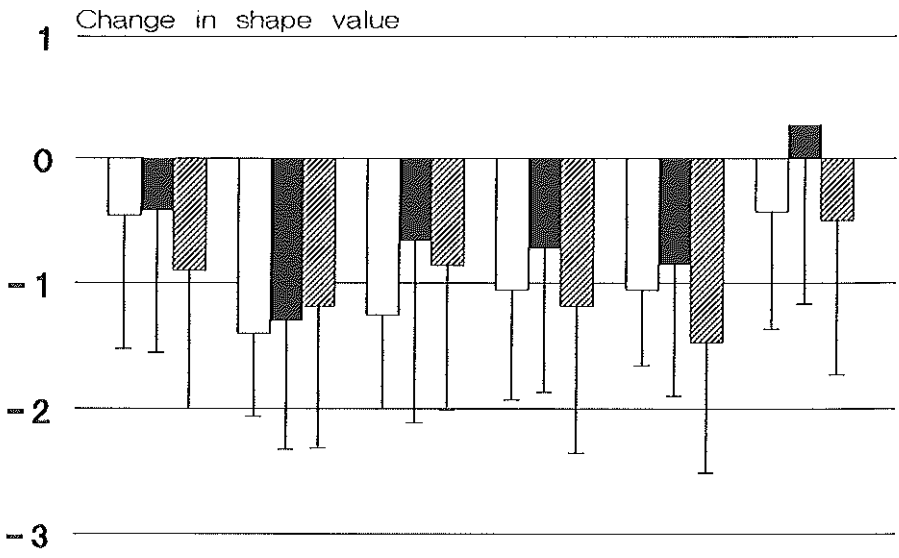

Fig. 4A

Figure 4. Mean (SD) of the change in all shape ($\int$)-values (H&SH=height vs sitting height, H&Ha=height vs hand, H&Fo=height vs foot, H&Biac= height vs biacromial diameter, H&Biil=height vs biiliacal diameter, and B&B=biacromial vs biiliacal diameter) for each GH treatment group (a) in the 4 years dose-response study (A: ☐; B: ▬; C: ▨) and (b) in the 2 years frequency-response study (OD=once daily: ☐; BID=twice daily injections: ▬)

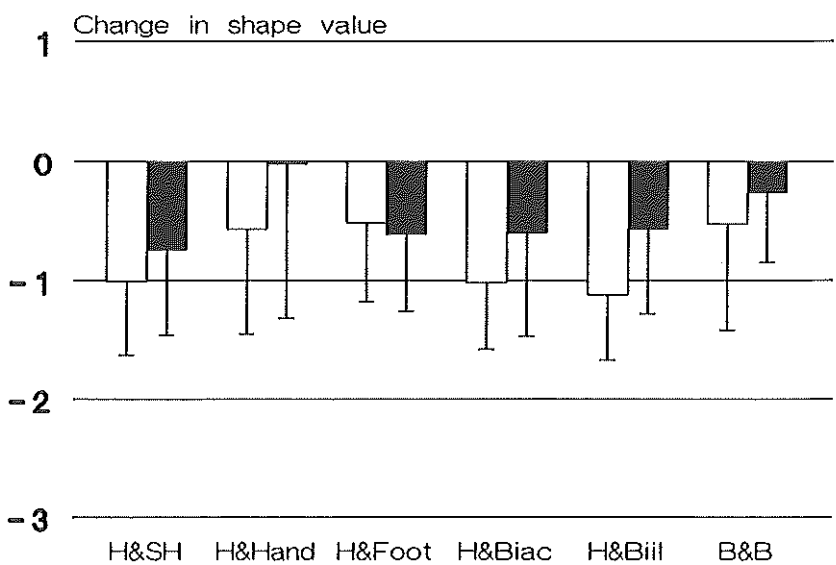

Fig. 4B

The mean *f*-value for H&Biil continued also in the second year to decrease towards the limit of normality. However, thereafter this *f*-value worsened again in Group A and B. As a result Group C was the only group to have a mean *f*-value within the normal limits. The development of the mean *f*-value for H&Biac was similar to that of H&Biil, be it less in magnitude. Again Group C showed the best results. The baseline mean *f*-value for Biac and Biil was already within the range of 'typicality' and showed during GH only minor changes. Group B even showed a slight worsening of this *f*-value over 4 years GH treatment.

The mean *f*-value for H&SH was only reduced by approximately 0.5. The change over 4 years for Group C was markedly higher compared with Group A and B, but no group reached the range of 'typicality'. The mean *f*-value for H&Hand and H&Foot showed a much better result and reached the range of 'typicality'. After the second year of treatment there was a slight worsening of the mean *f*H&Foot for Group B and C compared with Group A. Within treatment groups only *f*H&SH in Group B and *f*B&B in groups B and C did not change significantly.

Mean baseline *f*–values in the FR study were overall higher than in the DR study, except for Biac&Biil. The mean change over 2 years during the FR study was similar for the *f*–values for H&SH, H&Biac, and H&Biil; for those of H&Hand, H&Foot, and Biac&Biil this change was somewhat less, and the BID group showed hardly any improvement in *f* H&Hand. The improvements of the *f*-values in the OD group were in general more pronounced compared with those in the BID group, except for *f*H&Foot, which was higher in the BID group compared with the OD group. Within treatment groups only *f*H&Ha and *f*B&B in both groups did not change significantly.

GIRLS WITH TYPICAL OR ATYPICAL *f*-VALUES

Table 3 shows that in the DR study, the baseline percentages of *f*-values within the 'typical' range were low, except for *f*Biac&Biil. After 4 years GH these percentages increased, but hardly so for *f*Biac&Biil. About one third of the girls had changed from atypical to typical *f*-values, except for *f*H&SH (14%). There were no differences between groups.

In the FR study hardly any *f*-value was within the 'typical' range, neither at baseline nor after 2 years GH, except for *f*Biac&Biil. For *f*H&Foot and *f*H&Biil there were no age-matched 'typical' values at any point in time.

APPEARANCE CLASSES

Table 4 represents the (change in) 'Appearance' class. In the DR study the percentage of girls with typical *z*-scores, but atypical *f*-values (class A1) at baseline was very low, but increased with GH treatment, for *f*H&SH even above 50% of the class. Baseline percentages of girls in class A3 (normal *z*-scores and *f*-values) were similar to class A1, except for *f*Biac&Biil (68% of

Table 3. Percentages of girls in each study with a shape ($\int$)-value within the range of 'typicality' (≤ 1.96) for various body proportion characteristics at start and at the end of study. For each study the percentage of girls that changed from an abnormal to a normal $\int$-value is given.

	DR study			FR study		
	Baseline	After 4 yrs	Change to normal	Baseline	After 2 yrs	Change to normal
$\int$H&SH	4	14	14	0	21	21
$\int$H&Hand	14	55	40	5	11	11
$\int$H&Foot	11	46	35	0	0	0
$\int$H&Biac	6	26	23	5	11	11
$\int$H&Biil	9	42	32	0	0	0
$\int$Biac&Biil	68	72	25	53	84	22

the class). After 4 years GH class A3 also showed an increase, however for other shape values: for /H&Hand and /H&Foot upto ± 50% of the class, and for /Biac&Biil it remained about 70% of the class. At baseline ± 85% percent of the girls had atypical z and /-values (class A4) for all body proportions, except for /Biac&Biil (30% of the class). The mean /-values of almost all girls in class A4 'fitted' to another age. After 4 years GH these percentages dropped considerably in favour of classes A1 and A3. The remaining girls in class A4 had a high percentage of /-values 'fitting' to a younger age (see time difference). The only exception was /Biac&Biil, with a positive mean time difference.

The RUS BA was significantly older compared with the CA corrected for the time difference of /H&SH both at baseline and at the end of the DR study (P<0.0001).

In the FR study the A4 class dominates the general 'picture', indicating that almost all girls at baseline, but also about three quarters of the girls after 2 years GH treatment, had atypical z and /-values. The percentage of girls in class A4 with /-values 'fitting' to another age varied: for H&SH, H&Hand, and H&Biac it changed from ± 65% at baseline to ± 50% of the class after 2 years GH, for /H&Foot it did not change (± 65% of the class), for /H&Biil there was even a slight increase from 11% to 29% of the class. Again /Biac&Biil behaved differently. After 2 years GH the percentage of girls with normal z and /-values (A3) increased from 53% to 83% of the class, and thus the percentage of class A4 reduced. These remaining girls had body proportions not fitting to any age.

RELATIONSHIPS WITH BASELINE VALUES AND AGE

At baseline of the DR study there was a significant, negative relationship between the CA and all *z*-scores (r: -0.23 to -0.43), except for *z*Biil. Also, the change during 4 years of only the *z*-scores for H, SH, and Biac was significantly, negatively correlated with the age at start of treatment (r: -0.26 to -0.43) and for H, SH, Hand, and Biac there was a significant, negative correlation with the their baseline *z*-scores (r: -0.31 to -0.44). In contrast, all /-values at baseline of the DR study showed a significant, positive relationship with CA (r: 0.25 to 0.49). The 4 years change in /-value was only for /H&SH, /H&Biil and /Biac&Biil significantly, negatively related with age at start of treatment (r: -0.41 to -0.77). The changes in all /-values during the 4 years DR study were comparable and significantly, negatively related with the /-value at baseline (r: -0.53 to -0.77).

At baseline of the FR study there was only a significant, negative relationship between the CA and the *z*-scores for H, SH, and Hand (r: -0.45 to -0.72). In contrast, the change during 2 years of only the *z*-scores for H, SH, Biac, and Biil was significantly, positively correlated with the age at start of treatment (r: 0.41 to 0.59) and for H and Foot there was a significant, negative correlation with the their baseline *z*-scores (r: -0.5 to -0.59). All /-values at baseline of the FR study showed a significant, positive relationship with CA (r: 0.52 to 0.71).

Table 4. Percentage of girls at start and end of both the dose and frequency response study according to the 'Appearance' class. The percentage of girls in group A4 with a minimal shape ($\int$)-value (time difference between the age of the girl and the age which fits the combination of measurements best) ≤1.96 is between brackets. Also, for each study the mean (SD) time difference expressed in years is given for all $\int$-values of the girls with 'atypical' body proportions.

		DR study				FR study			
		A1	A3	A4	Time Difference (yrs)	A1	A3	A4	Time Difference (yrs)
$\int$H&SH	B	13	4	83 (88)		5	0	95 (67)	
	E	57	14	29 (47)	-1.0 (2.0)	5	21	74 (43)	-3.5 (1.4)
$\int$H&Hand	B	2	14	84 (91)		0	5	95 (67)	
	E	17	55	28 (67)	-1.9 (1.4)	11	11	78 (47)	-3.7 (1.4)
$\int$H&Foot	B	5	11	84 (98)		5	0	95 (61)	
	E	26	46	28 (50)	-1.7 (1.6)	26	0	74 (64)	-3.6 (1.1)
$\int$H&Biac	B	9	6	85 (72)		0	5	95 (67)	
	E	40	26	34 (41)	-0.6 (1.5)	16	11	74 (36)	-2.9 (1.3)
$\int$H&Biil	B	6	9	85 (93)		5	0	95 (11)	
	E	29	42	29 (32)	-0.9 (1.9)	26	0	74 (29)	-2.4 (1.6)
$\int$Biac&Biil	B	2	68	30 (95)		0	53	47(100)	
	E	6	72	22 (100)	1.6 (1.8)	5	84	11 (0)	-2.3 (2.8)

B: Baseline; E: End of study

A1: z-scores ≤, $\int$-value > 1.96; A2: z-scores >, $\int$-value ≤ 1.96 (very unlikely, no 'fits', not listed)

A3: z-scores and $\int$-value ≤ 1.96; A4: z-scores and $\int$-value > 1.96

The 2 years change in /-value was only for /H&SH, /H&Foot and /Biac&Biil significantly, negatively related with age at start of treatment (r: -0.44 to -0.69). The changes in all /-values during the 2 years FR study were comparable and significantly, negatively related with the /-value at baseline (r: -0.43 to -0.56).

CALCULATIONS WITH THE TURNER REFERENCE GROUP

If instead of a healthy female reference group a Turner reference group was used to calculate *z*-scores, baseline mean *z*-scores of all groups in both studies were close to zero (data not shown). This indicates that these girls were not very different from other girls with TS. Baseline mean /-values were also well within the 'typical' range for girls with TS. At the end of the two studies, there were marked increases in both *z*- and /-values of all groups, except for *z*Hand and *z*Foot. Only the change in *z*H, *z*Biac, and *z*Biil was significantly different between groups in the DR study; the mean /-values after 4 years were all abnormal compared with girls with TS, except for /Biac&Biil in Group A and B. In contrast, this was only the case in the BID group of the FR study, not significantly different from the OD group.

The percentage of girls in each study with a /-value within the 'typical' range for untreated girls with TS changed in the DR study for various body proportion characteristics from ± 90% at start of study to ± 20% of the class at the end of study; except for /Biac&Biil at end of study (50% of the class).

DISCUSSION

Baseline values of the girls with TS in our two studies clearly showed that the vast majority (>85%) had disproportionate short stature for all measurements in relation to height. Only when the Biacromial and Biiliacal diameters were compared with each other (/Biac&Biil) about two-thirds of the girls were 'typical' for their age.

GH treatment resulted in a marked improvement of *z*-scores. The changes by the end of studies were significant between groups in the dose-response (DR) study for *z*H (Group C larger than Group A), *z*Hand (Group C larger than Group A), and for *z*Foot (Group C and B larger than Group A; in the frequency response (FR) study only for *z*Foot (OD Group larger than than BID Group).

The improvement in /-values after GH therapy was less pronounced and not significantly different between groups, except for /H&Biil in both studies: Group C larger than Group A and B, and the OD Group larger than the BID Group. These results were to be expected since shape, defined by several measurements, not only demands 'typicality' of the individual

measurements but also a certain mutual dependency. Still, end of study *f*-values in relation to height were significantly lower compared with baseline in both studies, except for /H&SH in Group A and B of the DR study, and for /H&Hand in both groups of the FR study. The percentage of girls with *f*-values within the 'typical' range improved in the DR study, for /H&Hand, /H&Foot, and /H&Biac even upto ± 50%. The older girls enrolled in the FR study showed hardly any change in the percentages of girls with 'typical' *f*-values.

In general, baseline relationships with age had a negative correlation coefficient for *z*-scores and a positive one for *f*-values. The change at end of study from baseline in *z*-scores and *f*-values was negatively related with its baseline value and age, only in the FR study the change *z*-scores showed a positive relationship with baseline age.

BASELINE COMPARISON

In comparison with healthy girls, newborns with TS are in general already about 3 cm smaller. The postnatal growth rate is fairly normal during the first 2-3 years of life, but thereafter progressively decreases. Final adult height is further hampered by an (almost) absent pubertal growth spurt [18-20]. Baseline group data (Table 1) were comparable with the Dutch TS reference populations (mean $HSDS_{CA}$ about zero), except for the taller BID Group in the FR study. If the Turner reference population for body proportion was used, baseline mean *z*-scores in both studies were also about zero. Moreover, the *f*-values were well within the 'typical' range for TS. The percentage of the 45,X karyotype in the present population was rather high compared with other TS studies [18,20], 82% and 50-60%, respectively. The influence on growth is multifactorial and a difference in growth pattern of the various body proportions due to karyotype has not been shown [5,21].

Hughes *et al.* [6] in teenage TS girls and Varella *et al.* [7] in adult TS observed proportional short stature. On the other hand, and in line with the present studies, impaired growth of the lower extremities in TS was noted by Tanner *et al.* [1], Preus [2], and Neufeld *et al.* [3], in more comparable age ranges. In addition to this finding and also conform our observations, Ikeda *et al.* [4] reported, relatively large biacromial and biiliacal diameters in comparison to height.

Rongen presented growth charts for SH, subischial leg length (LL) and their ratio for Dutch girls with TS [5,20]. She confirmed that the disproportional short stature was due to short lower extremities, but with increasing age the girls became less disproportionate. Estrogen treatment increased the growth of the legs and to a lesser extent that of the trunk. However, the mean SH to LL ratio after one year estrogen treatment was not significantly different compared with untreated girls with TS. A difference in body proportions between small groups of estrogen treated

and untreated adult women with TS was not found [20].

Baseline *z*-scores in TS reported by Gerver *et al.* [8] were comparable with those in the present study, despite differences in age-range between the studies. Although the same reference population for healthy girls was used and a similar calculation method, a good comparison is impossible due to the negative correlation between *z*-scores and CA. The baseline mean *f*-values in that study were markedly lower than in the present studies (standard deviations were not reported). Except for an abnormal mean *f*H&Biil Gerver *et al.* reported only normal mean *f*-values in contrast to the abnormal mean baseline *f*-values with relation to H in the present studies.

INFLUENCE OF GH TREATMENT

Administration of GH generally resulted in a more pronounced growth of height and extremities such that the body proportion as well as height tend to 'typicality'. However, a substantial gain in *z*-scores does not always result in a better *f*-value, illustrated by the development of *z*Foot in the DR study. The baseline *f*-value for H&Foot in all three groups was 'atypical'. During GH therapy the development of *z*Foot in the higher dose groups B and C was not accompanied by a parallel improvement of *z*H. Therefore, at end of study mean *f*H&Foot in these groups was still 'atypical', whereas in the constant dose group A the mean *f*-value reached the 'typical' range. Due to the large standard deviation a significant difference between groups was not found.

Although mean baseline *z*-scores for SH, hands, and feet were higher than those for *z*H, their development during GH therapy resembled that of *z*H. The mean change after 4 years in the DR study varied in magnitude between these *z*-scores, resulting in different shapes. In general, the *f*-values improved most during the first year of GH treatment. In the FR study with an equal GH dose but differences in frequency regimen between groups, only small differences between groups could be observed in the change in *z*-scores after 2 years.

Gerver *et al.* [8] reported after 2 years GH (24 IU/m^2/week) only worsening of the mean *f*-values. In relation to height only length of hands, feet, and biacromial and biiliacal diameters resulted in evident abnormal mean *f*-values after 2 years GH. The girls with TS in that study had an age-range 6-19 years and received ethinyl estradiol (0.1 µg/Kg/day) when they were aged 12 years or older. A comparison of Gerver's study with the present studies is impeded by differences in age range and GH dose. More importantly, an increasing number of girls in that study received estrogens in a dose twice as high compared with the girls in the FR study. Still, the worsening trend of all *f*-values observed by Gerver *et al.* is in sharp contrast to the observed improving trend in Group A of the DR study (28 IU GH/m^2/week) and in the OD Group of the FR study (42 IU GH/m^2/week and 0.05 µg/Kg/day). Their final suggestion that 2 years GH caused a

relatively wide pelvis in TS could not be confirmed by the present studies. On the contrary, ∫H&Biil also normalized, in particular at higher dosages (Group C in the DR study) and during OD injections in the FR study. Since only 7 girls by the end of the DR study had progressed to Tanner breast stage 2, a relationship of these high GH dosages with a lipolytic effect of GH seems tempting.

In 10 TS girls aged 6-12 year earlier described by Gerver *et al.*, Rongen *et al.* [22] reported 4 years data on 4 IU GH/m^2/6 times week. SH and LL SD-scores (Dutch TS references) improved significantly, however, their ratio did not change.

BIOLOGICAL MATURITY

The RUS BA can be regarded as a measure of biological maturity and is in TS generally retarded [18,23]. The time difference to the minimal *f*-value (i.e. to the age at which the girl's shape would best fit within the 'typical' range the reference population) could also be indicative for the biological age-lag. However, this mean 'time difference corrected CA' for ∫H&SH was significantly younger than the mean RUS BA, both at baseline and at the end of the DR study ($P<0.0001$). This suggests that the retarded BA in these girls is not sufficient to explain the the time-lag for body proportions. It must be noted, though, that the variation in BA determinations is large [23].

TURNER REFERENCE POPULATION FOR BODY PROPORTIONS

If the Turner reference population for body proportion was used, GH treatment in the DR study resulted in 'atypical' body proportions, except for ∫Biac&Biil. If baseline *f*-values for TS were 'atypical' in contrast to those for healthy girls, it seems comprehensible that end of study values were 'atypical' for TS, because *f*-values calculated with reference to healthy age-matched girls normalized. Remarkably, only the BID Group in the FR study ended with 'atypical' body characteristics. In both studies no significant differences between groups were found for the change in *f*-values.

CONCLUSIONS

The vast majority of the girls with TS had 'atypical' baseline *z*-scores and *f*-values compared with age-matched healthy girls. In general, these values worsened with age. GH treatment resulted in significant improvements of most *z*-scores. The change from baseline to end of study was only significantly dose-dependent for *z*H, *z*Hand, and *z*Foot. During the GH studies *f*-values improved significantly towards the 'typical' range of the normal female reference population, in particular at younger ages and for ∫H&Hand, ∫H&Foot, and ∫H&Biil. A significant dose-dependent improvement was only noted for ∫H&Biil.

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APPENDIX

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Chapter 8

Carbohydrate and lipid metabolism during various growth hormone dosing regimens in girls with Turner syndrome

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CARBOHYDRATE AND LIPID METABOLISM DURING VARIOUS GROWTH HORMONE DOSING REGIMENS IN GIRLS WITH TURNER SYNDROME

ABSTRACT

Growth hormone (GH) treatment, in particular at supraphysiological dosages, may alter carbohydrate and lipid metabolism. In 87 girls with Turner syndrome, aged 2-17 years, metabolic effects were investigated during a four year GH dose-response (DR) study (upto 8 IU/m^2/day) and a two year administration frequency-response study (FR), comparing once (OD) and twice daily injections of a total GH dose of 6 IU/m^2/day, in combination with low-dose ethinyl estradiol (0.05 mcg/kg/day, orally).

Impaired glucose tolerance was present in 6% of the girls at baseline, at end of the studies this number even decreased. In the DR study, the area under the curve for time-concentration (AUC_{ab}) for glucose and its peak value showed no change over time and was never significantly different between any of the study groups. However, in all DR groups the AUC_{ab} for insulin, its peak value, fasting glucose, insulinogenic index, HbA_{1C} levels, and uCp levels were all significantly higher after four years compared with pretreatment. In the FR study, group differences were not observed, changes over time for the OD group were similar to those in the DR study.

Compared with healthy Dutch controls, median baseline levels for total cholesterol (TC) were similar and for high-density lipoprotein cholesterol (HDL) lower in the DR study, whereas in the FR study median TC levels were higher and HDL levels similar. With increasing GH dosages in the DR study, median TC, low-density lipoprotein (LDL), and apolipoprotein (apo-) B levels decreased; median HDL levels increased, apo-A1 decreased. The changes after 4 years were significant, except for LDL. Thus, the lipid profiles changed in a more cardioprotective direction, with a significant reduction of the total cholesterol to HDL-cholesterol ratio. In conclusion, GH therapy even at dosages upto 8 IU/m^2/day is a safe therapy in TS with respect to carbohydrate and lipid metabolism.

INTRODUCTION

Recombinant human growth hormone (GH) is widely used in girls with Turner syndrome (TS) to increase height velocity. Supraphysiological GH dosages are necessary to induce a positive nitrogen balance [1] in this disorder.

However, such dosages given for many years may also influence carbohydrate (CH) and lipid metabolism.

GH modulates tissue-responses to insulin in man. GH-deficiency (GHD) increases sensitivity to insulin [2]. Supraphysiologic concentrations of GH in acromegalic patients [3] and in normal [4,5] and diabetic [6] adults showed a decrease in glucose sensitivity to insulin, both in liver and in extrahepatic tissues. This insulin resistance can be explained on the basis of a postreceptor defect [7] and results in impaired ability of insulin to suppress (hepatic) glucose production and to stimulate glucose utilization [5]. Diabetogenic effects only occur if compensatory mechanisms fail, e.g. if insulin secretion is deficient.

Substantial concern has been expressed regarding the GH induced influence on CH metabolism in view of the association of diabetes mellitus in TS. The prevalence of CH intolerance and noninsulin-dependent diabetes mellitus (NIDDM) is common in adult women with TS, with frequencies upto 32.5% [8]. In girls with TS the reported frequency rate of impaired glucose tolerance (IGT) ranges from 15% [9,10] to 43% [11] and was associated with normal or increased insulin responses. Both insulin resistance and hyperglucagonemia have been implicated [11]. As in other conditions with insulin resistance, e.g. obesity and NIDDM, it involves nonoxidative pathways of glucose metabolism [12]. Age, karyotype, family history of DM, and estrogen replacement are important factors in aggrevating a pre-existent CH intolerance. CH tolerance is defective in young girls with TS but improves at pubertal ages due to the almost complete absence of estrogen-progestagen secretion [13]. Results after one or two years of GH therapy have indicated that in TS glucose homeostasis is maintained during GH therapy at the expense of a compensatory increase in insulin response [9,10,14].

In GHD adults GH was shown to have an intrinsic lipolytic activity, which improves after long-term GH administration [15]. This results in a reduction of adipose tissue and an increase in lean body mass [16]. Normalization of plasma lipids has been observed, in particular a reduction in triglycerides (TG), and to some extent low-density lipoprotein (LDL), and an increase in high-density lipoprotein (HDL) [17]. Other studies reported reductions in total cholesterol (TC) and LDL [18]. Contradictory effects of GH therapy in GHD children have been reported, but in general reduced or equal total cholesterol (TC) and TG levels have been found [19]. Even a triple GH dose-increase from 0.3 IU/kg/week to 0.9 IU/kg/week (TIW injections) [20], or an equal division of the total GH dose (20 IU/m^2/week) in a thrice daily regimen [21], did not have an effect on TG and TC plasma levels. Acromegalic patients often have increased serum TG (but not TC) levels which returned within the normal range after surgery [22].

Girls with TS at an adolescent age were shown to have higher TC levels, but similar TG levels compared with age-matched controls, even after adjustment for age and for obesity. These findings are likely to be related to the absent influence of estrogens, possibly mediated by GH [23], which lowers LDL and increases HDL levels [24]. In TS, although one group reported an increase in TG levels after 6 months GH therapy [25], other studies have not observed any

significant changes in TC or TG concentrations after one or two years GH administration [9,26].

In this study we investigated the changes in CH and lipid metabolism during two GH treatment studies in girls with TS: a four year dose-response study and a two year administration frequency-response study.

SUBJECTS AND METHODS

STUDY GROUPS

Only previously untreated girls with TS were enrolled in two nationwide, multicenter trials. The diagnosis was confirmed by lymphocyte chromosomal analysis. Table 1 lists baseline clinical data. Inclusion criteria were a height below the 50th percentile for chronological age (CA) for Dutch children [27] and a normal thyroid function. Exclusion criteria were: associated endocrine and/or metabolic disorders, growth failure due to other disorders or emotional deprivation, hydrocephalus, previous use of drugs that may interfere with GH therapy, Tanner puberty stage of the breasts ≥B2 [28]. Written, informed consent was obtained from the parents or custodians of each child. The study protocol was approved by the Ethics Committee of each participating centre.
In both studies biosynthetic hGH (Norditropin^{R}, Novo Nordisk A/S, Denmark) was injected daily subcutaneously by means of a pen injection system (Nordiject^{R} 24).

DOSE RESPONSE (DR) STUDY

Sixty-eight girls, aged 2-11 years, participated in a 4 year GH dose-response study. The girls were randomized into three GH dosing groups with stratification according to CA and height standard deviation score for CA ($HSDS_{CA}$):

- **A** (N=23) 4 IU GH/m^2 body surface (equivalent to 0.045 mg/kg)/day for four years,
- **B** (N=23) 4 IU/m^2 in the first year, followed by 6 IU/m^2/day during the second through the fourth year,
- **C** (N=22) 4 IU/m^2 in the first year, 6 IU/m^2/day in the second year, and 8 IU/m^2/day during the third and fourth year.

In each group one girl dropped out of the study: non-compliance (group B, after 24 months, presumed increase of muscle mass and decline in school performance according to the mother (group C, after 30 months, and wish to initiate estrogen therapy before the end of study (group A, after 36 months).

Table 1. Baseline clinical data (mean and SD) of the GH treatment groups in both studies.

	DR study Group A	Group B	Group C	FR study OD Group	BID Group
Number of girls	23	23	22	9	10
CA (yr)	6.3 (2.2)	6.8 (2.4)	6.5 (2.3)	13.1 (1.7)	13.6 (1.8)
$HSDS_{CA}$ (DSD)	0.1 (1.0)	0.3 (1.1)	0.1 (1.1)	0.2 (1.1)	1.1 (1.3)
BMI-SDS	0.2 (1.0)	0.3 (1.3)	0.2 (1.3)	1.3 (1.2)	1.2 (1.5)
karyotype:					
45,X	19	21	17	6	8
other	4	2	5	3	2

DR: dose-response
FR: administration frequency response
CA: chronological age
RUS BA: bone age according to Tanner & Whitehouse RUS-score
$HSDS_{CA}$ (DSD): height standard deviation score for CA according to the Dutch-Swedish-Danish Turner reference population
BMI-SDS: body mass index standard deviation score

FREQUENCY RESPONSE (FR) STUDY

Nineteen girls with a CA ≥11 years and a BA (RUS-score, see below) ≤13.5 years, took part in a two year study on the effect of two administration frequency regimens. After matching for BA (RUS-score, see below) and $HSDS_{CA}$, the girls were randomly divided into two GH injection frequency groups:

- **OD group** (N=9) received 6 IU GH/m^2 body surface once daily, and
- **BID group** (N=10) received the same total GH dose divided in one-third in the morning and two-thirds at bedtime.

In addition to GH therapy all girls received ethinyl estradiol (0.05 mcg/kg/day). Immediately prior to the present study and after a separate randomization procedure (i.e. baseline), all girls followed the same 10-week cross-over design as described earlier [29]. In brief, after 4 weeks only ethinyl estradiol the girls started with 2 weeks GH in either frequency group. Following a wash-out period of 2 weeks they received GH again, but in the alternate administration frequency.

STUDY PROTOCOL

Prior to any treatment (baseline) and every three months after start of continous GH therapy all girls were seen at their local hospital for a physical examination, including measurements of standing height (H) and weight (W), and every six months for a venapuncture for determination of glycosylated hemoglobin (HbA_{1C}). At baseline and at end of study all girls collected urine for measurement of urinary C-peptide (uCp) during three 24-hour periods as day and night portion. The early morning urine was included in the night portion. In addition, all girls from group C in the DR study, collected urine two days prior to and during a 24-hour GH profile test at baseline, six month after initiation of GH, and six month after each GH dosage increase. The urine was stored in the refrigerator during collection. All blood samples were stored on ice for no more than 3 hours. After centrifugation, the samples were frozen (-20° C) until assayed 1-3 weeks later. The urine samples were frozen (-20° C) until assayed after several months.

ORAL GLUCOSE TOLERANCE TEST (OGTT)

All girls in the DR study underwent an OGTT at baseline (T=0) and after 48 months. In addition, OGTT's were performed in a subgroup of 10 girls from Group C, every half year after initiation and increase of the GH dosage, i.e. T=6, 18, and 30 months. In all girls in the FR study an OGTT was performed prior to treatment, and after the first and second year of GH therapy (T=0, 12, 24 months). A single team carried out all OGTT's. After three days of unrestricted diet added with 100 g of carbohydrate forms (Fantomalt^R) and overnight fasting the girls were given an indwelling venous catheter. The time interval between the last GH injection and the OGTT was between 11 and 13 h. In girls from the BID group of the FR study the time interval was at least 2 h. A dose of 1.75 g glucose/kg body weight (maximum 50 g) was administered

orally within 5 min. Bloodsamples were collected at 0, 15, 30, 60, 90, 120, 150, and 180 min for measurement of plasma glucose, insulin, and glucagon.

To evaluate the overall responses to the oral glucose load, apart from the plasma levels at the various time-points, the following variables were described. Impaired glucose tolerance (IGT) for children was defined according to the National Diabetes Data Group [30]: fasting venous plasma glucose <7.8 mmol/L and the 2h level >7.8 mmol/L (140 mg/dl) and <11.1 (200 mg/dl). The 3h area under the curve for time-concentration, corrected for baseline values, (AUC_{ab}) during the OGTT was determined using the trapezoideal rule. The insulinogenic index -an indicator of insulin reserve capacity to the rise in blood glucose- was calculated as the ratio of the integrated rise of insulin over that of glucose [31].

LIPID FRACTIONS

After overnight fasting blood was collected from all girls in Group C of the DR study at T=baseline, 6, 18, 30, and 48 months and from all girls in the FR study at T=baseline, 12, and 24 months for the determination of total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), and the apo-lipoproteins (apo-) A_1, and B. The atherogenic index was calculated as the ratio of TC over HDL cholesterol.

ASSAYS

Plasma glucose was measured at the local hospital laboratories with automatic analysers using a hexokinase catalyzed glucose oxidase method. All other measurements were performed in one laboratory. Plasma insulin was determined with a RIA (Medgenix, Belgium). The intra assay CV was 6-10% and the inter assay CV was 6-11% (fasting normal range <20 mU/L). Glucagon was extracted from plasma with aethanol, dried on air, dissolved in albumine-phosphate buffer and measured with a RIA (Novo Nordisk, Denmark). The intra assay CV was 8-12% and the inter assay CV was 9-13% (normal range <80 ng/L). Urinary C-peptide was measured with a RIA (Novo Nordisk, Denmark). Samples were diluted twenty times with phosphate-albumine buffer and pre-incubated with antiserum during three days at 2° C. Incubation with ^{125}I-C-peptide during two days was followed by a bound-free separation with ethanol. The intra assay CV was 5-6% and the inter assay CV was 6-8% (normal range 0.7-87 nmol/24 h). Glycosylated hemoglobin was measured using an automatic HPLC analyser (DIAMAT™, BioRad, Edgemont, California, USA). The upper normal assay limit is < 6.6%.

Determination of fasting serum levels of the various lipid fractions was performed using the Kone Specific Analyzer (Kone Instruments, Espoo, Finland) for all automated analyses. Lipid analysis was subject to the quality assessment program of the WHO Regional Lipid Reference Center (Prague, Czechia). TC was measured with an automated enzymatic method [32], using the CHOD-PAP High Performance reagent kit from Boehringer Mannheim (Germany). HDL and LDL cholesterol were measured with the same method after precipitation. For HDL cholesterol the phosphotungstate method according

to Burstein *et al.* was modified [33]. For LDL cholesterol precipitation was carried out with polyvinylsulphate (Boehringer Mannheim, Germany). Overall coefficients of variation for total cholesterol, HDL cholesterol and LDL cholesterol were 2.9%, 3.7% and 5.8% respectively. TG's were determined after enzymatic hydrolysis with subsequent determination of liberated glycerol by colorimetry. No correction was made for serum free glycerol. The reagent kit from Boehringer Mannheim (Germany) was used. The overall coefficient of variation of this method did not exceed 3.2%. Apolipoprotein A1 and B were measured by an automated turbidimetric immuno-assay using the reagent kits of Orion Diagnostica (Espoo, Finland) [34]. The overall coefficients of variation for apolipoprotein A1 and B were 6.1% and 5.6% respectively.

STATISTICAL ANALYSIS

Results are expressed as median (range), unless indicated otherwise. In both the DR and the FR study, differences between groups were tested by the non-parametric, two-sample Kruskal-Wallis or Wilcoxon test. Differences between points in time were tested by Wilcoxon's signed rank test. The degree of obesity was expressed as body mass index (BMI) SD-score [35]. Correlations were tested with the non-parametric Spearman's rank correlation test. A P-value < 0.05 was considered significant.

RESULTS

A. CARBOHYDRATE METABOLISM

ORAL GLUCOSE TOLERANCE TEST (OGTT)

a. Comparison between (DR) groups at baseline and after 4 years (Table 2).

Impaired glucose tolerance was present in 4 out of 66 girls (6%) at baseline (2 girls had missing values at 2 h), divided over the groups. After 4 years GH therapy only 2 (other) girls out of 63 (3%; 3 girls dropped-out) had IGT, both in Group B.

Significant differences between groups were not found for any of the OGTT variables at baseline nor after 4 years GH. After 4 years GH treatment only the 120 and 150 min glucose levels were higher in Group B compared with Group A ($P<0.02$) and the 150 min insulin levels were

Table 2. Median (range) for carbohydrate variables in each group prior to start and after four years of GH treatment in the dose-response study.

	Baseline			After 4 yrs		
	Group A	Group B	Group C	Group A	Group B	Group C
IGT, N	2	1	1	0	2	0
AUC_{ab} glucose	399	357	452	388	441	389
min*mmol/L	(92;824)	(20;983)	(191;876)	(101;759)	(87;981)	(111;720)
AUC_{ab} insulin	4095	4320	3855	7365*	9855*	9180*
min*mU/L	(405;13500)	(1095;16695)	(1050;8550)	(3240;20430)	(3180;70350)	(4005;18285)
fasting glucose	4.6	4.6	4.4	4.8*	4.7*	5.0*
mmol/L	(3.1;6.0)	(3.6;5.5)	(3.4;7.0)	(3.6;6.1)	(3.7;6.7)	(3.1;8.2)
insulinogenic	8.5	11.8	9.1	19.6*	30.9*	25.5*
index	(1.9;19.9)	(3.6;856)	(3.1;18.3)	(12.1;67.3)	(9.3;104.0)	(14.2;60.8)
HbA_{1C}	4.8	4.9	4.8	5.2*	5.4*	5.1*
%	(3.6;5.5)	(4.0;6.1)	(4.0;6.2)	(3.1;6.0)	(3.7;6.2)	(4.3;6.1)
uCp	6.4	9.0	8.2	16.5*	25.9*	21.1*
nmol/24h	(1.3;17.6)	(2.6;17.5)	(3.1;22.6)	(7.5;65.9)	(9.3;43.8)	(13.2;45.5)

* significantly different from baseline ($P < 0.05$)
IGT: impaired glucose tolerance
AUC_{ab}: area under the curve above baseline
uCp: urinary C-peptide

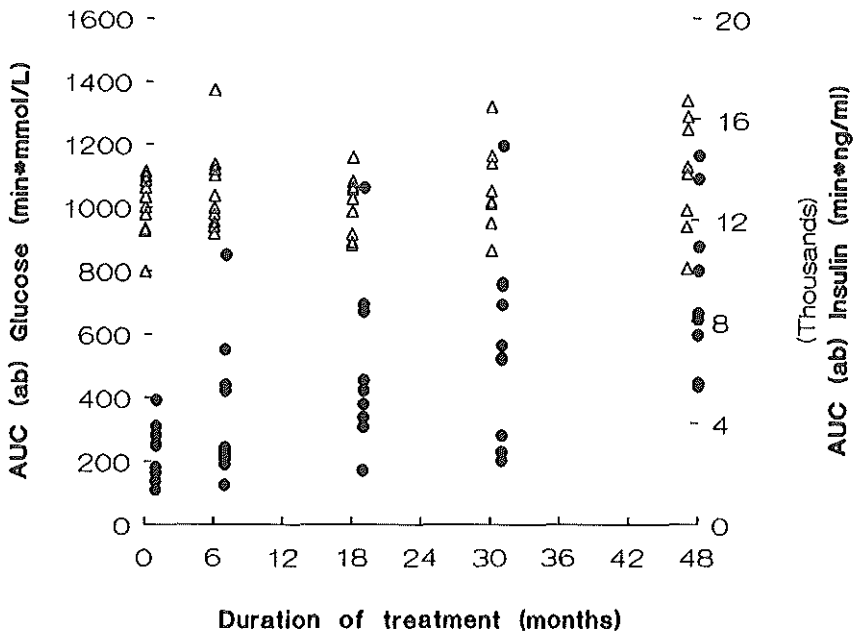

Figure 1. Integrated Area Under the Curve for glucose (△) and insulin (●) values during an oral glucose tolerance test in 10 girls of Group C at baseline and after 6, 18, 30, and 48 months of GH treament at 4, 6, 8, and 8 IU/m^2/day, respectively.

higher in Group B and C compared with Group A ($P<0.02$). The insulin values after 4 years in Group B were skewed due to one girl with very high insulin test levels (and a normal glucose response). All groups had a significantly higher median fasting glucose, AUC_{ab}, and insulinogenic index after 4 years GH compared with baseline. The baseline insulinogenic index and BMISDS were positively correlated ($r=0.54$;$P=0.01$).

b. Effects of GH dose increments in 10 girls of Group C in the DR study (Table 3).

Impaired glucose tolerance was present in one girl only during 4 and 6 IU GH/m^2/day, however, at baseline and during 8 IU GH/m^2/day she had a normal response to the glucose load. The median fasting glucose levels and its AUC_{ab} was similar at baseline and during the various GH dosages employed. The dose-related increase of the median AUC_{ab} insulin (significant from 18 months onwards) and the insulinogenic index (significant from 6 months onwards) seems to exceed the effect caused by the duration of the study, although the variation was considerable.

Table 3. (continued).

	T=0	T=6	T=18	T=30	T=48 months
cholesterol	4.7	4.7	4.2*	4.1*	3.8*
mmol/L	(3.0;6.4)	(3.5;5.8)	(3.0;4.9)	(2.9;5.0)	(3.2;6.6)
HDL-chol.	0.9	1.2*	1.2*	1.2*	1.3*
mmol/L	(0.6;1.4)	(0.7;2.1)	(0.8;1.8)	(0.7;1.9)	(0.9;1.9)
LDL-chol.	3.0	2.6	2.3	2.2	2.0
mmol/L	(1.4;4.7)	(1.4;4.0)	(0.7;3.4)	(1.7;3.3)	(1.4;4.8)
Triglycerides	0.8	1.0	0.8;	1.0	1.1*
mmol/L	(0.3;2.2)	(0.5;1.7)	(0.6;2.4)	(0.5;2.0)	(0.6;2.5)
apo A1	124	114*	119	119*	119*
mg/dl	(94;170)	(81;166)	(86;165)	(77;146)	(94;150)
apo B	74	68	66*	61*	59*
mg/dl	(48;108)	(53;88)	(53;88)	47;79)	(40;105)

*: significantly different from baseline ($P < 0.05$)
uCp: urinary C-peptide

Table 3. Median (range) for OGTT (N=10), HbA_{1C}, urinary C-peptide, and lipid (N=23) variables in Group C at baseline (T=0) and during GH treatment (T=6, 18, 30, 48 months) in the dose-response study.

	T=0	T=6	T=18	T=30	T=48 months
IGT, N	0	1	1	0	0
AUC_{ab} glucose	376	350	443	437	420
min*mmol/L	(317;609)	(203;867)	(200;657)	(138;930)	(179;720)
AUC_{ab} insulin	2468	3630	5910*	8145*	10860*
min*mU/L	(1050;5250)	(1545;13350)	(2025;13815)	(2835;17670)	(4590;18285)
fasting glucose	4.2	4.7	4.5	4.7	4.6
mmol/L	(3.4;7.0)	(4.3;4.9)	(3.4;7.0)	(2.2;5.2)	(4.3;5.1)
insulinogenic	5.9	10.7*	15.1*	19.6*	27.7*
index	(3.1;13.2)	(5.1;18.8)	(8.5;32.5)	(5.6;38.6)	(14.7;60.8)
HbA_{1C}	4.7	5.0	4.8	4.9	5.1*
%	(4;6.2)	(4.1;5.5)	(4.2;5.6)	(4.0;5.3)	(4.3;6.1)
uCp	8.2	14.1*	21.2*	26.7*	21.1*
nmol/24h	(3.1;22.6)	(8.2;37.9)	(7.5;51.4)	(12.0;52.7)	(13.2;45.5)

*: significantly different from baseline ($P < 0.05$)
IGT: impaired glucose tolerance
AUC_{ab}: area under the curve above baseline

Table 4. (continued).

	Baseline		After 2 yrs	
	OD Group	BID Group	OD Group	BID Group
cholesterol	5.4	5.2	5.0	5.3[&]
mmol/L	(3.4;6.4)	(3.8;6.5)	(3.4;6.0)	(4.0;7.0)
HDL-chol.	1.5	1.4	1.9[*]	1.4[&]
mmol/L	(1.2;1.7)	(1.0;1.7)	(1.2;2.3)	(0.9;1.7)
LDL-chol.	3.2	3.2	2.6	3.4[&]
mmol/L	(1.1;4.6)	(2.5;4.6)	(1.5;3.5)	(2.1;4.9)
Triglycerides	1.2	1.1	0.8	1.5
mmol/L	(0.6;1.8)	(0.6;2.5)	(0.5;1.7)	(0.5;2.8)
apo A1	137	126	143	130
mg/dl	(126;148)	(114;157)	(129;158)	(83;155)
apo B	85	76	73[*]	104[*&]
mg/dl	(64;109)	(61;123)	(59;83)	(72;119)

*: significantly different from baseline ($P < 0.05$)
&: change from baseline significantly different between groups ($P < 0.05$)
IGT: impaired glucose tolerance
AUC_{ab}: area under the curve above baseline
uCp: urinary C-peptide

Table 4. Median (range) for carbohydrate and lipid variables in each group prior to any treatment and after two years of GH treatment in the frequency-response study.

	Baseline		After 2 yrs	
	OD Group	BID Group	OD Group	BID Group
IGT, N	1	0	0	1
AUC_{ab} glucose	465	502	405	410
min*mmol/L	(263;738)	(254;890)	(270;584)	(323;1293)
AUC_{ab} insulin	6585	8783	6870	6383
min*mU/L	(1215;12210)	(1725;12615)	(2955;8865)	(2295;28830)
fasting glucose	4.8	4.8	5.0	4.1
mmol/L	(4.4;5.2)	(3.1;5.2)	(4.3;5.9)	(3.3;5.7)
insulinogenic	13.9	15.8	15.4	16.8
index	(4.6;26.3)	(3.1;38.0)	(7.4;25.3)	(7.1;53.4)
HbA_{1C}	4.9	4.9	5.1*	5.0
%	(4.3;5.5)	(4.4;5.3)	(4.4;5.6)	(4.2;5.7)
uCp	18.0	29.3	42.2*	39.4*
nmol/24h	(12.5;67.2)	(7.6;63.5)	(17.4;89.2)	(13.5;86.0)

*: significantly different from baseline ($P < 0.05$)
&: change from baseline significantly different between groups ($P < 0.05$)
IGT: impaired glucose tolerance
AUC_{ab}: area under the curve above baseline

c. Comparison between FR groups at baseline and after 2 years GH therapy (Table 4).

Impaired glucose tolerance was present in 1 out of the 19 girls (5%) at each point during the study, each time a different girl; at baseline in the OD group, after 1 year GH in the OD group, and after 2 years GH in the BID group. There were no significant differences between groups for any of the OGTT variables in Table 4 at any point in time. Within group comparisons revealed no significantly different AUC_{ab} for glucose and insulin in time. The insulinogenic index was similar before and after GH treatment. At baseline the insulinogenic index was correlated with BMISDS (r=0.61;P=0.006).

HBA_{1C}

In both studies glycosylated hemoglobin plasma levels were never outside the normal range. In all groups there was a progressive increase in HbA_{1C} levels during the subsequent years of GH therapy, resulting in significantly higher end of study levels compared with baseline, without a difference between groups, except for the BID group in the FR study. In the FR study there were no differences between groups.

URINARY C-PEPTIDE

At baseline there were no differences between groups in uCp amounts. Longitudinal follow-up within group C showed higher uCp levels with higher GH dosages, both during day and night (data not shown). End of study uCp values within each treatment group in both trials were significantly higher compared with baseline. The change from baseline to end of study was not significantly different between groups.

B. LIPID FRACTIONS

In group C of the DR study median serum levels of TC, LDL, apo-A1, and apo-B decreased with time, whereas HDL and TG increased with time. The main effects can be observed after initiation of GH therapy. After four years GH treatment in the DR study all lipid levels were significantly different from baseline. The atherogenic index (Fig. 2) decreased significantly (P=0.0005) from 4.8 (3.6;7.2) to 3.2 (2.0;6.0). Clinically relevant cut-off values for normal levels of TC (≤5.0 mmol/L) and HDL (>0.9 mmol/L) were exceeded by 6/21 (28%) and 10/21 (48%), respectively. The response to GH treatment as a percentage of baseline values was significantly higher in the 'low-HDL' group compared with the 'normal-HDL' group (P=0.01).

The development of the median lipid levels in the OD group of the FR study was comparable with that in the DR study for TC, HDL, LDL,

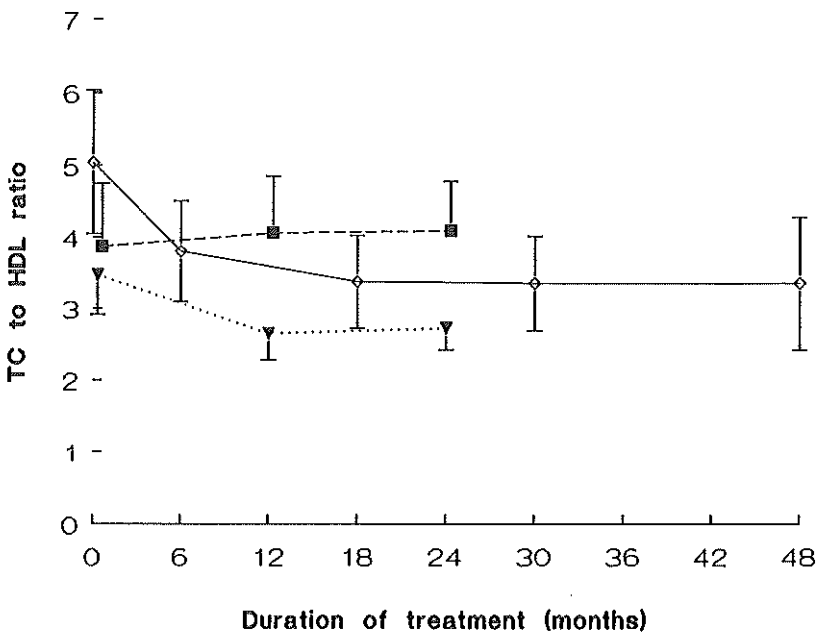

Figure 2. Ratio of Total Cholesterol to HDL cholesterol (mean and SD) at baseline and after 6, 18, 30, and 48 months of GH treament at 4, 6, 8, and 8 IU/m^2/day for group C (—◇—) of the 4 year dose-response study and after 12 and 24 months of GH treament at 6 IU/m^2/day of the once daily (OD: ····▼····) and twice daily (BID: – ■ –) GH injection groups in the 2 year administration frequency-response study .

and apo-B. In contrast, TG decreased and apo-A1 levels showed an increase. After two years GH in the FR study only levels HDL levels in the OD group, and apo-B levels in both groups were significantly different from baseline. In the FR study the change from baseline after two years GH treatment was significantly different between groups for TC, HDL, LDL, and apo-B. The atherogenic index decreased significantly (P=0.008) only in the OD group from 3.4 (2.8;4.3) to 2.7 (2.2;3.1); in the BID group values were stable (Fig. 2). Clinically relevant cut-off values for normal levels of TC (≤5.0 mmol/L) and HDL (>1.2 mmol/L) were exceeded by 12/19 (63%) and 6/19 (32%), respectively. For both lipids 'abnormal' values were predominantly present in the BID group, 7/12 and 5/6 girls, respectively. The response to GH treatment as a percentage of baseline values was not significantly different between the groups with 'normal/abnormal' baseline levels.

CHAPTER 8

DISCUSSION

A. CARBOHYDRATE METABOLISM

The prevalence of IGT at baseline and during both the DR and FR study was low and without differences between study groups. At the end of the four year DR study a decrease from baseline was even noted from 6 to 3%. None of the girls developed diabetes. The AUC_{ab} for glucose and its peak value showed no change over time and was never significantly different between any of the study groups, not even the study group treated upto a dose of 8 IU GH/m^2/day. However, in all groups the AUC_{ab} for insulin, its peak value, fasting glucose, insulinogenic index, HbA_{1C} levels, and uCp levels were all significantly higher after four years compared with pretreatment. After 4 years the 120 and 150 min median glucose levels in groups B were significantly higher than in group A. The longitudinal follow-up in ten girls of group C showed that the increase of insulin, expressed as absolute levels, AUC or as the insulinogenic index, were dose-related, albeit with a large interindividual variation. Only after 18 months of GH treatment, i.e. after the second GH dose-increment, levels became significantly different from baseline.

Group differences in the FR study were not observed. After two years GH treatment the AUC for glucose and its peak value were not significantly different from baseline. Although in the OD group HbA_{1C} and peak insulin levels showed a significant increase, both peak insulin levels and the AUC of the BID group showed slightly lower values (NS). In contrast, the AUC for insulin and the insulinogenic index showed no significant change after two years compared with baseline.

GLUCOSE TOLERANCE AND HBA_{1C} VALUES

The IGT prevalence at baseline was definitely lower in the present studies than in other TS studies [9,11,36]. Even during high-dose GH treatment only a few of our girls -others than at baseline- had an IGT compared with TS girls in other studies [9,10,14,37]. Apart from a relatively young age-cohort in the DR study, these low numbers may be influenced by the procedure of the OGTT: the calculation of the glucose load per kg bodyweight, instead of ideal body weight, and by allowing a lower maximum amount of glucose in the present study, 50 g in stead of 75 g advocated by the National Diabetes Data Group [30]. In normal subjects the use of 100 g resulted in 2h bloodglucose levels which were 0.83 mmol/L higher compared with the use of 50 g [30].

Several studies [9,26,37-39] reported no significant change in HbA_{1C} levels in upto 3 years of GH treatment. And although Haesler *et al.* [10] observed a slight increase on 3 IU GH/m^2/day, and Weise *et al.* [14] observed transient increases, in

the DR study a small, but significant increase in Hb_{A1C} levels was found after four years GH, not different between the dose groups. In the FR study only the OD group had significantly higher Hb_{A1C} levels after two years GH. It should be emphasized, though, that none of the girls in the present studies exceeded the assay reference range. Higher Hb_{A1C} values could well reflect the slight increase seen at a pubertal age in a normal population, accompanying the insulin resistance [40].

BLOODGLUCOSE, INSULIN, AND GLUCAGON LEVELS, AND URINARY C-PEPTIDE

In agreement with the present studies at even higher GH dosages, most studies in TS showed after 1-4 years GH treatment in doses varying from 2-4 IU/m^2/d (5-15 mg/week) no significant changes in fasting plasma glucose levels or after an oral glucose load [9,10,26,37-39,41]. Only Weise *et al.* [14] found a significant increase in integrated glucose levels after two years on 4 IU GH/m^2/day as opposed to the two lower dosages of 2 and 3 IU GH/m^2/day.

Some studies -even using GH dosages upto the lowest one in the present study- report no significant change in insulin levels after 1-2 years GH [9,10]. So did Weise *et al.* only on the lowest GH dose used (2 IU/m^2/day). However, on 3 and 4 IU GH/m^2/day significant, dose-dependent increases in insulin levels were observed during this two year study [14]. Wilson *et al.* found only fasting insulin levels to be significantly highe after 4 years GH at 4 IU/m^2/day, but not the 2h levels [38]. Takano *et al.* [37] reported only on 4 IU/m^2/day, but not on 2 IU/m^2/day, a significant increase of insulin levels, without development of glucose intolerance. In the DR study the two higher GH dosages resulted in higher increases of the AUC for insulin and insulinogenic index compared with the lowest dose group. Moreover, the longitudinal follow-up showed a clear-cut dose-dependent increase of the insulin response.

The AUC for insulin and the insulinogenic index in the FR study were somewhat higher at baseline than in the DR study, reflecting the age-dependency of insulin. After initiation of GH and estrogen treatment, these variables did not change significantly due to the large interindividual variation. The BID group even showed a decrease of these insulin variables, in agreement with a Belgian study in which a 50/50 division of the daily dose was used [39].

Plasma glucagon levels and its AUC after an oral glucose load was significantly greater in TS compared with controls [11]. In the present studies, although with large interindividual variation, dose-dependent increases were observed, in conformity with the expected conterregulatory response. Interpretation of this data is hampered by the lack of other hormones involved.

Urinary C-peptide, reflecting endogenous insulin secretion, showed at baseline and during GH therapy higher levels during the day than during the night and followed the pattern of the insulin plasma levels, without significant differences between treatment groups. Comparison of 24h uCp levels with Dutch reference values [42] confirmed age-dependency in Turner girls prior to GH treatment ($r=+0.35$;$P=0.005$). Also, pretreatment values were consistent with reference values within 95% limits of the proposed age-classes, except for

the youngest age-class in which 3 of the six girls had higher values. After 4 years GH treatment 24h uCp values were higher than the 95% limit compared with the reference population in the 6-8 year age-class (6 out of 10 girls) and in the age-class ≥ 10 years (7 out of 39 girls).

The consequence of long-term hyperinsulinism in otherwise healthy subjects is unknown. Induction of insulin dependent diabetes could be possible in susceptible individuals. Cardiovascular effects, with an increased risk of hypertension and non-insulin dependent diabetes [43], however, are reversible in acromegalic patients even after long-term GH exposure [22].

B. LIPID FRACTIONS

Baseline median HDL level of the younger girls (DR study), aged 2-11 years, was substantially lower (0.9 mmol/L) and of the older girls (FR study), aged 2-17 years, similar (1.4 mmol/L) compared with Dutch controls of a similar age measured in the same laboratory (1.5 and 1.4 mmol/L, respectively) [44]. In contrast, baseline median TC level of the girls in the DR study was similar (4.7 mmol/L) and in the FR study substantially higher (5.3 mmol/L) compared with Dutch controls with a comparable age (4.8 and 4.7 mmol/L, respectively) [44]. In agreement, although with systematically lower median values, lipid levels in untreated American girls with TS [45] showed only during adolescence higher TC levels compared with controls matched for age and obesity. This phenomenon could be related to the near complete absence of the reducing influence of estrogens on TC and HDL levels [9,46] in girls with TS of that age. The findings might be confounded by the fact that in the FR study most girls were obese. Generally obesity is associated with a decrease in HDL cholesterol [47].

With increasing dosages in the DR study, and in agreement with findings in GH treated GHD adults, median serum levels of TC decreased with time, along with a decrease in LDL levels and its major constituent apo-B. A favorable change in median HDL level was seen, although apo-A1, the major constituent of the HDL particle, unexpectedly decreased. The main effects could be observed after initiation of GH therapy, in particular for HDL and apo-A1; for TC and apo-B only after the first dosage increase. The GH effects over time in TS are interesting, since a Dutch female control group showed for TC a gradual rise until the age of 10-11 years; and for HDL the controls showed, after a 'dip' around the age of 9 years, a gradual decrease until the age of 16-17 years (to 1.3 mmol/L) [44]. For these lipids a progressive difference emerged with GH therapy. Remarkably, the response to GH treatment in the group with 'low-HDL' baseline values was significantly higher compared with the 'normal-HDL' group.

While in two other GH intervention studies in TS no significant changes were found [9,26], in the DR study all lipid levels were significantly different from baseline after four year GH treatment, except for LDL levels. The differences with the earlier findings may be due to the duration of GH treatment (four years in the present study vs one year in the other studies) and the use of incremental steps for the GH dose. Furthermore, variability was reduced by using a single

laboratory. But, apart from the large inter-individual variation in some of the measurements, still a large biological intra-individual variation exists for lipid measurements with a short interval [48]. Intervention, therefore, is only thought to be of clinical relevance when the change exceeds 15%. The lipolytic effects of long-term GH treatment could have important beneficial implications for the development of cardiovascular disease also in TS.

In GHD adults GH treatment resulted in normalization of plasma lipids [18]. In untreated GHD children the change towards abnormal plasma lipid levels may not have developed yet. This may also be the reason that contradictory effects on lipid levels of GH therapy in these children have been reported. However, in general lowered or equal TC and TG levels have been observed [19,49]. In GHD children, even a triple GH dose-increase from 0.3 IU/kg/week to 0.9 IU/kg/week (TIW injections) [20], or an equal division of the total GH dose (20 IU/m^2/week) in a thrice daily regimen, did not have an effect on TG and TC plasma levels after 6-12 months of treatment. A decrease in the atherogenic index after 9 months GH treatment in 12 prepubertal GHD boys has been reported [50], in accord with the present long-term results in the DR study and the OD group of the FR study.

It seems difficult to interpret the lipid data of FR study, since the BID group consisted at baseline of more girls with 'abnormal' TC levels. The treatment response, though, for TC and HDL was not different between 'normal/abnormal' girls within each treatment group. Both groups received an identical low dosage of ethinyl estradiol with a known hypocholesterolemic effect. After two years GH therapy the beneficial pattern of change in cholesterol fractions and apo-B in the OD group of the FR study was similar to that of group C in the DR study.

CONCLUSIONS

The present studies showed no significant effect of GH, even at a high, supraphysiological dose of 8 IU/m^2/day, on carbohydrate metabolism. The dose-dependent increase in insulin levels was similar to what can be expected during puberty in a control population. Together with the genetic predisposition, this implies that long-term follow-up of CH metabolism is warranted. In general, the lipid profiles changed in a more cardioprotective direction, with a significant reduction of the total cholesterol to HDL-cholesterol ratio. This indicates that r-hGH even at high dosages is a safe therapy in TS with respect to CH and lipid metabolism.

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APPENDIX

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Chapter 9

Effect of long term growth hormone treatment in high dosages on left ventricular cardiac dimensions in girls with Turner syndrome

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EFFECT OF LONG TERM GROWTH HORMONE TREATMENT IN HIGH DOSAGES ON LEFT VENTRICULAR CARDIAC DIMENSIONS IN GIRLS WITH TURNER SYNDROME

ABSTRACT

Objectives: To evaluate possible effects of growth hormone (GH) on echocardiographically measured left ventricular (LV) dimensions, electrocardiography, and systemic blood pressure (BP) in girls with Turner syndrome (TS).

Study design: During a four year dose-response (DR) study, comparing 4, 6, and 8 IU GH/m^2/day and during a two year administration frequency-response study (FR), comparing once daily with twice daily GH injections in combination with low-dose ethinyl estradiol, baseline and yearly measurements were performed in 87 girls with TS, aged 2-17 years.

Results: At baseline 23% of the girls presented with cardiovascular abnormalities and somewhat lower mean values of the LV septum, posterior wall, and internal diameter compared with normal Dutch girls, probably due to increased weight of the girls with TS. Mean values for end-diastolic relative wall thickness and shortening fraction both at baseline and after completion of the studies remained within the reference range. LV activity, heart axis, and BP showed an age-dependent development, with values well within the normal range, except for diastolic BP with values >90th age-specific percentile in 15% of the cases at baseline and 31% after four years in the DR study, without group differences.

Conclusions: Long-term GH therapy, even at dosages upto 8 IU/m^2/day, did not result in LV hypertrophy in girls with TS. The number of girls with an elevated diastolic BP increased from 15% at baseline to 31% after four years GH treatment in the DR study.

INTRODUCTION

GH increases height velocity in TS [1]. Its anabolic action is not only restricted to the epiphyseal growth plate, but animal studies also suggest that GH can increase heart size and enhance DNA synthesis of heart muscle [2].

In humans, GH is related to cardiovascular morbidity and mortality, since life expectancy in acromegaly [3] and in GHD [4] is shortened. LV hypertrophy is a feature of GH hypersecretion in acromegaly [5]. It is directly correlated with the

duration of the disease [6] and usualy reverses after treatment [7]. Asymmetrical septal hypertrophy may primarily be found in acromegalic patients with longstanding disease complicated by hypertension [8]. This in turn may lead to important alterations of systolic and diastolic function of both ventricles and thus to impaired cardiac performance [9]. A direct inotropic effect of GH is also possible, since short-term GH administration in normal subjects increases heart rate, cardiac output, and myocardial contractility [10].

Adult GHD patients show decreased lean body mass, skeletal muscle volume and oxygen uptake [11]. Long term follow-up (until 38 months) of GHD adults on GH treatment (2 IU/m^2/day) [12] showed that LV diastolic dimensions increased and seemed to normalize, while heart rate and cardiac output increased to supranormal levels. Fractional shortening increased only after 16 and 38 months. The initially reduced systolic BP did not change during GH therapy. Two years GH therapy in non-GHD short children resulted in modest changes in BP and LV dimensions, within normal limits [13].

TS, in particular the 45,X karyotype, is associated with cardiac anomalies (upto 45%), e.g. aortic coarctation, aortic valve abnormalities, and dilated ascending aorta [14,15]. Systemic hypertension has also been reported in TS [16]. After one year GH treatment a small increase in diastolic LV internal diameter was found, remaining within norm. BP showed no significant changes [17].

We evaluated the possible effects of GH on echocardiographically measured LV dimensions, electrocardiography, and systemic BP in girls with TS, aged 2-17 yrs, at baseline and at yearly intervals during GH treatment in two multicenter trials: a four year dose-response study and a two year administration frequency-response study.

SUBJECTS AND METHODS

STUDY GROUPS

Only previously untreated girls with TS were enrolled in two nationwide, multicenter trials. The diagnosis was confirmed by lymphocyte chromosomal analysis. Clinical data for the girls, all Tanner stage breast stage B1), are listed in Table 1. Inclusion criteria for the girls were a height below the 50th chronological age centile for Dutch children [18] and a normal thyroid function. Exclusion criteria were: associated endocrine and metabolic disorders, growth failure due to other disorders or emotional deprivation, hydrocephalus, the (previous) use of drugs that may interfere with GH therapy.

Girls with previous coarctation repair, but without a residual gradient and without LV hypertrophy (n=7) and girls with a non-stenotic abnormal aortic valve (n=2) were included; two girls (group B and C) were excluded because of aortic valve stenosis. At start of study the ecg tracings of two girls (one in group A and one in the BID group) and the echocardiogram of four girls (one in

Group A and three in Group C) were not performed due to restlesness of the patients. One girl (group B) was operated upon her mitral valve stenosis. Written, informed consent was obtained from the parents or custodians. The study protocol was approved by the Ethics Committee of each participating centre (see Appendix). In both studies biosynthetic hGH (Norditropin^R, Novo Nordisk A/S, Denmark) was injected daily subcutaneously by means of a pen injection system (Nordiject^R 24).

DOSE-RESPONSE (DR) STUDY

Sixty-eight girls, aged 2-11 years, participated in a four year GH dose-response study. The girls were randomized into three GH dosing groups with stratification according to CA and $HSDS_{CA}$:

- **A** (N=23) 4 IU rhGH/m^2 body surface/day for 4 years,
- **B** (N=23) 4 IU in the first year and during the second through the fourth year 6 IU/m^2,
- **C** (N=22) 4 IU in the first year, 6 IU in the second year, and during the third and fourth year 8 IU GH/m^2/day.

In each group only one girl dropped out of the study: non-compliance (group B, at 24 months, alleged increase of muscle mass and decline in school performance according to the mother (group C, at 30 months, and desire to initiate estrogen therapy before the end of study (group A, at 36 months).

FREQUENCY-RESPONSE (FR) STUDY

Nineteen girls with a CA ≥11 years and a BA (RUS-score [19]) ≤13.5 years, took part in a two year study on the effect of two administration frequency regimens. After matching for BA and $HSDS_{CA}$, the girls were randomly divided into two GH injection frequency groups:

- **OD group** (N=9) received 6 IU GH/m^2 body surface once daily, and
- **BID group** (N=10) received the same total GH dose divided in one-third in the morning and two-thirds at bedtime.

In addition to GH therapy all girls received ethinyl estradiol (0.05 mcg/kg/day). Immediately prior to the present study and after a separate randomization procedure, all girls followed the same 10-week cross-over design as described earlier [20]. In brief, after 4 weeks only ethinyl estradiol the girls started with 2 weeks GH in either frequency group. Following a wash-out period of 2 weeks they received GH again, but in the alternate administration frequency.

STUDY PROTOCOL

Prior to start of treatment (baseline) and subsequently every year of continous GH therapy all girls were seen at their local hospital for

Table 1. Baseline clinical data of the groups in both studies.

	DR study			FR study	
	Group A	Group B	Group C	OD Group	BID Group
Number of girls	23	23	22	9	10
CA (yr)	6.3 (2.2)	6.8 (2.4)	6.5 (2.3)	13.3 (1.7)	13.8 (1.8)
RUS BA (yr)	5.6 (2.3)	6.2 (2.6)	5.9 (2.4)	12.2 (1.0)	12.7 (0.9)
$HSDS_{CA}$ (DSD)	0.1 (1.0)	0.3 (1.1)	0.1 (1.1)	0.2 (1.1)	1.1 (1.3)
BMISDS	0.2 (1.0)	0.3 (1.3)	0.2 (1.3)	1.3 (1.2)	1.2 (1.5)
karyotype:					
45,X	19	21	17	6	8
other	4	2	5	3	2

Data is expressed as mean (SD)

DR: dose-response

FR: frequency-response

CA: chronological age

RUS BA: bone age according to Tanner & Whitehouse RUS-score

$HSDS_{CA}$ (DSD): height standard deviation score for CA (according to the Dutch-Swedish-Danish Turner reference population)

BMISDS: body mass index standard deviation score

measurements of height and weight, an electrocardiogram (ecg) and an echocardiography (echo) performed by an experienced technician. The degree of obesity was expressed as BMISDS [21]. All ecg and echo tracings were evaluated by a single observer (AT). Ecg LV activity was judged on the R in lead V6 plus S in lead V1 (normal values <42 [22], the heart axis on leads I and AVF (normal values -20° to 110°. M-mode echocardiography along the parasternal axis was used to assess end-diastolic and end-systolic septal and posterior wall thickness and LV internal diameter according to Gutgesell *et al.* [23]. In general, the coefficient of variation for serial echocardiographic measurements is 10% [24]. Standard deviation scores for IVS_{ed}, PW_{ed}, $LVID_{ed}$ and $LVID_{es}$ were calculated using equations based on the LV dimensions and weight values of healthy Dutch children [25]. These LV dimensions were used to calculate end-diastolic and end-systolic relative wall thickness (RWT=[(PW+IVS)/2]/LVID) [26] and shortening fraction ($SF=(LVID_{ed}-LVID_{es})/LVID_{ed}*100\%$) [23]. Normal ranges were reported for RWT_{ed} upto 0.25 and 0.3, for RWT_{es} to 0.5, and for SF to 0.45 [26,27]. Asymmetrical septal hypertrophy was defined as end-diastolic IVS/LVPW >1.3 [28]. BP was determined with a single Dinamap Critikon 1846SX with the girls in sitting position using a cuff size corresponding to the size of their arm. Only the second to fourth readings were used to calculate the mean systolic and diastolic BP, which were compared with age-specific reference values [29].

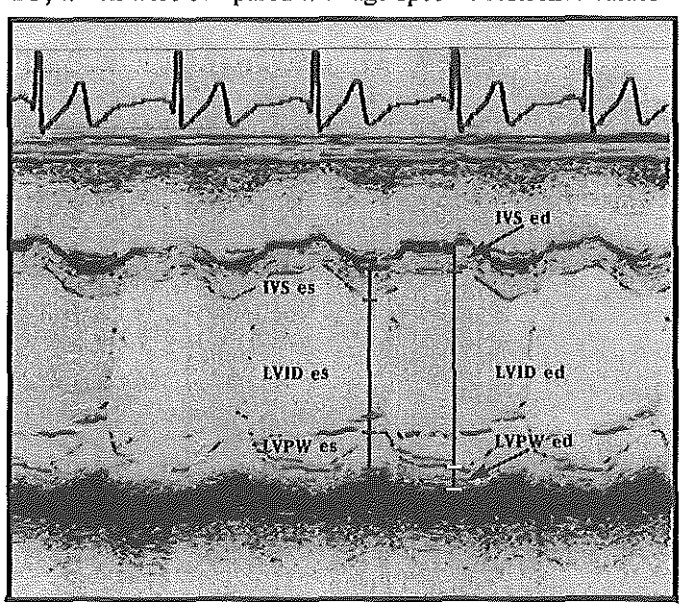

Figure 1. Example of left ventricular (LV) echocardiographic M-mode trace. Cursor positions for end-diastolic (ed) and end-systolic (es) measurements, just below the mitral valve, are indicated. IVS=interventricular septum; LVID=LV internal diameter; PW=LV posterior wall.

Table 2A. Mean (SD) of the ecg, echo, and BP findings of the three GH treatment groups (A, B, and C) at start and at end of the dose-response study. P-values are given for significant changes after four years GH treatment from baseline between all three groups.

	Baseline			After 4 yrs			P
	Gr.A	Gr.B	Gr.C	Gr.A	Gr.B	Gr.C	
RWT_{es}	0.37 (.07)	0.38 (.13)	0.41 (.07)	0.38 (.07)	0.39 (.08)	0.38* (.04)	0.05#
RWT_{ed}	0.14 (.03)	0.14 (.04)	0.16 (.03)	0.16 (.03)	0.16* (.03)	0.16 (.02)	0.03&
SF (%)	36.5 (3.8)	35.0 (6.7)	38.1 (3.4)	36.8 (3.7)	33.1 (5.9)	33.2* (4.1)	NS+
BPs (mmHg)	112 (16)	105 (11)	101 (14)	116 (14)	114 (12)	112 (15)	NS
BPd (mmHg)	69 (8)	69 (8)	64 (10)	73 (6)	71 (6)	68 (6)	NS

*: within group significantly different from baseline

Non-parametric (Mann-Whitney) tests between two groups:

#: A > C (P=0.02)

&: A > C (P=0.04); B > C (P=0.02)

+: B > C (P=0.05)

Gr Group

(e)d (end) diastolic

(e)s (end) systolic

RWT relative wall thickness

SF shortening fraction

BP blood pressure

STATISTICAL ANALYSIS

Results are expressed as mean (SD), unless indicated otherwise. Differences between groups were tested by the non-parametric Kruskal-Wallis (for three groups) or Mann-Whitney (for two groups) test. Differences between points in time were tested by paired Student's t-tests. The non-parametric MnNemar test was used to test paired dichotomous variables. Correlations were tested with the Pearson's linear correlation coefficient. A P-value < 0.05 was considered significant.

RESULTS

Table 1 summarizes baseline clinical data of the groups in both studies. The distribution of BMISDS for all girls was slightly skewed with a median (range) 0.23 (-1.8 to 4.3), in either study without substantial differences between groups. BMISDS was positively related with age at start of treatment ($r=0.44$; $P<0.0001$). At start of treatment, in the DR study three (5%) and in the FR four (20%) girls had a BMISDS >2.5.
Cardiovascular anomalies were found in 20 of the 87 girls (23%): operated aortic coarctation (10), aortic valve abnormalities (11), ligated ductus Botalli (2), surgically corrected type II ASD (1), mitral valve abnormality. Five girls had two anomalies. The number of cardiovascular anomalies was not significantly different between girls with a 45,X and other karyotypes.

ELECTROCARDIOGRAPHY

Mean LVact values at baseline and during all treatment years in both studies were well within the reference range. In the DR study, mean LVact values after 4 years GH were significantly higher compared with baseline only in group B and C ($P\leq0.005$). However, there were no significant differences between groups. In the FR study no significant differences between groups or time-points were found.

The heart axis showed a wide variation. Group means at baseline were within the reference range, although some girls in the DR study had abnormal values (baseline: 10%; after 4 years 2%; higher numbers in groups B and C). In neither study, group differences nor changes from baseline reached significance.

ECHOCARDIOGRAPHY (TABLE 2A AND 2B)

Baseline SD-scores for IVS_{ed}, PW_{ed}, $LVID_{ed}$, and $LVID_{es}$ were in general somewhat lower compared with a Dutch female referencepopulation: mean values varied between -1.0 and +0.5 SDS. In either study the changes from baseline to end of study for each of these four LV dimensions were not significantly different between groups. A general tendency could only be

Table 2B. Mean (SD) of the ecg, echo, and BP findings of the two GH treatment groups (OD and BID) at start and at end of the frequency-response study. P-values are given for significant changes after two years GH treatment from baseline between the two groups.

	Baseline		After 2 yrs		P
	OD	BID	OD	BID	
RWT_{es}	0.37 (.06)	0.33 (.06)	0.45 (.11)	0.38 (.10)	NS
RWT_{ed}	0.15 (.04)	0.13 (.03)	0.19* (.04)	0.15 (.03)	0.06
SF (%)	34.5 (4.5)	34.4 (3.4)	35.8 (7.1)	35.2 (4.7)	NS
BPs (mmHg)	119 (12)	116 (11)	120 (11)	113 (11)	NS
BPd (mmHg)	73 (7)	69 (7)	72 (7)	71 (5)	NS

*: within group significantly different from baseline
OD: once daily
BID: twice dialy
(e)d: (end) diastolic
(e)s: (end) systolic
RWT: relative wall thickness
SF: shortening fraction
BP blood pressure

noted for IVS_{ed} (upward in both studies) and PW_{ed} (downward only in the DR study). At end of study compared with baseline significant differences were in the DR study only found for $LVID_{es}$ in group C (higher) and PW_{ed} in all groups (lower); in the FR study for IVS_{ed} in the OD group (higher) and for PW_{ed} in the BID group (higher). Baseline PW_{ed}, $LVID_{ed}$, and $LVID_{es}$ values were significantly, negatively related with BMISDS (r=-0.7,-0.2,-0.3,respectively).

Mean RWT_{ed} values were within the reference range. The percentage of abnormal RWT_{ed} values fluctuated around 7%, except for year 3 (15%), with numbers equally spread between the groups. The mean change in RWT_{ed} values during 4 years GH treatment in the DR study was significantly higher in group B than those in group C (P=0.03). Mean RWT_{ed} values of groups A and B showed an increase during the first three years of treatment, significantly so for group B after the second year and for group A after the third year. In the final year of study a small decrease was observed for group A (significant) and group B. Only group B mean RWT_{ed} values after 4 years were significantly higher compared with baseline. Mean RWT_{ed} values of group C hardly changed during the course of study. In the FR study, mean RWT_{ed} values after 2 years GH in the OD group were significantly higher compared first year or baseline values, but still within the reference range. They were also higher compared with mean values in the BID group. The change from baseline after two years was not significantly different between groups.

Mean RWT_{es} values were within the reference range. The change in mean RWT_{es} values after 4 years treatment was in the DR study significantly higher in groups A and B compared with those in group C (P=0.05). Mean RWT_{es} values showed only minor changes during treatment, except for group C values in the DR study which were significantly lower compared with baseline after the second year of GH therapy onwards. In the FR study significant differences between groups or time-points were not found, except for higher values in the OD group for the change during the second year.

Mean SF values were within the reference range. There were only minor changes in mean values during GH treatment in either study. However, mean group C values in the DR study were significantly lower compared with baseline from 2 years GH onwards. They were also lower compared with mean values in group A. The difference in mean values after 4 years of GH therapy between group A and groups B and C was close to the 0.05 level of significance. In the FR study no significant differences between groups or time-points were found.

The change from baseline to end of each study for all four SD-scores as well as for RWT_{ed}, RWT_{es}, and SF was not significantly different between girls with and without cardiac abnormalities.

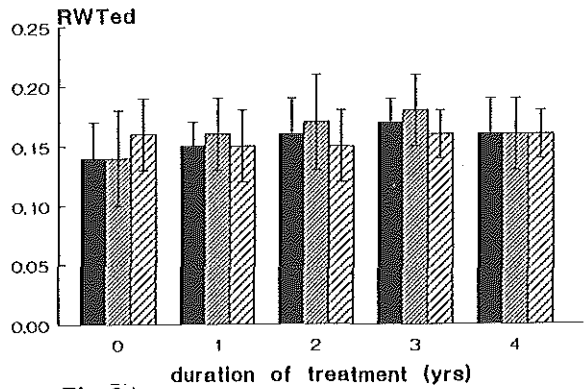

Fig. 2A

Figure 2. Development of the end-diastolic relative wall thickness RWTed) for each GH treatment group in the (a) dose-response study (A: ▒; B: ▒; C: ▒) and in the (b) frequency-response study (once daily: ▒; twice daily: ▒ injections)

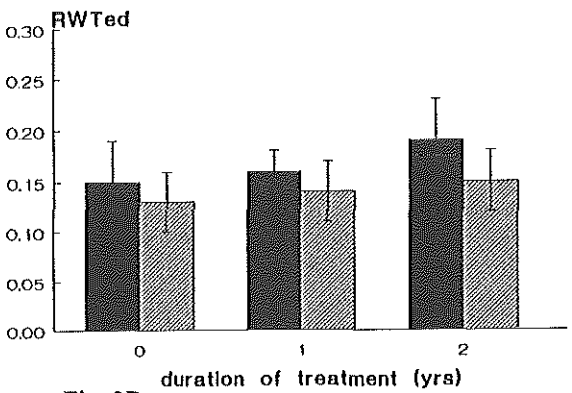

Fig. 2B

Asymmetric septumhypertrophy was found to be present at baseline in 6 girls (9%) of the DR study and 4 (21%) of the FR study. At end of study the numbers had increased to 12 (18%) and 6 (32%), respectively. In both studies, only one girl had asymmetric septumhypertrophy at both measurement points. The numbers were evenly distributed between study groups.

BLOOD PRESSURE

In the DR study the mean systolic BP was not significantly different between groups at any point in time. The mean baseline diastolic BP of group C was lower compared with groups A and B at any point in time. In the FR study there were no significant differences between groups or time-points during the study.

Both mean systolic and diastolic BP levels were in concordance with age-matched healthy girls. In comparison with the reference population 15% of the girls in the DR study presented with a baseline diastolic BP >90th age-specific

percentile. After 4 years this percentage increased significantly to 31% (P=0.013). Group differences were not found. Observations with regard to systolic BP were almost identical. In the FR study, with a smaller number of girls, 10% of the girls presented with an elevated diastolic BP. After 2 years treatment this percentage decreased to 5% (NS). In contrast an elevated systolic BP was found at baseline in 16% of the cases and after 2 years in 21% (NS). Again, group differences were not found.

DISCUSSION

PREVALENCE CONGENITAL HEART DISEASE IN TS

Twenty-two percent of all girls in the two studies had cardiovascular anomalies. Recently, an impressive number of 179 of 393 females with TS diagnosed in Denmark were cardiologically evaluated [14]. In 26% of the females a total 69 cardiovascular malformations was found. This percentage is comparable with another recent study [17,30], but lower compared with other reports [15,31]. In agreement with earlier reports [15,30,31] and our findings, aortic valve abnormality (18%) and aortic coarctation (10%) were most common in the Danish study. In the Danish study and previous reports [15,30,31] a significantly higher prevalence of CV malformations was found in 45,X females compared with mosaic monosomy (38% vs 11%), primarily due to aortic valve abnormalities and aortic coarctation. Patients with structural abnormalities of the X chromosome had no CV malformations. In the present studies, the number of cardiovascular anomalies was not significantly different between girls with a 45,X and other karyotypes. The differences between the prevalence of various CV anomalies in TS are likely to be explained by selection bias (hospital referral, karyotyping,etc.) and methodological differences.

INFLUENCES OF GH TREATMENT

In accord with an age-dependent development an increase in LVact and decrease in heart axis angle was found. Values did remain well within the normal range for children of these ages [22].

On echo mean baseline values for all SD-scores were somewhat lower compared with normal Dutch girls. A possible explaination is that the SD-scores are calculated with reference to weight and BMISDS values indicated a higher number of obese girls. Both in the DR and FR study the SD-scores for IVS_{ed} increased (normalized) over values expected in normal age-matched Dutch girls. PW_{ed}-SDS decreased (worsened) only in the DR study. Since changes, in particular the increasing values, of the individual girls occured predominantly within the normal range, LV hypertrophy appears not to be present, even at high GH dosages for an extended period. The calculated RWT and SF values should remain constant with age [27]. At start of treatment, at end of study, and the change in between indeed was not related to CA. Thus, as described in the general

population [27], the structure of the heart muscle seemed to increase proportionally with general development.

The RWT calculations use both IVS and PW measurements and thus assume concentric LV changes and, in relation to GH treatment, possible hypertrophy. However, both in the DR and FR study the SD-scores for IVS_{ed} increased over values expected in normal age-matched Dutch girls. PW_{ed}-SDS decreased only in the DR study. Mean baseline values for all SD-scores were all somewhat lower compared with their normal Dutch girls. This seems not surprising since BMISDS values were skewed to the right, indicating a higher number of obese girls, particularly when older of age. The septum thus showed a tendency to normalize in contrast to the posterior wall. The latter only so in the younger girls (DR study). The discrepancy in development between IVS and PW also explaines the (increase of the) number of girls with an asymmetrical LV development. In addition, measurement variation is higher for IVS than for PW [24]. The girls with asymmetrical LV values at end of study were others than the ones at baseline, except for one girl in each study. Since with GH treatment mean PW_{ed} values decreased, this asymmetry should not be considered as asymmetrical septumhypertrophy.

Only one other study involving GH treatment in TS has reported cardiological results [17]. A small increase was found in diastolic LVID, although higher than in reference population, remaining within the norm. Also, LV mass, when corrected for body surface area, showed no significant change, in agreement with the present studies. Girls with cardiac abnormalities behaved similar to others during GH treatment, as was the case in the present studies. Barton *et al.* investigated 27 children with non-GHD short stature [13]. After two years GH treatment with 20 or 40 IU/m^2/wk LV mass showed a modest increase within 95% confidence interval.

In GHD adults Amato *et al.* reported echocardiographic impairment, with reduced LV mass and function before and restoration after six months of GH treatment. Six months after withdrawal of low-dose GH replacement therapy the improvements disappeared again [32]. Several short term (placebo controlled) studies have been performed using replacement GH dosages in adult GHD patients. In a cross-over study LV mass increased [33], and since wall thickness did not increase, this was mainly due to an increased LVID. The latter contrasts to another study [34] without significant changes in LV mass. In adult-onset GHD baseline LV dimensions and mass were found to be normal [35]. This reversed after one year GH therapy. All GH induced changes disappeared within 3 months after withdrawal, only LV mass was still increased. A four months GH (±2 IU/m^2/d) cross-over study with 21 GHD adults showed that LV diastolic dimensions increased and seemed to normalize [12]. SF increased only after long term therapy (16 and 38 months). This was not regarded as an increase in contractility of the heart, since the calculated afterload was unchanged and, therefore, the author's suggested that the preload had to have increased.

Thus, the effect of GH treatment studies in GHD seems to depends on the onset and duration of the GH deficiency -childhood or adult onset-, previous use of GH and the dosage used [32,33,35,36]. In contrast to increased echocardiographic

measurements in GHD adults, studies in non-GHD children, including the present ones, show no significant influences on LV dimensions.

Following GH therapy expansion of extracellualr volume and fluid retention, possibly due to activation of the RAA system, has been reported, but also an increased glomerular filtration and resting heart rate. There is no well established link between GH excess or deficiency and arterial blood pressure [37]. In TS an elevated BP has been reported [16]. Another group found BP values to be within the normal range for age, and following one year GH treatment no significant change [17]. In contrast, the present studies showed that 10-15% of the baseline diastolic and systolic BP values exceeded the 90th age-specific percentile for healthy girls. With GH treatment these percentages increased to 31% in the DR study, without differences between groups.

In conclusion, long term GH therapy in three dosage regimens up to 8 IU/m^2/day and in two administration frequencies did not result LV hypertrophy. In view of the increase in end-diastolic RWT in two groups on 6 IU GH/m^2/day, although still within the normal range, further follow-up is advocated. Although mean diastolic and systolic blood pressure remained within normal age related limits, a considerable number of girls with TS had elevated values already at baseline and this number increased after four years GH, without a difference between treatment groups.

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APPENDIX

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Chapter 10

General discussion, conclusions, recommendations

GENERAL DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

Hormonal intervention studies directed at improvement of final height require substantial treatment periods. Therefore, interim analyses are appropriate to evaluate the validity of continuing treatment. Judgement of the improvement in height velocity, the gain in height or in predicted final height are then necessary. This raises several critical issues as the results can only be interpreted in relation to an untreated disease specific population.

CRITICAL ISSUE (1):
BONE AGE DETERMINATION

A number of investigators have reported out that the biological maturity in TS can differ from the chronological age of the girls [1-3]. There is also no disagreement about the fact that 'reading' the radiographs of the hand to determine the bone age, even in series, is subject to substantial interindividual variation and is furthermore time consuming and dull. Of these BA methods the TW2 method is widely used, although the reliability is limited [4-7]. 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 [8,9]. 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). In Chapter 2 it was shown that this computerized method is a valid method and applicable in children with a growth disturbance, such as girls with TS. Some of the mean differences between manual and computerized readings were statistically significant. However, since these mean differences were less than 0.4 BA 'year', they may not be clinically relevant. 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. After the validation procedure, bone ages from over 800 radiographs from more than 300 untreated girls with TS gathered from 11 paediatric centers in the Netherlands served to construct a mixed longitudinal/cross-sectional curve over the full chronological age range.

We conclude that in the hands of an experienced observer, the computerized methods result in a fast and objective determination of the TW2-RUS bone age. One of the drawbacks is the need for individual positioning of each bone. Full automization of the system would further enhance its practical implementation. A reliable measure of biological maturity is crucial to predict final height.

CRITICAL ISSUE (2):
FINAL HEIGHT PREDICTION

In order to judge success of hormonal growth promoting interventions one essential question remains difficult to answer: what would have happened without treatment? A definite answer is not available. Ideally, each prospective study should include a control group. Given the accepted indication for the use of GH in girls with TS in the Netherlands and the drop-out rate with such a long-term study, this seems not feasible. Using historical controls would be another option. However, to identify sufficient girls in each age class with an adequate long-term follow-up is also difficult. Another potential cause of errors with historical controls is the possible existence of a secular trend.

Therefore, a number of attempts have been made to develop final height prediction methods: [1] Bayley and Pinneau [10] designed tables with percentages of achieved final height at an observed bone age determined according to the atlas of Greulich and Pyle [11]. Drawbacks using the BP method are that it can only be used after a GP BA of 6 years. More importantly, separate tables for girls with a BA retardation of more than one year may lead to marked differences in the FH prediction; e.g. when the GP BA retardation changes from just over to just under one year. [2] In the Tanner and Whitehouse method [12] FH is calculated using regression coefficients for each age group. Although TW provides models with more coefficients to improve accuracy, the pre-menarcheal 3-variate equations, for height, CA, and RUS, are most widely used. Both the BP and the TW method are essentially based on healthy children and, therefore, overpredict final height in particular from the age of 9 years onwards, as was demonstrated in Chapter 3.

The relative inaccuracy of these methods (and others) have led us to develop Turner specific FH prediction methods: [3] the Projected Adult Height (PAH), based on the assumption that the SDS of FH is equal to the SDS of H for CA ($HSDS_{CA}$) of girls with TS at any moment before FH is reached, and [4] the Modified Projected Adult Height (mPAH), developed by Lyon *et al.* [13], using linear regression analysis on growth data of untreated Turner girls for the relationship between FHSDS and $HSDS_{CA}$.

Based on the assumption that the FHSDS in TS is equal to the HSDS for BA ($HSDS_{BA}$, both for RUS and GP BA) rather than the HSDS for CA, the [5] Index of Potential Height (IPH) was developed [14]. It should be stated that merely substituting BA for CA is incorrect; yearly classes for the development in height should be based on the relation during a BA 'year'. Lenko *et al.* [15,16] and Naeraa *et al.* [17,18] developed modifications of IPH, [6] the Modified Index

of Potential Height (mIPH), by defining the difference between CA and BA in another way. In Chapter 3 it was shown that the simplest methods - projected height (PAH) and its modification (mPAH) - were remarkably reliable at most ages. Thus, if population-specific Turner reference data are available, a number of calculations - each with possible errors - will result in a smaller mean prediction error and a higher accuracy.

For this reason a new Turner specific FH prediction method, PTS, was constructed, using 235 measurement points of 57 Dutch women with TS, including women with spontaneous menarche and estrogen treatment. Analogous to the TW for normal children, smoothed regression coefficients were tabulated for PTS for height, CA, and BA (both RUS and GP). Although the validity of PTS_{RUS} and PTS_{GP} remains to be tested below the age of 6 years, both gave small mean prediction errors (maximum 1.7 cm). In particular at 'early' ages, the accuracy was at least as high as existing FH prediction methods. As expected, accuracy increased with age. The PTS methods are, in principle, applicable in other populations of the world as a constant deficit of 20 cm has been found between the FH and mid-parental height of Turner women [18-21]. Correction can also be made for a secular trend.

We conclude that the PTS methods are valuable additions to existing methods for FH prediction in girls with TS. Moreover, they may be of help when setting the indication for growth promoting treatment and evaluating its effects. Since the reference sample included women with spontaneous menarche and estrogen treatment these effects are accounted for.

CRITICAL ISSUE (3):
EVALUATING AUXOLOGICAL GROWTH HORMONE EFFECTS

The efficacy of growth promoting therapy has mainly been judged on short-term results. Although growing along with one's peers is psychologically very important, the main outcome variable remains final adult height. After becoming unlimited available, rDNA human GH was used on a larger scale in TS from 1986 onwards. Since, initially, predominantly girls of 6 years or over were included, data on final height of these girls has been reported in recent years. The general outcome was disappointing: the mean gain in height was in the order of 3-5 cm [22-24].
Possible explanations for the disappointing results are:

1. Earlier studies predominantly concerned girls who started GH therapy at the age of 6 years or over with possibly a more substantial growth retardation than younger girls.
2. The wide range of the gain in height, from a loss of several cm to a gain of about 15 cm.
3. The reports did not include all patients enrolled in the study. Information about those who are still growing and those that dropped-out of study should not be withheld.

4. The methods to determine spontaneous growth without growth promoting treatment may be questioned. A region or cultural specific Turner reference population should be used as well as the appropriate final height prediction method (see above). Note that all methods have been designed for untreated subjects and may not be automatically applicable to estimate the effect of treatment. Bone maturation may also be influenced by growth promoting hormonal therapy. As a result final height could be achieved at an earlier age.
5. The study design of the various studies was not always stable over the years, e.g. GH dose or administration frequency, estrogen dose. And finally,
6. Height criteria to stop GH therapy are not necessarily equal to final adult height. After cessation of growth promoting treatment many girls will continue growing at a very low rate, and thus may add millimeters but some even centimeters, as in untreated girls with TS (personal experience).

Since the studies described in this thesis do not report final height results, this discussion will focus on the optimization of GH therapy (without additional use of androgens such as oxandrolone) on the interim growth response, expressed as height or HV (SDS) and estimated gain in cm.

A. DOSE-RESPONSE EFFECTS OF GH THERAPY

FIRST AIM DR STUDY:

A. COUNTERACTING THE 'WANING' EFFECT

The question to be answered is whether a yearly stepwise increment of the GH dose from 4 (group A) to 6 (group B) to 8 IU/m^2/day (group C) could maintain or augment the initial increase in height velocity and thereby improve FH prediction.

The answer is clearly affirmative. Our study showed that each increase of the GH dose resulted in a significantly higher HV compared with the previous dose. However, during the fourth year, only group C mean HV - but not group B - remained significantly higher than group A. The change of growth pattern expressed as $HSDS_{CA}$ (Dutch-Swedish-Danish Turner references) was identical with that of HV; however, in year 4 the mean change in $HSDS_{CA}$ in group B also remained significantly higher than group A. The mean change in $HSDS_{CA}$ after 4 years GH treatment was significantly higher for groups B and C compared with group A, but not significantly different between groups B and C. Individual growth curves are depicted in Appendix 1a. Figure 1 shows a plot of the standing heights at baseline and after 4 years GH treatment for all girls in the DR study.

The DR study design was best comparable with a French study described by Chaussain *et al* [25]. In this study the GH dose of 0.7 IU/kg/week (about 3 IU/m^2/day) was increased by the same amount (to a maximum of 2.1 IU/kg/week) if HV after 6, 12, or 24 months had not doubled. After 6 months, 14 of the 24 girls received a higher dose (58%). Using these adjustments on an individual basis the waning effect of HV was considerably attenuated during 3 years, the range of individual variation was low, and the effect of CA on the amplitude of HV was suppressed.

An important difference was that the French study design was tailored to the individual situation. Ideally this should result in a favorable outcome, since those girls with insufficient growth response to GH treatment will likely benefit most from a further increase of the GH dosage. However, this is not expressed by the interindividual variation: for $HSDS_{CA}$ (compared with normal peers) it was comparable with that in our highest dose group (about 1 SDS). Also, the 'waning' effect of HV seems similar to our group C: pretreatment and first through third year values were 3.9, 7.6, 6.5 and 6.2 compared with 5.9, 10.1, 8.4, and 7.9, respectively, for the French and our study. On the other hand, the bone maturation of about 3 BA years over the 3 study years was less than that in our study.

In the past years, the most commonly used GH dosages in TS varied from 2 to 4 IU/m^2 body surface/day subcutaneously [25-29]. Five GH dose-response studies in TS have meanwhile reported interim results. All studies were in favor of the higher dosage tested. However, the highest dose in four studies was comparable to the lowest dose in the present study: Takano *et al.* [29] and Bertrand *et al.* [30] compared 2 and 4 IU/m^2/day, Stahnke *et al* [27] 2, 3, and 4 IU/m^2/day, and Spoudeas *et al* [28] 3 and 4 IU GH/m^2/day. These dose-comparison studies simply showed that the higher dosages reduced the waning effect of the short-term growth response. Bertrand *et al.* [30] recently described comparison of a constant GH dose of 4 IU/m^2/day (group 2) with an increase in the GH dose from 2 to 4 IU/m^2/day after the first year of treatment (group 1). In agreement with our results, the waning of HV after the first year of treatment was reduced in group 1. The increase in HV during the first year was significantly lower in group 1 compared with group 2.

We conclude that a stepwise approach of the GH dose from 4 to 6 and to 8 IU/m^2/day reduced the 'waning' effect of the growth response after four years treatment in girls with TS aged 2 to 11 years.

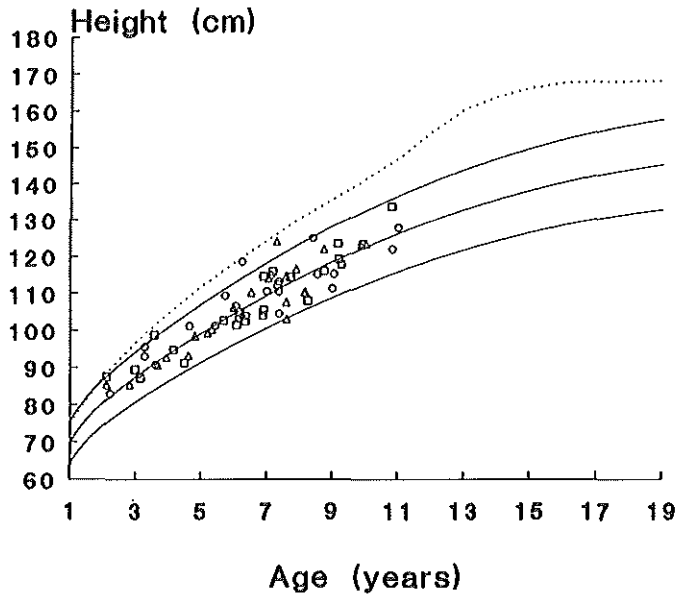

Fig. 1A

Figure 1. Plot of standing heights at baseline (a) and after 4 years GH treatment (b) of all girls in the dose-response study according to their randomization group A (△), B (○), or C (□) against the Dutch-Swedish-Danish Turner references (mean +/- 2 SD) and the 50th percentile for healthy Dutch girls (·····).

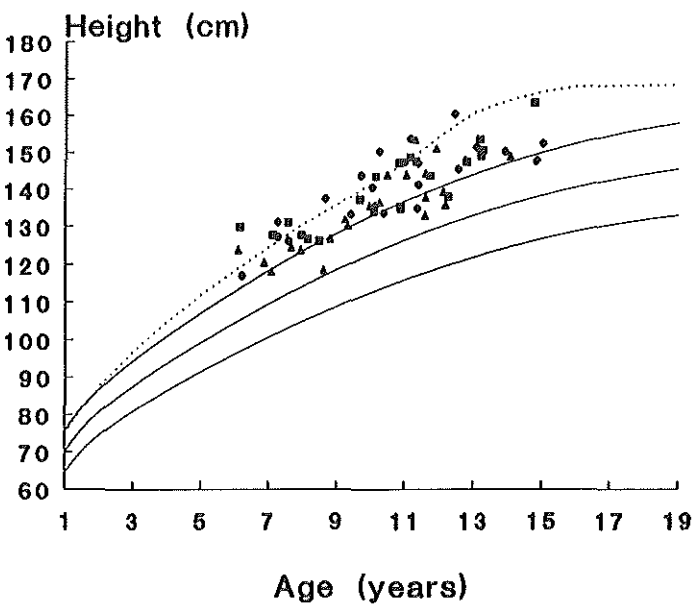

Fig. 1B

FIRST AIM DR STUDY:
B. IMPROVEMENT OF FH PREDICTION

As pointed out in critical issue 2, estimation of long-term growth promoting treatment effects should be performed with methods including bone maturation. A common denominator seems to emerge from the above dose-response and other studies using higher GH dosages: with GH therapy bone maturation progresses at a certain advanced 'velocity' independent of the dose used, since none of the above studies (including ours) showed significant differences in bone maturation between GH dosage groups. Here the word 'advanced' needs to be stressed.

To be able to compare the bone maturation of the girls in the GH treatment studies with untreated Dutch Turner girls, a Turner specific bone age curve was developed. The X-rays of the hand during the study were read by a single observer to eliminate between observer variance. Figure 2 shows a plot of the bone ages at baseline and after 4 years GH treatment of all girls in the DR study.

In studies using GH dosages only up to 4 IU/m^2/day the growth rate outweighed the accelerated bone maturation[31,32], and thus, FH prediction improved more than with lower GH doses[26-29]. This contrasts with the findings after four years in the present study, although values in the higher dose groups were higher than in the constant dose group. In Figure 3 the bone maturation per study year for each GH treatment group in the DR study is given.

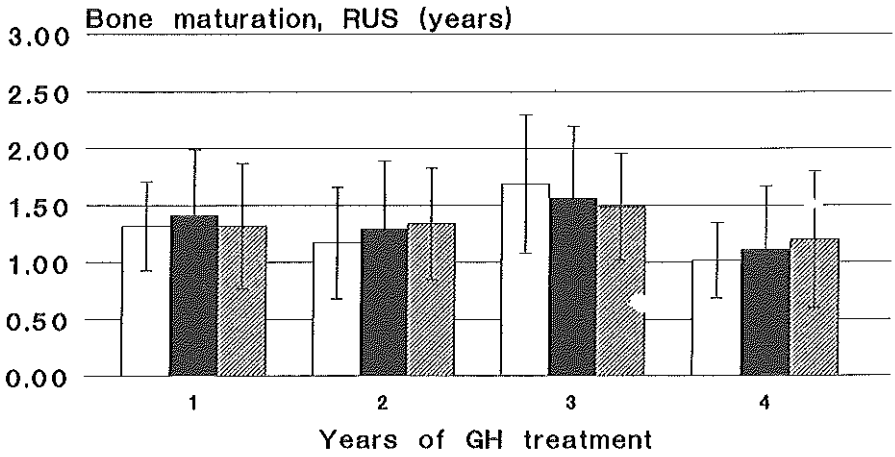

Figure 2. Mean (SD) bone maturation per study year for each GH treatment group A (□), B (▩), or C (▩) in the dose-response study.

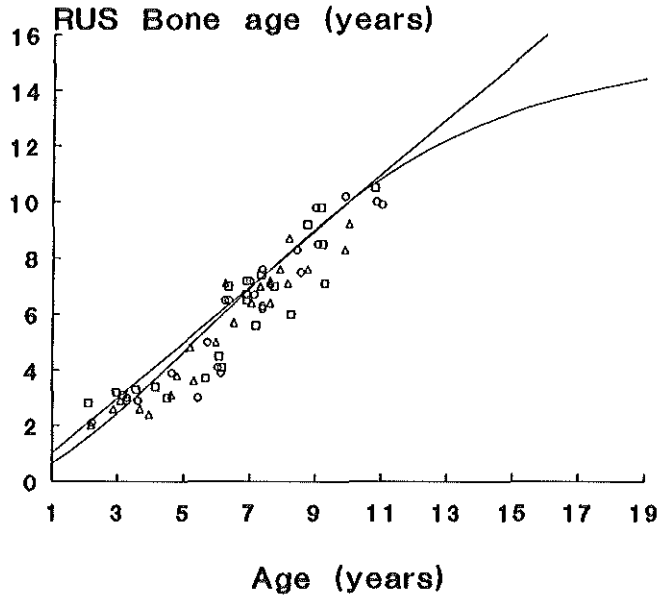

Fig. 3A

Figure 3. Plot of bone age RUS-score at baseline (a) and after 4 years GH treatment (b) of all girls in the dose-response study according to their randomization group A (△), B (○), or C (□) against the mean Dutch Turner reference and the assumed $y=x$ line for bone age and chronological age.

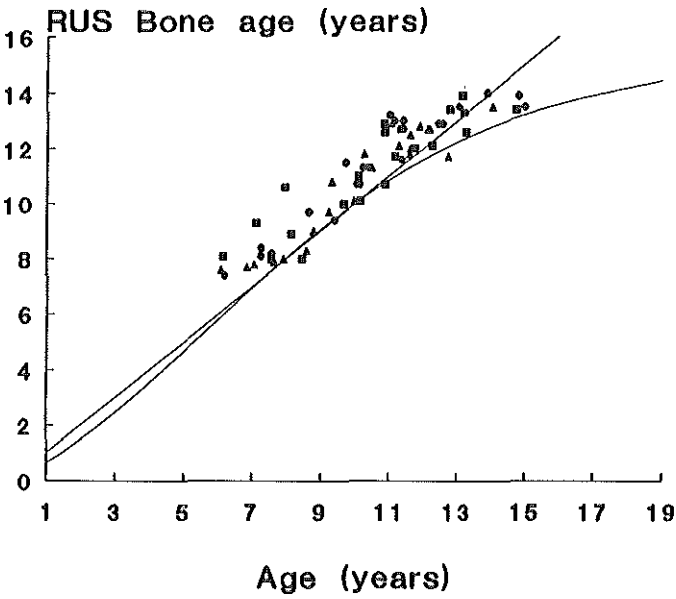

Fig. 3B

The values using the modified Index of Potential Height ($mIPH_{RUS}$) were markedly lower than those using the PTS_{RUS} method. It should be noted that [a] the $mIPH_{RUS}$ method comprises two separate calculations, each with their margin of error. And more importantly for this method, [b] the expression of CA ('CA') as a function of BA should be adjusted during GH treatment, since bone maturation increases. The same is true for the second calculation in this method, [c] the relationship between FHSDS and the initial HSDS. In general, bone maturation during GH treatment was faster compared with untreated values. Therefore, the treated girls had an older 'CA' and thus a lower $HSDS_{'CA'}$, since the current height of these girls was compared with an older age class. This resulted in lower $mIPH_{RUS}$ values during treatment. For an adequate comparison treatment specific 'CA' classes should be developed for this method.

The PTS_{RUS} method is also based on girls with TS without growth promoting treatment. Only one simple calculation has to be performed. Differences in bone age maturation could lead to altered regression coefficients.

We were able to demonstrate that bone maturation, although not significantly different between the treatment groups, was significantly faster compared with estimated values for untreated Dutch girls with TS. Furthermore, bone maturation during GH therapy was positively related to the degree of BA retardation at start of study and negatively with baseline CA. Surprisingly, there was no relationship between baseline BA retardation and age. This indicates that the younger girls at baseline in the DR study responded with a faster bone age development compared with 'older' girls and that if baseline bone age retardation was larger the bone age progression was faster.

After four years GH treatment both $mIPH_{RUS}$ and PTS_{RUS} showed significant increases in mean FH prediction, without significant differences between groups.

SECOND AIM DR STUDY:
INFLUENCE OF AGE AT START OF THERAPY

The second objective of the DR study was to establish whether treatment from a young age onwards could improve FH prediction. In an earlier Dutch TS study [26] a negative relationship between the FH gain over initial projected FH and age at start of therapy was found. We found that irrespective of the GH dose used, initiation of GH treatment at a younger age was beneficial after four years GH when expressed as actual cm gained over estimated untreated values or as gain in FH prediction, but not statistically significant when expressed as change in $HSDS_{CA}$ over the study period (see Figure 4). It thus seems that if one standardizes (the change in) height for age during GH therapy, the positive influence of a younger age at baseline is lost. Again, it must be stressed that the

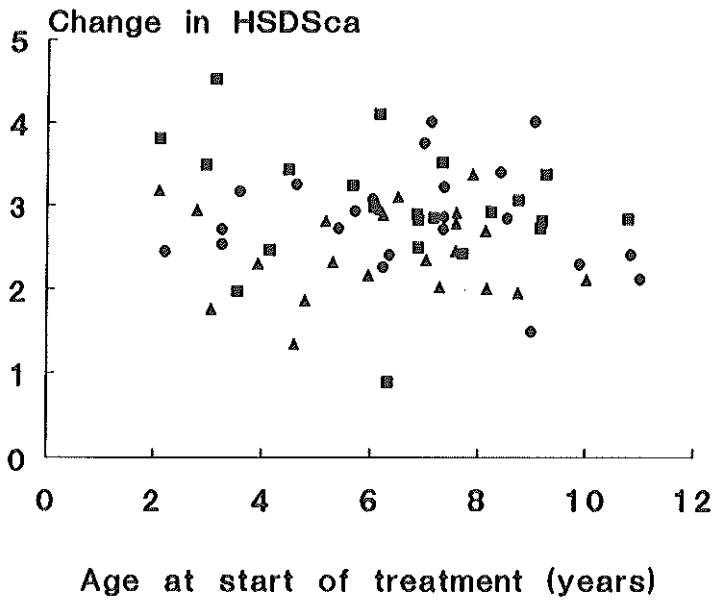

Figure 4. Plot of the change in $HSDS_{CA}$ after 4 years GH treatment for all girls in the dose-response study according to their randomization group A (▲), B (●), or C (■) versus the age at start of study.

age range at start of GH therapy in our study (2 to 11 years) was much lower compared with other studies.

We have also shown that the change in $HSDS_{CA}$ after four years GH treatment was not related to its baseline value, in agreement with earlier reports [26]. In contrast, others did find a negative correlation between baseline $HSDS_{CA}$ and the FH gain over initial projected FH [22] or between baseline $HSDS_{CA}$ and gain in cm or $HSDS_{CA}$ at FH [24] in girls with TS who started GH treatment after the age of 10 years.

In conclusion, it appears that after four years study, initiation of GH treatment at these very young ages was not more beneficial (expressed as change in $HSDS_{CA}$) compared with somewhat older girls, irrespective of the GH dose used.

THIRD AIM DR STUDY: PROPORTIONATE GROWTH

The third aim of the DR study addressed the question whether the use of different growth hormone dosages would result in changes in body proportions. To be able to answer this question we first looked for the most appropriate method to determine body proportions (see Appendix 2). In general, all proposed descriptions are satisfying when describing the typical cases, but differ

strongly in the description of atypical cases. Nonparametric description of the variables is indicated but for reasons of simplicity the use of a parametric description is common. Basically, they are chosen such that they describe the majority of the data rather well. We opted for the method described by Gerver*et al.* [33,34]. Apart from SD (or *z*-) scores, the concept of 'shape (*f*-) values' was used to describe combinations of measurements. Also, their earlier work allowed us to use a large healthy Dutch female reference population[35]. In addition, we gathered data from untreated girls with TS to construct Turner specific reference curves for various combinations of measurements, see Appendix 3.

Baseline *z*-scores and *f*-values were well within the reference range for age-matched girls with TS. However, the vast majority of the girls (>85%) in both the DR and the FR study had disproportionate short stature for all measurements in relation to height compared with a healthy Dutch reference population. Only when the biacromial and biiliacal diameters were compared with each other about two-thirds of the girls were 'typical' compared with healthy age-matched peers. GH treatment resulted in significant improvements of most *z*-scores. The change from baseline to end of study was only significantly dose-dependent for *z*H(eight), *z*Hand, and *z*Foot. During the GH studies *f*-values improved significantly towards the 'typical' range of the normal female reference population, in particular at younger ages and for *f*H&Hand, *f*H&Foot, and *f*H&Biiliacal diameter. A significant dose-dependent improvement was only noted for *f*H&Biiliacal diameter. The development of the shape value for H&Hand and for H&Foot of the girls in the DR study is shown in Figure 5.

Gerver *et al.* [36] described contradictory results after two years GH treatment (24 IU/m^2/week) in TS. Their study particularly suggested the development of a relatively wide pelvis (*f*H&Biiliacal diameter). A comparison with the present studies is impeded by differences in age range and GH dose. More importantly, an increasing number of girls in that study received estrogens in a dose twice as high compared with the girls in the FR study. In agreement with the results in the present studies, Rongen*et al.* found no significant change in the Sitting Height to Subischial Leg Length[26].

The present studies show that GH treatment, even at high dosages up to four years, does not result in disproportionate growth in girls with TS.

Safety aspects, i.e. the fourth aim of the DR study, will be discussed below.

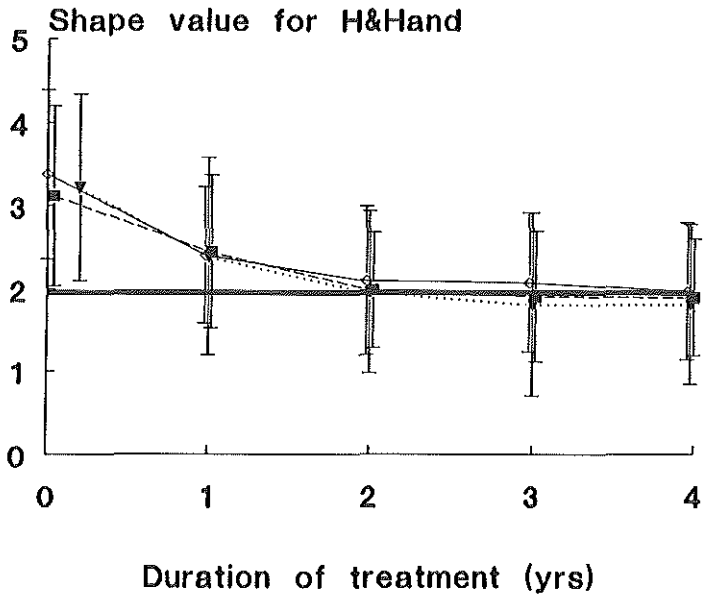

Fig. 5A

Figure 5. Development of the mean (SD) shape (f)-value for Height (H) & Hand (a) and for Height (H) & Foot (b) for each GH treatment group (A: –◇–; B: ···▼···; C: –■–) in the dose-response study. The horizontal line at f=1.96 represents the mean for age-matched healthy girls (limit of typicality).

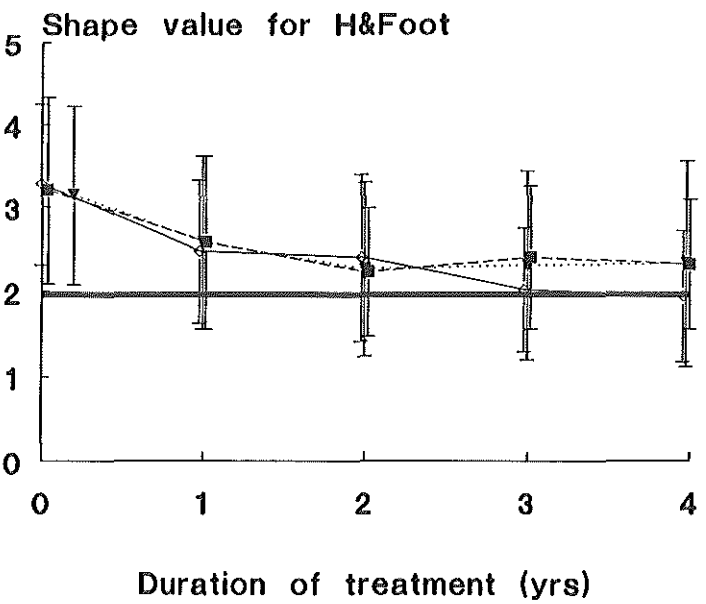

Fig. 5B

B. FREQUENCY EFFECTS OF GH ADMINISTRATION

GH profile studies during a 24-hour period showed that endogenous GH in healthy individuals, as well as in girls with TS, is secreted episodically [37-39]. The major peak occurred in the early phase of sleep. This was also confirmed in 10 girls participating in the DR study. As a result of these findings GH has traditionally been injected in the evening, preferably just before bedtime. As pointed out in Chapters 4 and 5, there is some evidence to suggest that GH therapy administered more frequently than once daily might be beneficial.

The second GH study was performed in 19 girls with TS, aged 11 years or over, and concurrently receiving low-dose ethinyl estradiol (0.05 µg/kg/day, orally). An attempt was made to mimick to some extent the physiological GH secretion, although it was recognized that this will not be achieved through subcutaneous administration. In this study 2/3 of a total GH dose of 6 IU/m^2/day was injected in the evening and 1/3 in the morning (BID regimen).

AIM FR STUDY:
COMPARISON OF EFFICACY AND SAFETY OF TWO GH INJECTION FREQUENCIES

The growth response after two years of GH treatment in the two administration frequency regimens was in conformity with the findings in the cross-over study. The growth response expressed as HV, HVSDS, the change in $HSDS_{CA}$, the gain in height over estimated untreated values and in FH prediction all showed significant improvements. Individual growth curves are depicted in Appendix 1b. Figure 6 shows a plot of the standing heights at baseline and after 4 years GH treatment for all girls in the FR study. Although mean values in auxological parameters tended to be higher with OD injections, significant differences between the OD and BID regimen were not observed. Figure 7 shows a plot of the bone ages at baseline and after 2 years GH treatment of all girls in the FR study. Bone maturation after two years was similar between groups and not significantly different from untreated estimated values. Therefore, FH prediction also improved. In a Belgian study in girls with TS [40], comparing an OD regimen with an equal division of a lower daily GH dose (25 IU/m^2/wk), the change in HV after one year treatment was somewhat, but not significantly, lower with OD compared with BID GH injections. In most studies the trends are in favor of undivided doses. However, at this point in time it may be too early to fully abandon the idea of GH administration in divided doses, since treatment groups may have been rather small and/or the treatment period may have been too short. Furthermore, the possiblity of endogenous GH production in GH treated short children and girls with TS cannot be ruled out.

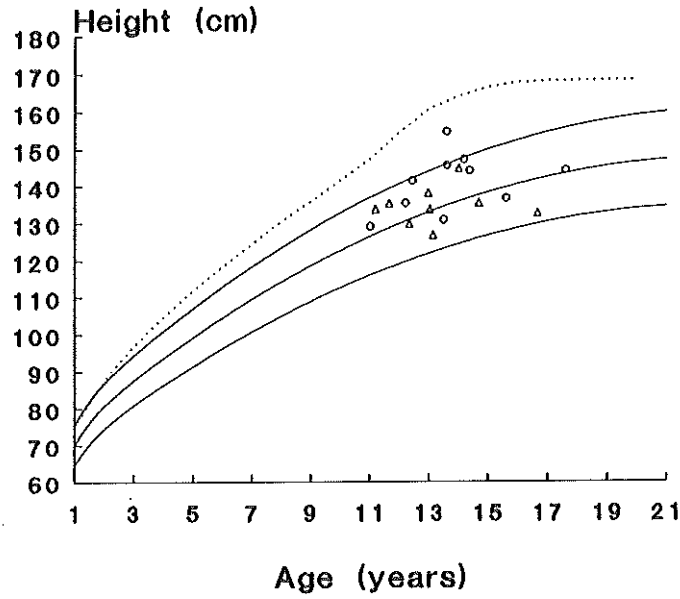

Fig. 6A

Figure 6. Plot of standing height at baseline (a) and after 2 years GH treatment (b) of all girls in the GH administration frequency-response study according to their randomization group with OD (△) or BID (○) injections against the Dutch-Swedish-Danish Turner references (mean +/- 2 SD) and the 50th percentile for healthy Dutch girls (·····).

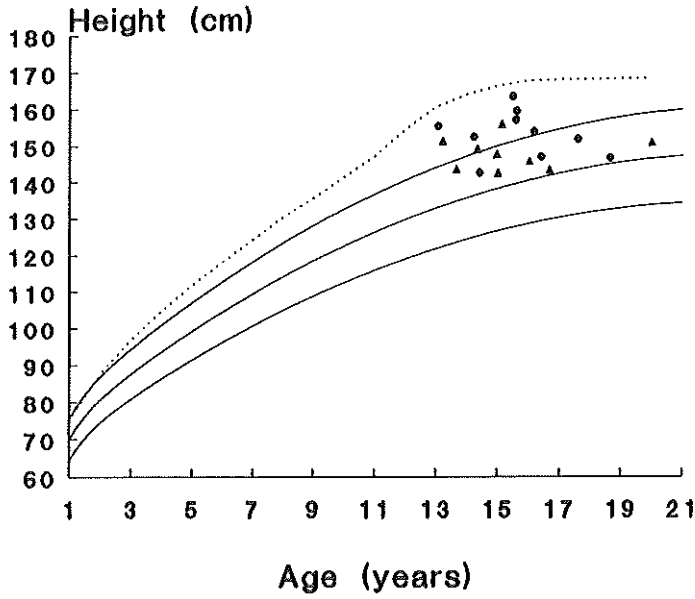

Fig. 6B

Finally, different GH injection regimens may have other long-term effects on carbohydrate and lipid metabolism [41].

Since the BID regimen was well accepted and the girls were compliant all agreed to continue GH therapy in their randomized groups until FH. Safety aspects of this study will be discussed below.

In conclusion, after two years GH treatment no significant differences between administration frequency groups were found.

ESTROGENS

Lack of estrogens is the main endocrine defect in Turner girls resulting in the difference in pubertal development with their healthy peers. It should be noted that although their female physical appearance may not be comparable with their peers, all girls in the FR study with an age range of 11-17 years did show clear signs of pubertal development in their hand-writing before estrogen therapy was started (courtesy of Mrs C.M.A. Wijs-ter Linden).

A key role for estrogens in the control of the pubertal growth spurt has been established, and several short-term and long-term studies of their growth promoting potential have been published [42-45]. The short-term 6-months cross-over placebo controlled study by Ross and coworkers demonstrated that height velocity in girls with TS aged 5-15 years could be increased by 69% with ethinyl estradiol in a daily dosage of 0.1 μg/kg body weight [46]. The long-term studies of Kastrup *et al.* [47] in which the estrogen treatment consisted of 17-ß-ethinylestradiol, showed a dose related, increased growth rate in the first year of treatment and a decrease in the following years. They also observed an accelaration in bone maturation, especially in the group with a bone age below 10 years. Pubertal development progressed at a normal rate. Salti and coworkers [48] presented data on the accelerating effect on skeletal maturation of a dosage of 0.1 μg/kg body weight per day. Therefore, several investigators favor an even lower dosage of approximately 0.05 μg/kg/day.

The latter dose was also used in our FR study. The combined effect of GH and estrogens may affect the final outcome in a favorable way [49], although some studies reported only a limited additional effect of estrogen [50,51]. Since both treatment groups received equal ethinyl estradiol dosages it is assumed that effects on auxological and biochemical parameters is comparable. In the FR study the progression through puberty on this low estrogen dose (0.05 μg/kg/day) seemed slower in a subgroup of nine girls compared with the British reference population, as indicated by the mean interval from B2-B3, 1.72 (SE 0.27) vs 0.93 (SE 0.06) year for TS and reference girls, respectively. Only one girl experienced her menarche.

We conclude that oral ethinyl estradiol at a daily dosage of 0.05 μg/kg in combination with GH does not accelerate skeletal maturation or pubertal development in girls with TS of 11 years or older.

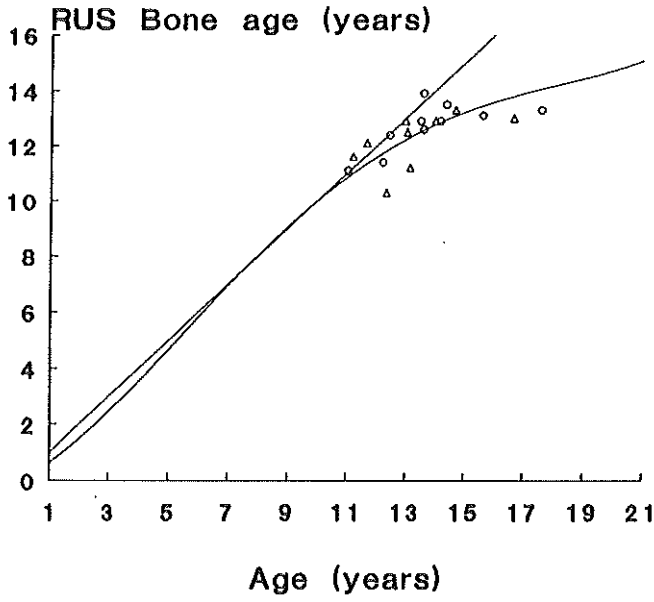

Fig. 7A

Figure 7. Plot of bone age RUS-score at baseline (a) and after 2 years GH treatment (b) of all girls in the GH administration frequency-response study according to their randomization group OD (△) or BID (○) injections against the mean Dutch Turner reference and the assumed $y=x$ line for bone age and chronological age.

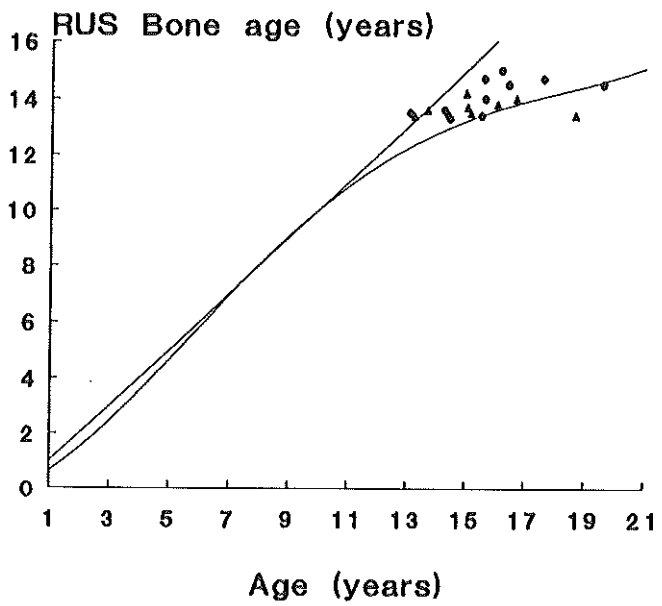

Fig. 7B

SAFETY ASPECTS OF GH TREATMENT AT HIGHER DOSAGES

INFLUENCE OF GH TREATMENT ON THE GH-IGF AXIS

An additional aim of the DR study was intended to relate biochemical parameters of 24-hour growth hormone profile testing during each growth hormone dose in a subgroup of 12 girls with the auxological response. Dose-dependency could also be shown for the area under the curve (AUC) for GH during 24-hour profiles, but the change in $HSDS_{CA}$ was not linearly related with AUC.

The bioavailability of GH and IGF-I is modified by their main binding proteins (BP), GHBP and IGFBP-3. Baseline GHBP levels were in 84% of the girls in the DR study within the normal age range. In contrast to the older girls in the FR study, but in agreement with another report on TS[52], the decrease in mean levels after 6 months GH in the DR study was not significant. As expected, mean IGF-I and IGFBP-3 plasma levels increased significantly, without significant differences between dose groups. In contrast, after three years GH treatment in Japanese girls with TS [53] mean IGF-I levels were statistically higher with 1 IU GH/kg/wk than with 0.5 IU/kg/wk.

The change in $HSDS_{CA}$ during GH was dependent on IGF-I plasma levels at baseline and during the study period. A progressive rise of the IGF-I to IGFBP-3 ratio has been proclaimed to be an indicator of the growth response[54]. We showed a significant increase of this ratio over time (see Figure 8), without significant differences between dose groups. However, after 30 or 48 months of treatment no significant relationship was observed between the change in IGF-I to IGFBP-3 ratio and the change in $HSDS_{CA}$, nor was there a significant difference between groups of the change of this ratio, although a trend was apparent after 30 months. Also, neither IGF-I nor IGFBP-3 levels, nor their ratio were related to the pretreatment HV(SDS). Taken together, there is little evidence to support an explanation of the differences in growth response between the groups by differences in the change of the IGF-I to IGFBP-3 ratio. Nevertheless, a repeated measures model with the dose-increment steps and duration of treatment as covariants, showed that each change in $HSDS_{CA}$ correlated significantly with IGF-I plasma levels.

Preceeding the auxological follow-up period of two years, 10 girls in the FR study underwent 24-hour profile testing during each GH treatment regimen in a cross-over design (see Chapter 5). The pharmacokinetic data reflected the mode of GH administration. Maximum GH levels were significantly higher and the area under the curve larger with OD than with BID GH treatment. Again, a consistent diurnal variation with either regimen in GHBP, IGF-I, or IGFBP-3 levels could not be demonstrated.

Pretreatment GHBP levels tended to be high compared with values in healthy prepubertal children. These levels decreased with GH therapy, significantly so with BID GH only. It was hypothesized that, as GHBP corresponds to the extracellular domain of the GH-receptor [55], a possible

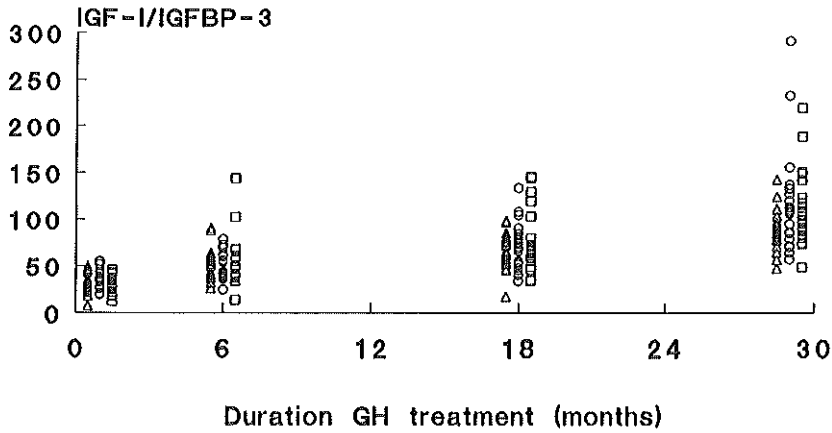

Figure 8. IGF-I to IGFBP-3 ratio at baseline and after 6, 18, and 30 months of GH treament with 4, 6, 8, and 8 IU/m^2/day for all girls in group A (△), B (○), and C (□) of the dose-response study.

explanation for our findings might be that TS is associated with a state of relative (intra-cellular) resistance to GH, which might result in compensatory up-regulation of GH-receptor/GHBP. Exogenous GH would then result in down-regulation of receptor leading to a decrease of GHBP levels towards the normal range. After 18 months of GH treatment in the FR study, the significant decrease in GHBP plasma levels observed after 6 months in all girls was no longer significant.

The mean pretreatment IGF-I plasma level in 10 girls of the cross-over study was in the subnormal and that of IGFBP-3 in the high-normal range, in conformity with other TS studies[54]. Short-term GH therapy induced a dramatic rise, without significant differences between frequency regimens. The IGF-I to IGFBP-3 ratio rose significantly and comparable with both regimens. During the FR study in all 19 girls, IGF-I and IGFBP-3 plasma levels and the IGF-I to IGFBP-3 ratio increased significantly. None of these growth related factors showed a difference between groups in their 18 months change.

In conclusion, our studies show, in agreement with other studies, a decrease in GHBP levels and an increase in the IGF-I to IGFBP-3 ratio, which may explain treatment effects of exogenous GH by a larger bioavailability of free GH and IGF-I. However, a dose or an administration frequency dependency was not established.

GH TREATMENT AND CARBOHYDRATE METABOLISM

The prevalence of impaired glucose tolerance at baseline and during the present studies was low (about 6%) compared with other studies (up to 43%). Apart from a relatively young age cohort in the DR study, these low numbers may be influenced by the procedure of the OGTT: the calculation of the glucose load per kg bodyweight, instead of ideal body weight, and by allowing a lower maximum amount of glucose in the present study, i.e. 50 g instead of 75 g advocated by the National Diabetes Data Group [56]. In normal subjects the use of 100 g glucose resulted in 2 hour bloodglucose levels which were 0.83 mmol/L higher compared with the use of 50 g [57,58].

Figure 9 shows the blood glucose and plasma insulin levels during OGTT's at baseline and during the DR study at varying dosages for 10 girls in group C. In the DR study, the area under the curve for time-concentration (AUC_{ab}) for glucose and its peak value showed no change over time and was never significantly different between any of the study groups. However, in all DR groups the AUC_{ab} for insulin, its peak value, fasting glucose, insulinogenic index, HbA_{1C} levels, and urinary C-peptide levels were all significantly higher after four years compared with pretreatment. In the FR study group differences were not observed, changes over time for the OD group were similar to those in the DR study. Higher Hb_{A1C} values might reflect the slight increase seen at a pubertal age in a normal population, accompanying the insulin resistance [59]. The increase in insulin and urinary C-peptide has not always been described in earlier reports on exogenous GH effects [53,60-62]. The consequence of long-term hyperinsulinism in otherwise healthy subjects is unknown. Theoretically, induction of insulin dependent diabetes might be possible in susceptible individuals. In addition, there may be cardiovascular effects, with an increased risk of hypertension and non-insulin dependent diabetes [63]. Interestingly, these effects may be reversible in acromegalic patients even after long-term GH exposure [64].

It is concluded that, although supraphysiological GH dosages in TS do not result in a larger proportion of girls with impaired glucose tolerance, the observed hyperinsulinism is reason to perform further follow-up on carbohydrate metabolism during and after prolonged GH treatment.

GH TREATMENT AND LIPID METABOLISM

Compared with healthy Dutch controls, median baseline levels of TC were similar and HDL levels lower in the DR study, whereas in the FR study median TC levels were higher and HDL levels similar. Similar results were previously reported in an American study [65] and could be related to the almost complete

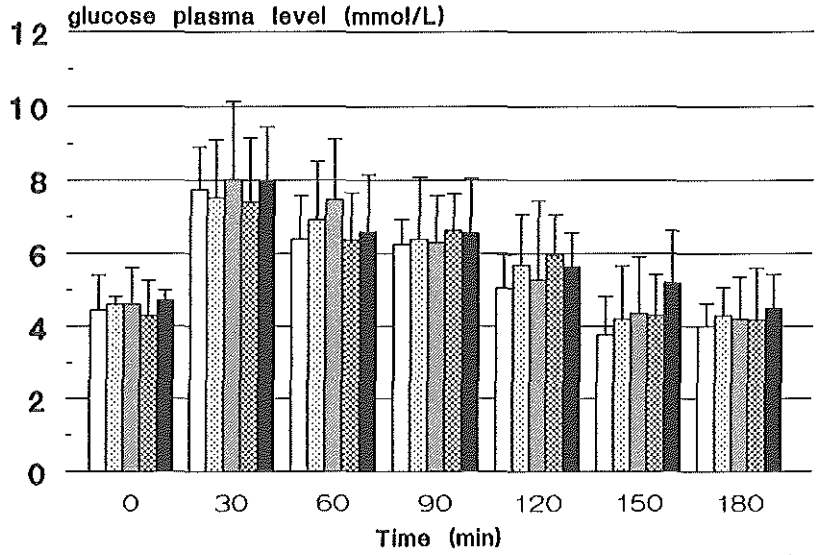

Fig. 9A

Figure 9. Mean (SD) of blood glucose (a) and plasma insulin (b) levels during oral glucose tolerance tests (T=0, 30, 60, 90, 120, 150, and 180 min) at baseline (□) and after 6 (▨), 18 (▩), 30 (■), and 48 (■) months of GH treament with 4, 6, 8, and 8 IU/m^2/day for 10 girls in group C of the 4 year dose-response study.

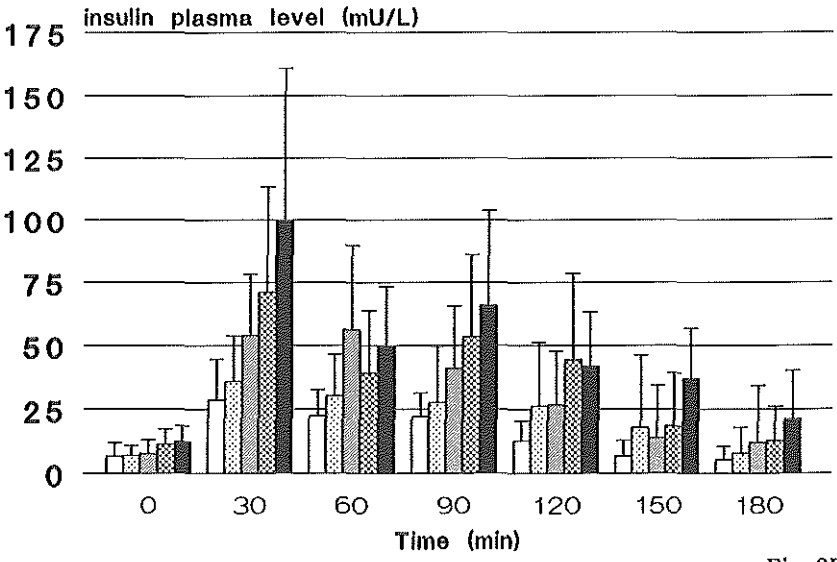

Fig. 9B

absence of the reducing influence of estrogens on TC and HDL levels [60,66] in girls with TS of a 'pubertal' age. With increasing GH dosages in the DR study, median TC, LDL, and apo-B levels decreased; median HDL levels increased and apo-A1 decreased. While in two other GH intervention studies in TS no significant changes were found [60,66], in the DR study all lipid levels were significantly different from baseline after four year GH treatment, except for LDL levels. The differences with the earlier findings may be due to the duration of GH treatment (four years in the present study vs one year in the other studies) and the use of incremental steps for the GH dose. Furthermore, variability was reduced by using a single laboratory. But, apart from the large inter-individual variation in some of the measurements, a large biological intra-individual variation still exists for lipid measurements with a short interval [67]. Intervention, therefore, is only thought to be of clinical relevance when the change exceeds 15%.

In conclusion, the lipid profiles changed in a more cardioprotective direction, with a significant reduction of the total cholesterol to HDL-cholesterol ratio.

CARDIAC EFFECTS OF GH

In agreement with a large Danish study, 22% of the girls in our studies presented at baseline with cardiovascular abnormalities. Mean baseline SD scores of the LV septum, posterior wall, and internal diameter were somewhat lower compared with normal Dutch girls. A possible explaination is that the SD scores are calculated with reference to weight and BMISDS values indicated a higher number of obese girls. Since changes of end-diastolic relative wall thickness and shortening fraction, in particular the increasing values, of the individual girls occurred predominantly within the normal range, LV hypertrophy appears not to be present, even at high GH dosages for an extended period. The calculated RWT and SF values should remain constant with age. Indeed, no relationship was found between these values and CA, neither at start of treatment, nor at the end of the study. The change in RWT and SF from baseline to end of study was also not related with age.

Thus, as described in the general population [68], under GH treatment the structure of the heart muscle seemed to increase proportionally with general development. LV activity, heart axis, and BP showed an age-dependent development, with values well within the normal range, except for diastolic BP with values >90th age-specific percentile in 15% of the cases at baseline and 31% after four years in the DR study, without group differences. In untreated girls with TS hypertension has been described.

CHAPTER 10

RECOMMENDATIONS

A stepwise approach of the GH dosage increase, reduced the 'waning' effect of the growth response after four years treatment without undue bone maturation. The increase in FH prediction was not significantly different between treatment groups, possibly due to the inter-individual variation. Stepwise increments of the GH dose thus seem beneficial for girls with TS, if not as a group than in individual cases. Also, whether a GH dose of 6 or 8 IU/m^2/day will prove to be a better dose than 4 IU/m^2/day can not yet be answered. Only attained FH will provide an adequate answer.

Irrespective of the GH dose used, initiation of GH treatment at a younger age does not seem not beneficial when expressed as the change in $HSDS_{CA}$ over the four-year study period. It should be stressed that this study comprised a four-year period with girls from 2 to 11 years of age, as opposed to other studies with somewhat older cohorts of girls.

After two years therapy, fractionated twice-daily GH injections do not seem beneficial over single injections of the total dose in girls with TS concurrently receiving low-dose estrogens. On the contrary, trends were in favor of once-daily GH injections and thus only extension of this study design until FH will reveal whether one regimen is advantageous over the other.

In general, these girls with TS aged 11 years or over responded better to a GH dose of 6 IU/m^2/day compared with other studies using GH dosages up to 4 IU/m_2/day. In the light of a higher GH secretion during puberty in healthy girls, the effect of higher GH dosages at these ages deserves further study.

The use of computerized systems to determine bone age is a welcome addition to the the endocrinological department of a paediatric clinic. However, the future use in daily practice depends on optimization of the system. In particular, the accuracy could be improved and the system could be fully automated.

Recent reports on cohorts of girls with TS who started GH therapy at an 'older' age and reached FH should be interpreted with caution. Treatment of girls at an 'earlier' age and the use of appropriate, Turner-specific FH prediction methods which include bone age may result in a better quantification of the growth response for a larger proportion of the girls. Possibly this will result in a more favorable outcome. Prospective, matched control studies would be helpful, but are likely to be hampered by a high drop-out rate in the end. At present a controlled study is performed in Canada. Recently, early experiences in a non-randomized design with 31 patients were published [69]. Contingency table analysis of attained versus projected FH showed significantly higher values in treated patients, although only 4 of 17 had a FH of 5 cm or more over projection. Completion of the randomized trial is anxiously awaited.

Decisions on the health economics of this expensive therapy should include age-related aspects of quality of life. This is a difficult exercise [70] since other pathological aspects act as confounders. In particular, infertility can become increasingly important with age. To test aspects of qualilty of life before adulthood seems even more challenging.

Considering the use of higher GH dosages we were also interested in safety aspects of this therapy. After a four-year study period, GH therapy even at dosages up to 8 IU/m^2/day does not result in disproportionate growth and is a safe therapy in TS with respect to carbohydrate and lipid metabolism. Also, LV hypertrophy was not observed. Nevertheless, given the high GH dosages it seems necessary to perform long-term follow-up of these safety variables.

Growth and pubertal development are important in paediatric endocrinological practice. In particular since several treatment options exist. A wide variety of clinical characteristics is associated with Turner syndrome. Although the severity of these features also varies, each one of them can present a medical or psychosocial problem to the girl and/or their custodians. For the latter, acceptance of the diagnosis is important to be able to give appropriate guidance to the girl. Therefore, effective management of girls with TS depends on [1] a timely diagnosis, [2] awareness of the potential anatomic and endocrinologic abberations, and the psychosocial burden of the syndrome, and [3] counseling and support.

Pubertal development in girls with TS is delayed compared with their healthy peers. The assessment of the optimal age to initiate estrogen replacement should take into account: the girls age and school grade, growth pattern, and the girl's wishes. While the mean age for the onset of puberty is between 10 and 11 years in the Netherlands, estrogen replacement can be delayed well beyond this age. Others have advocated to start estrogen therapy at the latest possible age, that is until growth will hardly be affected. However, at the currently used low dosages height development does not have to be hampered and an individual approach should be followed. In cases without physical response the dose should be increased. After a period of about two years of unopposed estrogen use, cyclic progesterone should be added to prevent 'spotting' and to reduce the risk of endometriosis and adenocarcinoma of the uterus.

The use of oxandrolone at a low dose in addition to GH is still under investigation in the Netherlands. Results in the United States of America are promising [71]. When the applicability of GH releasing peptides is proven, girls with TS may also be candidate as they have, in general, no pituitary deficiency.

Based on our interim results as well as on reports of others with FH results it seems justified to treat Turner girls with GH. The outcome of growth promoting treatment in children with idiopathic short stature and GH deficiency bears uncertainties due to the timing and course of puberty. In contrast, in girls with TS this outcome can be predicted with greater accuracy because puberty can be manipulated pharmacologically.

CHAPTER 10

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Summary

SUMMARY

This thesis presents two prospective, nationwide clinical trials with a large cohort of previously untreated girls with Turner syndrome (TS). In an attempt to optimize subcutaneous growth hormone (GH) treatment, both studies assessed the efficacy and safety of recombinant DNA manufactured human GH at higher than currently used dosages. The first study focused on a dose-response relationship in 68 girls, chronological age (CA) 2-11 years. They were randomized into 3 dosage regimen groups A, B, and C, with a daily GH dose during four study years of 4-4-4-4, 4-6-6-6, and 4-6-8-8 IU/m^2 body surface, respectively. The second study with 19 girls, aged 11 years or older and concurrently receiving low-dose ethinyl estradiol (0.05 µg/kg/day, orally), tested the GH administration frequency-response. In an attempt to more closely mimick the normal pulsatile GH secretion, fractionated twice daily (BID) injections were compared with once daily (OD) in a total GH dose of 6 IU/m^2/day. BID injections were administered as two-thirds at bedtime and one-third in the morning.

Chapter 1 describes the first report of the syndrome and the variety in genetics and characteristic features. Subsequently, the natural growth pattern, ovarian demise, the somatotropin-IGF-I axis, as well as the influence of these hormones with respect to the growth disturbance are discussed. The interaction of GH with carbohydrate and lipid metabolism is briefly outlined together with the GH effects on cardiac dimensions. Growth promoting treatment with GH is extensively discussed in relation to the aims of the present studies.

In order to better evaluate GH treatment effects it is necessary to know the development of height and biological maturation in untreated girls with TS. Dutch Turner-specific growth charts were already available. However, radiographs of the hand and wrist bones as a measure of biological maturation were still to be assessed. **Chapter 2** describes the use of a recently developed computerized method to determine bone age according to the Tanner and Whitehouse RUS score. After validating the system for such a measure of biological maturity, the use of X-rays from children with a disturbance in their biological development was assessed with a positive result. Only then was it possible to determine bone ages in a sample of over 800 radiographs from more than 300 girls with TS gathered from 11 pediatric centers in the Netherlands. Based on these readings a mixed longitudinal/cross-sectional curve for the development of the Tanner and Whitehouse (TW) RUS bone age over the full chronological age range could be constructed. The first derivative of bone age with CA on the regression equations gives the biological maturation which has taken place during a full year.

A subset of the above sample, 57 Turner women with 235 measurement points of height and bone age, served to develop a new Turner-specific final height prediction method, PTS (**Chapter 3**). Analogous to the Tanner and Whitehouse Mark 2 method for normal children, smoothed regression

coefficients are tabulated for PTS for height, CA, and bone age, both TW RUS and Greulich and Pyle. Although the validity of PTS_{RUS} and PTS_{GP} remains to be tested below the age of 6 years, both gave small mean prediction errors and a high accuracy. Comparison between existing methods indicated a tendency to overpredict final height. Overall, the simplest methods - projected height and its modification - were remarkably good at most ages. Final height prediction in TS is important in the consideration of growth promoting therapy or in the evaluation of its effects.

In **Chapter 4** the auxological results of the dose-response study are given. A dosage increase resulted in a significant increase of the growth response. After four years GH treatment 65 girls could be evaluated: [1] the mean change in height standard deviation score ($HSDS_{CA}$) was significantly higher for groups B and C compared with group A, but not significantly different between groups B and C. Although significantly higher compared with estimated values for untreated Dutch girls with TS, [2] bone maturation of the GH treated girls was not significantly different between groups and [3] it was positively related with the degree of bone age retardation at start of study and negatively related with baseline CA. [4] Both the modified Index of Potential Height ($mIPH_{RUS}$) and the PTS_{RUS} method showed significant increases in mean final height prediction, without significant differences between groups. PTS_{RUS} values were markedly higher than the $mIPH_{RUS}$ values.

Dose-dependency could be shown for the area under the curve (AUC) for GH, but the change in $HSDS_{CA}$ was not linearly related with AUC. Baseline GH binding protein (BP) levels were in 84% of the cases within the normal age range; the decrease in mean levels after 6 months GH was not significant. Mean IGF-I and IGFBP-3 plasma levels increased significantly, without significant differences between groups. The change in $HSDS_{CA}$ during GH was dependent on IGF-I plasma levels at baseline and during the study period. Thus, a stepwise GH-dosing approach reduced the 'waning' effect of the growth response after four years treatment without undue bone maturation. Final height prediction was not significantly different between treatment groups. Irrespective of the GH dose used, initiation of GH treatment at a younger age is beneficial after four years GH when expressed as actual cm gained or as gain in FH prediction, but not statistically significant when expressed as the change in $HSDS_{CA}$ over the study period.

Preceeding the auxological administration frequency-response study, a pharmacokinetic 2-week period crossover study with a 2-week washout interval was performed in 10 of the 19 girls (**Chapter 5**). Four weeks after initiation of 0.05 µg/kg/day ethinyl estradiol, BID was compared with OD subcutaneous injections of 6 IU GH/m^2/day. Each treatment period was concluded with 24-hour GH profile tests. There were significantly higher maximum GH levels and a larger area under the curve with OD than with BID GH, while GH clearance was larger with BID. The pharmacokinetic values with OD injections were in conformity with values for healthy and GH-deficient children. Pretreatment GHBP levels tended to be high compared with values in healthy prepubertal children. These levels decreased with GH therapy, significantly so with BID GH

only. There was a significant increase in levels of IGF-I and IGFBP-3, irrespective of regimen. The IGF-I to IGFBP-3 ratio, a possible indicator of the growth response, increased significantly and comparable with both regimens. There was no consistent diurnal variation with either regimen in GHBP, IGF-I, or IGFBP-3 levels. Four-hourly levels of GH, GHBP, IGF-I, and IGFBP-3 were not correlated. It was concluded that although the 24-hour profiles differed during once or twice daily administration of the same total GH dose, the diurnal pattern and mean levels of factors involved in the biological effects of GH are comparable for both frequency regimens.

All 19 girls followed the cross-over schedule and were, after a second randomization, treated for two years in the frequency-response study (**Chapter 6**). The growth response expressed as HV, HVSDS, the change in $HSDS_{CA}$, the gain in height over estimated untreated values and in FH prediction, all showed significant improvements. Although mean values tended to be higher with OD injections, significant differences between groups were not found. Bone maturation was similar between groups and compared with untreated estimated values. After 18 months of GH treatment the significant decrease in GHBP plasma levels observed after 6 months was no longer significant. In contrast, IGF-I and IGFBP-3 plasma levels and the IGF-I to IGFBP-3 ratio increased significantly during 18 months GH therapy. The change in 18 months of these growth-related factors did not showe a difference between groups. In summary, the growth data are in conformity with the data of the earlier 24-hour GH profiles. The growth response and plasma levels of growth-related factors after two years GH on a total dose of 6 IU/m^2/day in combination with low-dose estrogens was not significantly different between the once daily and the twice daily GH injection regimen.

In Chapters 7 through 9, all 87 girls participating in both trials are considered. **Chapter 7** addresses the concerns about disproportionate growth with long-term GH treatment by using *z*-scores and shape (*f*)-values for combinations of measurements. Comparison was made with healthy Dutch girls and with untreated girls with TS.

Although baseline *z*-scores and *f*-values were 'typical' compared with age-matched girls with TS, the vast majority of the girls in the two studies (>85%) had 'atypical' baseline *z*-scores and *f*-values compared with age-matched healthy girls. In general, these values worsened with age. GH treatment resulted in significant improvements of most *z*-scores. The change from baseline to end of study was only significantly dose-dependent for *z*H(eight), *z*Hand, and *z*Foot. During the GH studies *f*-values improved significantly towards the 'typical' range of the normal female reference population, in particular at younger ages and for *f*H&Hand, *f*H&Foot, and *f*H&Biiliacal diameter. A significant dose-dependent improvement was noted only for *f*H&Biiliacal diameter. At end of study, the change from baseline in *z*-scores and *f*-values was negatively related with their baseline value and with age; only in the FR study did the change in *z*-scores show a positive relationship with baseline age.

SUMMARY

Chapter 8 addresses carbohydrate and lipid metabolism during the studies. Impaired glucose tolerance was present in 6% of the girls at base-line; at end of the studies this number even decreased. In the dose-response study, the area under the curve for time-concentration (AUC_{ab}) for glucose and its peak value showed no change over time and was never significantly different between any of the study groups. However, in all dose-response groups the AUC_{ab} for insulin, its peak value, fasting glucose, insulinogenic index, HbA_{1C} levels, and urinary C-peptide levels were all significantly higher after four years compared with pretreatment. In the frequency-response study, group differences were not observed; changes over time for the OD group were similar to those in the dose-response study.

Compared with healthy Dutch controls, median baseline levels for total cholesterol (TC) were similar and for high-density lipoprotein cholesterol (HDL) lower in the dose-response study, whereas in the frequency-response study median TC levels were higher and HDL levels similar. With increasing GH dosages in the dose-response study, median TC, low-density lipoprotein (LDL), and apolipoprotein (apo-) B levels decreased; median HDL levels increased, while apo-A1 decreased. The changes after four years were significant, except for LDL. Thus, the lipid profiles changed in a more cardioprotective direction, with a significant decrease of the total cholesterol to HDL-cholesterol ratio. In conclusion, GH therapy even at dosages up to 8 IU/m^2/day is a safe therapy in TS with respect to carbohydrate and lipid metabolism.

The effects of GH on echocardiographically measured left ventricular (LV) dimensions, electrocardiography (ecg), and systemic blood pressure are discussed in **Chapter 9**. At baseline 23% of the girls presented with cardiovascular abnormalities and somewhat lower mean values of the LV septum, posterior wall, and internal diameter compared with normal Dutch girls. Mean values for end-diastolic relative wall thickness and shortening fraction, both at baseline and after completion of the studies, remained within the reference range. LV activity, heart axis, and blood pressure showed an age-dependent development, with values well within the normal range, except for diastolic blood pressure with values >90th age-specific percentile in 15% of the cases at baseline and 31% after four years in the dose-response study, without group differences.

It was concluded that long-term GH therapy, even at dosages up to 8 IU/m^2/day, does not result in LV hypertrophy in girls with TS. The number of girls with an elevated diastolic blood pressure increased from 15% at baseline to 31% after four years GH treatment in the dose-response study.

An overall discussion of this work is given in **Chapter 10**.

Samenvatting

SAMENVATTING

Dit proefschrift beschrijft twee prospectieve, klinische onderzoeken in een groot aantal centra in Nederland met een groot cohort van tot dan toe nog onbehandelde meisjes met syndroom van Turner. In een poging om de subcutane groeihormoon (GH) behandeling te optimaliseren werd de werkzaamheid en veiligheid van humaan GH, vervaardigd met behulp van recombinant DNA techniek, in hogere doseringen dan tot nog toe gebruikelijk, onderzocht.

De eerste studie richtte zich op de dosis-respons relatie bij 68 meisjes met een kalender leeftijd van 2-11 jaar. Zij werden gerandomiseerd in 3 doserings groepen A, B en C met een dagelijkse GH dosering gedurende 4 studie jaren van respectievelijk 4-4-4-4, 4-6-6-6, and 4-6-8-8 IE/m^2 lichaams oppervlak. De tweede studie betrof 19 meisjes van 11 jaar en ouder die tevens ethinyl oestradiol (0.05 µg/kg/dag) oraal gebruikten en richtte zich op het effekt van twee GH toedienings frequenties. In een poging om de normale pulsatiele GH secretie dichter te benaderen werd het effekt van tweemaal daagse injecties vergeleken met eenmaal daagse, avondlijke injecties bij een GH dosering van 6 IE/m^2/dag. Bij het tweemaal daagse schema werd tweederde van het totaal voor het slapen gaan en eenderde bij het wakker worden gegeven.

Hoofdstuk 1 geeft een kort historisch overzicht en beschrijft de variëteit in de genetische achtergrond en karakteristieke eigenschappen van het syndroom van Turner. Vervolgens worden het natuurlijke groei-patroon, het te gronde gaan van de ovaria, alsmede de relatie van de somatotropine-IGF-I as met betrekking tot de groeistoornis besproken. De interaktie van GH met het koolhydraat- en vetmetabolisme wordt kort geschetst alsmede de GH effekten op de cardiale afmetingen. Uitgebreid wordt de groei bevorderende behandeling met GH besproken in relatie tot de doelen van de onderhavige studies.

Om de GH behandelings-effekten beter te kunnen beoordelen is het noodzakelijk om de ontwikkeling van de lengte en de biologische rijping bij onbehandelde meisjes met het syndroom van Turner te kennen. Turner-specifieke, Nederlandse groeicurves waren reeds beschikbaar. Echter, het beloop van de biologische rijping aan de hand van de bot ontwikkeling moest nog worden vastgesteld met behulp van analyse van Röntgenfoto's van de hand- en pols. **Hoofdstuk 2** beschrijft het gebruik van een recent ontwikkelde gecomputeriseerde methode om de botleeftijd volgens de Tanner en Whitehouse RUS score te bepalen. Na de validatie van het systeem als een maat van biologische rijpheid werd het gebruik ervan getoetst bij kinderen met een stoornis in hun biologische ontwikkeling, met een positief resultaat. Pas daarna was het mogelijk om het beloop van de botleeftijd te bepalen op grond van 800 Röntgenfoto's van meer dan 300 meisjes met het syndroom van Turner, verzameld in 11 pediatrische centra in Nederland. Op basis van deze interpretaties kon een gemengde longitudinale/cross-sectionele curve voor de bot ontwikkeling volgens de methode van Tanner en Whitehouse over het volledige kalenderleeftijds interval worden samengesteld. De eerste differentiaal van botleeftijd met kalenderleeftijd op de vergelijking van de regressie coëfficienten

geeft de biologische rijping gedurende het verstrijken van een jaar weer. Een deel-populatie van de bovengenoemde groep, 57 vrouwen met het syndroom van Turner met 235 meetpunten van de lengte en botleeftijd, dienden om een nieuwe Turner-specifieke eindlengte voorspellings-methode, PTS, te ontwikkelen **(Hoofdstuk 3)**.

Analoog aan de Tanner en Whitehouse 'Mark 2' methode voor gezonde kinderen werden vloeiend verlopende regressie coëfficienten voor PTS voor lengte, kalender- en botleeftijd (zowel voor de RUS-score als voor de Greulich & Pyle methode) getabuleerd. Alhoewel de validiteit van PTS_{RUS} en PTS_{GP} beneden de leeftijd van 6 jaar nog moet worden vastgesteld, gaven beiden slechts kleine voorspellingsfouten en een goede nauwkeurigheid. Vergelijking tussen bestaande methoden liet een tendens tot overpredictie van de eindlengte zien. Over het algemeen waren de eenvoudigste methoden -de geprojecteerde lengte methode en de modificatie hierop- op de meeste leeftijden opvallend goed. Eindlengte voorspellingsmethoden bij het syndroom van Turner zijn belangrijk bij de overweging om een groeibevorderende behandeling in te stellen en bij de evaluatie van de effekten van zo'n behandeling.

In **Hoofdstuk 4** worden de auxologische resultaten van de dosis-respons studie gegeven. Ophoging van de dosering resulteerde in een significante verbetering van de groeirespons. Na 4 jaar GH behandeling konden 65 meisjes worden geëvalueerd: [1] de gemiddelde verandering in de lengte standaard deviatie score was significant groter voor groepen B en C vergeleken met groep A, maar niet significant verschillend tussen groepen B en C. Alhoewel significant sneller vergeleken met geschatte waarden bij onbehandelde meisjes met het syndroom van Turner, [2] was de botrijping van de met GH behandelde meisjes niet significant verschillend tussen de groepen en [3] was deze positief gerelateerd met de mate van botleeftijd retardatie bij de start van de studie en negatief gerelateerd met de kalenderleeftijd bij de start. [4] Zowel de gemodificeerde 'Index of Potential Height' ($mIPH_{RUS}$) en de PTS_{RUS} methode lieten significante stijgingen zien van de gemiddelde voorspelde eindlengte, zonder significante verschillen tussen de groepen. De PTS_{RUS} waarden waren beduidend groter dan de $mIPH_{RUS}$ waarden.

Dosis-afhankelijkheid kon worden aangetoond voor het oppervlak onder de curve voor de GH concentratie, maar de verandering in de lengte standaard deviatie score was niet lineair gerelateerd aan het oppervlak onder de GH curve. De spiegels van GH bindende eiwit (GHBP) bij start van de studie waren bij 84% van de meisjes binnen het normale gebied, terwijl de verlaging van de gemiddelde niveau's ervan na zes maanden GH niet significant was. De gemiddelde IGF-I en IGFBP-3 plasma waarden stegen significant, zonder significante groeps verschillen. De verandering in lengte standaard deviatie score onder GH behandeling was afhankelijk van de IGF-I plasma spiegels bij de start en gedurende de studie periode. Een stapsgewijze benadering van de GH dosering verminderde het fenomeen van het 'teruglopen' van de groeirespons na 4 jaar behandeling zonder een te overmatige botrijping. De voorspelde eindlengte was niet significant verschillend tussen de behandelingsgroepen. Onafhankelijk van de gebruikte dosering is start van de GH behandeling op een

jongere leeftijd gunstig na 4 jaar therapie uitgedrukt als winst in cm of als winst in de voorspelde eindlengte, maar niet statistisch significant wanneer het wordt uitgedrukt als de verandering in lengte standaard deviatie score over de studie periode.

Voorafgaand aan de auxologische GH toedienings frequentie-respons studie werd een farmacokinetische crossover studie bij 10 van de 19 meisjes uitgevoerd. De 2 weekse GH perioden werden gescheiden door een 2 weken durende uitwas periode (**Hoofdstuk 5**). Hierbij werden, vier weken na de start van behandeling met 0.05 ug/kg/dag ethinyl oestradiol, tweemaal daagse injecties vergeleken met eenmaal daagse subcutane GH injecties met 6 IU/m^2/dag. Elke behandelingsperiode werd afgesloten met 24 uur durende GH profiel tests. Met eenmaal daags injecties waren er significant hogere maximum GH plasma spiegels en een groter oppervlak onder de GH curve dan met tweemaal daagse GH injecties, terwijl de GH klaring groter was met tweemaal daagse injecties. De farmacokinetische waarden met eenmaal daagse injecties waren in overeenstemming met die van gezonde en van GH deficiënte kinderen. De GHBP spiegels voor de start van de behandeling waren enigszins hoger waarden dan die bij gezonde prepubertaire kinderen. Deze spiegels werden lager met GH therapie, alleen significant met tweemaal daagse injecties. Er was, onafhankelijk van het GH regime, een significante stijging van de IGF-I en IGFBP-3 plasma spiegels. De IGF-I/IGFBP-3 ratio, een mogelijke indicator van de groeirespons, steeg significant en vergelijkbaar voor beide doseringsschema's. De GHBP, IGF-I en IGFBP-3 spiegels vertoonden bij beide regimes geen consistente variatie over een etmaal. De vier uurs spiegels van GH, GHBP, IGF-I en IGFBP-3 waren niet gecorreleerd. Wij concludeerden dat, alhoewel de 24-uurs profielen verschilden tijdens de een- en tweemaal daagse injecties van eenzelfde totale GH dosis, het patroon per etmaal en de gemiddelde spiegels van faktoren betrokken bij de biologische effekten van GH vergelijkbaar zijn tussen de twee GH toedienings schema's.

Alle 19 meisjes volgden hetzelfde crossover-schema en werden, na een tweede randomisatie, behandeld gedurende twee jaar in de frequentie respons studie (**Hoofdstuk 6**). De groeirespons uitgedrukt als groeisnelheid of de standaard deviatie score ervan, de verandering in lengte standaard deviatie score, de winst in lengte ten opzichte van geschatte onbehandelde waarden en in de voorspelde eindlengte, lieten alle significante verbeteringen zien. Alhoewel de gemiddelde waarden met eenmaal daagse injecties enigszins hoger waren, werden er geen significante verschillen tussen de groepen gevonden. De botrijping was vergelijkbaar tussen de groepen en met waarden van onbehandelde meisjes. De significante daling van de GHBP spiegels die na 6 maanden GH behandeling werd waargenomen was na 18 maanden niet meer significant. In tegenstelling hiermee stegen de IGF-I en IGFBP-3 plasma spiegels en de IGF-I/IGFBP-3 ratio significant gedurende 18 maanden therapie. De verandering in 18 maanden was voor geen van deze groei gerelateerde faktoren verschillend tussen de groepen. Samenvattend, waren de groei gegevens in overeenstemming met de gegegens van de eerdere 24-uurs profiel studie. De groeirespons en plasma spiegels van groei gerelateerde faktoren waren na twee

jaar GH met een totale dosering van 6 IE/m^2/dag in kombinatie met een lage dosering oestrogenen niet significant verschillend tussen het eenmaal daagse en tweemaal daagse GH injectie regime.

In hoofdstukken 7 tot 9 worden alle 87 meisjes uit beide studies beschouwd. **Hoofdstuk 7** gaat in op de ongerustheid over de mogelijkheid van gedisproportioneerde groei na langdurige GH behandeling. Door gebruik te maken van *z*-scores en vorm (*f*)-getallen voor combinaties van metingen wordt een vergelijking gemaakt met gezonde Nederlandse meisjes en met onbehandelde meisjes met het syndroom van Turner. Alhoewel de *z*-scores en *f*-getallen bij start van de behandeling 'typisch' waren voor leeftijdsgenoten met het syndroom van Turner, had de meerderheid van de meisjes in de studies (>85%) 'afwijkende' *z*-scores en *f*-getallen bij start van de behandeling vergeleken met gezonde leeftijdsgenoten. In het algemeen waren deze waarden meer afwijkend met het vorderen van de leeftijd. GH behandeling gaf een significante verbetering van de meeste *z*-scores. De verandering van start van behandeling tot het einde van de studie was alleen significant dosis-afhankelijk voor de *z*-scores van lengte, handen en voeten.

Gedurende de GH studies verbeterden de *f*-getallen significant richting 'typische' waarden voor de normale vrouwelijke referentie populatie, met name op een jongere leeftijd en voor *f*-getallen van handen, voeten en de heupbreedte (biiliacale diameter) in relatie tot de lengte. Een significante dosis-afhankelijke verbetering werd alleen gezien voor het laatste *f*-getal. De verandering van start tot einde van de studie in *z*-scores en *f*-getallen was negatief gerelateerd aan hun waarden en de leeftijd bij start van de behandeling, terwijl alleen in de frequentie-response studie een positieve relatie tussen de verandering van de *z*-scores en de leeftijd bij de start werd gezien.

In **Hoofdstuk 8** wordt het koolhydraat- en vetmetabolisme tijdens de studies beschreven. Een verminderde glucose tolerantie was aanwezig bij 6% van de meisjes bij de start van de behandeling, terwijl bij het einde van de studies dit percentage zelfs daalde. In de dosis-respons studie lieten het oppervlak onder de glucose curve en de maximale glucose waarden geen verandering in de tijd zien en was nooit significant verschillend tussen de groepen. Echter, in alle dosis-respons groepen waren het oppervlak onder de insuline curve, de maximale insuline waarde, de nuchtere bloed glucosespiegel, de insulinogene index, de HbA_{1C} spiegels en het C-peptide gehalte in de urine significant hoger na vier jaar behandeling vergeleken met waarden voor de start. In de frequentie-respons studie werden geen significante groepsverschillen gezien, terwijl de veranderingen in de tijd in de eenmaal daags injectie groep vergelijkbaar waren met die in de dosis-respons studie. Ten opzichte van gezonde Nederlandse controles waren bij de start de mediane waarden van het totaal cholesterol (TC) vergelijkbaar en van 'high-density lipoprotein cholesterol' (HDL) lager in de dosis-respons studie, terwijl bij de iets oudere meisjes in de frequentie-respons studie de mediane TC waarden hoger en de HDL waarden vergelijkbaar waren met de controles. Met hogere GH doseringen in de dosis-

respons-studie daalden de mediane TC, 'low-density lipoprotein' (LDL) en apolipoproteine (apo-) B spiegels; de mediane HDL spiegels stegen, terwijl de apo-A1 spiegels daalden. De veranderingen na 4 jaar waren significant, behalve voor LDL. Al met al veranderde het lipiden profiel met een significante verlaging van de ratio van het TC/HDL-cholesterol naar de meer cardioprotectieve kant. Hieruit kan geconcludeerd worden dat GH therapie zelfs bij doseringen tot 8 IE/m^2/dag een veilige therapie bij meisjes met het syndroom van Turner is wat betreft het koolhydraat- en vet metabolisme.

De effekten van GH op echocardiografisch gemeten linker ventrikel (LV) afmetingen, op het electrocardiogram (ecg) en de bloeddruk wordt besproken in **Hoofdstuk 9**. Bij de start van de behandeling presenteerde 23% van de meisjes zich met cardiovasculaire afwijkingen en ietwat lagere gemiddelde waarden van de septumdikte, LV achterwand en interne diameter vergeleken met normale Nederlandse meisjes. De gemiddelde waarden van de eind-diastolische relatieve wanddikte en verkortingsfraktie zowel bij de start als bij het einde van de studies bleven binnen het referentie gebied. De LV aktiviteit, de hart-as en de bloeddruk vertoonden een leeftijds-afhankelijke ontwikkeling met waarden binnen het referentie gebied, behalve de diastolische bloeddruk. Hiervoor werden waarden boven de 90ste leeftijds-specifieke percentiel in 15% van de gevallen bij de start en in 31% bij het einde in de dosis-repons studie gevonden, zonder groepsverschillen. Er werd geconcludeerd dat langdurige GH therapie, zelfs bij doseringen tot 8 IE/m^2/dag, niet resulteerde in LV hypertrofie bij meisjes met syndroom van Turner. Het percentage meisjes met een verhoogde diastolische bloeddruk nam toe van 15% bij de start tot 31% na vier jaar GH behandeling in de dosis-respons studie.

In **Hoofdstuk 10** worden de resultaten van de diverse studies in samenhang met recente literatuurgegevens besproken. Tevens worden de belangrijkste conclusies en aanbevelingen ten aanzien van GH behandeling bij meisjes met het syndroom van Turner gegeven.

Dankwoord

DANKWOORD

Multicentrisch onderzoek is alleen mogelijk dankzij intensieve samenwerking met een groot aantal mensen in alle betrokken centra. Een aantal van hen wil ik graag persoonlijk bedanken, zonder de overigen tekort te doen.

Mijn grootste dank gaat uit naar alle meisjes en hun families. Zonder hun bereidwilligheid om deel te nemen aan de studies met alle onderzoeken die daar bij hoorden, zou het onmogelijk zijn geweest om onze kennis over de groeihormoon behandeling verder uit te breiden en was dit proefschrift niet tot stand gekomen. Lieve meiden, ik heb bewondering voor jullie moed en volhoudendheid. Jullie karakters en gezelligheid hebben gezorgd dat het 'werk' veranderde in een 'hobbie'.

In een adem zou ik ook Anne van de Wiel willen bedanken. Anne, eigenlijk is dit een boekje van ons samen, want samen gingen wij op pad naar alle uithoeken van het land, bij nacht en ontij, tijdens vakanties, enz. om 'onze' meisjes te 'zien'. Je aanpassingsvermogen was bijna grenzeloos. Je benadering van hen was professioneel en passend bij elke leeftijd, soms niet altijd even soepel, maar wel getuigend van een enorm inzicht. Ik hoop dat ook jij bereikt in je werk en je prive-leven wat je hoopt te bereiken.

Dr. S.M.P.F de Muinck Keizer-Schrama, mijn co-promotor, Sabine, heel veel dank gaat uit naar jou voor je prima begeleiding, de vele uren waarin wij de meest uiteenlopende aspekten van alle onderzoeken bespraken, je snelle en minitueuze reviewen, je kritische vragen, je gastvrije fraaie huis, je man. Je brede zorg voor de meisjes was een voorbeeld mij. Je betrokkenheid bij mijn studies en gezin waren erg belangrijk voor de voltooiing van dit proefschrift.

Prof.dr. S.L.S. Drop, mijn promotor, Sten, graag wil ik je bedanken dat je mij hebt benaderd voor deze studies. Een wens om een afgerond stuk onderzoek te doen is daardoor uitgekomen. Je wetenschappelijke blik, je snelle en gedegen reviewen, je humor en je professoriale verstrooidheid waren een grote inspiratie voor mij. Dat ik uiteindelijk toch een andere weg ben ingeslagen heeft zeker niet aan jou en Sabine gelegen.

Dr. Th. Stijnen, Theo, ook jij hebt een aparte rol vervuld bij het opzetten en beschrijven van de studies. Ik wil je graag bedanken voor je geweldige vermogen om de statistiek dichter bij de praktijk te brengen, voor je zorg en tijd die je in mij wilde steken en niet in de laatste plaats dat je bereid was plaats te nemen in de kleine commissie.

DANKWOORD

Prof.dr. H.A. Delemarre-van de Waal, Henriëtte, graag wil ik jou bedanken voor je aanstekelijke enthousiasme, je vertrouwen in de zorg voor jouw meisjes, je interesse in mijn toekomst en voor je deelname in de kleine commissie.

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Dank ook aan alle leden van (voorheen) de Werkgroep Groeihormoon van de Nederlandse Groeistichting, en naast de elders genoemden, met name prof.dr. N.M. Drayer, dr. M. Gons, drs. J.J. Gosen, dr. J.J.G. Hoorweg-Nijman, dr. M. Jansen, drs. W. Oostdijk, dr. B.J. Otten, dr. C.W. Rouwé, dr. H.M. Reeser, dr. C. Rongen-Westerlaken, dr. T. Vulsma, dr. J.J.J. Waelkens, prof.dr. J.M. Wit. Beste Niek, Margreet, Jos, Geja, Maarten, Wilma, Barto, Catharinus, Maarten, Ciska, Tom, Johan, Jan Maarten, een simpel woord van dank lijkt onvoldoende voor de fantastische samenwerking die het mogelijk maakte om zulke kwaliteits 'data' te genereren. Niet alleen de vele voorbereidende discussies over de uit te voeren protocollen, maar ook jullie betrokkenheid en diversiteit in de benadering van het kontakt met patienten was van zeer grote waarde. Dank voor het vertrouwen dat jullie mij schonken om met jullie meisjes in jullie omgeving te mogen werken. Het heeft de kindergeneeskunde voor mij heel speciaal gemaakt.

Dr. W.J.M. Gerver en dr. R. de Bruijn, Willem Jan en Rob, jullie nemen een aparte plaats in dit proefschrift in. Jullie enthousiasme en benadering op het gebied van de lichaamsproporties heeft mij aangespoord om me verder in dit lastige aspekt van de groei te verdiepen. Zonder jullie begeleiding was dit boekje een stuk minder interessant geworden. Willem, jij tevens bedankt voor je gastvrijheid tot in de late uurtjes.

Dr. A.H. Cromme, Adri, je recht-door-zee benadering, technische vaardigheden en kennis van met name de echocardiografie waren van zeer grote waarde om deze data te interpreteren. In het verlengde van jouw betrokkenheid zou ik je collega cardiologen, willen bedanken voor hun tijd en interesse in dit onderzoek. Het toont eens te meer de integratie tussen de verschillende aandachtsgebieden in de kindergeneeskunde. Tenslotte gaat mijn dank ook uit naar Maarten Witsenburg, immers jij was sterk betrokken bij de opzet van de cardiologische 'poot' van de studies.

En dan natuurlijk de 'fellow-kamer'. Anneke Saarloos, Janneke van Nieuwkasteele, Annemieke Boot, Ingrid van Slobbe, Lydia Velt, jullie wil ik bedanken voor jullie inschikkelijkheid in onze overvolle kamer, de gezellige werksfeer, de afleiding en de hulp die op bepaalde momenten zo nodig was.

Theo Sas, dank voor je keuze om 'mijn' meisjes verder te willen vervolgen en wel zo snel na het behalen van je bul. Ik ben er van overtuigd dat zij in goede handen zijn en hoop dat jij net zoveel plezier zult beleven aan de kontakten met hen en de uitvoering van het onderzoek als ik. Ik wens je sterkte met het analyseren van de enorme hoeveelheid gegevens en het opschrijven van de resultaten.

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A special relationship has develop over time with Dr. W.F. Blum. Werner, after our first meeting in Frankfurt at the second International Symposium on Turner syndrome I feel fortunate to have met you. Your laboratory knowledge and your own expertise on the insulin-like growth factors and their binding proteins were invaluable for my studies and my personal development. I hope that we will be able to continue our discussions, sometimes held at late hours, in our current working environment.

Prof.dr. G. Baumann, Gerry, I thank you for the cooperation with the determination of the growth hormone binding proteins. Our discussions about the interpretation of the findings were inspiring. They were only interrupted by the big pancakes in Zaandijk!

DANKWOORD

Graag wil ik ook al diegenen in de diverse centra bedanken die behulpzaam zijn geweest bij de organisatie van allerhande aktiviteiten met betrekking tot de studies. Door enkelen in het Sophia te noemen hoop ik mijn erkenning naar de anderen (ook in de andere centra) weer te geven: Geja Reuver, Manon van Paasen en Sophie Dumas van de polikliniek betrokken bij de drie-maandelijkse bezoekjes van de meisjes; Babs Keller van de dagbehandeling (maar ook Erik Herdes, VU), een belangrijke setting bij de uitvoering van de testen; prof.dr. M.Meradji, Simon Robben en alle laboranten van de Röntgenafdeling; Emiel Ronda, Koos Lezer en Maarten van de Lippe voor de hulp op de diverse archief-afdelingen; Laura Verhagen van de cardiologische funktie-afdeling. Allen bedankt voor de prettige samenwerking.

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Ares Serono S.A. bedank ik voor het beschikbaar stellen van de software en de camera om de CASAS beoordelingen te kunnen verrichten. I also would like to thank Professor Jim Tanner and Robert Gibbons for their support and their input in the discussions about the use of this new technology and interpretation of the data.

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Ook Eli Lilly Nederland BV ben ik veel dank verschuldigd. Het proefschrift had niet afgerond kunnen worden zonder de mogelijkheid die ik door hen geboden kreeg.

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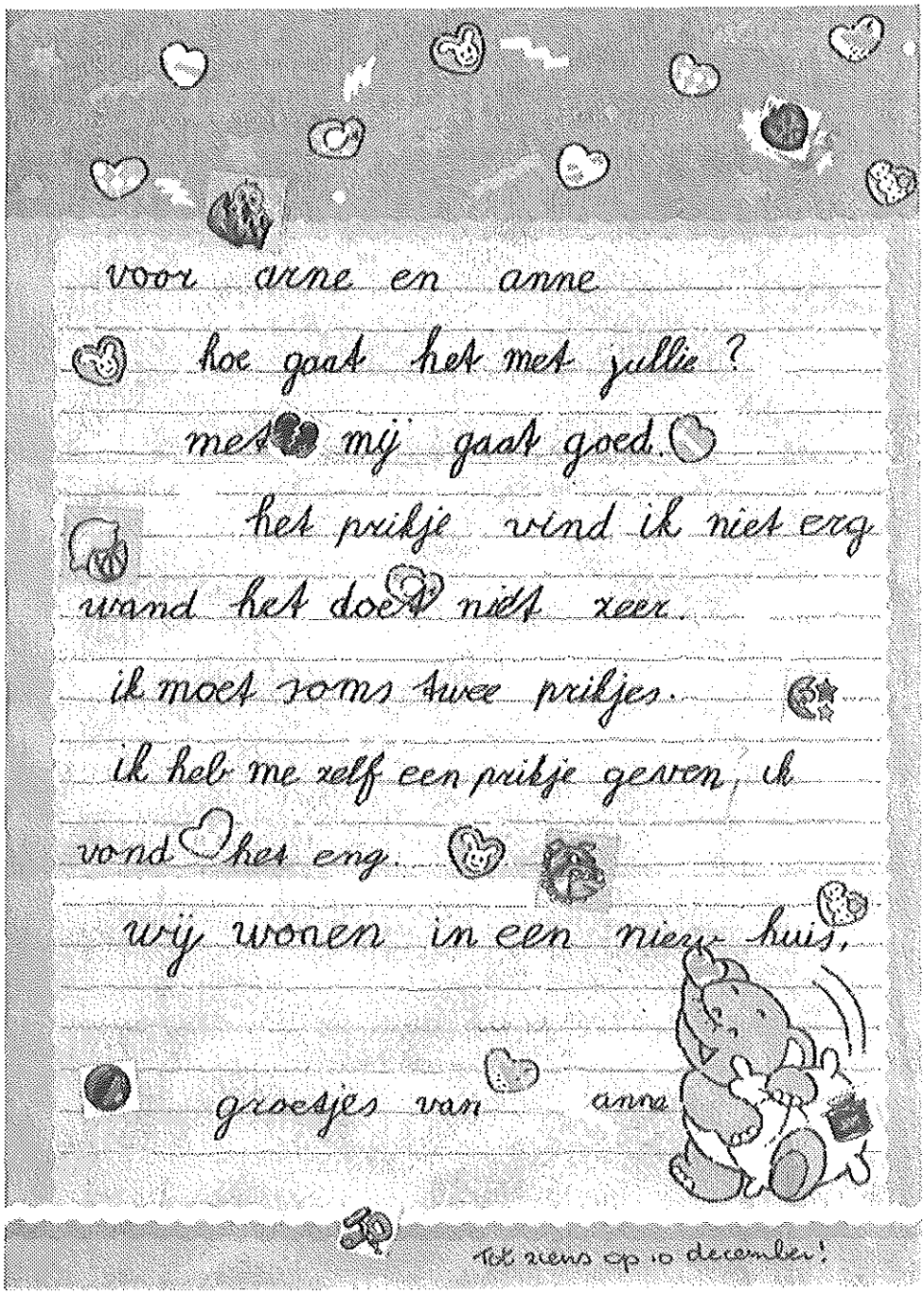
Tenslotte mijn gezinnetje. Roos, ik hoef maar een alleszeggende zin hier weer te geven: 'Het is af'. Alhoewel jij nooit hebt aangegeven dat het allemaal te lang ging duren of dat het je teveel werd, was je opluchting de laatste tijd wel merkbaar. De tijd die ik jou en de kinderen tekort heb gedaan kan ik niet teruggeven. Ter compensatie hoop ik dat ik er ook een 'beter' mens door ben geworden. Lieve Mark en Lara, ik hoop dat jullie net zoveel plezier aan 'leren' zullen beleven als ik heb gehad.

Uithoorn
augustus 1996


voor arne en anne
hoe gaat het met jullie ?
met mij gaat goed.
het prikje vind ik niet erg
want het doet niet zeer.
ik moet soms twee prikjes.
ik heb me zelf een prikje gezet, ik
vond het eng.
wij wonen in een nieuw huis,
groetjes van anne
tot ziens op 10 december!

Curriculum Vitae

CURRICULUM VITAE

The author was born November 18th 1958 in Amsterdam. In 1978 he passed his Gymnasium beta examination at the Spinoza Lyceum in Amsterdam. After a delay of one year due to the limited entry system he commenced Medical school in 1979 at the University of Amsterdam. In June 1984 he graduated for the Bachelor's degree, in March 1986 for the Master's degree, and in August 1988 passed the final examinations in Medicine. He started as a research assistant/Assistent Geneeskunde (niet in opleiding, AGNIO) at the Department of Neonatology, Academic Medical Centre, Amsterdam (head: Mrs. Prof. Dr. J.G. Koppe) in September 1988. From February until October 1989 he worked as a Resident (niet in opleiding, AGNIO) in the Department of Pediatrics at the University Hospital of Groningen (head: Prof. Dr. H.S.A. Heymans). He was then asked to become clinical research fellow in the Department of Pediatrics (head: Prof. Dr. H.K.A. Visser), subdivision Endocrinology (head: Prof. Dr. S.L.S. Drop), at the Sophia Children's Hospital, Rotterdam. During this period he was a member of the Dutch Working Group on Growth Hormone. He passed the Oxford Examination in English as a Foreign Language (higher level) and in 1991 attended the course on Classical Statistical Methods for Data Analysis at the Netherlands Institute for Health Sciences of the Erasmus University Medical School, and in 1993 the Erasmus Autumn School on Endocrinology and Immunology of the Erasmus University Medical School, Rotterdam. From April 1995 until July 1996 he worked as an Associate Clinical Research Physician for Diabetes and Growth Hormone with Eli Lilly Nederland B.V., Nieuwegein. Thereafter, he became Associate Medical Director.

The author married Roos-Marie F.E.N. Wijs in 1989. They are very happy with their son Mark (born June 5th 1990) and daughter Lara (born December 13th 1992). The author takes a special interest in sports, in particular football, tennis, skiing, and cricket.

Appendices

APPENDIX 1

INDIVIDUAL GROWTH CURVES

APPENDIX 1A

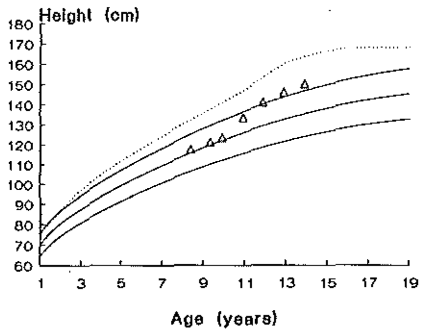
This appendix shows growth curves for each girl in the dose-response study. The markers indicate two pretreatment measurement points followed by the baseline and yearly measurements of standing height. Reference is made to the Dutch-Swedish-Danish Turner references (mean +/- 2 SD) and the 50th percentile for healthy Dutch girls (·····).

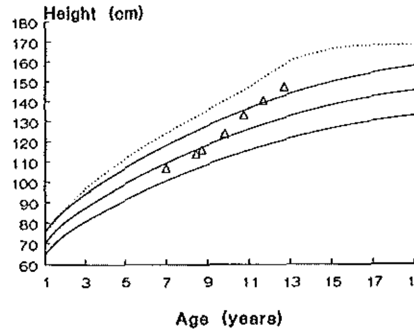
APPENDIX 1B

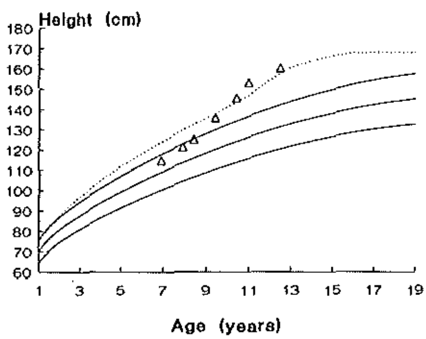
This appendix shows growth curves for each girl in the GH adminstration frequency-response study. The markers indicate two pretreatment measurement points. The subsequent measurements have been obtained at start of the 24-hour GH profile study, 10 weeks later at start of continuous GH treatment and after 1 and 2 years GH therapy. Reference is made to the Dutch-Swedish-Danish Turner references (mean +/- 2 SD) and the 50th percentile for healthy Dutch girls (·····).

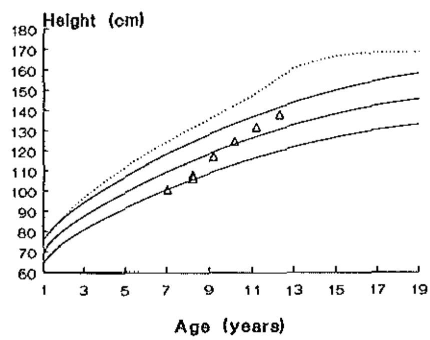
Appendix 1A

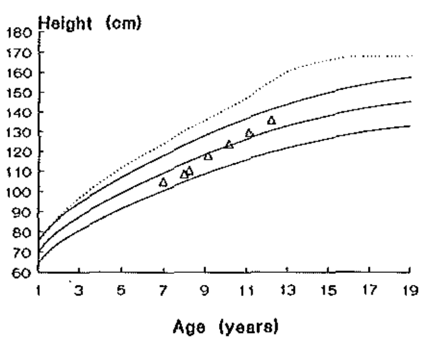
Appendix 1A

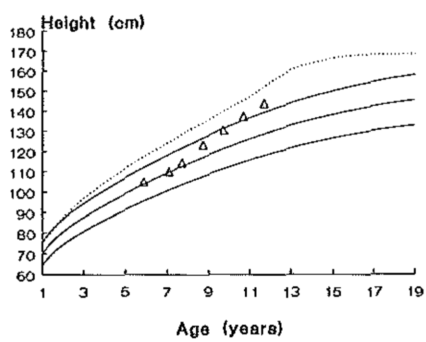


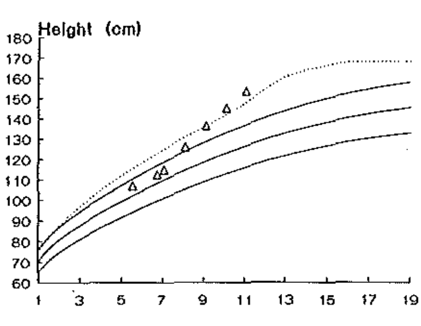


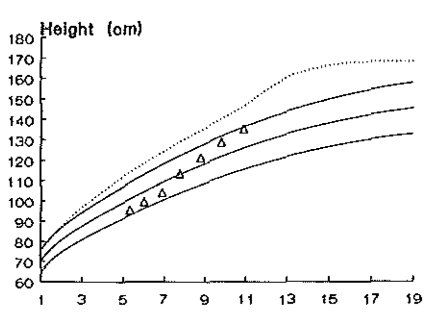


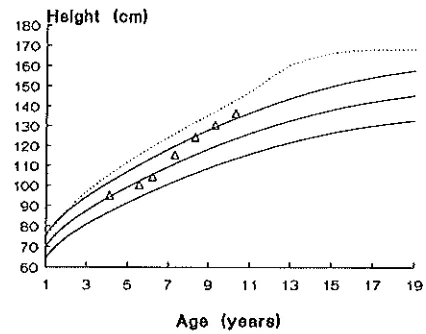








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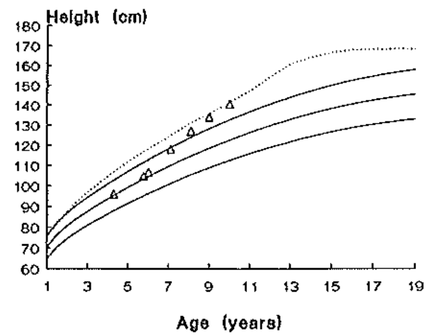
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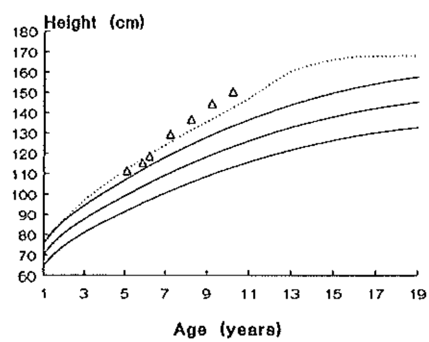
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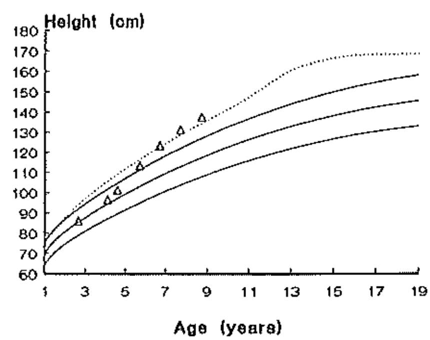
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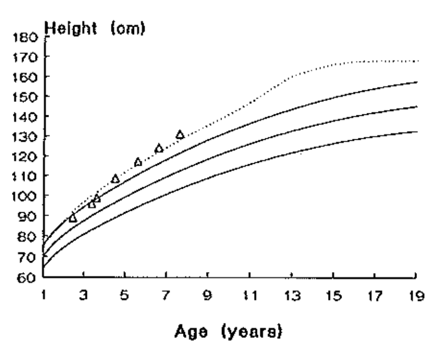
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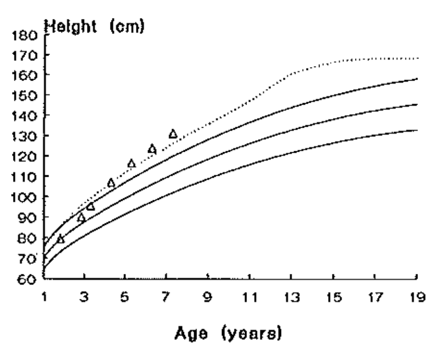
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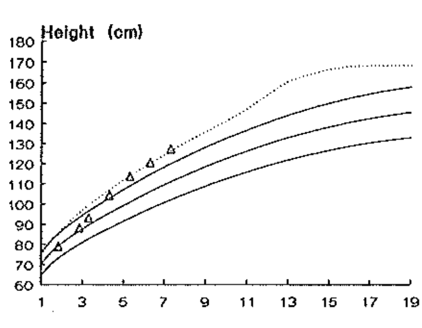
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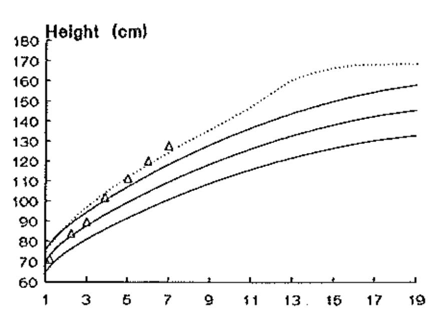
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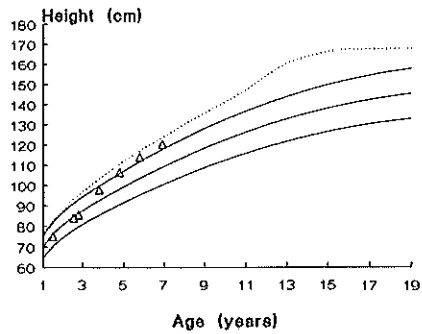
Appendix 1A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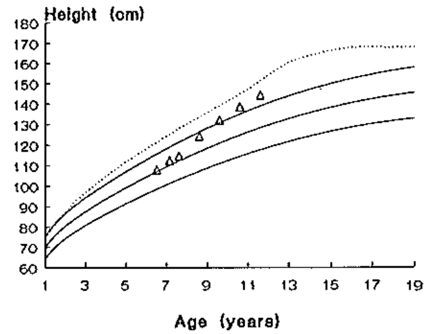
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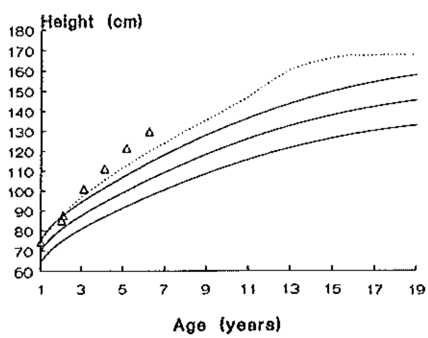
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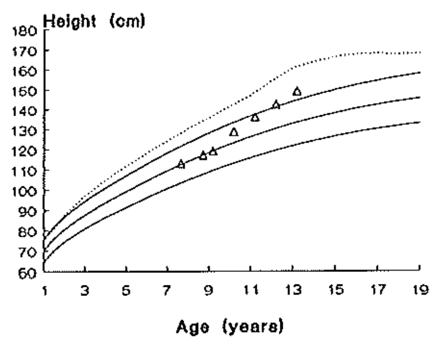
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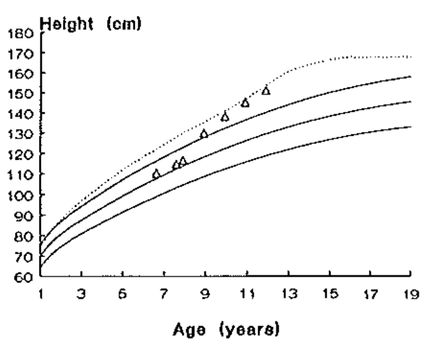
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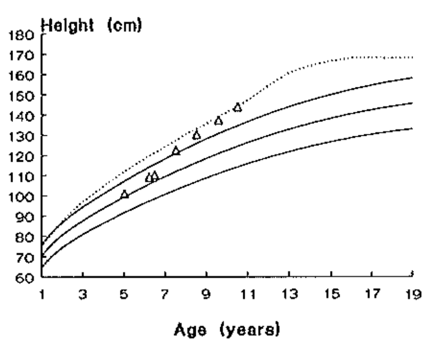
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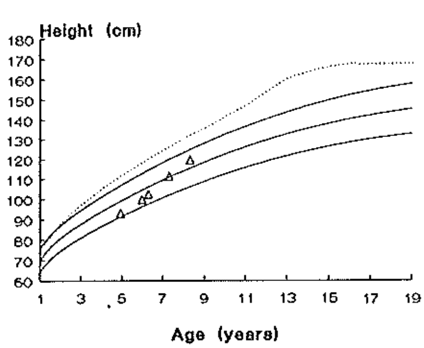
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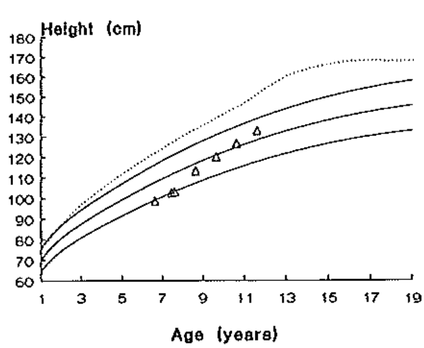
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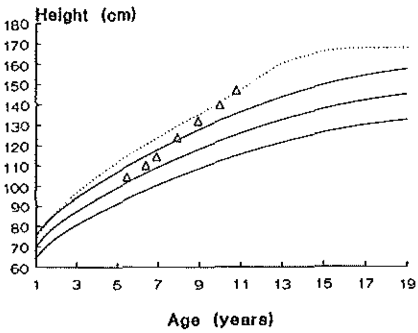
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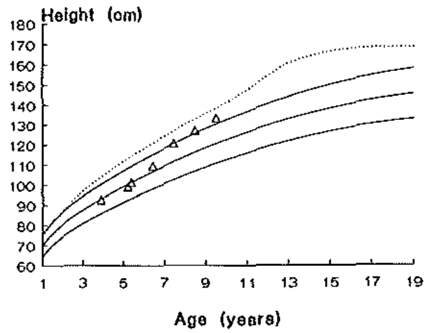
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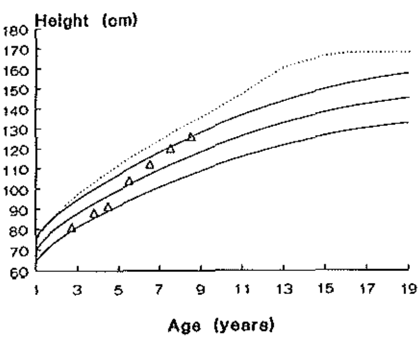
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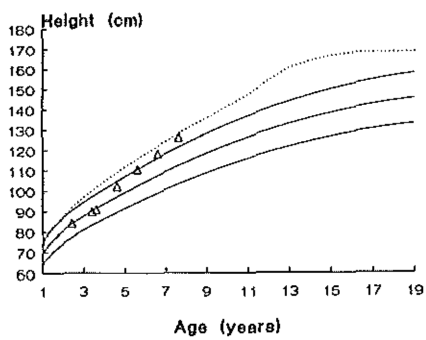
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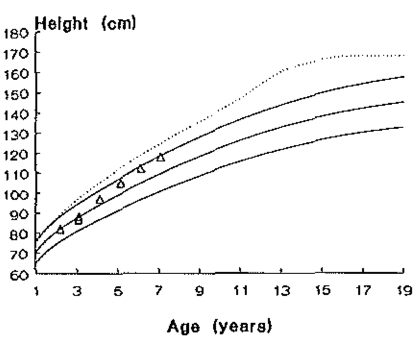
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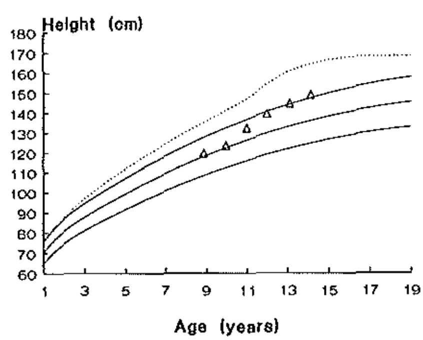
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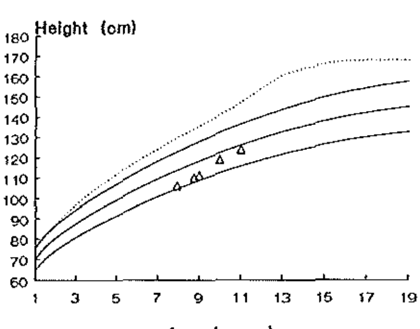
1 3 5 7 9 11 13 15 17 19
Age (years)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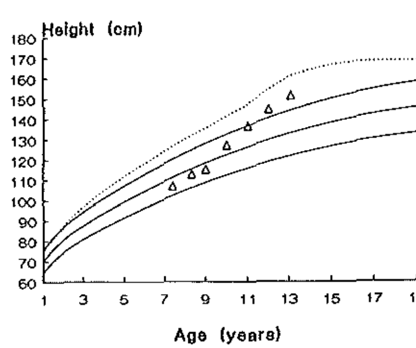
1 3 5 7 9 11 13 15 17 19
Age (years)


Height (cm)
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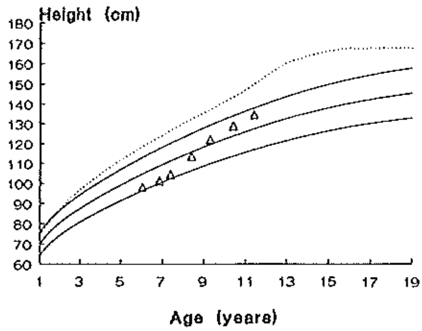
1 3 5 7 9 11 13 15 17 19
Age (years)

Appendix 1A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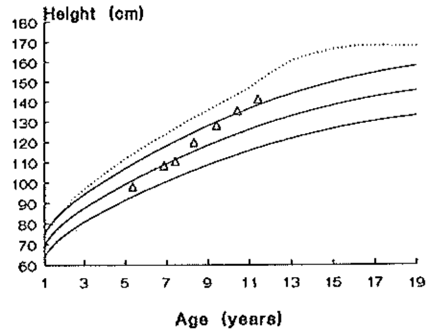
Age (years)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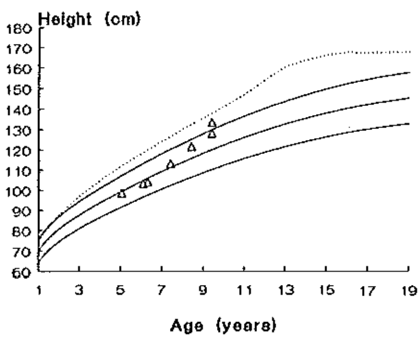
1 3 5 7 9 11 13 15 17 19
Age (years)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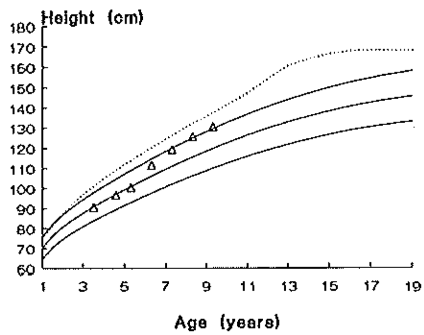
Age (years)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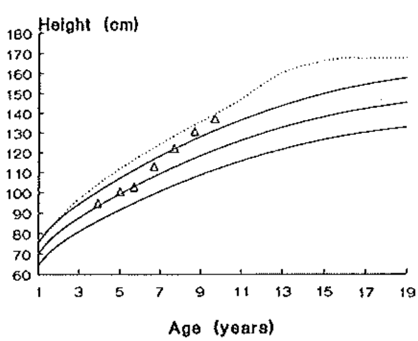
1 3 5 7 9 11 13 15 17 19
Age (years)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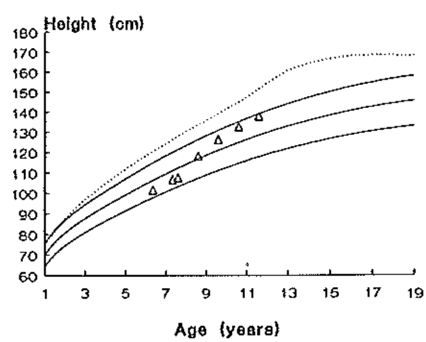
1 3 5 7 9 11 13 15 17 19
Age (years)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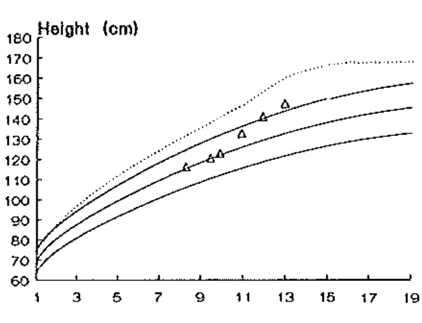
Age (years)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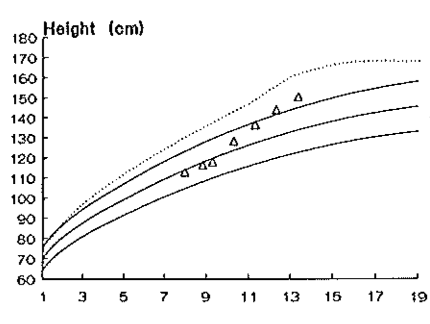
Age (years)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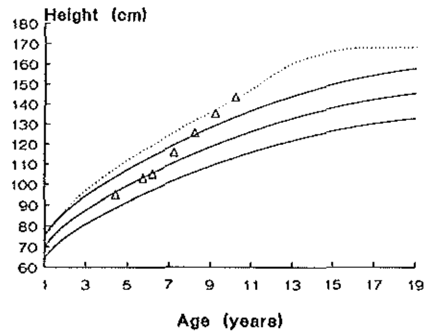
Age (years)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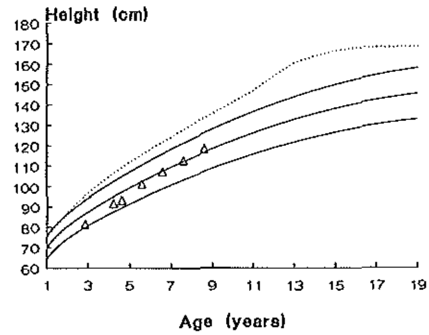
Age (years)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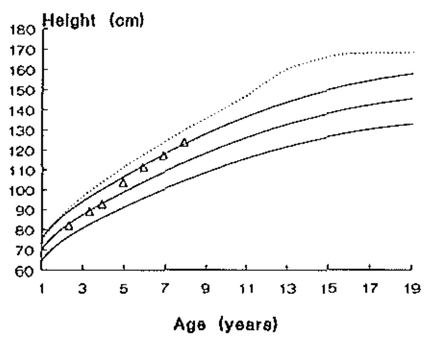
Age (years)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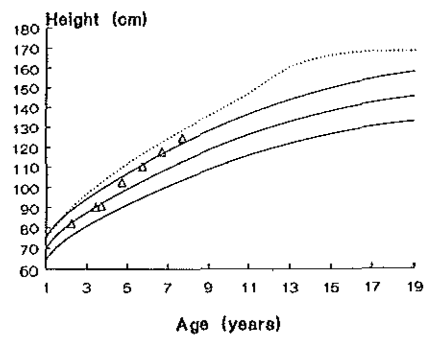
Age (years)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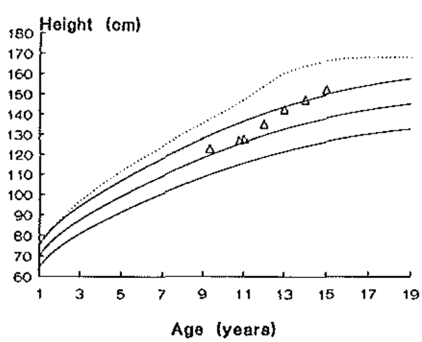
Age (years)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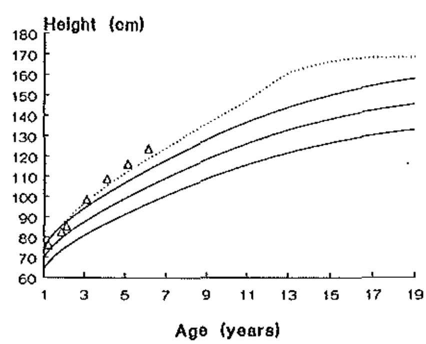
Age (years)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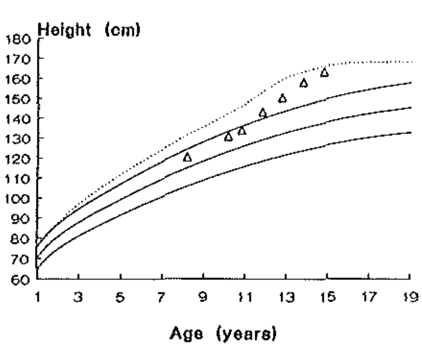
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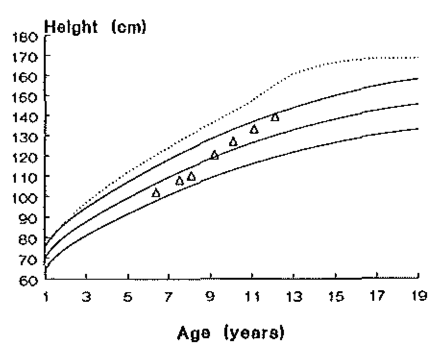
Age (years)


Height (cm)
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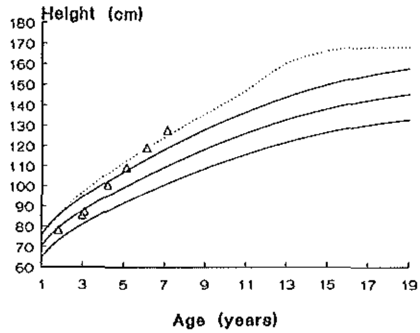
Age (years)

Appendix 1A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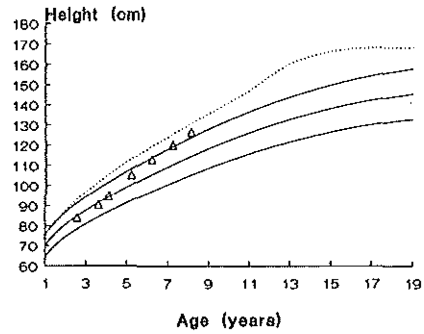
Age (years)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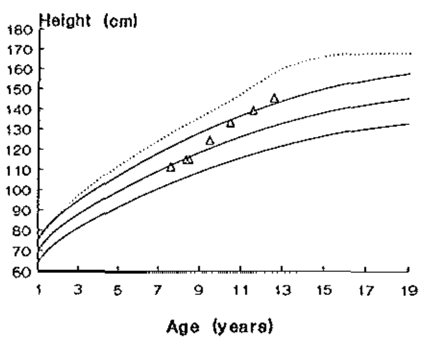
Age (years)


Height (cm)
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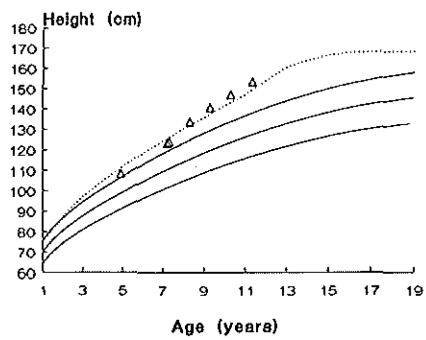
1 3 5 7 9 11 13 15 17 19
Age (years)


Height (cm)
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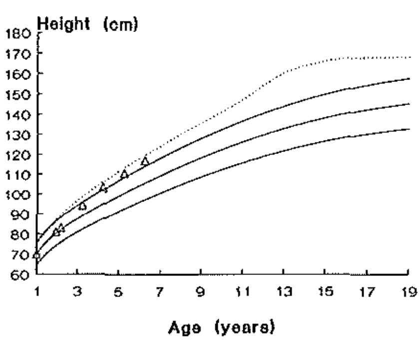
Age (years)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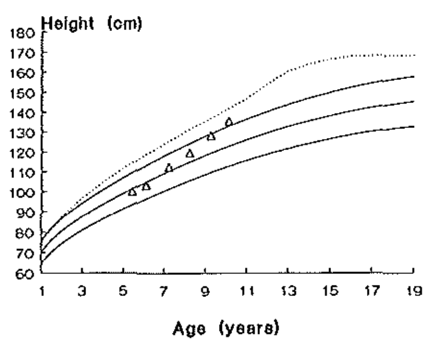
Age (years)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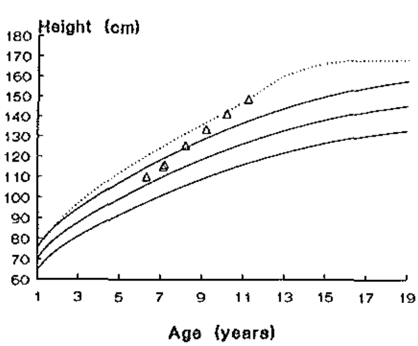
Age (years)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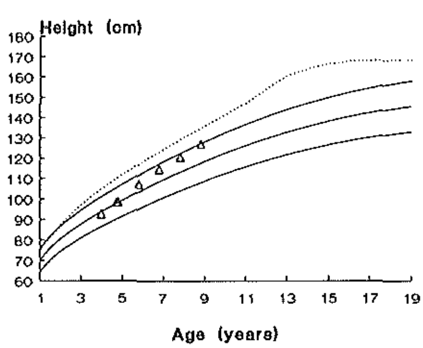
Age (years)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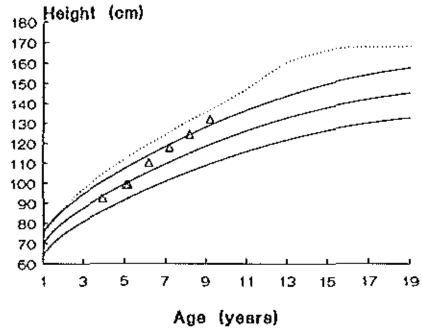
Age (years)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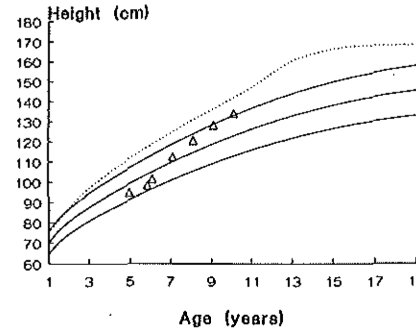
Age (years)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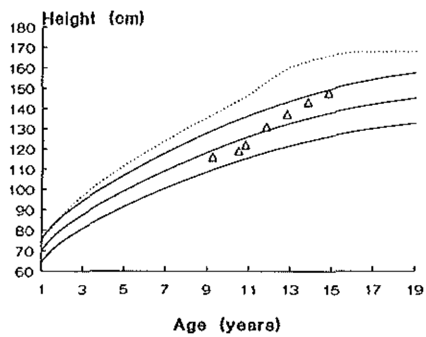
Age (years)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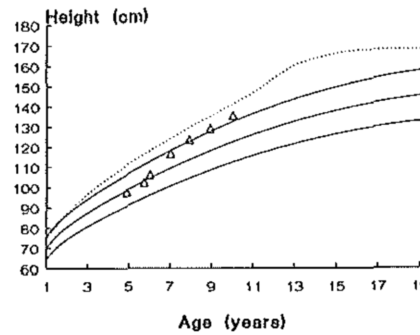
Age (years)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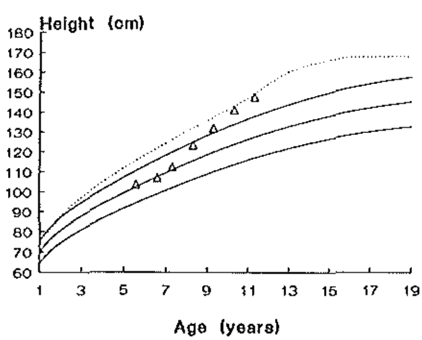
Age (years)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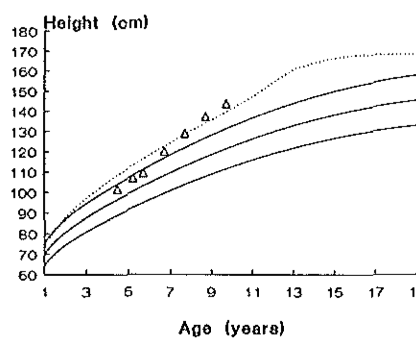
Age (years)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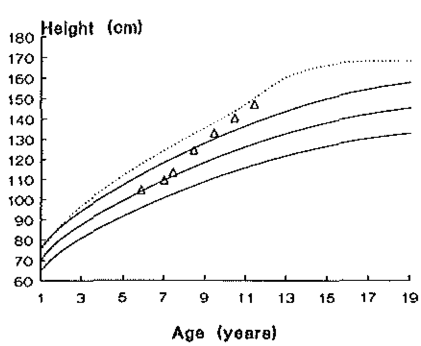
Age (years)


Height (cm)
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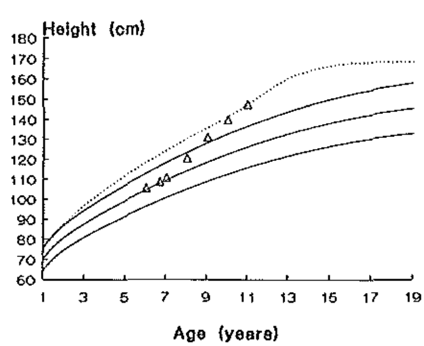
Age (years)


Height (cm)
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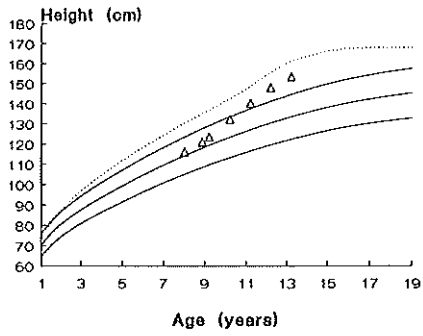
Age (years)

APPENDIX 1A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150
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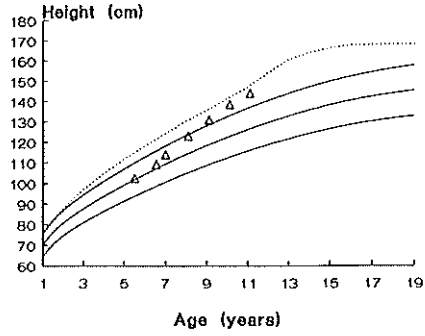
120
110
100
90
80
70
60
1 3 5 7 9 11 13 15 17 19
Age (years)


Height (cm)
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100
90
80
70
60
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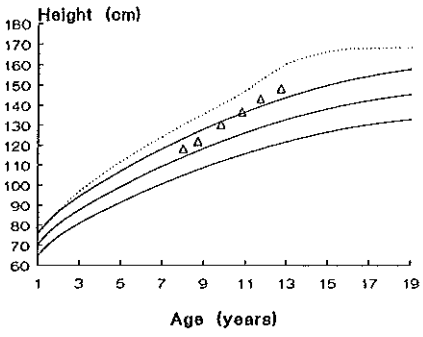
Age (years)


Height (cm)
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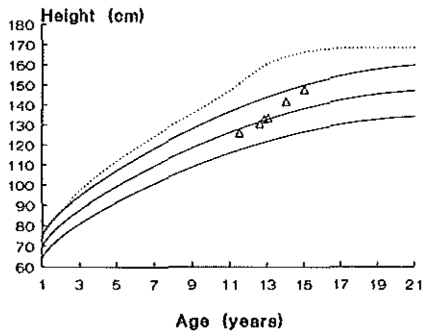
120
110
100
90
80
70
60
1 3 5 7 9 11 13 15 17 19
Age (years)

Appendix 1b


Height (cm)
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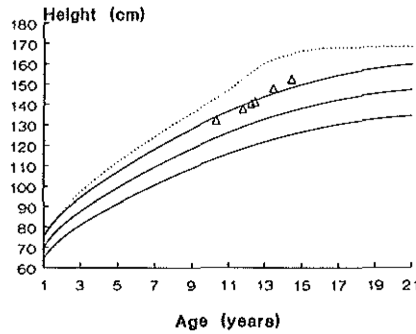
Age (years)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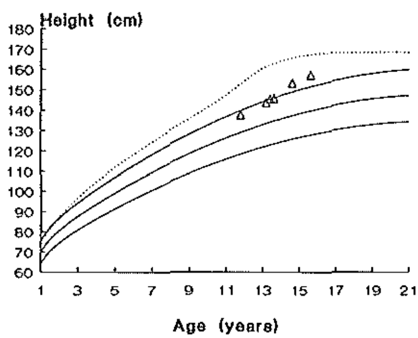
Age (years)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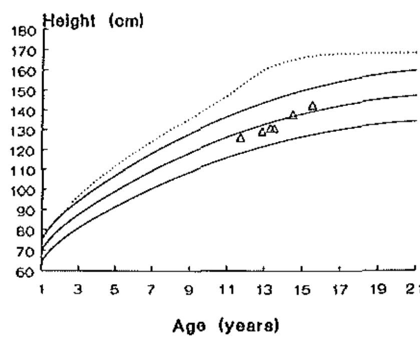
Age (years)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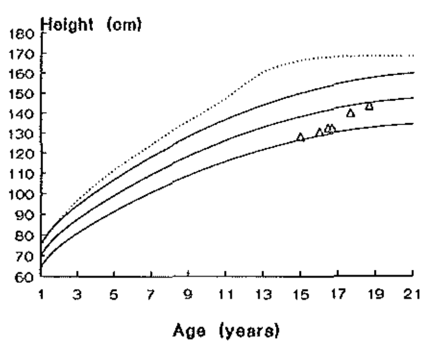
1 3 5 7 9 11 13 15 17 19 21
Age (years)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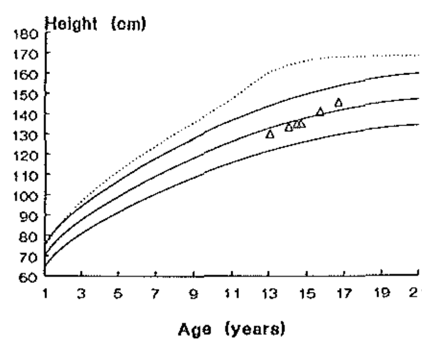
Age (years)


Height (cm)
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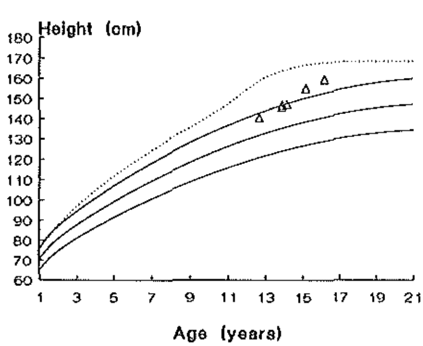
Age (years)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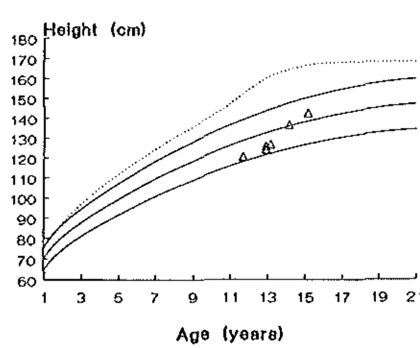
Age (years)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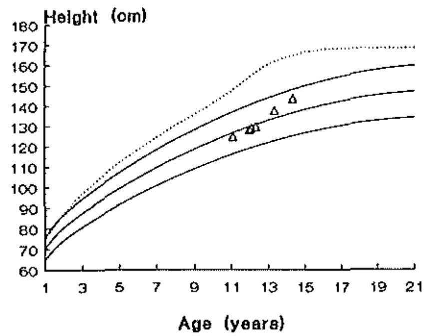
Age (years)

Appendix 1B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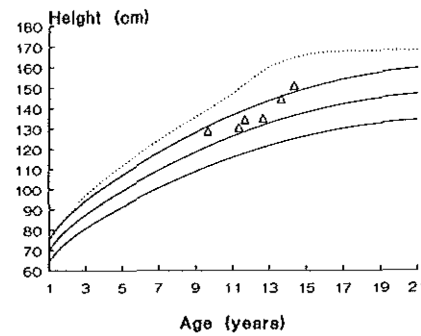
Age (years)


Height (cm)
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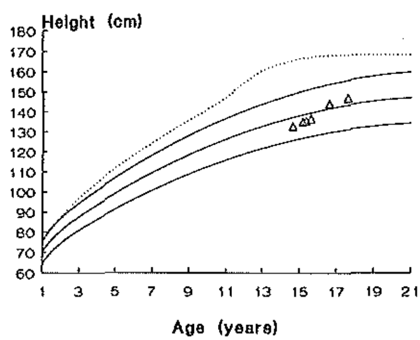
1 3 5 7 9 11 13 15 17 19 21
Age (years)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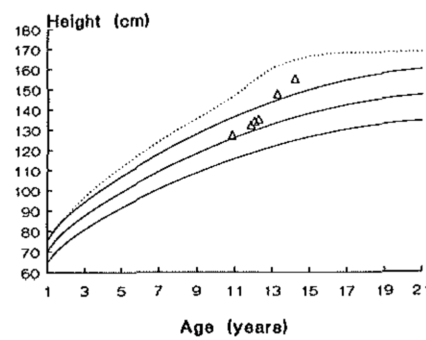
Age (years)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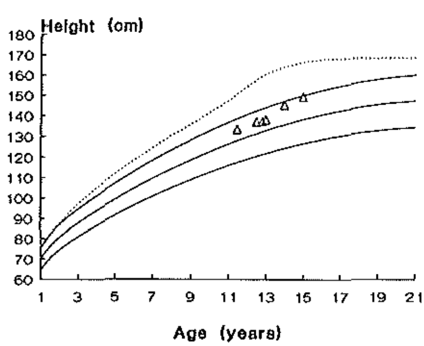
Age (years)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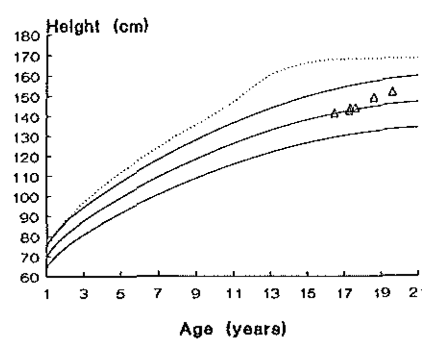
Age (years)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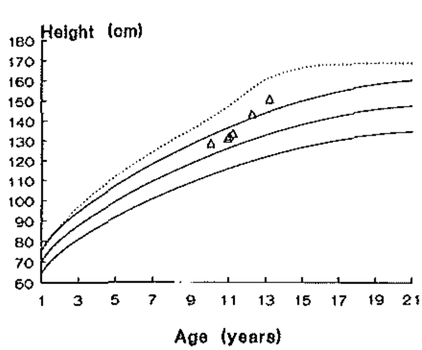
Age (years)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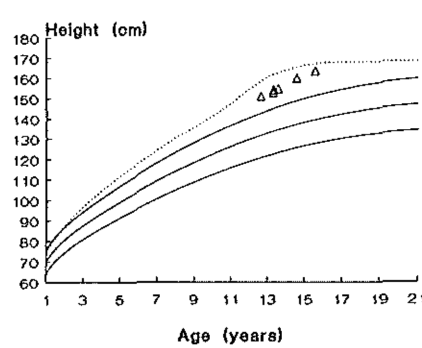
1 3 5 7 9 11 13 15 17 19 21
Age (years)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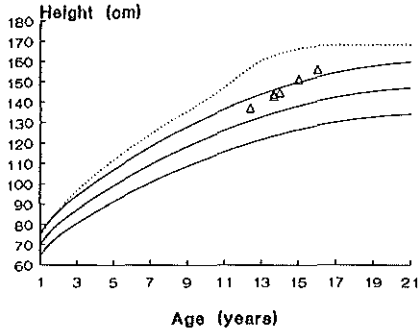
1 3 5 7 9 11 13 15 17 19 21
Age (years)


Height (cm)
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90
80
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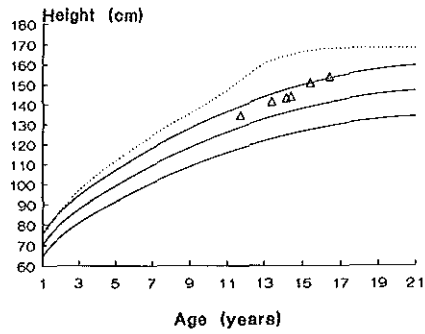
1 3 5 7 9 11 13 15 17 19 21
Age (years)


Height (cm)
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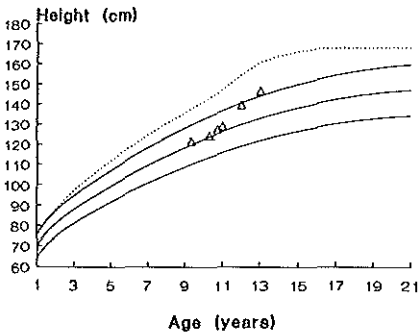
1 3 5 7 9 11 13 15 17 19 21
Age (years)


Height (cm)
180
170
160
150
140
130
120
110
100
90
80
70
60
1 3 5 7 9 11 13 15 17 19 21
Age (years)

DISCREPANCIES IN JUDGING ATYPICALLITY OF BODY PROPORTIONS

ABSTRACT

Consider a multivariate set of percentiles, $\vec{P}_j(t)$, where e.g. j=5, 10, 50, 90, 95, serving as an age-dependent description of growth and based on a series of cross-sectional multivariate measurements $\{(t_i;\vec{x}_i)\}_{i=1}^{n}$, say anthrometric variables to fix thoughts, taken from some reference population consisting of n individuals. A description like this enables the quantification of (a)typicallity of an individual as compared to this reference population. Obviously, a description is but an approximation of the `true' distribution. A range of popular descriptions exists yielding, by definition, different results and hence discrepancies in judging (a)typicallity of an individual's proportion. In general, all proposed descriptions are satisfying when describing the typical cases, but differ strongly in the description of atypical cases. In this paper (1) some simple descriptions are summarized, (2) discrepancies are discussed and a reasonable choice from these descriptions is decided on and (3) as an example some types of patients are compared to a reference population.

INTRODUCTION

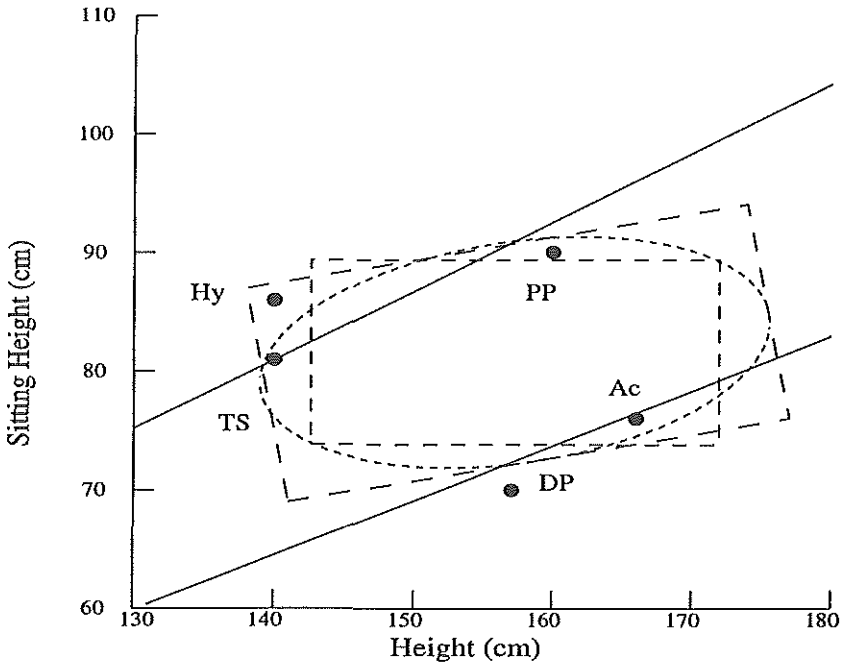
A gross simplification of the dynamics of growth is considering a set of age-dependent quantiles which are generally based on data collected on a series of fixed ages (or thought, or interpreted as such). The description of the distribution of the data at one such point of age is unfortunately a matter of taste. Nonparametric density estimation is indicated but has practical draw backs in that, especially for multivariate distributions, tables or graphs that present the density estimates become complicated and, in fact useless without the aid of a computer. Moreover, nonparametric density estimation demands huge datasets to estimate atypicallity, e.g., $\vec{P}_5$ and $\vec{P}_{95}$.

For simplicity reasons the use of parametric density estimates is common. Basically, they are chosen such that they describe the majority of the data rather well. The number of such estimates is without end. Let us fix our thoughts to parametric density estimation and discuss some commonly used methods.

The description of data by a few parameters only, implicitely introduces smoothening. The highest density is usually located centrally in these densities. This, in general, provides a satisfactory description of the majority of data. Put differently, the peaks of all density functions will be close together for whatever convenient parametric density function one chooses. This means that for the description of the typical growth dynamics `any' parametric density estimate

may serve. A one-dimensional standardized sample statistic p may provide a means to judge (a)typicallity of the shape or form of an individual, where the shape or form is expressed in a higher dimensional space. Unfortunately, the description of atypical cases, i.e. individuals whose measurements are outside a certain boundary deduced from a chosen density estimate, is highly dependent on the choice of that density estimate. What may appear as a perfectly typical shape or form for one density estimate may be judged as an atypical case for another choice of density function. In Figure 1 four common density functions are depicted describing one set of data. The discrepancies that arise from these choices are readily observed by eye.

Figure 1. A two-dimensional dataset (Sitting Height against Height for girls at 12 years of age) is described in four ways. The 95% confidence intervals are depicted. The rectangle (long dashes: see section A) depicts the joint univariate normal distribution, the ellipse (dots: see section B) depicts the bivariate normal distribution, the cone (solid lines: see section C) shows the ratio-based distribution and finally the rotated rectangle (short dashes: see section D) graphs the principle axes based distribution (i.e. size and shape numbers). Some examples of atypical shape are indicated by dots (Hypochondroplasia (Hy), Acromegaly (Ac), Precocious Puberty (PP), Delayed Puberty (DP) and Turner's Syndrome (TS)). See the text for further explanation.



Judging atypicallity, expressed as a one-dimensional distance function or sample statistic p, is of course hazardous since the complexity of the shape consists of more than one variable. Even more hazardous it is to follow an individual through time and try to make a statement about the development of this individual. Cross-sectional reference data alone cannot provide a clue about individual development. Put bluntly, if an individual is 'small' at a certain point of age, nothing can be concluded about the future development. So the mean value may be indicated as the expectation. Yet, in practice, one often expects an individual to follow its percentile. Longitudinal studies have shown that the 'truth' is somewhere in between these two statements. There is a tendency for atypical cases to develop toward the mean. This tendency to the mean can be modelled in a simple way (one-parametric regression to the mean) or with higher order adaptations [1,2].

Both aspects, choice of density function and lack of longitudinal data, do not provide a solid basis to judge atypicallity of body proportions and the development of such an atypical individual. However, given the limitation of information and the fundamental lack of uniqueness in describing this information, a reasonable approximation may be provided bearing in mind that especially atypical cases are hard to classify.

METHODS

In this section some usual density estimates are discussed. Figure 1 clearly depicts the discrepancies that arise when comparing several choices of density estimates. Especially, the further away from the centre of the distribution, i.e. for the more interesting cases from a clinical perspective, the more differences one will find due to the choice of density function.

The data set that is considered essentially consists of n individuals represented by a

m+1 dimensional vector $\{(t_i,y_{1i},....,y_{mi})\}$i , $m \geq 2$, where t_i denotes the age, $t_i \leq t_{i+1}$, and y_{ji} the j-th kind of measurement of individual i, i=1,....,n, under investigation. The distribution of the vectors of scores Y_1,....,Y_m is age-dependent. To bypass this age-dependence the distribution at some point of age t_r is thought independent of age on some interval containing t_r, e.g. $[t_r - \varepsilon, t_r + \varepsilon]$ or $[t_{r-q}, t_{r+q}]$.

A. JOINT UNIVARIATE NORMAL DISTRIBUTION

A joint distribution of variables is considered, where each variable is normally distributed independently. The 100 (1 - ε)% confidence interval is given by

$$|Y_j - \mu_j| \leq \alpha\sigma_j,$$

for j=1,....,m, with μ_j and σ_j the mean and standard deviation of the j-th measurement respectively and $P(\alpha)$=1 - ½ε, where P is the standard normal probability function. Typically α=1.96 for ε=0.05. The joint confidence interval takes the form of a hyper-rectangle. The joint 100 (1 - ε)% confidence interval trivially contains less than 100 (1 - ε)% of the data points. Since the distribution of the measurements is thought indepently, often the situation is encountered that data points within such a confidence interval are quite atypical from a biological point of view. The description is similar to multiple z-scores.

B. MULTIVARIATE NORMAL DISTRIBUTION

The variables are considered to be normally distributed. The 100 (1 - ε)% confidence interval is given by

$$(\vec{Y} - \vec{\mu})^T \Sigma^{-1} (\vec{Y} - \vec{\mu}) \leq \chi^2_m;\ \varepsilon = \alpha,$$

where Σ is the covariance matrix and χ^2_m the 100 (1 - ε)% confidence interval of the Chi-squares function for m degrees of freedom. Typically α=5.99 for ε=0.05 and m=2. The confidence interval takes the form of an ellipsoid. Depending on the measurements used to describe shape or form this cigar-like distribution may be a reasonable candidate for the description of body proportion.

C. MULTIPLE RATIO-BASED DISTRIBUTION

The measurements involved are studied as a linear expression of a specific other one, Y_r, i.e. the distribution of the m - 1 derived variables $U_j = Y_j / Y_r$, j=1,....,m and $j \neq r$. The distribution of the variables U_j are considered to be normally distributed. The 100 (1 - ε)% confidence interval is given by

$$|U_j - \mu_j| \leq \alpha\sigma_j,$$

or equivalently,

$$Y_r(\mu_j - \alpha\sigma_j) \leq Y_j \leq Y_r(\mu_j + \alpha\sigma_j),$$

j=1,....,m, $j \neq r$ with μ_j and σ_j are the mean and standard deviation of the j-th derived measurement respectively and $P(\alpha)$=1 - ½ε, where P is the standard normal probability function. The joint confidence interval takes the form of a pyramidoid. The confidence interval is an overestimation, just as in other multiple univariate distributions. For the description of measurements collected at more or less the same age this method is not recommended.

D. JOINT PRINCIPLE AXES BASED DISTRIBUTION

This distribution is a mixture of the first two. It is a multiple univariate distribution after a rotation of the data such that the axes of the multivariate ellipsoid (the eigenvectors of Σ^{-1}) are mapped on the normal coordinate axes. Then it is proceeded as in the first case. The confidence interval takes the form of a hyper-rectangle [3].

SOME EXAMPLES

Figure 1 describes the 95% confidence interval of the above mentioned density estimates for measurements Height and Sitting Height. Similar considerations could be drawn for any combination of measurements at any age. The data are from a random group of healthy Dutch children [4], at the age of 12 (11.5 - 12.5) years. Added to the graph are five anomalies: Hp, Ac, PP, DP and TS.

Hypochondroplasia (Hp) is characterized by relatively short extremities compared to trunk (and head). With increasing age the patient will diverge progressively from normality since growth of the legs is hampered. The point Hp is quite typical for this kind of anomalies. In contrast, the point Ac could well be a patient with Acromegaly which is characterized by enlargement of the distal parts of the body, although it may involve all body portions.

In prepuberty the ratio Sitting Height/Subischial Leg Length tends to one. In puberty, sex-steroids result not only in rapid growth with predominance toward the extremities, but also in an increase in bone maturation with a earlier closure of the epifyseal growth plates. Therefore the ratio first decreases and since growth of the legs ceases earlier than growth of the trunk, the ratio subsequently rises again. Body growth in Precocious Puberty (PP) is increased, but bone maturation even more, resulting in adult heights in low normal ranges with the main deficit in the extremities. In contrast, for Delayed Puberty (DP), adolescents with hypogonadism tend to have disproportionally long legs. Primary hypogonadism in Turner's Syndrome (TS) is also accompanied with short stature. Recent semi-longitudinal Dutch studies [5,6] with mainly estrogen-treated girls with Turner's Syndrome showed a tendency for relatively short legs.

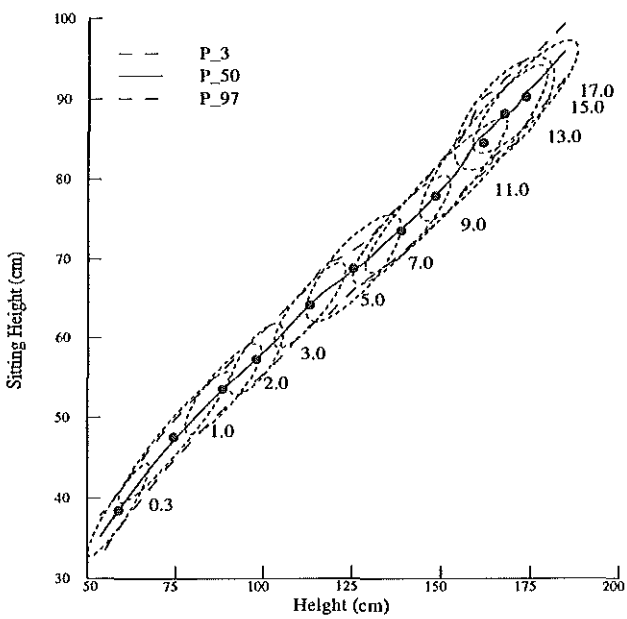
The figure illustrates that the classification of anomalies (definied as points outside the 95% confidence interval) is strongly subject to the choice of distribution function.

DISCUSSION

The description of a reference population depends in general on the choice of the density function that is used to smooth the data. Some nonparametric density estimation (e.g. using kernel functions) may be indicated but is somewhat troublesome and demands extensive data collection. Simple and commonly used descriptions such as indicated above are easy to concieve but lead to discrepancies when regarding atypical cases. Especially, methods such as multiple z-scores or ratio's have the advantage of simplicity, but they appear too rough an approximation to the 'true' distribution.

To describe the developing shape or form involves an age-dependent distribution (see the introduction of *Methods*). In Figure 2 an age-dependent bivariate normal distribution, $N(\mu(t), \Sigma(t))$, $t > 0$, is depicted for a series of fixed ages. Such an age-dependent distribution enables to judge timing of growth, e.g. growth retardation may be studied. The p-values outside a certain given interval are said to show an appearance of atypical form or shape for their age, t. Individuals which are growth retarded and have an atypical appearance for their age may however be

Figure 2. A two-dimensional dataset (Sitting Height against Height for girls). The 95% confidence intervals of the bivariate normal distribution are given for some fixed ages based on surrounding one-year intervals. For comparison also the percentiles P_3, P_{50} and P_{97} are depicted.



well- proportioned when compared to younger children in the reference populations. The value

$$t_m = \min_t p(t)$$

provides the age which is most likely to correspond with the given body proportions. The time $\tilde{t} - t_m$ is an indication for the amount of retardation.

In conclusion, an age-dependent multivariate normal distribution, which is nothing but another approximation, appears somewhat more `accurate' than other common approximations. Scepsis about the classification into typical or atypical is indicated.

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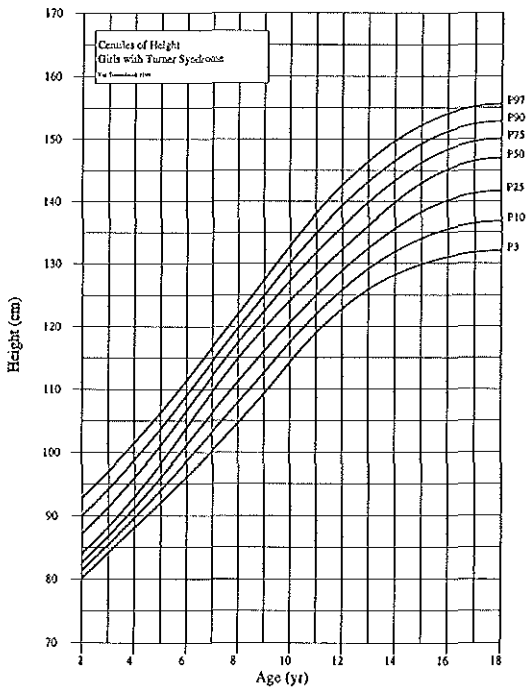
REFERENCE TABLES AND CURVES FOR MEASUREMENTS OF BODY PROPORTIONS IN TURNER SYNDROME

APPENDIX 3

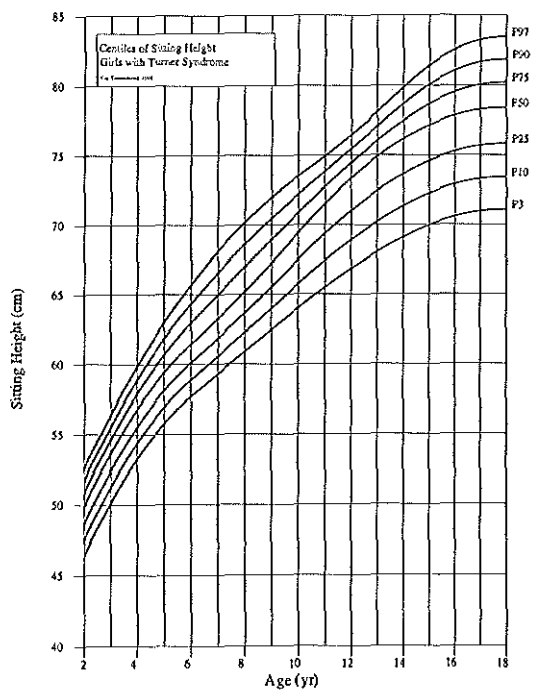
APPENDIX 3A: HEIGHT

AGE	P3	P10	P25	P50	P75	P90	P97
2	80.13	81.38	82.64	84.03	87.18	90.04	92.86
3	84.13	85.45	86.79	88.27	91.39	94.23	97.02
4	88.13	89.68	91.25	92.99	96.02	98.78	101.50
5	92.12	94.06	96.02	98.19	101.07	103.70	106.29
6	96.11	98.58	101.10	103.86	106.55	108.99	111.40
7	100.23	103.21	106.24	109.57	112.11	114.42	116.69
8	104.64	107.90	111.22	114.86	117.42	119.75	122.04
9	109.32	112.64	116.02	119.73	122.48	124.98	127.44
10	114.27	117.43	120.64	124.17	127.28	130.11	132.90
11	118.97	121.95	124.99	128.32	131.80	134.96	138.06
12	122.86	125.88	128.94	132.31	135.98	139.31	142.59
13	125.94	129.20	132.51	136.14	139.82	143.17	146.47
14	128.23	131.92	135.68	139.80	143.33	146.55	149.71
15	129.88	134.04	138.28	142.94	146.26	149.29	152.27
16	131.05	135.56	140.14	145.17	148.35	151.25	154.10
17	131.76	136.47	141.26	146.52	149.61	152.42	155.19
18	131.99	136.77	141.63	146.96	150.03	152.81	155.56



APPENDIX 3B: SITTING HEIGHT

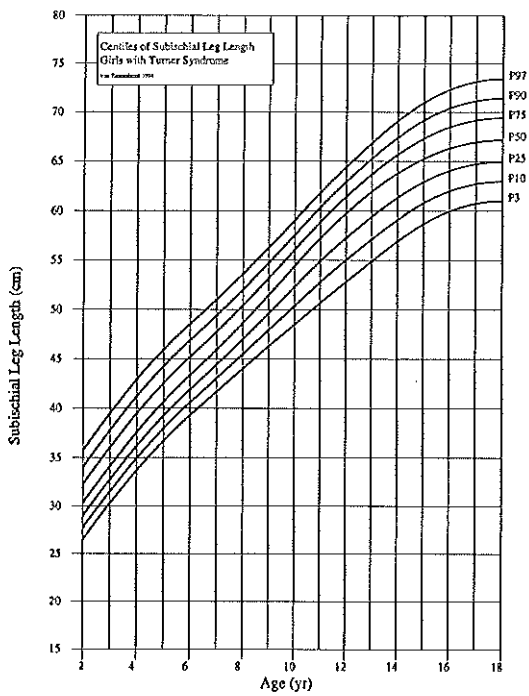
AGE	P3	P10	P25	P50	P75	P90	P97
2	46.44	47.55	48.69	49.94	50.89	51.76	52.61
3	50.14	51.26	52.40	53.66	54.64	55.54	56.43
4	53.25	54.39	55.54	56.81	57.91	58.90	59.88
5	55.77	56.93	58.11	59.41	60.68	61.84	62.97
6	57.69	58.89	60.11	61.45	62.97	64.35	65.70
7	59.31	60.57	61.86	63.27	64.99	66.56	68.10
8	60.90	62.28	63.67	65.21	66.99	68.61	70.20
9	62.48	64.00	65.56	67.26	68.96	70.50	72.01
10	64.03	65.76	67.51	69.44	70.90	72.22	73.52
11	65.50	67.43	69.39	71.55	72.76	73.86	74.94
12	66.84	68.92	71.04	73.37	74.48	75.48	76.47
13	68.04	70.23	72.46	74.91	76.06	77.09	78.12
14	69.11	71.37	73.66	76.18	77.50	78.69	79.87
15	69.98	72.28	74.61	77.17	78.70	80.09	81.45
16	70.61	72.93	75.29	77.88	79.55	81.08	82.58
17	70.98	73.32	75.69	78.30	80.07	81.68	83.26
18	71.11	73.45	75.83	78.44	80.24	81.88	83.49



APPENDIX 3

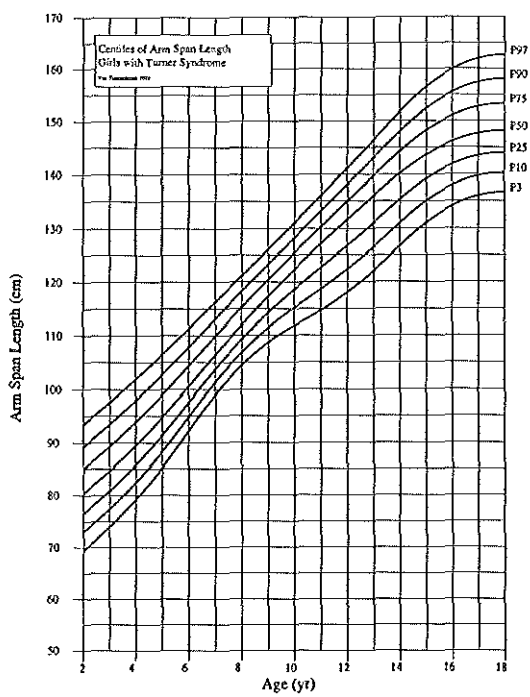
APPENDIX 3C: SUBISCHIAL LEG LENGTH

AGE	P3	P10	P25	P50	P75	P90	P97
2	26.49	27.72	28.96	30.32	32.24	33.99	35.71
3	30.30	31.52	32.77	34.14	36.05	37.80	39.52
4	33.70	34.94	36.20	37.58	39.48	41.22	42.92
5	36.70	37.96	39.24	40.65	42.54	44.25	45.94
6	39.30	40.60	41.91	43.36	45.21	46.90	48.56
7	41.68	43.04	44.42	45.93	47.75	49.40	51.03
8	44.01	45.48	46.99	48.64	50.41	52.02	53.61
9	46.28	47.93	49.61	51.46	53.18	54.75	56.29
10	48.49	50.38	52.30	54.41	56.07	57.58	59.07
11	50.66	52.76	54.90	57.25	58.88	60.36	61.82
12	52.79	55.01	57.27	59.76	61.42	62.93	64.41
13	54.88	57.13	59.42	61.94	63.68	65.27	66.83
14	56.92	59.11	61.34	63.78	65.67	67.39	69.08
15	58.70	60.79	62.91	65.25	67.29	69.15	70.98
16	59.97	61.99	64.04	66.30	68.45	70.41	72.34
17	60.73	62.70	64.71	66.92	69.14	71.16	73.15
18	60.98	62.94	64.94	67.13	69.38	71.42	73.42



APPENDIX 3D: ARM SPAN

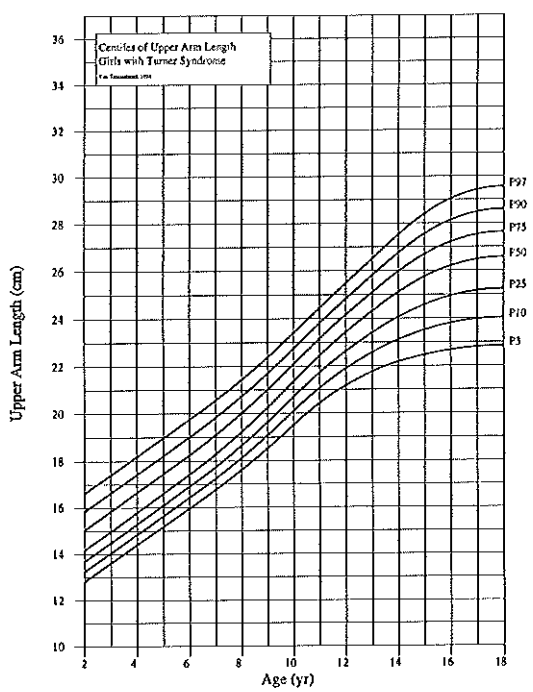
AGE	P3	P10	P25	P50	P75	P90	P97
2	69.48	72.97	76.52	80.42	85.05	89.26	93.40
3	73.90	77.34	80.84	84.68	89.26	93.42	97.52
4	79.16	82.44	85.78	89.45	93.88	97.91	101.88
5	85.25	88.28	91.35	94.72	98.91	102.72	106.47
6	92.19	94.85	97.54	100.51	104.35	107.85	111.30
7	98.94	101.32	103.74	106.41	109.91	113.10	116.24
8	104.46	106.87	109.32	112.01	115.28	118.26	121.18
9	108.77	111.50	114.28	117.33	120.46	123.31	126.12
10	111.86	115.21	118.62	122.36	125.46	128.28	131.05
11	114.65	118.64	122.69	127.14	130.31	133.20	136.04
12	118.06	122.41	126.83	131.69	135.06	138.14	141.16
13	122.08	126.53	131.05	136.02	139.72	143.09	146.41
14	126.72	131.00	135.35	140.14	144.29	148.07	151.79
15	131.04	135.06	139.15	143.64	148.24	152.43	156.55
16	134.13	137.97	141.86	146.14	151.06	155.54	159.95
17	135.99	139.71	143.49	147.64	152.76	157.41	161.99
18	136.61	140.29	144.03	148.15	153.32	158.04	162.67



APPENDIX 3

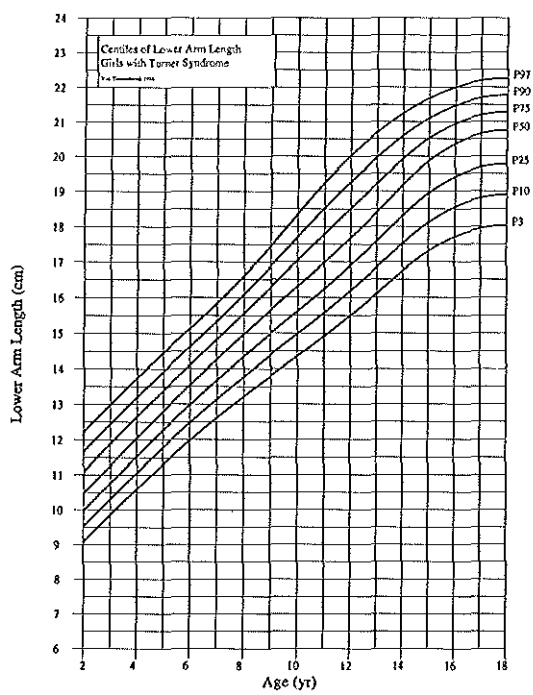
APPENDIX 3E: UPPER ARM

AGE	P3	P10	P25	P50	P75	P90	P97
2	12.81	13.25	13.69	14.17	15.04	15.83	16.61
3	13.61	14.05	14.49	14.97	15.84	16.63	17.40
4	14.40	14.84	15.29	15.78	16.64	17.42	18.19
5	15.18	15.63	16.09	16.60	17.45	18.22	18.98
6	15.96	16.42	16.90	17.42	18.25	19.01	19.75
7	16.76	17.24	17.74	18.28	19.09	19.83	20.56
8	17.62	18.13	18.65	19.23	20.01	20.73	21.44
9	18.53	19.08	19.64	20.25	21.01	21.70	22.38
10	19.51	20.10	20.70	21.36	22.09	22.75	23.40
11	20.43	21.07	21.73	22.45	23.16	23.81	24.45
12	21.18	21.90	22.63	23.44	24.17	24.83	25.49
13	21.77	22.58	23.41	24.32	25.10	25.82	26.52
14	22.18	23.12	24.06	25.11	25.97	26.76	27.53
15	22.47	23.52	24.58	25.75	26.70	27.56	28.41
16	22.68	23.80	24.95	26.20	27.22	28.14	29.05
17	22.80	23.98	25.17	26.48	27.53	28.48	29.42
18	22.84	24.03	25.24	26.57	27.63	28.60	29.55



APPENDIX 3F: LOWER ARM

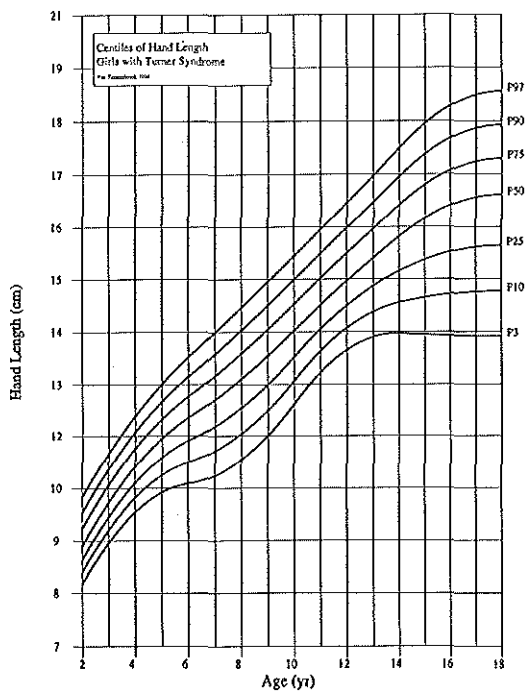
AGE	P3	P10	P25	P50	P75	P90	P97
2	9.08	9.52	9.98	10.48	11.09	11.66	12.21
3	9.86	10.31	10.77	11.27	11.88	12.44	12.99
4	10.60	11.06	11.53	12.04	12.65	13.20	13.74
5	11.31	11.78	12.27	12.80	13.39	13.93	14.46
6	11.97	12.48	12.99	13.55	14.11	14.63	15.14
7	12.61	13.14	13.68	14.27	14.83	15.34	15.84
8	13.22	13.78	14.35	14.97	15.56	16.08	16.61
9	13.80	14.39	14.99	15.65	16.29	16.87	17.45
10	14.36	14.98	15.62	16.31	17.04	17.70	18.36
11	14.92	15.57	16.24	16.98	17.79	18.52	19.25
12	15.50	16.19	16.90	17.68	18.52	19.28	20.03
13	16.11	16.84	17.59	18.41	19.23	19.97	20.70
14	16.74	17.52	18.31	19.18	19.92	20.59	21.25
15	17.31	18.12	18.95	19.87	20.52	21.11	21.69
16	17.71	18.55	19.41	20.36	20.95	21.48	22.01
17	17.95	18.81	19.69	20.65	21.20	21.70	22.19
18	18.03	18.90	19.78	20.75	21.29	21.78	22.26



APPENDIX 3

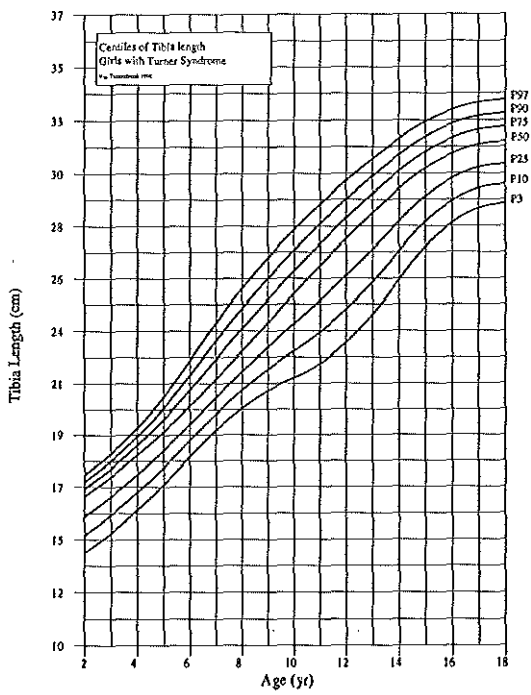
APPENDIX 3G: HAND

AGE	P3	P10	P25	P50	P75	P90	P97
2	8.32	8.58	8.85	9.14	9.51	9.85	10.18
3	9.21	9.48	9.76	10.07	10.44	10.78	11.12
4	9.86	10.17	10.49	10.83	11.23	11.59	11.94
5	10.28	10.65	11.03	11.45	11.87	12.26	12.64
6	10.48	10.93	11.39	11.90	12.37	12.80	13.22
7	10.64	11.17	11.71	12.30	12.82	13.28	13.74
8	10.99	11.55	12.12	12.75	13.29	13.79	14.27
9	11.51	12.06	12.62	13.24	13.80	14.31	14.82
10	12.20	12.71	13.22	13.78	14.35	14.86	15.37
11	12.89	13.34	13.81	14.32	14.90	15.42	15.94
12	13.37	13.84	14.31	14.84	15.43	15.98	16.51
13	13.64	14.18	14.73	15.33	15.96	16.53	17.09
14	13.71	14.37	15.05	15.79	16.46	17.08	17.68
15	13.68	14.48	15.29	16.18	16.90	17.55	18.20
16	13.67	14.56	15.46	16.46	17.21	17.90	18.57
17	13.65	14.60	15.56	16.62	17.40	18.10	18.79
18	13.65	14.62	15.60	16.68	17.46	18.17	18.87



APPENDIX 3H: TIBIA

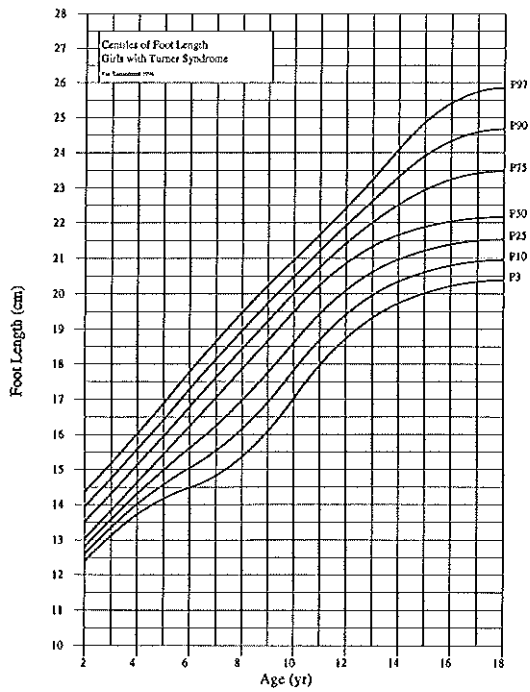
AGE	P3	P10	P25	P50	P75	P90	P97
2	13.93	14.70	15.49	16.35	16.68	16.99	17.28
3	14.79	15.56	16.34	17.19	17.55	17.87	18.19
4	15.76	16.52	17.29	18.13	18.55	18.94	19.32
5	16.85	17.59	18.33	19.15	19.69	20.19	20.67
6	18.05	18.76	19.48	20.27	20.97	21.61	22.24
7	19.21	19.92	20.64	21.44	22.30	23.08	23.85
8	20.17	20.96	21.75	22.63	23.59	24.46	25.32
9	20.93	21.86	22.81	23.85	24.85	25.76	26.65
10	21.49	22.64	23.80	25.08	26.07	26.96	27.85
11	22.09	23.44	24.80	26.30	27.24	28.09	28.93
12	22.99	24.42	25.87	27.46	28.34	29.15	29.94
13	24.19	25.58	27.00	28.55	29.38	30.13	30.87
14	25.68	26.92	28.19	29.59	30.35	31.04	31.72
15	27.11	28.18	29.27	30.46	31.17	31.81	32.43
16	28.13	29.08	30.04	31.09	31.75	32.35	32.94
17	28.74	29.61	30.50	31.47	32.10	32.68	33.25
18	28.95	29.79	30.65	31.59	32.22	32.79	33.35



APPENDIX 3

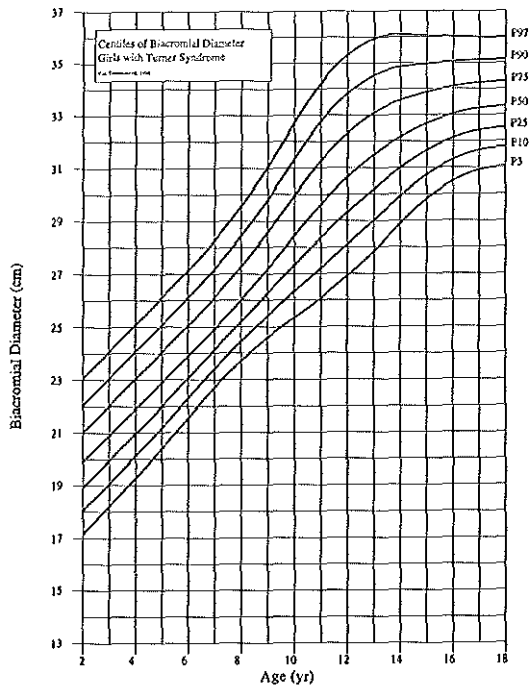
APPENDIX 3I: FOOT

AGE	P3	P10	P25	P50	P75	P90	P97
2	12.40	12.61	12.82	13.04	13.52	13.95	14.38
3	13.13	13.36	13.59	13.84	14.32	14.76	15.19
4	13.72	14.02	14.32	14.64	15.14	15.59	16.03
5	14.18	14.58	14.99	15.44	15.96	16.43	16.89
6	14.49	15.05	15.62	16.25	16.79	17.29	17.78
7	14.83	15.54	16.26	17.05	17.62	18.14	18.66
8	15.37	16.16	16.97	17.86	18.44	18.96	19.48
9	16.10	16.92	17.76	18.68	19.24	19.74	20.25
10	17.03	17.82	18.62	19.50	20.02	20.49	20.96
11	17.97	18.70	19.44	20.26	20.76	21.22	21.67
12	18.74	19.42	20.11	20.87	21.43	21.93	22.43
13	19.32	19.97	20.62	21.34	22.01	22.63	23.24
14	19.74	20.35	20.97	21.66	22.53	23.32	24.10
15	20.02	20.61	21.22	21.88	22.94	23.91	24.86
16	20.22	20.80	21.39	22.04	23.24	24.34	25.41
17	20.34	20.91	21.49	22.13	23.42	24.59	25.74
18	20.38	20.95	21.53	22.16	23.48	24.67	25.85



APPENDIX 3J: BIACROMIAL DIAMETER

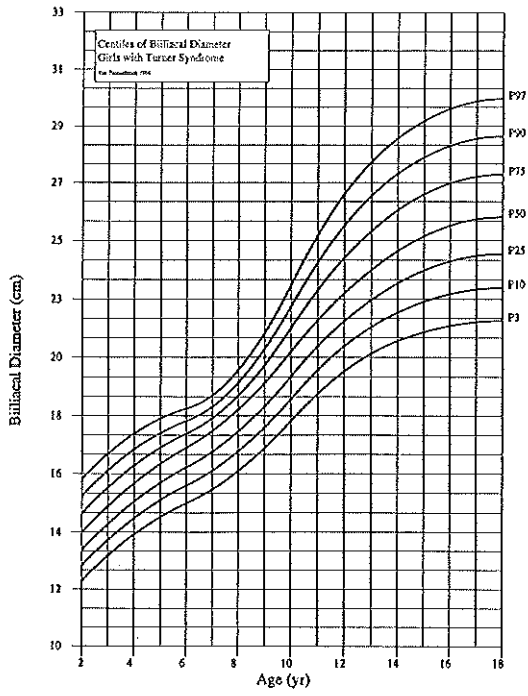
AGE	P3	P10	P25	P50	P75	P90	P97
2	17.18	18.05	18.94	19.91	21.04	22.06	23.07
3	18.21	19.07	19.95	20.91	22.04	23.06	24.07
4	19.28	20.12	20.97	21.91	23.04	24.07	25.08
5	20.40	21.20	22.01	22.90	24.04	25.08	26.11
6	21.57	22.31	23.06	23.89	25.05	26.10	27.14
7	22.70	23.41	24.13	24.92	26.11	27.20	28.27
8	23.71	24.44	25.19	26.02	27.28	28.44	29.57
9	24.59	25.42	26.26	27.19	28.57	29.82	31.06
10	25.34	26.33	27.33	28.44	29.97	31.36	32.73
11	26.08	27.22	28.37	29.64	31.28	32.78	34.26
12	26.92	28.11	29.33	30.66	32.32	33.84	35.33
13	27.85	29.02	30.20	31.50	33.09	34.53	35.95
14	28.89	29.93	31.00	32.17	33.58	34.85	36.11
15	29.83	30.74	31.67	32.68	33.88	34.98	36.06
16	30.51	31.32	32.14	33.05	34.10	35.07	36.02
17	30.91	31.66	32.42	33.26	34.24	35.12	35.99
18	31.05	31.78	32.52	33.34	34.28	35.14	35.98



APPENDIX 3

APPENDIX 3K: BIILIACAL DIAMETER

AGE	P3	P10	P25	P50	P75	P90	P97
2	12.39	12.96	13.54	14.17	14.86	15.48	16.09
3	13.32	13.89	14.47	15.11	15.78	16.40	17.00
4	14.10	14.68	15.28	15.93	16.57	17.15	17.72
5	14.73	15.34	15.96	16.63	17.22	17.75	18.27
6	15.22	15.86	16.50	17.22	17.72	18.18	18.63
7	15.71	16.39	17.07	17.82	18.28	18.70	19.11
8	16.37	17.08	17.80	18.60	19.10	19.55	20.00
9	17.19	17.94	18.70	19.54	20.17	20.75	21.32
10	18.18	18.97	19.77	20.66	21.51	22.28	23.05
11	19.15	19.99	20.84	21.78	22.87	23.86	24.83
12	19.96	20.85	21.76	22.76	24.03	25.18	26.31
13	20.58	21.54	22.52	23.59	24.98	26.25	27.49
14	21.04	22.07	23.12	24.27	25.73	27.06	28.37
15	21.36	22.45	23.57	24.80	26.30	27.66	29.01
16	21.58	22.73	23.90	25.17	26.70	28.09	29.46
17	21.72	22.90	24.09	25.40	26.94	28.35	29.73
18	21.77	22.95	24.16	25.48	27.03	28.44	29.82



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