Treatment of early puberty in adopted and non-adopted children: when, why and how.

Auxological, psychological and ethical aspects of growth promoting treatment around puberty

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Treatment of early puberty in adopted and non-adopted children: when, why and how.

Behandeling van vroege puberteit bij geadopteerde en niet-geadopteerde kinderen: wanneer, waarom en hoe.

Auxological, psychological and ethical aspects of growth promoting treatment around puberty

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Today is a gift,
that's why we call it present

Voor jou, Annemiek

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General introduction

1. NORMAL PUBERTY

1.1 The hypothalamo-pituitary-gonadal (HPG) axis

Normal puberty is the result of the maturation of the hypothalamo-pituitary-gonadal (HPG) axis. Primary and secondary sexual characteristics develop by the increase in the serum levels of gonadal steroids: oestradiol in girls and testosterone in boys.

The main players in the process of normal puberty are the hypothalamus, by producing pulsatile Gonadotrophin Releasing Hormone (GnRH), and the pituitary. GnRH influences the pituitary via the portal vein system and the pituitary secretes the gonadotrophins Luteinizing Hormone and Follicle Stimulating Hormone (LH and FSH). LH and FSH in turn stimulate the gonads to develop the cells essential for reproduction and to secrete the gonadal steroids. (Figure 1)

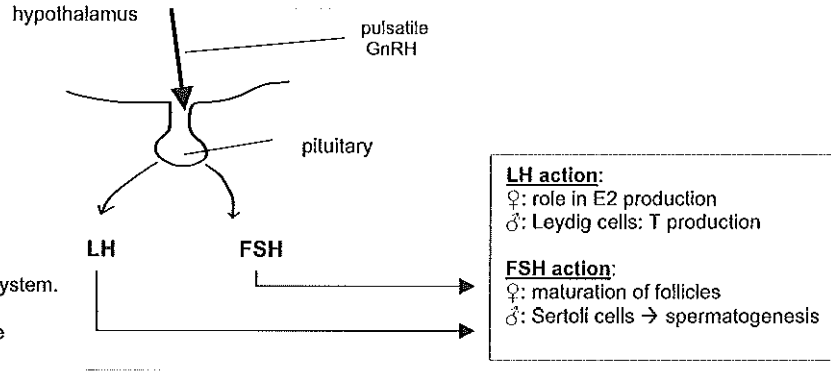

Figure 1:
Reproductive system.
E2= oestradiol;
T= testosterone

In girls this maturational process primarily leads to the development of the breasts and internal genitalia. Menarche follows as a result of ongoing maturation. In boys it results in growth of the testes, pubic and axillary hair, lowering of the voice and spermarche. In both sexes, a pubertal growth spurt occurs as well as further psychosocial and psychosexual development.

1.2 Onset of puberty

The onset of puberty is one of the intriguing yet unresolved issues in paediatric endocrinology. In recent years progress in basic science has led to further insight in the control of the onset of puberty (1). There is a central role for the GnRH cells, unusual cells that originate from outside the brain and migrate in early development from the olfactory placode to the hypothalamic region. Later in fetal development the GnRH cells stimulate fetal gonadotrophic activity. In late fetal life this GnRH activity is suppressed by fetoplacental steroids. From birth up to puberty, except for the early neonatal period, the GnRH activity appears to be suppressed or compromised (2, 3). However, when using ultrasensitive LH assays some activity is measurable in prepubertal children (4-7).

The general view on the onset of puberty has been that increased maturation of GnRH neurons leads to pulsatile GnRH release. This view has been debated in the last few years (8).

The issue of the pubertal 're-awakening ' of the GnRH pulse generator is complex and many mediators have been described to have a particular role:

I. Glutamate

Studies in explanted hypothalamic tissue of rats have shown the involvement of glutamate receptors of the N-methyl-D-aspartate (NMDA) subtype in the increase in GnRH pulse frequency at the onset of puberty (9, 10). NMDA administration was shown to have a stimulatory effect with regard to the onset of puberty (11, 12). However, both an inhibitory and a facilitatory role of NMDA receptors have been described (10). A possible mechanism is that glutamate reduces the inhibitory effect of NMDA receptors on GnRH secretion.

II. Gamma aminobutyric acid (GABA)

Gamma aminobutyric acid (GABA) is an inhibitory neurotransmitter to GnRH release in vivo. It has extensively been studied in primates. A reduction in endogenous GABA release is suggested to lead to the pubertal increase in GnRH release (8, 13). The mechanisms for this reduction may be a decrease in the number of GABA-ergic neurons, a reduced GABA synthesis or enhanced degradation, or a reduction in the neural activity of GABA-ergic neurons during hypothalamic development. Recent research connects the findings on glutamate and GABA by showing that release of glutamate follows the disinhibition of the GnRH neuronal system to further increase GnRH release at the onset of puberty (13). Glutamic-acid-decarboxylase 67 (GAD67) is a catalytic enzyme that synthesises GABA from glutamate, thus probably an important player in the mechanism of the onset of puberty (13).

III. Transforming growth factor–alpha (TGF-α) and other glial-derived substances

The concept that GnRH neuronal function is not only influenced by neurotransmitters, but also by molecules of astrocytic origin has created a lot of research and the support for this hypothesis is emerging.

Clinically, the occurrence of precocious puberty in children with hypothalamic hamartomas (14-16) has led to the hypothesis that hamartomas contain an ectopic GnRH pulse generator. However, histologic examination of tumour tissue showed no GnRH neurons present. Astroglial cells producing TGF-α were demonstrated in the tumour, thus suggesting that lesion-induced precocity is mediated by increased expression of TGF-α in glial cells causing activation of GnRH neuronal network in the hypothalamus (17-19). TGF-α affects the GnRH neuronal network inducing glia to produce bio-active substances, such as prostaglandin-E2, that stimulates the release of GnRH (20, 21). TGF-α production is stimulated by glutamate (22). Oestradiol seems to enhance TGF-α gene expression in hypothalamic astrocytes (22). TGF-α is probably not involved in the inhibitory effect of oestradiol on GnRH neurons (23).

IV. Leptin and neuropeptide Y (NPY)

An overwhelming amount of literature on leptin has been published in the last few years, focussing on its relation to obesity. Indeed, the original *ob/ob* mice in whom the leptin gene

was cloned were obese (24). On the other hand the relationship of leptin with the onset of puberty has extensively been studied since clinical data suggest a link between peripheral energy stores and central regulation of reproductive capacity (25-28). The potential role of leptin is demonstrated by the fact that leptin deficient mice fail to undergo pubertal development, while administration of leptin restores this process (29).

The general view by now about the role of leptin in pubertal development is that leptin acts as a permissive signal for the onset of puberty (25, 30-33).

" Many investigators have now reported that leptin increases gradually in both sexes over the prepubertal years. At each age, girls tend to have higher levels than boys. The leptin peak is reached at Tanner genital stage (G) 2-3 in boys, but in girls leptin continues to rise through puberty with a particular increase after menarche" (25).

Leptin is regulated by several hormones (e.g. sex steroids, growth hormone and melatonin) and proinflammatory cytokines (31). Several hypothalamic neuropeptides are involved in leptin action: orexigenic neuropeptides, including for example neuropeptide Y (NPY) and alpha-melanocyte stimulating hormone (α-MSH) and anorexigenic neuropeptides including corticotropin releasing hormone (CRH) and cocaine- and amphetamine regulated transcript (CART).

Leptin activates pro-opiomelanocortin (POMC) and CART neurons that project to the lateral hypothalamic area (J. Elmquist, Proceedings 5th international conference on the control of the onset of puberty, Luik 1999), thus linking leptin with neurons that regulate feeding behaviour, energy expenditure and body weight homeostasis. CART seems to be involved in the leptin effects on GnRH pulsatility in rat hypothalamic explants (34).

NPY has importance in dictating the ontogeny of the GnRH release. Removal of the inhibitory NPY input to the hypothalamic GnRH neurons may lead to the onset of puberty (35)

V. Insulin-like Growth Factor (IGF) system

In the hypothalamus and pituitary IGF receptors have been localised and in vitro studies showed possible effects on GnRH expression. Probable mechanisms involve interactions with sex steroids and other hormones, extensively reviewed in (36). From a clinical point of view the elevated IGF-I levels during puberty and precocious puberty suggest a role of IGF-I (37-40). Since IGF-I is nutrition-dependent refeeding after malnutrition may cause a rise in IGF-I acting as a stimulating signal for the timing of sexual maturation, which is probably age dependent (41).

VI. Other mediators

Nitric oxide is reported to be a mediator in the induction of GnRH release; norepinephrine, glutamic acid and oxytocin may stimulate the release of GnRH by activation of nitric oxide synthase (42). Recently, local regulatory effects of GnRH on the hypothalamus were described suggesting that local regulatory effects of the neuropeptide could supplement the primary hypothalamic mechanism for the control of episodic gonadotrophin secretion. Changes in melatonin secretion were suggested to have influence on the timing of puberty as well (43, 44).

Based on recent literature reviewed above, the following tentative scheme is suggested (22, 25) (see Fig. 2)

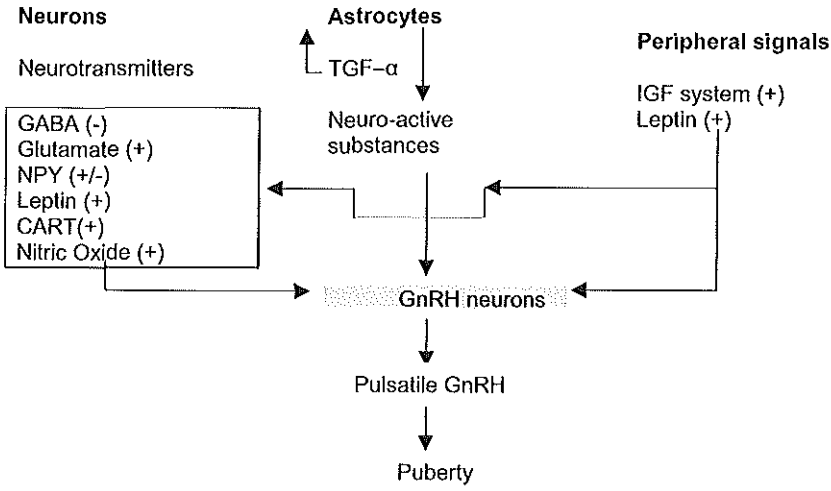

Figure 2: Mediators with a role in the onset of puberty; summary from animal and human data. In brackets: proposed direction of action, see text for details.

1.3 Timing of puberty

In The Netherlands four consecutive growth studies have been performed showing a continuing secular change towards tall stature. Fredriks *et al.* reported another increase of final height in the most recent survey of 1997 (45). From 1955 to 1980 this increase in adult height was accompanied by a decrease in the age of onset of puberty and of menarche (46-48). In 1997 the age of onset of puberty in girls has been reported to be 10.7 years, in boys 11.5 years, suggesting a stabilisation in the age of onset (45). Menarcheal age in this study was 13.14 years, slightly lower than in 1980.

The timing of puberty in South America, Asia or Africa differs from the timing in Western Europe or the United States. In Asian and African countries menarche is often later than in the western world. This is understandable from a biological point of view, since fertility demands a healthy biologic status and acceptable body composition. In table 1 the age of menarche is shown for a variety of countries outside Europe.

In The Netherlands each year many children have been adopted from developing countries. The Dutch government reports that in 1999 most adopted children originate from China (n=271), Colombia (n=196), South Korea (n=77) and Haiti (n=61). Between 1980 and

1995 many children were adopted from India, Sri Lanka and Indonesia. The earlier onset of puberty of children adopted from Asia or Africa to Europe is described more extensively in section 2.2.3.

Table 1: Age of menarche (mean or P50) from different countries reported in the literature

Country	Age menarche (yr)	Reference
India (urban)	13.4	(49)
India (rural)	14.0	
India (highest SES)	13.2	
India (lowest SES)	14.6	
SriLanka (Tamil girls)	13.8	(50)
Thailand (urban)	12.4	(51)
Bangladesh	13.0	(52)
Nepal (high altitude)	16.2	(53)
Senegal	16.1	(54)
China	13.7	(55)
Hong Kong	12.4	(56)
Argentina (urban)	12.5	(57)
Bolivia (all SES)	12.7	(58)
Nigeria (urban)	13.5	(59)
The Netherlands		
1980	13.3	(48)
1997	13.1	(45)

2. CENTRAL PRECOCIOUS PUBERTY (CPP)

2.1 Definition

In central precocious puberty, the age at onset of puberty is below the mean of normal onset minus 2 or 2.5 SD. In practice the cut-off limit of 8 years for girls and 9 years for boys is used. Because of the mathematical relation of the cut-off age with the mean age at onset of puberty of the normal population, one might argue that the secular trend of earlier onset of puberty in many countries should lead to adaptation of the age limits. The Lawson Wilkins Pediatric Endocrine Society advised to take a cut-off value of 7 years for white girls and 6 years for African-American girls to look for a pathologic aetiology of precocious puberty (60). This was based on a cross-sectional population study that showed earlier onset of signs of puberty than in the data used for the common definition of precocious puberty (61). In The Netherlands the 1997 growth study did not show a further significant change towards earlier occurrence of puberty (45) (see chapter 2.2). Therefore, in this thesis the former definition of precocious puberty is used. *Early* puberty is defined as the onset of puberty in girls between 8 and 10 years of age and in boys between 9 and 11 years of age.

2.2 *Aetiology*

This thesis deals with CPP, or gonadotrophin dependent precocious puberty, which means that there is a normal mechanism of activation of the pituitary and subsequent gonadotrophin production. In girls the incidence of CPP is higher than in boys.

2.1.1 *Idiopathic CPP*

In idiopathic CPP no organic or external factor can be demonstrated that causes the GnRH pulse generator to be re-activated. In boys the percentage of idiopathic precocious puberty is lower than in girls (62). Recently it was reported that in 45 boys with CPP the aetiology was idiopathic in 60% and in 40% neurogenic (e.g. hamartoma or neurofibromatosis I) (63). An earlier report from the NIH described idiopathic precocious puberty to be present in 6% of the boys and 63% of girls (62). In girls the overall incidence of neurogenic abnormalities varied from 18 to 37% (62, 64).

2.1.2 Organic CPP

Organic causes of CPP are given in table 2. The mechanisms underlying the onset of puberty in these conditions are not known in detail. It can be assumed that local pressure or increased intracranial pressure stimulates the GnRH neurons in the case of hydrocephalus and meningo-myelocele (MMC), as well as in brain tumours. Another hypothesis is that the compression and stretch may cause disruption of the inhibitory fibers, thus removing their inhibitory actions (65). Decompression of arachnoid cysts was described to reverse the precocious pubertal development (66).

Table 2: Organic causes of CPP

Causes of organic CPP
- Hamartoma
- Neurofibromatosis I
- Braintumors: craniopharyngeoma, astrocytoma
- Hydrocephalus and meningo-myelocele
- Cerebral infections
- Cerebral trauma
- Arachnoid cyst

In hamartomas recent findings suggest local production of TGF-α stimulating the GnRH neuronal network (17). The diagnosis of hamartoma is established neuroradiologically (67). In general a hamartoma needs no neurosurgical intervention.

Exposure to oestrogen containing substances may mimic precocious puberty with accelerated growth and advanced bone age (68, 69).

MRI evaluation of the hypothalamo-pituitary region showed that in cases, clinically regarded as idiopathic, an intracerebral process may be present, in most cases a hamartoma (15, 63, 70). In 304 girls evaluated by CT or MRI a neurogenic abnormality was demonstrated in 18.4% of cases, of which 26/56 were just diagnosed at presentation of CPP (64).

2.2.3 *Early puberty and CPP in adopted children*

In several countries in Europe it was described that adopted children from developing countries are overrepresented among children suffering from CPP or early puberty (71-77). Compared to an incidence of about 1/10.000 for idiopathic CPP, in Belgium 20-25% of children with CPP were adopted from developing countries (41, 78), estimating the incidence of CPP in adopted children 1 per 54 adoptions (J-P Bourguignon, communication Copenhagen Workshop on Endocrine Disrupters 2000).

In auxological studies in adopted children catch-up growth was demonstrated (72, 79-81). Theoretically, this should result in a higher mean final height than in the country of origin. However, due to the early occurrence of menarche mean final height did not substantially exceed the final height in the country of origin (82, 83).

The high prevalence of early onset of puberty in adopted children may serve as an experiment of nature. However, the factors involved remain unclear as long as we do not know the perinatal medical history of the child, nor the health status of the mother.

In comparison to the normal age of menarche in the countries of origin, an early menarche is observed when the girls migrate to the western world. Proos et al. showed that menarcheal age was 11.6 years (median) in a sample of 107 girls from India, whereas the normal age of menarche in India varies from 12.8 years in privileged girls to 14.4 yrs in girls in rural areas (71).

The same pattern of early menarche was observed by Oostdijk in the Netherlands. In 446 girls adopted from four different countries, mean age at menarche was 12.0 (SD 1.5) years, varying from 11.2 years in Indian girls to 12.4 years in girls from South Korea (82). The mean age of menarche was significantly lower than the mean for Dutch girls. In the studies of Oostdijk and Proos a positive correlation was found between menarcheal age and final height. Although in bivariate correlations later arrival was associated with earlier menarche, in multiple regression analysis the menarcheal age was associated with height at arrival, but not with age at arrival (83, 84). Height at arrival, the velocity of height catch-up growth and menarcheal age appeared to be significantly correlated with FH (83).

Proos studied the natural growth of 66 adopted children in Sweden. Their height at arrival was –2.8 SDS, increasing to –0.8 SDS 2 years before the peak height velocity. At FH, however, H-SDS was –1.8 SD. These data suggest that despite the promising magnitude of catch-up growth FH-SDS is substantially lower. Pubertal height gain was reported to be equivalent to that of Swedish girls (85), suggesting a negative effect of shortening of the prepubertal growth phase. The study of Oostdijk showed that in 34 adopted children H-SDS at arrival was –1.8 and increased to a mean H-SDS of –0.5 between 6 and 8 years of age, where FH-SDS was around –1 SD (76).

Some authors speculate on genetic reasons for the fall in H-SDS, for example that a certain height potential is already fulfilled at an earlier age than in the country of origin, as a result of *genomic imprinting* of a growth pattern appropriate for that country (81).

The differences in expression of genes derived from the father and the mother are already marked in early development (86). The mechanism of this **imprinting** might be differential methylation of specific gene sites. Imprinted genes are involved in many aspects of development including fetal and placental growth (87). Genomic imprinting of regions of chromosome 14, especially of a gene involved in the onset of puberty, was related to uniparental disomy in a boy with precocious puberty, low birth weight and growth abnormalities and some dysmorphic features and developmental delay (88). In a later report intrauterine growth retardation was linked to a comparable uniparental disomy of chromosome 14 (89). The combination of phenotypes with intrauterine growth retardation and early onset of puberty in chromosome 14 abnormalities may stimulate further research on the genetic basis of early puberty.

However, several studies show that environmental factors can increase final height in a population, as was shown in the consecutive Dutch growth studies.

One might speculate early puberty to occur as a result of transition from a situation with low socio-economic conditions to the highly affluent western world during a specific, maybe even critical period after birth. Specifically, Virdis suggests a role of the dietary change in adopted children from low protein and low energy vegetarian diet to a balanced enriched diet after adoption (73). The presence of oestrogen- like substances in food (phytoestrogens) (90) and other endocrine disrupters in their new environment influencing the pubertal development of adopted children remains to be studied (91). The influence of environmental factors on the timing of puberty in healthy Dutch children seems minimal, as no further decrease in the age at onset of puberty was shown (chapter 2.1).

The occurence of early puberty in adopted children might be related to the improvement of nutritional status in early life, possibly facilitated by an increase in leptin levels acting as permissive factor for the onset of puberty by interacting with several neuropeptides (see section **1** of this introduction). In male rats it was demonstrated that unrestricted feeding after nutritional deprivation in a specific period after birth resulted in accelerated hypothalamic and testicular maturation (41). In that study the role of increased IGF-I is suggested as an important mediator.

On the other hand, Engelbregt *et al.* studied the role of early undernutrition pre- or postnatally on pubertal development in rats (92). Food restriction in each of these periods resulted in a change of programming of pubertal development: early malnutrition resulted in *delayed* onset of puberty in intra-uterine growth retarded (IUGR) male and female rats, as well as in male food-restricted rats. In female food restricted rats the onset of puberty was normal. This study and other data suggest that there may be environmental factors in the pre- or postnatal period having long term effects on the hypothalamo-pituitary action (93).

As it can be assumed that many adopted children are born from mothers from the lower social classes and thus at risk for unfavourable circumstances in utero. The comparison of these adopted children with children born small for gestational age (SGA) is challenging. The long-term influence of intra-uterine growth retardation on several endocrine axes has been described, especially by Barker and co-workers (94-99). With regard to the HPG axis an earlier menopause and altered hypothalamic control of LH release were described to have an association with IUGR (94, 95). It can therefore be assumed that programming of the setting of the GnRH pulse generator is influenced by prenatal factors.

2.2 *Effects of CPP*

2.2.1 *Height*

The oldest reports of the deteriorating effects of CPP on final height (FH) date back from the nineteen sixties, when Thamdrup reported a series of 56 untreated patients with a mean final height of 151.3 (SD: 8.8) cm in 26 girls and of 155.4 (8.3) cm in 8 boys. Mean parental height was 166.6 (4.6) cm and 166.8 (5.1) cm respectively (100). Summarising the available data in girls, FH in untreated CPP without slow progressive forms, range from 149.6 to 161.7 cm (101). The decrease in final height is due to acceleration of the fusion of the epiphyseal plates as a result of the sex steroid exposure, combined with the early interruption of the prepubertal growth period.

Factors negatively influencing FH in girls were reported to be an early age at start of puberty and menarche, short parental height and a large difference between bone age and height age (100).

2.2.2 *Reproduction*

The reports of the effects of precocious sexual maturation on reproductive capacity are scarce. A relation with early polycystic-ovary-like symptoms was suggested since an abnormal adrenal response after adrenocorticotropic hormone (ACTH) stimulation was observed in 55.4% of girls in early stages of CPP. However, it did not result in clinical signs of a hyperandrogenic state, nor in abnormal elevation of baseline androgen levels (102, 103). Thamdrup described the occurrence of pregnancies in girls with CPP from literature dating back from the 18th to 20th century (100).

Ovarian ultrasound in children with CPP revealed an increased prevalence of polycystic ovarian appearance, related to either precocious puberty itself or its treatment (104). However, other reports did not confirm the presence of PCO (105, 106) after GnRHa treatment.

2.2.3 *Body proportions*

The studies of Thamdrup also address body proportions in untreated precocious puberty. In most cases the ratio of upper to lower segment is above normal for age during the course of puberty and also after completion of growth. It is concluded that in most cases the patients preserve the childlike body proportions with relatively short limbs and a large head in proportion to the trunk; the later onset of puberty, the more chance of attaining normal proportions (100). The observation of the relative large trunk compared to the limbs was confirmed in a later study, although in that study several children received treatment (107).

2.2.4 *Malignancy*

The precocious exposition to natural oestrogens in girls with CPP might theoretically lead to oestrogen induced tumors in later life. However, no prospective study was performed to evaluate this relationship. There are epidemiologic studies that suggest a relationship of breast cancer with an earlier menarche (108). The mutagenic or carcinogenic properties of oestradiol are weak, but induction of genetic lesions might occur with low frequency (109).

3. DIAGNOSIS OF CPP

3.1 Physical examination

Physical examination is the basis for the clinical diagnosis of CPP. The child is carefully examined for the presence of secondary sexual characteristics and these are scored according to the Tanner criteria (110). In boys testicular volume is assessed using an orchidometer. In girls palpation and measurement of the diameter of the mammary gland is a valuable option. To define the onset of puberty B2 stage in girls should be present, and in boys the testicular volume should be 4 ml or more. A 3 ml cut-off is suggested by some authors (111, 112).

The general physical examination further includes careful inspection of the skin for signs of any dysplasia, café au lait spots or signs of neurofibromatosis. Neurological examination should be performed as well. The visual fields need attention in case of possible brain tumours. Other signs include the presence of acne, oily hair and transpiration odour. Furthermore, height, weight and sitting height should be recorded in order to evaluate the effect of possible interventions and the progression of pubertal development. Increase in height velocity is an important means to demonstrate that there is progression of pubertal development over time.

3.2 Radiological evaluation

3.2.1 Bone age (BA)

Bone maturation is used as a marker for the biological maturation of a child. An X-ray of the left hand is made for BA assessment. In CPP bone age assessment is usually performed by the Greulich and Pyle method (113, 114) and the difference between chronological age (CA) and BA is assessed. The more BA is advanced over CA, the more height will be lost in case of no treatment (100).

To predict adult height the tables of Bayley and Pinneau (BP) are used for height prediction (115). Most children with CPP have a BA advance of 1 year or more. In this condition, the traditional method of height prediction used the accelerated BP tables. For a long time, it was questioned to what extent the abnormal growth process in CPP was to be analysed by tables for the normal population. Kauli showed that using the accelerated tables the predicted adult height (PAH) was overestimated. She suggested that it would be better to use the average tables in children with CPP (116).

---------- Intermezzo: Bone age and final height predictions ----------

The assessment of bone age (BA) is used as a marker of biological maturation of a child (113, 117). In all techniques an X-ray of the left hand is used and maturity indicators are scored (118).

- The Greulich and Pyle (GP) method assumes that all bones have identical skeletal age so that a skeletal age can be assigned to an X-ray as a whole.
- The Tanner & Whitehouse 2 (TW2) method uses a bone-specific approach, assessing the maturity of 20 bones that are matched to a series of written criteria. The RUS (radius, ulna, short bones) score is a shortened form of TW2 without scoring of the carpal bones.
- The FELS method introduced by Roche, is based on 98 maturity indicators. In only few studies this method was used.

In the evaluation of children with precocious puberty the GP method is most common and it is linked with the Bayley and Pinneau tables for prediction of adult height (115).

For several reasons there is a need for new standards: the standards of GP and TW2 BA assessment date back from nineteen thirties and fifties, and the samples consisted of relatively lower class children in the TW2 sample and upper-middle class children in the GP sample. Now, after several decades with a secular trend towards earlier maturation and taller stature the reliability of the old standards might be waning. Furthermore, BA assessments are largely dependent on the observator's experience and inter-individual differences in BA assessment are inevitable.

For research purposes all BA assessments over a long period of time should be performed by the same observer. An improvement of the generalising GP method would be a bone age based upon assessing the maturity of each of the 20 bones. The average of these ages then should be calculated to establish bone age (S.I. Pyle referenced in (118)). This time-consuming approach has been reported to have an inter-observer-difference between 0.1 and 0.34 'years', and mean intra-observer difference ranging from 0.15 to 0.63 years. King reported a mean difference between 3 observers of 0.74 years for the TW2 method and of 0.96 years for the GP method, whereas the intra-observer variation in TW2 method was 0.33 years (95% CI: -0.87 – 1.53) (119). Comparing TW2 and GP the 95% confidence interval of intra-observer variation amounts to 2.28 to –1.52 years (120).

New methods in BA determination using computer imaging have been developed, but still positioning and exposure of the image cannot fully be automated (121-127).

Several studies showed that GP method was preferable in clinical conditions, such as precocious puberty (118, 128). Height predictions in untreated CPP patients were performed with BP predictions, showing a correlation of 0.85 with the attained final height (114). In precocious puberty in girls it was shown by Kauli that the use of the accelerated tables of Bayley and Pinneau resulted in *over*estimation of final height. Therefore, it would be more appropriate to use the average tables (116).

The problem in children with early or precocious puberty is that BA maturation is beyond the physiologic range, thus reducing the expected final height. In contrast, early maturing girls –still in the normal range – show a more intensive growth reaching their FH earlier, but also with a greater gain in height per bone age advance (116).

In general, predicting final height suffers from a limited accuracy, with a mean prediction error of 7 – 9 cm depending on the bone age (129). An additional error could be introduced by using western world standards to children from Africa, Asia or South-America, as in adopted children, refugees or immigrants. No data is available on the bone age progression during catch-up growth in adopted children or during recovery of undernutrition.

In our clinical practice in adopted children we frequently observed dissociated maturation between carpal and phalangeal bones, as was also observed in children with precocious puberty and congenital adrenal hyperplasia (130). Possibly, the early exposure to androgens and/ or estrogens has different effects on the ossification of carpal bones than on phalangeal bones. Thus, in children from other ethnicity who have increased risk of the development of early puberty, one should be aware that height prediction is very complicated and should be performed with caution. In the group of adopted children studied in chapter 6 we observed a mean difference between carpal and phalangeal bones of 0.5 'years' (range 0.1- 1.1).

The literature on the relationship between skeletal maturation in non-European countries and western world standards for bone age assessment shows variable results (131-133), suggesting that children of the higher socio-economic classes can better be compared with the European standards than can children in situations of lower socio-economic circumstances, malnutrition and poor health.

To assess the chronological age in cases where the birth date is uncertain one should always be careful to focus on bone age only. Other methods include for example determination of dental age, which was described to be reliable regardless of sex and race but still difficult in cases with uncertain birth date (134). Another marker for biological maturation is the closure of the epiphyseal plates of the clavicle, which is highly independent of external factors (135). Its use however is limited to forensic age determination, for example in young adolescent refugees.

3.2.2 Magnetic Resonance Imaging (MRI)

The use of MRI in the diagnosis of CPP is mainly of interest in those cases in which no known underlying disorder is present. In those patients labelled as having idiopathic CPP intracranial pathology can be excluded by MRI (15, 64, 70).The relevance of these findings for follow-up of these patients can be disputed, as most of the hamartomas do not increase in size.

The indication for MRI is especially important in young girls and in boys with precocious puberty. However, Cisternino reported that neurogenic abnormalities not previously known were also detected by MRI in 7.4 % of girls between 7 and 7.9 years of age (64).

3.2.3 Ultrasound

Ultrasound is used in the evaluation of CPP for assessing the size and aspects of uterus and ovaries. Several studies in normal children provided reference values for the ovarian volumes or uterine size (136-139). In CPP the sizes of ovaries and uterus increase in a comparable sequence as in normal puberty, follicles become visible in the ovaries and the delineation between endometrial and myometrial tissue will be present in later stages.

A considerable overlap between ovarian size of normal girls and girls with precocious puberty has been observed (106, 140)

Another application of ultrasound is the assessment of glandular tissue in the breast in case of doubt whether the breast volume is increased by adipose tissue or by the mammary gland (141, 142).

3.3 Biochemical evaluation

3.3.1 Basal values of gonadotrophins

The assessment of basal values of gonadotrophins with the conventional assays does not contribute to the diagnosis of CPP. More sensitive assays could be used to demonstrate early activation of the hypothalamo-pituitary axis (143-145).

3.3.2 Stimulated values of gonadotrophins in the GnRH stimulation test

Classically, the GnRH stimulation test has been used to demonstrate the central origin of precocious puberty by stimulating the pituitary to produce LH and FSH. LH serum levels above a certain cut off (dependent on the assay) or a ratio of LH/FSH > 1 have been considered criteria for central activation (146). However, the use of this test especially in children with recent onset of puberty has been challenged (see chapter 3).

3.3.3 Stimulated values of gonadotrophins in the GnRH agonist stimulation test

To improve the diagnostic process in early pubertal children agonists of GnRH with higher potency were used. As in clinical practice a considerable number of children with Tanner stage 2 showed no pubertal response in the GnRH stimulation test, the more potent GnRH agonist was used for diagnostic use. Ibanez *et al.* demonstrated that a pubertal response of > 8 IU/L for LH had a good correlation with clinical progression of puberty (147).

3.3.4 Basal and stimulated sex steroid levels in serum

Thusfar, the levels of oestradiol and testosterone in the diagnosis of precocious puberty can not be regarded as essential, although they can be indicative in the diagnosis of pseudo-precocious puberty. The levels can be used for the follow-up of treatment of precocious puberty. In stimulation tests no reference values for the sex steroids are available. In a GnRH agonist stimulation test clinically relevant oestradiol levels were found in absence of 'pubertal' LH responses (148). The time interval between the stimulus and production of ovarian oestradiol is probably between 6 and 9 hours, due to the time required for aromatization (149).

4. TREATMENT

4.1 Treatment options

4.1.1 *Agonists of GnRH*

Currently, potent agonists of GnRH are the treatment of choice in central precocious puberty. The publication of Belchetz *et al.* in 1978 showed that intermittent administration of potent GnRH agonists initially stimulated but subsequently inhibited LH and FSH release (150). This led to the development of several GnRH agonists with addition of an aminoacid at position 6 or replacement of the Gly-NH_2 terminal group by N-EtNH_2 (151). GnRH agonists are widely used in reproductive medicine to achieve ovarian suppression before stimulation with exogenous gonadotrophins (152, 153). In 1981 the use of a daily administered GnRH analogue was reported in children with precocious puberty (154).

To improve the suppressing capacity of GnRH agonists slow-release depot preparations were developed (155-159). These preparations were shown to suppress pubertal activity better then short-acting preparations (160). An extensive review on GnRH agonists in paediatrics is given by Lahlou *et al.* (161).

In the Netherlands triptorelin and leuprolide-acetate depot preparations are available as monthly injections with 3.75 mg to be given each 28 days. A depot with leuprolide-acetate 11.25 mg for injections once in 3 months has been introduced in patients with prostate carcinoma (162, 163). The use of this preparation in children with CPP is still under investigation. In the United States a 7.5 mg preparation is commonly used as IM injection every 28 days.

Recently, the use of GnRHa for delay of pubertal development in a transsexual adolescent has been reported (164).

4.1.2 Acute and long-term side effects of GnRHa

During treatment with depot GnRHa several minor side effects were reported. Most common is the occurence of local reactions during leuprolide acetate treatment (165-167). Other reports with only very small numbers of patients included hair loss (168), prolonged vaginal bleeding (169) and hyperprolactinaemia (170).

After long-term use of GnRH agonist resumption of the activity of the reproductive axis was reported (171-173) with normal pregnancies (171, 174). The mean period beween discontinuation of treatment and menarche is about 12 months (174-176). No changes in pituitary size or shape during GnRHa treatment in girls with precocious puberty were observed (177).

4.1.3 Antagonist of GnRH

Antagonists of GnRH competitively bind to the pituitary GnRH receptors and block the receptors immediately. They do not induce gonadotropin release (178). These antagonists are currently used in reproductive medicine, as they prevent the occurence of an premature LH surge (179). Theoretically, they could be used for pubertal suppression in precocious puberty, but thusfar no publications have been describing this application. Further they can be used for androgen blockade in prostate cancer (180).

4.1.4 Aromatase inhibitors

Recently, aromatase inhibitors were introduced in the hormonal therapy of breast cancer as treatment of advanced breast cancer after tamoxifen treatment or after failure of other hormonal therapies in postmenopausal women (181, 182). These drugs inhibit the conversion of androstenedione into oestrone and of testosterone into oestradiol. So, theoretically, they could be used in precocious puberty in order to inhibit the oestrogen driven effects of early puberty on skeletal maturation. The manufacturer reports that exemestane did not affect cortisol or aldosterone secretion at baseline or after ACTH stimulation (183). An advantage of this method of hormonal therapy is the oral route of administration. No data on the use in paediatric patients is available.

4.2 ***Evaluation of the effect of treatment***

4.2.1 *Physical examination and BA assessment*

During treatment with GnRHa the physical signs of puberty will be arrested or even regress. No increase in Tanner breast stage stage should be observed, and testicular volume should halt or regress during treatment. During treatment height velocity (HV) should not exceed prepubertal limits.

In precocious puberty the ratio ΔBA/ΔCA is > 1. During treatment a decrease to values < 1 should be obtained. In practice, the ratio ΔBA/ΔCA in the first 6 months of treatment is often > 1 even in case of effective suppression (see chapter 4). Probably, the actual BA is the calcified representation of growth plate physiology of approximately 6 months before.

4.2.2 Biochemical methods

During treatment, the levels of gonadotrophins and sex steroids are regularly measured at the end of the injection interval. Most often a GnRH stimulation test is used in which a prepubertal response is expected after the stimulus. Values of sex steroids have to be at castration levels. The use of GnRHa testing during treatment might be useful (184).

4.2.3 Adaptation of treatment

In case of evidence of ineffective suppression the injection method and frequency should be checked. When these show no problems, the interval between 2 injections could be shortened to 3 weeks or the dose might be doubled to 7.5 mg each month. This is the commonly applied dose in the United States.

4.2.4 *Rationale for addition of GH during GnRHa treatment.*

During treatment with GnRH agonists it is frequently observed that height velocity decreases, even below normal prepubertal levels (185, 186). The effects of GnRHa treatment on the GH axis, both on GH secretion and IGF-I levels remain controversial as reviewed by Walvoord (187). However, the reports on alterations in the GH axis in children with low growth velocities during GnRHa treatment have led to the hypothesis of a functional GH deficiency.

4.3 ***Results of treatment***

4.3.1 *Parameters of effect*

In the evaluation of the effect of treatment for precocious puberty several methodological issues arise. First, the issue of bone age assessment and the method of prediction; can they be applied as in healthy children in which the standards were developed? Second, what is the best marker for effect: is it the difference between initial height prediction and attained final height, the difference between FH and TH, or FH itself? Third, how can predictive factors for effect of treatment be assessed in view of the obvious interdependence between BA and height prediction?

As a rule of thumb: in randomised studies with FH data both the difference between FH and initial prediction and the difference between FH and TH should be presented. When FH is not yet reached, the best option is to compare initial prediction with height prediction after discontinuation of treatment. In the latter situation one should take into account that FH will be lower than PAH at discontinuation of treatment ((105, 188). Table 3 summarises possible effect parameters in the evaluation of GnRHa treatment.

Table 3: Parameters of effect in the evaluation of GnRHa treatment

Parameter of effect	**Comment**
FH	To be used in comparative studies with untreated controls or differently treated patients; provided comparable baseline parameters
FH-TH	For comparison with parental growth potential; provided healthy parents without growth disorder
FH-PAH start	For comparison within the patients; provided the best prediction method is used and BA assessments performed by 1 observer
PAHstop – PAH start	For estimation of effect in case no FH is attained and no untreated control group is available

4.3.2 Results of GnRHa treatment

In the last few years several reports on the effects of GnRHa treatment have been published. Only the reports on FH in girls with CPP will be summarised in table 4.

Table 4: Results of GnRHa treatment in girls with CPP at final height

1st author	Number of girls	Treatment duration (yr)	FH – PAHstart (cm)	Ref
Arrigo	71	4.0	2.9	(189)
Carel	58	3.7	4.8	(176)
Heger	50	4.4	5.9	(174)
Oostdijk	31	3.4	3.4	(105)
Galluzzi	22	4.0	3.2	(190)
Bertelloni	14	3.9	4.6	(191)
Kauli	8	2.4	5.8	(116)

4.3.3 Results of GnRHa treatment in combination with growth hormone (GH)

The combination of GnRHa and GH was studied in several patient groups. Thus far, only a few studies have reported FH results (219).

In adopted children, Tuvemo *et al.* showed that the combination of GnRHa and GH results in an increase in height velocity. After 2 years of treatment the increase in prediction of adult height is + 1.8 cm in the combination group and – 0.9 cm in the group with GnRHa alone (192). We report the results of a study in adopted children in chapter 6.

In children with *precocious puberty* and low HV short term results show that HV increases and that PAH increases (185, 193).

The application of GnRHa and GH in *short girls* or girls with idiopathic short stature with normally timed or early puberty was reported to result in a final height not different from controls (194) or from initial prediction (195). In contrast, Pasquino *et al.* reported a mean height gain at FH of 10 cm (SD: 2.9), compared to 6.1 (4.4) cm in a control group with GH alone (196). Short-term studies (treatment period 1 to 2 years) report that the PAH increases during combined treatment (197, 198). In chapter 7 a Dutch study with untreated controls is described.

4.3.4 Effects on body composition, body proportions and reproductive function

Many clinicians report their clinical impression that children become fat during GnRHa treatment. Measured by Dual Energy X-ray Absorptiometry (DEXA) fat mass-SDS and percentage fat SDS increased during GnRHa treatment (199). Heger *et al.* found that obesity is a common problem in children with CPP already at start of treatment, but that GnRHa treatment does not aggravate this (174). The assessment of a pre-treatment Body Mass Index (BMI) can predict the development of obesity during GnRHa treatment, but the development of obesity appears to be unrelated to GnRHa treatment (200).

The treatment with GnRHa theoretically results in a decrease in bone mineral density (BMD) as a result of sex steroid depletion. The studies addressing this issue report either a decrease in BMD (201, 202) or, when expressed in comparison to healthy controls, normal BMD for chronological age, but low BMD for bone age after 2 years of treatment (199). At final height Heger *et al.* found a normal BMD for age in women after GnRHa treatment (174). In male patients at FH bone mineral density was normal too (203).

CPP may lead to relatively short limbs compared to trunk length. After GnRHa treatment an improvement of body proportions was described at FH (174).

Only few data is available on reproductive function or fecundity in women who had received GnRHa treatment for precocious puberty. After treatment the reversibility of gonadal suppression is demonstrated by spontaneous menses after about one year after discontinuation of treatment (105, 171, 173, 176, 204). Normal pregnancies have been described (171, 174).

The PCO-syndrome is one of the forms of ovarian hyperandrogenism. In premature adrenarche the development of functional ovarian hyperandrogenism (clinical signs of androgen excess, and elevated ovarian 17 – hydroxy-progesterone (17-OHP) response after GnRHa stimulation) is frequently seen (205). The combination of hyperandrogenism and insulin resistance in this condition might be caused by a gain-of-function mutation of a single kinase causing hyperphosphorylation of the insulin receptor and of P450c17 causing hyperinsulinism and hyperandrogenism, respectively (206). In precocious puberty with GnRHa treatment ovarian ultrasound data demonstrated polycystic ovaries (PCO) in varying percentages (summarised in reference (174)). In summary, PCO may occur in increased percentage after GnRHa treatment, but a clinical development of PCO-syndrome has not been demonstrated thus far. In this context, it has to be considered whether the right diagnosis of CPP was made at start of GnRHa treatment or that a premature adrenarche should have been diagnosed. Premature adrenarche is associated with PCO (207).

5. PSYCHOLOGICAL ASPECTS OF CPP AND ITS TREATMENT

It is known from an earlier report that, using the Child Behaviour Checklist (CBCL), early or precocious puberty leads to elevated scores on the internalising syndrome (withdrawal and anxious /depressed) up to 2 years after start of treatment (208). Furthermore, it was reported that precocious puberty in girls is associated with a long-term risk of minor psychopathology (209-211). With respect to psychosexual development, early pubertal development was associated with earlier, but not extremely advanced psychosexual development (209, 210) During treatment with GnRH agonist problematic behaviour and functioning decreased slightly, particularly in the girls showing regression of breast development. In another study, perceived self-esteem was within the normal limits, but anxiety levels were increased (212).

The delay between start of pubertal development and start of treatment may be lengthened as a result of a cultural taboo on sexuality and pubertal development (208). The initial total IQ score in CPP patients is not different from normal (208), although an effect of sex steroids on brain development, especially the left hemisphere is suggested, resulting in a higher verbal IQ score (213).

In a recent publication a relation between earlier menarche and later onset of schizofrenia – only in girls- was described. The authors speculate on oestrogen's protection against nerve cell loss and the preservation of neuronal connectivity (214). A glutamate dysfunction in schizophrenia is suggested with a role of the NMDA glutamate receptor (215, 216). Whether this implies a dual involvement of NMDA receptor or an association of the onset of puberty with schizophrenia remains to be elucidated.

6. Ethical aspects

The treatment of short children with growth hormone has long been restricted to clearly defined groups, for example those with GH deficiency. However, the wide availability of recombinant GH has widened the administration to many groups of children with any growth disorder without GH deficiency. As long-term results of GH treatment become available, questions arise whether we should treat "all" short children (217). There is no clear picture on the psycho-social effects of GH treatment in short children, and the lack of validated instruments to estimate the burden of short stature or the improvement of a variety of psycho-social indicators inhibits proper research in this field.

In adopted children growth promoting treatment either by GH or GnRHa was disputed specifically, as it was felt that early puberty was 'normal' for adopted children and that it was unethical to try to make adopted children as tall as their non-adopted peers (218).

7. Structure and scope of the thesis

The thesis is divided into 5 parts, addressing a wide spectrum of questions with regard to the treatment of adopted and non-adopted children with early or precocious puberty.

The *auxology* is presented in chapters 1 to 8 and contains effects of treatments with GnRHa with or without GH, short-term results and evaluation of GnRHa treatment, diagnostic aspects and final height data after GnRHa treatment.

Psychological aspects and motivation for treatment were studied in adopted chidren treated with GnRHa alone or in combination with GH. The results of these studies are reported in chapters 6.2. and 6.3.

Ethical aspects of growth promoting and puberty delaying treatment come up for disussion in chapter 9.

In the final part of the thesis (chapter 10) the results will be summarised and discussed, and recommendations for future research and clinical work will be provided.

REFERENCES

1. Plant TM. Concluding remarks: fourth international conference on the control of the onset of puberty. In: Plant TM, Lee PA, editors. The neurobiology of puberty. Bristol UK: Society for Endocrinology; 1995. p. 337-342.
2. Delemarre-Van De Waal HA, Wennink JM, Odink RJ. Gonadotrophin and growth hormone secretion throughout puberty. Acta Paediatr Scand Suppl 1991;372:26-31; discussion 32.
3. Schmidt H, Schwarz HP. Serum concentrations of LH and FSH in the healthy newborn. Eur J Endocrinol 2000;143(2):213-215.
4. Wennink JM, Delemarre-van de Waal HA, Schoemaker R, Schoemaker H, Schoemaker J. Luteinizing hormone and follicle stimulating hormone secretion patterns in boys throughout puberty measured using highly sensitive immunoradiometric assays. Clin Endocrinol (Oxf) 1989;31(5):551-64.
5. Wennink JM, Delemarre-van de Waal HA, Schoemaker R, Schoemaker H, Schoemaker J. Luteinizing hormone and follicle stimulating hormone secretion patterns in girls throughout puberty measured using highly sensitive immunoradiometric assays. Clin Endocrinol (Oxf) 1990;33(3):333-44.
6. Wennink JMB, Delemarre-Van de Waal HA, Kessel H van, Mulder GH, Foster JP, Schoemaker J. Luteinizing Hormone secretion patterns in boys at the onset of puberty measured using a highly sensitive immunoradiometric assay. J Clin Endocrinol Metab 1988;67:924-928.
7. Wu FCW. GnRH pulse generator activity during human puberty. In: Plant TM, LeePA, editors. The neurobiology of puberty. Bristol UK: Society for Endocrinology Ltd; 1995. p. 185-197.
8. Mitsushima D, Hei DL, Terasawa E. gamma-Aminobutyric acid is an inhibitory neurotransmitter restricting the release of luteinizing hormone-releasing hormone before the onset of puberty. Proc Natl Acad Sci USA 1994;91(1):395-9.
9. Bourguignon JP, Gerard A, Alvarez Gonzalez ML, Purnelle G, Franchimont P. Endogenous glutamate involvement in pulsatile secretion of gonadotropin- releasing hormone: evidence from effect of glutamine and developmental changes. Endocrinology 1995;136(3):911-6.
10. Bourguignon JP, Gerard A, Alvarez Gonzalez ML, Purnelle G, Fanchimont P. The role of excitatory amino acids in triggering the onset of puberty. In: Plant TM, Lee PA, editors. The neurobiology of puberty. Bristol: Society of Endocrinology Ltd; 1995. p. 129-138.
11. Plant TM, Gay VL, Marshall GR, Arslan M. Puberty in monkeys is triggered by chemical stimulation of the hypothalamus. Proc Natl Acad Sci USA 1989;86(7):2506-10.
12. Urbanski HF, Ojeda SR. Activation of luteinizing hormone-releasing hormone release advances the onset of female puberty. Neuroendocrinology 1987;46(3):273-6.
13. Terasawa E. Mechanisms controlling the onset of puberty in primates: the role of GABAergic neurons. In: Plant TM, Lee PA, editors. The neurobiology of puberty. Bristol: Society for Endocrinology; 1995. p. 139-151.
14. Brito VN de, Latronico AC, Arnhold IJP, et al. Treatment of gonadotropin dependent precocious puberty due to hypothalamic hamartoma with gonadotropin releasing hormone agonist depot. Arch Dis Childh 1999;80:231-234.
15. Cacciari E, Frejaville E, Cicognani A, et al. How many cases of true precocious puberty in girls are idiopathic? J Pediatr 1983;102(3):357-60.
16. Sharafuddin MJ, Luisiri A, Garibaldi LR, et al. MR imaging diagnosis of central precocious puberty: importance of changes in the shape and size of the pituitary gland. AJR Am J Roentgenol 1994;162(5):1167-73.
17. Jung H, Carmel P, Schwartz MS, et al. Some hypothalamic hamartomas contain transforming growth factor alpha, a puberty-inducing growth factor, but not luteinizing hormone-releasing hormone neurons. J Clin Endocrinol Metab 1999;84(12):4695-701.
18. Rage F, Hill DF, Sena-Esteves M, et al. Targeting transforming growth factor alpha expression to discrete loci of the neuroendocrine brain induces female sexual precocity. Proc Natl Acad Sci USA 1997;94(6):2735-40.
19. Junier MP, Wolff A, Hoffman GE, Yun Ma Y, Ojeda SR. Effect of hypothalamic lesions that induce precocious puberty on the morphological and functional maturation of the Luteinizing Hormone-Releasing Hormone neuronal system. Endocrinol. 1992;131:787-798.
20. Ma YJ, Berg-von der Emde K, Rage F, Wetsel WC, Ojeda SR. Hypothalamic astrocytes respond to transforming growth factor-alpha with the secretion of neuroactive substances that stimulate the release of luteinizing hormone-releasing hormone. Endocrinology 1997;138(1):19-25.
21. Ma YJ, Hill DF, Creswick KE, et al. Neuregulins signaling via a glial erbB-2-erbB-4 receptor complex contribute to the neuroendocrine control of mammalian sexual development. J Neurosci 1999;19(22):9913-27.
22. Ma YJ, Ojeda SR. Neuroendocrine control of female puberty: glial and neuronal interactions. J Investig Dermatol Symp Proc 1997;2(1):19-22.

23. El Majdoubi M, Sahu A, Plant TM. Effect of estrogen on hypothalamic transforming growth factor alpha and gonadotropin-releasing hormone gene expression in the female rhesus monkey. Neuroendocrinology 1998;67(4):228-35.
24. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372(6505):425-32.
25. Clayton PE, Trueman JA. Leptin and puberty. Arch Dis Child 2000;83(1):1-4.
26. Georgopoulos N, Markou K, Theodoropoulou A, et al. Growth and pubertal development in elite female rhythmic gymnasts. J Clin Endocrinol Metab 1999;84(12):4525-30.
27. Warren MP, Voussoughian F, Geer EB, Hyle EP, Adberg CL, Ramos RH. Functional hypothalamic amenorrhea: hypoleptinemia and disordered eating. J Clin Endocrinol Metab 1999;84(3):873-7.
28. Frisch RE. Body fat, menarche, fitness and fertility. Hum Reprod 1987;2(6):521-33.
29. Chehab FF, Lim ME, Lu R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat Genet 1996;12(3):318-20.
30. Flier JS. What's in a name? In search of leptin's physiologic role. J Clin Endocrinol Metab 1998;83:1407-1413.
31. Ahima RS, Saper CB, Flier JS, Elmquist JK. Leptin Regulation of Neuroendocrine Systems. Front Neuroendocrinol 2000;21(3):263-307.
32. Kiess W, Reich A, Meyer K, et al. A Role for Leptin in Sexual Maturation and Puberty? Horm Res 1999;51(Suppl S3):55-63.
33. Foster DL, Nagatani S. Physiological perspectives on leptin as a regulator of reproduction: role in timing puberty. Biol Reprod 1999;60(2):205-15.
34. Lebrethon MC, Vandersmissen E, Gerard A, Parent AS, Junien JL, Bourguignon JP. In vitro stimulation of the prepubertal rat gonadotropin-releasing hormone pulse generator by leptin and neuropeptide Y through distinct mechanisms. Endocrinology 2000;141(4):1464-9.
35. El Majdoubi M, Sahu A, Ramaswamy S, Plant TM. Neuropeptide Y: A hypothalamic brake restraining the onset of puberty in primates. Proc Natl Acad Sci U S A 2000;97(11):6179-84.
36. Lackey BR, Gray SL, Henricks DM. The insulin-like growth factor (IGF) system and gonadotropin regulation: actions and interactions. Cytokine Growth Factor Rev 1999;10(3-4):201-17.
37. Juul A, Bang P, Hertel NT, et al. Serum insulin like growth factor-I in 1030 healthy children,adolescents, and adults: relation with age,sex stage of puberty, testicular size and body mass index. J Clin Endocrinol Metab 1994;78:744-752.
38. Juul A, Flyvbjerg A, Frystyk J, Muller J, Skakkebaek NE. Serum concentrations of free and total insulin-like growth factor-I, IGF binding proteins -1 and -3 and IGFBP-3 protease activity in boys with normal or precocious puberty. Clin Endocrinol (Oxf) 1996;44(5):515-23.
39. Juul A, Scheike T, Nielsen CT, Krabbe S, Muller J, Skakkebaek NE. Serum Insulin-like Growth Factor I (IGF-I) and IGF-Binding Protein 3 levels are increased in central precocious puberty: effects of two different treatment regimens with Gonadotropin-Releasing Hormone agonists, without or in combination with an antiandrogen (Cyproteron Acetate). J Clin Endocrinol Metab 1995;80:3059-3067.
40. Suter KJ, Pohl CR, Wilson ME. Circulating concentrations of nocturnal leptin, growth hormone, and insulin-like growth factor-I increase before the onset of puberty in agonadal male monkeys: potential signals for the initiation of puberty. J Clin Endocrinol Metab 2000;85(2):808-14.
41. Bourguignon JP, Gerard A, Alvarez Gonzales ML, Fawe L, Franchimont P. Effects of changes in nutritional conditions on timing of puberty: clinical evidence from adopted children and experimental studies in the male rat. Horm Res 1992;38(suppl 1):97-105.
42. McCann SM, Kimura M, Walczewska A, Karanth S, Rettori V, Yu WH. Hypothalamic control of FSH and LH by FSH-RF, LHRH, cytokines, leptin and nitric oxide. Neuroimmunomodulation 1998;5(3-4):193-202.
43. Cavallo A. Melatonin and human puberty: current perspectives. J Pineal Res 1993;15(3):115-21.
44. Commentz JC, Helmke K. Precocious puberty and decreased melatonin secretion due to a hypothalamic hamartoma. Horm Res 1995;44(6):271-5.
45. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 2000;47(3):316-23.
46. Wijn JF de, Haas JH de. Groeidiagrammen van 1-25 jarigen in Nederland [Growth diagrams for ages 1-25 years in The Netherlands]. Leiden: Nederlands Instituut voor Praeventieve Geneeskunde; 1960.
47. Wieringen JC van, Wafelbakker F, Verbrugge HP, Haas JH de. Groeidiagrammen 1965 Nederland [Growth diagrams 1965 The Netherlands]. Leiden: Nederlands instituut voor praeventieve geneeskunde TNO; 1968.

48. Roede MJ, Wieringen JC van. Growth diagrams 1980. Netherlands third nation-wide survey. Tijdschr Soc Gezondheidszorg 1985;63 (suppl.):11-32.
49. Indian Council of Medical Research. Indian average population. Growth and physical development of Indian infants and children. Technical report Series 1989(18).
50. Prakash S, Pathmanathan G. Age at menarche in Sri Lankan Tamil girls in Jaffna. Ann Hum Biol 1984;11(5):463-6.
51. Chompootaweep S, Tankeyoon M, Poomsuwan P, Yamarat K, Dusitsin N. Age at menarche in Thai girls. Ann Hum Biol 1997;24(5):427-33.
52. Chowdhury S, Shahabuddin AK, Seal AJ, et al. Nutritional status and age at menarche in a rural area of Bangladesh. Ann Hum Biol 2000;27(3):249-56.
53. Beall CM. Ages at menopause and menarche in a high-altitude Himalayan population. Ann Hum Biol 1983;10(4):365-70.
54. Simondon KB, Simon I, Simondon F. Nutritional status and age at menarche of Senegalese adolescents. Ann Hum Biol 1997;24(6):521-32.
55. Graham MJ, Larsen U, Xu X. Secular trend in age at menarche in China: a case study of two rural counties in Anhui Province. J Biosoc Sci 1999;31(2):257-67.
56. Huen KF, Leung SS, Lau JT, Cheung AY, Leung NK, Chiu MC. Secular trend in the sexual maturation of southern Chinese girls. Acta Paediatr 1997;86(10):1121-4.
57. Lejarraga H, Sanchirico F, Cusminsky M. Age of menarche in urban Argentinian girls. Ann Hum Biol 1980;7(6):579-81.
58. Farid-Coupal N, Contreras ML, Castellano HM. The age at menarche in Carabobo, Venezuela with a note on the secular trend. Ann Hum Biol 1981;8(3):283-8.
59. Uche GO, Okorafor AE. The age of menarche in Nigerian urban school girls. Ann Hum Biol 1979;6(4):395-8.
60. Kaplowitz PB, Oberfield SE. Reexamination of the age limit for defining when puberty is precocious in girls in the United States: implications for evaluation and treatment. Drug and Therapeutics and Executive Committees of the Lawson Wilkins Pediatric Endocrine Society. Pediatrics 1999;104(4 Pt 1):936-41.
61. Herman-Giddens ME, Slora EJ, Wasserman RC, et al. Secondary sexual characteristics and menses in young girls seen in office practice: A study from the pediatric research in office settings network. Pediatrics. 1997;99:505-512.
62. Pescovitz OH, Comite F, Hench K, et al. The NIH experience with precocious puberty: diagnostic subgroups and response to short-term luteinizing hormone releasing hormone analogue therapy. J Pediatr 1986;108(1):47-54.
63. De Sanctis V, Corrias A, Rizzo V, et al. Etiology of central precocious puberty in males: the results of the Italian study group for physiopathology of puberty. J Ped Endocrinol Metab 2000;13(Suppl 1):687-693.
64. Cisternino M, Arrigo T, Pasquino AM, et al. Etiology and age incidence of precocious puberty in girls: a multicentric study. J Ped Endocrinol Metab 2000;13(Suppl1):695-701.
65. Abdolvahabi RM, Mitchell JA, Diaz FG, McAllister JP, 2nd. A brief review of the effects of chronic hydrocephalus on the gonadotropin releasing hormone system: implications for amenorrhea and precocious puberty. Neurol Res 2000;22(1):123-6.
66. Mohn A, Schoof E, Fahlbusch R, Wenzel D, Dorr HG. The endocrine spectrum of arachnoid cysts in childhood. Pediatr Neurosurg 1999;31(6):316-21.
67. Harada K, Yoshida J, Wakabayashi T, Okabe H, Sugita K. A super long-acting LH-RH analogue induces regression of hypothalamic hamartoma associated with precocious puberty. Acta Neurochir 1995;137(1-2):102-5.
68. Felner EI, White PC. Prepubertal gynecomastia: indirect exposure to estrogen cream. Pediatrics 2000;105(4):E55.
69. Fara GM, Del Corvo G, Bernuzzi S, et al. Epidemic of breast enlargement in an Italian school. Lancet 1979;2(8137):295-7.
70. Robben SGF, Oostdijk W, Drop SLS, Tanghe HLJ, Vielvoye GJ, Meradji M. Idiopathic isosexual central precocious puberty magnetic resonance findings in 30 patients. Br J Radiol 1995;68:34-38.
71. Proos LA, Hofvander Y, Tuvemo T. Menarcheal age and growth pattern of Indian girls adopted in Sweden I menarcheal age. Acta Paediatr Scand 1991;80:852-858.
72. Proos LA, Hofvander Y, Tuvemo T. Menarcheal age and growth pattern of Indian girls adopted in Sweden. II. Catch-up growth and final height. Indian J Ped 1991;58:105-114.
73. Virdis R, Street ME, Zampolli M, et al. Precocious puberty in girls adopted from developing countries. Arch Dis Childh 1998;78:15-154.

74. Marx M, Schoof E, Kapferer L, Dorr HG. Pubertas praecox vera bei einem indischen Mädchen nach Adoption durch deutsche Eltern. Monatsschr Kinderheilk 1998;146:957-960.
75. Oostdijk W, Yap YN, Doude van Troostwijk ML, et al. Final height and timing of puberty of adopted children in The Netherlands. Horm Res 1991;35(2):24.
76. Oostdijk W, Yap YN, Rekers-Mombarg LTM, Massa GG, Brand R, Drop SLS. The impact of early puberty on final height in foreign born, adopted children in The Netherlands. In: Central Precocious Puberty and gonadotropin releasing hormone agonist treatment. Thesis Erasmus University Rotterdam; 1996. p. 135-150.
77. Bureau JJ, Maurage C, Bremond M, Despert F, Rolland JC. [Children of foreign origin adopted in France. Analysis of 68 cases during 12 years at the University Hospital Center of Tours]. Arch Pediatr 1999;6(10):1053-8.
78. Lebrethon MC, Bourguignon JP. Management of central isosexual precocity: diagnosis, treatment, outcome. Curr Opin Pediatr 2000;12(4):394-9.
79. Proos LA, Hofvander Y, Wennqvist K, Tuvemo T. A longitudinal study on anthropometric and clinical development of Indian children adopted in Sweden. II. Growth, morbidity and development during two years after arrival in Sweden. Upsala Journal of medical Science 1992;97(1):93-106.
80. Sorgedrager N. Medical examination and growth of foreign born adoptive children. Groningen: State University Groningen, [thesis in Dutch]; 1988.
81. Golden MH. Is complete catch-up possible for stunted malnourished children? Eur J Clin Nutr 1994;48(Suppl 1):S58-70; discussion S71.
82. Oostdijk W, Yap YN, Slijper FME, Wit JM, Drop SLS. Puberteit en eindlengte bij uit het buitenland geadopteerde kinderen. Tijdschrift Kindergeneeskunde 1996;64:39-43.
83. Proos LA. Growth and development of Indian children adopted in Sweden. Uppsala; 1992.
84. Proos LA. Anthropometry in adolescence - Secular trends, adoption, ethnic and environmental differences. Horm Res 1993;39 (suppl 3):18-24.
85. Proos LA, Karlberg J, Hofvander Y, Tuvemo T. Pubertal linear growth of Indian girls adopted in Sweden. Acta Paediatrica 1993;82:641-644.
86. Preece MA, Moore GE. Genomic Imprinting, Uniparental Disomy and Foetal Growth. Trends Endocrinol Metab 2000;11(7):270-275.
87. Falls JG, Pulford DJ, Wylie AA, Jirtle RL. Genomic imprinting: implications for human disease. Am J Pathol 1999;154(3):635-47.
88. Tomkins DJ, Roux AF, Waye J, Freeman VC, Cox DW, Whelan DT. Maternal uniparental isodisomy of human chromosome 14 associated with a paternal t(13q14q) and precocious puberty. Eur J Hum Genet 1996;4(3):153-9.
89. Miyoshi O, Hayashi S, Fujimoto M, Tomita H, Sohda M, Niikawa N. Maternal uniparental disomy for chromosome 14 in a boy with intrauterine growth retardation. J Hum Genet 1998;43(2):138-42.
90. Mazur W. Phytoestrogen content in foods. Baillieres Clin Endocrinol Metab 1998;12(4):729-42.
91. Sharara FI, Seifer DB, Flaws JA. Environmental toxicants and female reproduction. Fertil Steril 1998;70(4):613-22.
92. Engelbregt MJT, Houdijk ECAM, Popp-Snijders C, Delemarre-van de Waal HA. The effects of intra-uterine growth retardation and postnatal undernutrition on onset of puberty in male and female rats. Ped Res 2000(In press).
93. Cooper C, Kuh D, Egger P, Wadsworth M, Barker D. Childhood growth and age at menarche. Br J Obstet Gynaecol 1996;103(8):814-7.
94. Cresswell JL, Barker DJ, Osmond C, Egger P, Phillips DI, Fraser RB. Fetal growth, length of gestation, and polycystic ovaries in adult life. Lancet 1997;350(9085):1131-5.
95. Cresswell JL, Egger P, Fall CH, Osmond C, Fraser RB, Barker DJ. Is the age of menopause determined in-utero? Early Hum Dev 1997;49(2):143-8.
96. Clark PM, Hindmarsh PC, Shiell AW, Law CM, Honour JW, Barker DJ. Size at birth and adrenocortical function in childhood. Clin Endocrinol (Oxf) 1996;45(6):721-6.
97. Fall CH, Pandit AN, Law CM, et al. Size at birth and plasma insulin-like growth factor-1 concentrations. Arch Dis Child 1995;73(4):287-93.
98. Phillips DI, Barker DJ, Osmond C. Infant feeding, fetal growth and adult thyroid function. Acta Endocrinol (Copenh) 1993;129(2):134-8.
99. Law CM, Barker DJ, Osmond C, Fall CH, Simmonds SJ. Early growth and abdominal fatness in adult life. J Epidemiol Community Health 1992;46(3):184-6.
100. Thamdrup E. Precocious sexual development. Copenhagen: Munksgaard; 1961.
101. Partsch C-J, Heger S, Sippell WG, Group GDS. Treatment of central precocious puberty:lessons from a 15 years prospective trial. J Ped Endocrinol Metab 2000;13(Suppl1):747-758.

102. Lazar L, Kauli R, Bruchis C, Nordenberg J, Galatzer A, Pertzelan A. Early polycystic ovary-like syndrome in girls with central precocious puberty and exaggerated adrenal response. Eur J Endocrinol 1995;133:403-406.
103. Lazar L, Kauli R, Bruchis C, Nordenberg J, Galatzer A, Pertzelan A. High prevalence of abnormal adrenal response in girls with central precocious puberty at early pubertal stages. Eur J Endocrinol 1995;133:407-411.
104. Bridges NA, Cooke A, Healy JR, Hindmarsh PC, Brook CGD. Ovaries in sexual precocity. Clinic Endocrinol 1995;42:135-140.
105. Oostdijk W, Rikken B, Schreuder S, et al. Final height in central precocious puberty after long-term treatment with a slow-release GnRH agonist. Arch Dis Childh 1996;75:292-297.
106. Baek Jensen A-M, Brocks V, Holm K, Laursen EM, Muller J. Central precocious puberty in girls: internal genitalia before, during and after treatment with long-acting gonadotropin- releasing hormone analogues. J Pediatr 1998;132:105-108.
107. Martinez L, Preece MA, Grant DB. Body proportions in precocious puberty. Acta Paediatr Scand 1984;73(2):185-8.
108. Rockhill B, Moorman PG, Newman B. Age at menarche, time to regular cycling, and breast cancer (North Carolina, United States). Cancer Causes Control 1998;9(4):447-53.
109. Liehr JG. Is estradiol a genotoxic mutagenic carcinogen? Endocr Rev 2000;21(1):40-54.
110. Tanner JM. Growth and endocrinology of the adolescent. In: Gardner L, editor. Endocrine and genetic diseases of childhood. Philadelphia and London: Saunders; 1969.
111. Zachmann M, Prader A, Kind HP, Hafliger H, Budliger H. Testicular volume during adolescence. Cross-sectional and longitudinal studies. Helv Paediatr Acta 1974;29(1):61-72.
112. Biro FM, Lucky AW, Huster GA, Morrison JA. Pubertal staging in boys. J Pediatr 1995;127(1):100-2.
113. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. 2 ed. Stanford, California: Stanford University Press; 1959.
114. Bar A, Linder B, Sobel EH, Saenger P, DiMartino-Nardi J. Bayley-Pinneau method of height prediction in girls with central precocious puberty: correlation with adult height. J Pediatr 1995;126(6):955-8.
115. Bayley N, Pinneau SR. Tables for predicting adult height for skeletal age: revised for use with the Greulich-Pyle hand standards. J Ped 1952;40:423-41.
116. Kauli R, Galatzer A, Kornreich L, Lazar L, Pertzelan A, Laron Z. Final height of girls with central precocious puberty, untreated versus treated with cyproteron acetate or GnRH analogue. A comparative study with re-evaluation of predictions by the Bayley-Pinneau method. Horm Res 1997;47:54-61.
117. Andersen E. Skeletal development as a measure of biological development. Acta Paediatr Scand 1967;Suppl(177):73-4.
118. Gilli G. The assessment of skeletal maturation. Horm Res 1996;45 (suppl 2):49-52.
119. King DG, Steventon DM, O'Sullivan MP, et al. Reproducibility of bone ages when performed by radiology registrars: an audit of Tanner and Whitehouse II versus Greulich and Pyle methods. Br J Radiol 1994;67(801):848-51.
120. Bull RK, Edwards PD, Kemp PM, Hughes IA. Bone age assessment: a large scale comparison of the Greulich and Pyle, and Tanner and Whitehouse (TW2) methods. Arch Dis Childh 1999;81:172-173.
121. Albanese A, Hall C, Stanhope R. The use of a computerized method of bone age assessment in clinical practice. Horm Res 1995;44 (suppl 3):2-7.
122. Argemi J, Badia J. A new computerised method for the assessment of skeletal maturity in the newborn infant. Ped Radiol 1997;27:309-314.
123. Cox LA. Tanner-Whitehouse method of assessing skeletal maturity: problems and common errors. Horm Res 1996;45(Suppl 2):53-5.
124. Gibbons RD, Patterson D. Application of computerized image analysis to estimating bone age. In: Hauspie R, Lindgren G, Falkner F, editors. Essays on auxology. Welwyn Garden City: Castlemead publications; 1995. p. 106-118.
125. Schwarze CP, Arens D, Haber HP, et al. Bone age in 116 untreated patients with Turner's syndrome rated by a computer-assisted method(CASAS). Acta Paediatr 1998;87:1146-1150.
126. Tanner JM, Tanner-Lindgren G. TW2 skeletal age read by computer (CASAS): a comparison between results from six and thirteen bones. Studies in Human Biology 1996:45-53.
127. Teunenbroek A van, Waal W de, Roks A, et al. Computer-aided skeletal age scores in healthy children, girls with Turner Syndrome, and in children with constitutionally tall stature. Ped Res 1996;39:360-367.

128. Zachmann M, Sobradillo B, Frank M, Frisch H, Prader A. Bayley-Pinneau, Roche-Wainer-Thissen, and Tanner height predictions in normal children and in patients with various pathologic conditions. J Pediatr 1978;93:749-755.
129. Hintz RL. Issues in final height prediction [comment]. Acta Paediatr Suppl 1996;417:51-2.
130. Vejvoda M, Grant DB. Discordant bone maturation of the hand in children with precocious puberty and congenital adrenal hyperplasia. Acta Paediatr Scand 1981;70(6):903-5.
131. Prakash S, Cameron N. Skeletal maturity of well-off children in Chandigarh, North India. Ann Hum Biol 1981;8:175-180.
132. Lejarraga H, Guimarey L, Orazi V. Skeletal maturity of the hand and wrist of healthy Argentinian children aged 4-12 years, assessed by the TWII method. Ann Hum Biol 1997;24(3):257-61.
133. Singer R, Kimura K. Body height, weight, and skeletal maturation in Hottentot (Khoikhoi) children. Am J Phys Anthropol 1981;54(3):401-13.
134. Crossner CG, Mansfeld L. Determination of dental age in adopted non-European children. Swed Dent J 1983;7(1):1-10.
135. Kreitner KF, Schweden FJ, Riepert T, Nafe B, Thelen M. Bone age determination based on the study of the medial extremity of the clavicle. Eur Radiol 1998;8(7):1116-22.
136. Griffin IJ, Cole TJ, Duncan KA, Hollmann AS, Donaldson MDC. Pelvic ultrasound measurements in normal girls. Acta Paediatr 1995;84:536-543.
137. Haber HP, Mayer EI. Ultrasound evaluation of uterine and ovarian size from birth to puberty. Ped Radiol 1994;24:11-13.
138. Stanhope R, Adams J, Jacobs HS, Brook CGD. Ovarian ultrasound assessment in normal children, idiopathic precocious puberty, and during low dose pulsatile gonadotrophin releasing hormone treatmentof hypogonadotrophic hypogonadism. Arch Dis Childh 1985;60:116-119.
139. Holm K, Mosfeldt Laursen E, Brocks V, Muller J. Pubertal maturation of the internal genitalia: an ultrsound evaluation of 166 healthy girls. Ultrasound Obstet Gynecol 1995;6:175-181.
140. Griffin IJ, Cole TJ, Duncan KA, Hollmann AS, Donaldson MDC. Pelvic ultrasound findings in different forms of sexual precocity. Acta Paediatrica 1995;84:544-549.
141. Orley JN, Goblyos P. Modern imaging methods in paediatric breast examination. Ceska Gynekol 1995;60(3):153-7.
142. Nakamura M, Okabe I, Shimoizumi H, Yanagisawa M, Taniguchi N, Itoh K. Ultrasonography of ovary, uterus and breast in premature thelarche. Acta Paediatr Jpn 1991;33(5):645-8.
143. Neely EK, Wilson DM, Lee PA, Stene M, Hintz RL. Spontaneous serum gonadotropin concentrations in the evaluation of precocious puberty. J Pediatr 1995;127:47-52.
144. Neely EK, Hintz RL, Wilson DM, et al. Normal ranges for immunochemiluminometric gonadotropin assays. J Pediatr 1995;127:40-46.
145. Brito VN, Batista MC, Borges MF, et al. Diagnostic value of fluorometric assays in the evaluation of precocious puberty. J Clin Endocrinol Metab 1999;84(10):3539-44.
146. Partsch CJ, Hümmelink R, Lorenzen F, Sippell WG, Studiengruppe dD-D. Bedeutung und Charakteristika des LHRH-Testes in der Diagnostik der vorzeitigen Pubertatsentwicklung bei Madchen: Der stimulierte LH/FSH-Quotient differenziert zwischen zentraler Pubertas praecox und pramaturer Thelarche. Monatssch Kinderheilkd 1989;137:284-288.
147. Ibanez L, Potau N, Zampolli M, et al. Use of Leuprolide acetate response patterns in the early diagnosis of pubertal disorders: comparison with the Gonadotropin Releasing Hormone test. J Clin Endocrinol Metab 1994;78:30-35.
148. Garibaldi LR, Aceto T, Weber C, Pang S. The relationship between Luteinizing Hormone and estradiol secretion in female precocious puberty: evaluation by sensitive gonadotropin asays and the leuprolide stimulation test. J Clin Endocrinol Metab 1993;76:851-856.
149. Goji K. Twenty-four-hour concentration profiles of gonadotropin and estradiol (E2) in prepubertal and early pubertal girls: the diurnal rise of E2 is opposite the nocturnal rise of gonadotropin. J Clin Endocrinol Metab 1993;77(6):1629-35.
150. Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E. Hypophysial responses to continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone. Science 1978;202(4368):631-3.
151. Conn PM, Crowley WF. Gonadotropin-Releasing hormone and its analogues. N Eng J Med 1990;324:93-103.
152. Hugues JN, Cedrin Durnerin IC. Revisiting gonadotrophin-releasing hormone agonist protocols and management of poor ovarian responses to gonadotrophins. Hum Reprod Update 1998;4(1):83-101.

153. Daya S. Gonadotropin releasing hormone agonist protocols for pituitary desensitization in in vitro fertilization and gamete intrafallopian transfer cycles. Cochrane Database Syst Rev 2000(2):CD001299.
154. Comite F, Cutler GB, Jr., Rivier J, Vale WW, Loriaux DL, Crowley WF, Jr. Short-term treatment of idiopathic precocious puberty with a long- acting analogue of luteinizing hormone-releasing hormone. A preliminary report. N Engl J Med 1981;305(26):1546-50.
155. Parker KL, Lee PA. Depot Leuprolide acetate for treatment of precocious puberty. J Clinl Endocrinol Metab 1989;69:689-691.
156. Oostdijk W, Hümmelink R, Odink RJH, et al. Treatment of children with central precocious puberty by a slow- release gonadotropin-releasing hormone agonist. Eur J Ped 1990;149:308-313.
157. Tanaka T, Hibi I, Kato K, et al. A dose finding study of a super long-acting Luteinizing Hormone- Releasing Hormone Analog (Leuprolide Acetate Depot, TAP-144-SR) in the treatment of central precocious puberty. Endocrinology Japon 1991;38:369-376.
158. Clemons RD, Kappy MS, Stuart TE, Perelman AH, Hoekstra FT. Long-term effectiveness of depot Gonadotropin-Releasing Hormone Analogue in the treatment of children with central precocious puberty. Am J Dis Child 1993;147:653-657.
159. Lee PA. Treatment of central precocious puberty with depot Lupron. 1993. p. 139-150.
160. Partsch CJ, Hümmelink R, Peter M, et al. Comparison of complete and incomplete suppression of pituitary- gonadal activity in girls with central precocious puberty: influence on growth and predicted final height. J Clin Endocrinol Metab 1993;39:111-117.
161. Lahlou N, Carel JC, Chaussain JL, Roger M. Pharmacokinetics and pharmacodynamics of GnRH agonists: clinical implications in pediatrics. J Ped Endocrinol Metab 2000;13(Suppl1):723-737.
162. Wechsel HW, Zerbib M, Pagano F, Coptcoat MJ. Randomized open labelled comparative study of the efficacy, safety and tolerability of leuprorelin acetate 1M and 3M depot in patients with advanced prostatic cancer. Eur Urol 1996;30(Suppl 1):7-14; discussion 19-21.
163. Zerbib M, Lucas C, Leblanc V. [Effectiveness and tolerance of three month sustained release leuprorelin in the treatment of metastatic prostatic cancer (comparative, randomized, multicentric study]. Prog Urol 1997;7(2):246-53.
164. Cohen-Kettenis PT, van Goozen SH. Pubertal delay as an aid in diagnosis and treatment of a transsexual adolescent. Eur Child Adolesc Psychiatry 1998;7(4):246-8.
165. Manasco PK, et al. Local reactions to depot Leuprolide therapy for central precocious puberty. [letter]. J Pediatr 1993;123:334-335.
166. Carel JC, Lahlou N, Guazzarotti L, et al. Treatment of central precocious puberty with depot leuprorelin. Eur J Endocrinol 1995;132:699-704.
167. Neely EK, Hintz RL, Parker B, et al. Two-year results of treatment with depot leuprolide acetate for central precocious puberty. J Pediatr 1992;121:634-640.
168. Kauschansky A, Lurie R, Ingber A. Hair loss in children on long-acting gonadotropin-releasing hormone agonist triptorelin treatment [letter]. Acta Derm Venereol 1997;77(4):333.
169. Yeshaya A, Kauschansky A, Orvieto R, Varsano I, Nussinovitch M, Ben-Rafael Z. Prolonged vaginal bleeding during central precocious puberty therapy with a long-acting gonadotropin-releasing hormone agonist. Acta Obstet Gynecol Scand 1998;77(3):327-9.
170. Kauschansky A, Nussinovitch M, Frydman M, Yeshaya A, Ben-Rafael Z. Hyperprolactinemia after treatment of long-acting gonadotropin-releasing hormone analogue Decapeptyl in girls with central precocious puberty. Fertil Steril 1995;64(2):285-7.
171. Feuillan PP, Jones JV, Barnes K, Oerter Klein K, Cutler Jr GB. Reproductive axis after discontinuation of Gonadotropin- Releasing Hormone analog treatment of girls with precocious puberty: long term follow-up comparing girls with hypothalamic hamartoma to those with idiopathic precocious puberty. J Clin Endocrinol Metab 1999;84:44-49.
172. Oostdijk W, Gevers EF, Drop SLS, et al. Growth and pubertal development during and after treatment with a slow-release Gonadotropin-Releasing Hormone Agonist in central precocious puberty. Horm Res 1991;36:121-125.
173. Oostdijk W, Partsch CJ, Drop SLS, Sippell WG. Hormonal evaluation during and after long-term treatment with a slow-release GnRH agonist of children with central precocious puberty; effect on final height. In: Plant TM, Lee PA, editors. The neurobiology of puberty. Bristol: J Endocrinol; 1995. p. 319-325.
174. Heger S, Partsch CJ, Sippell WG. Long-term outcome after depot gonadotropin-releasing hormone agonist treatment of central precocious puberty: final height, body proportions, body composition, bone mineral density, and reproductive function. J Clin Endocrinol Metab 1999;84(12):4583-90.

175. Mul D, Oostdijk W, Otten BJ, et al. Final height after Gonadotrophin Releasing Hormone agonist treatment for Central Precocious Puberty: The Dutch experience. J Ped Endocrinol Metab 2000;13(Suppl 1):765-772.
176. Carel JC, Roger M, Ispas S, et al. Final height after long-term treatment with Triptorelin slow release for central precocious puberty: Importance of statural growth after interruption of treatment. J Clin Endocrinol Metab 1999;84:1973-1978.
177. Van Beek JT, Sharafuddin MJA, Kao SCS, Luisiri A, Garibaldi LR. Prospective assessment of pituitary size and shape on MR imaging after suppressive hormonal therapy in central precocious puberty. Ped Radiol 2000;30(7):444-446.
178. Reissmann Th, Felberbaum R, Diedrich K, Engel J, Comaru-Schally AM, Schally AV. Development and applications of luteinizing hormone-releasing hormone antagonists in the treatment of infertility: an overview. Hum Reprod 1995;10:1974-1981.
179. Felberbaum R, Diedrich K. Ovarian stimulation for in-vitro fertilization/intracytoplasmic sperm injection with gonadotrophins and gonadotrophin-releasing hormone analogues: agonists and antagonists. Hum Reprod 1999;14(Suppl 1):207-21.
180. Cook T, Sheridan WP. Development of GnRH antagonists for prostate cancer: new approaches to treatment. Oncologist 2000;5(2):162-8.
181. Paridaens R, Thomas J, Wildiers J, et al. Safety, activity and estrogen inhibition by exemestane in postmenopausal women with advanced breast cancer: a phase I study. Anticancer Drugs 1998;9(8):675-83.
182. Kvinnsland S, Anker G, Dirix LY, et al. High activity and tolerability demonstrated for exemestane in postmenopausal women with metastatic breast cancer who had previously failed on tamoxifen treatment. Eur J Cancer 2000;36(8):976-82.
183. Aromasin: a new advance in hormonal breast cancer therapy: Pharmacia Oncology; 2000.
184. Saterno M, Di Maio S, Gasparini N, Mariano A, Macchia V, Tenore A. Central precocious puberty: a single blood sample after gonadotropin-releasing hormone agonist administration in monitoring treatment. Horm Res 1998;50:205-211.
185. Saggese G, Pasquino AM, Bertelloni S, et al. Effect of combined treatment with gonadotropin releasing hormone analogue and growth hormone in patients with central precocious puberty who had subnormal growth velocity and impaired height prognosis. Acta Paediatr 1995;84:299-304.
186. Oostdijk W, Drop SLS, Odink RJH, Hümmelink R, Partsch CJ, Sipell WG. Long-term results with a slow release gonadotrophin-releasing hormone agonist in central precocious puberty. Acta Paediatr Scand 1991;372(suppl):39-45.
187. Walvoord EC, Pescovitz OH. Combined use of growth hormone and gonadotropin-releasing hormone analogues in precocious puberty: theoretic and practical considerations. Pediatrics 1999;104(4 Pt 2):1010-4.
188. Oerter KE, Manasco PK, Barnes KM, Jones J, Hill SC, Cutler GB. Adult height in precocious puberty after long-term treatment with deslorelin. In: Grave GD, Cutler GB, editors. Sexual precocity: Etiology, diagnosis and management. New York: Raven Press Ltd.; 1993. p. 45-54.
189. Arrigo T, Cisternino M, Galluzzi F, et al. Analysis of the factors affecting auxological response to GnRH agonist treatment and final height outcome in girls with idiopathic central precocious puberty. Eur J Endocrinol 1999;141(2):140-4.
190. Galluzzi F, Salti R, Pasquini E, La Cauza C. Adult height comparison between boys and girls with precocious puberty after long-term gonadotrophin-releasing hormone analogue therapy. Acta Paediatr 1998;87:521-527.
191. Bertelloni S, Baroncelli GI, Sorrentino MC, Perri G, Saggese G. Effect of central precocious puberty and gonadotropin-releasing hormone analogue treatment on peak bone mass and final height in females. Eur J Pediatr 1998;157(5):363-7.
192. Tuvemo T, Gustafsson J, Proos LA. Growth hormone treatment during suppression of early puberty in adopted girls. Swedish Growth Hormone Advisory Group. Acta Paediatr 1999;88(9):928-32.
193. Tatò L, Saggese G, Cavallo L, et al. Use of combined Gn-RH agonist and hGH therapy for better attaining the goals in precocious puberty treatment. Horm Res 1995;44 (suppl.3):49-54.
194. Lanes R, Gunczler P. Final height after combined growth hormone and gonadotrophin-releasing hormone analogue therapy in short healthy children entering into normally timed puberty. Clin Endocrinol 1998;49:197-202.
195. Balducci R, Toscano V, Mangiantini A, et al. Adult height in short normal adolescent girls treated with Gonadotropin-Releasing Hormone analog and growth hormone. J Clin Endocrinol Metab 1995;80:3596-3600.

196. Pasquino AM, Pucarelli I, Roggini M, Segni M. Adult height in short normal girls treated with gonadotropin-releasing hormone analogs and growth hormone. J Clin Endocrinol Metab 2000;85(2):619-22.
197. Job JC, Toublanc JE, Landier F. Growth of short normal children in puberty treated for 3 years with growth hormone alone or in association with Gonadotropin-releasing Hormone agonist. Horm Res 1994;41:177-184.
198. Saggese G, Cesaretti G, Barsanti S, Rossi A. Combination treatment with growth hormone and gonadotropin-releasing hormone analogs in short normal girls. J Pediatr 1995;126(3):468-73.
199. Boot AM, Muinck Keizer - Schrama SMPF de, Pols HAP, Krenning EP, Drop SLS. Bone mineral density and body composition before and during treatment with Gonadotropin-Releasing Hormone Agonist in children with central precocious and early puberty. J Clin Endocrinol Metab 1998;83:370-373.
200. Palmert MR, Mansfield MJ, Crowley WF, Jr., Crigler JF, Jr., Crawford JD, Boepple PA. Is obesity an outcome of gonadotropin-releasing hormone agonist administration? Analysis of growth and body composition in 110 patients with central precocious puberty. J Clin Endocrinol Metab 1999;84(12):4480-8.
201. Saggese G, Bertelloni S, Baroncelli GI, Battini R, Franchi G. Reduction of bone density: an effect of gonadotropin releasing hormone analogue treatment in central precocious puberty. Eur J Pediatr 1993;152(9):717-20.
202. Antoniazzi F, Bertoldo F, Zamboni G, et al. Bone mineral metabolism in girls with precocious puberty during gonadotrophin-releasing hormone agonist treatment. Eur J Endocrinol 1995;133:412-417.
203. Bertelloni S, Baroncelli GI, Ferdeghini M, Menchini-Fabris F, Saggese G. Final height, gonadal function and bone mineral density of adolescent males with central precocious puberty after therapy with gonadotropin-releasing hormone analogues. Eur J Pediatr 2000;159:369-374.
204. Schroor EJ, Van Weissenbruch MM, Delemarre-van de Waal HA. Long-term GnRH-agonist treatment does not postpone central development of the GnRH pulse generator in girls with idiopathic precocious puberty. J Clin Endocrinol Metab 1995;80(5):1696-701.
205. Ibanez L, Potau N, De Zegher F. Endocrinology and metabolism after premature pubarche in girls. Acta Paediatr Suppl 1999;88(433):73-7.
206. Miller WL. The molecular basis of premature adrenarche: an hypothesis. Acta Paediatr Suppl 1999;88(433):60-6.
207. Ibanez L, de Zegher F, Potau N. Premature pubarche, ovarian hyperandrogenism, hyperinsulinism and the polycystic ovary syndrome: from a complex constellation to a simple sequence of prenatal onset. J Endocrinol Invest 1998;21(9):558-66.
208. Xhrouet-Heinrichs D, Lagrou K, Heinrichs C, et al. Longitudinal study of behavioral and affective patterns in girls with central precocious puberty during long-acting triptorelin therapy. Acta Paediatr 1997;86:808-815.
209. Ehrhardt AA, Meyer-Bahlburg HF. Idiopathic precocious puberty in girls: long-term effects on adolescent behavior. Acta Endocrinol Suppl (Copenh) 1986;279:247-53.
210. Ehrhardt AA, Meyer-Bahlburg HFL. Psychosocial aspects of precocious puberty. Horm Res 1994;41 (suppl 2):30-35.
211. Mazur T, Clopper RR. Pubertal disorders. Psychology and clinical management. Endocrinol Metab Clin North Am 1991;20(1):211-30.
212. Jackson PL, Ott MJ. Perceived self-esteem among children diagnosed with precocious puberty. J Pediatr Nurs 1990;5(3):190-203.
213. Galatzer A, Beth-Halachmi N, Kauli R, Laron Z. Intellectual function of girls with precocious puberty. Pediatrics 1984;74(2):246-9.
214. Cohen RZ, Seeman MV, Gotowiec A, Kopala L. Earlier puberty as a predictor of later onset of schizophrenia in women. Am J Psychiatry 1999;156(7):1059-64.
215. Hirsch SR, Das I, Garey LJ, de Belleroche J. A pivotal role for glutamate in the pathogenesis of schizophrenia, and its cognitive dysfunction. Pharmacol Biochem Behav 1997;56(4):797-802.
216. Tamminga C. Glutamatergic aspects of schizophrenia. Br J Psychiatry Suppl 1999;37:12-5.
217. Guyda HJ. Four decades of growth hormone therapy for short children: what have we achieved? J Clin Endocrinol Metab 1999;84(12):4307-16.
218. Dupuis HM. De groeihormonen. In: Op het scherp van de snede. Amsterdam: Balans; 1998. p. 176-178.
219. Pasquino AM, Pucarelli I, Segni M, Matrunola M, Cerrone F. Adult height in girls with central precocious puberty treated with Gonadotropin-Releasing Hormone analogues and growth hormone. J Clin Endocrinol Metab 1999;84:449-452

I INTRODUCTION

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Chapter 2.1

Pubertal development in The Netherlands 1965 - 1997

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ABSTRACT

We investigated pubertal development of 3909 boys and 3454 girls ≥ 9 years of age participating in a cross-sectional nation-wide survey in the Netherlands and compared the results to those of two previous surveys using similar methodology.

Reference curves for all pubertal stages were constructed. The 50th percentile of Tanner breast stage 2 was 10.7 years, and 50% of the boys had reached a testicular volume of 4 ml at 11.5 years of age. Median age at menarche was 13.15 years.

The median age at which the various stages of pubertal development were observed has stabilised since 1980. The increase of the age at stage G2 between 1965 and 1997 is probably due to different interpretations of its definition. The age limits for the definition of precocious puberty that are currently in use (8 years for girls and 9 years for boys), are close to the 3rd percentile of these references.

A high agreement was found between the pubic hair stages and stages of pubertal (genital and breast) development, but slightly more in boys than in girls. In 11.3% of the girls in stage B1 pubic hair was present. In 10.2% of the boys in stage G1 pubic hair was observed.

Menarcheal age was dependent on height, weight and BMI. At a given age tall or heavy girls have a higher probability to have menarche compared to short or thin girls. A body weight exceeding 60 kg (+1 SDS), or a BMI of > 20 (+1 SDS) does not further increase the chance to have menarche, while for height such ceiling effect was not observed.

In conclusion, in The Netherlands the age at onset of puberty or menarche has stabilised since 1980. Height, weight and BMI have a strong influence on the chance of menarche.

INTRODUCTION

The development and first appearance of secondary sexual characteristics can be regarded as reflection of the overall physiological development in adolescence (1). The subdivision of the continuous process of pubertal development into discrete numerical stages, as proposed by Marshall and Tanner (2, 3), has been widely used and serves to label the phases of various elements of pubertal development more or less objectively.

However, the assessment of pubertal stages in the individual child or adolescent in the clinic is only useful if reliable reference data from the same population is available for comparison. In many European countries a positive secular trend with regard to height has been accompanied by a decrease of the age at onset of puberty (1, 4, 5). This would imply that the definition of precocious and delayed puberty should change over time. In fact, in the United States it was recently proposed to revise the guidelines for the evaluation of girls with precocious puberty (6, 7).

Besides clinical reasons, there are also scientific reasons to study pubertal development in a large population-based sample of healthy children and adolescents. First, it is unclear if the secular trend with regard to body stature is invariably associated with a trend towards earlier pubertal development. Second, there is little data on the association between the markers of the maturation process of the hypothalamo-pituitary-gonadal axis (breast development in girls and genital stage in boys) and pubic hair as a marker of adrenarche in the female (pubic hair) and of the combination of adrenarche and genital development in the male. While gonadal development and adrenarche usually occur in the same age period, the endocrine regulation of these processes is different (2, 8). Based on this background, one would hypothesise that the agreement between gonadal (G- or B-stage) and pubic hair development (P-stage) should be higher in boys than in girls. Third, there are observations suggesting that pubertal development is influenced by anthropometric variables, particularly body weight (9, 10), but the exact nature of the correlation is unknown.

In the Netherlands four consecutive growth studies have been performed since 1955 (1, 11-13). These studies provide the opportunity to thoroughly study the secular changes in height, weight and pubertal development. In two earlier papers on the study performed in 1997 we concentrated on the secular trend of body stature, weight and body mass index (BMI) and only shortly discussed pubertal development (13, 14).

In this paper we present the reference data in more detail, as well as a comparison with the previous growth studies. Furthermore, we investigated the degree of concurrence of breast or genital stages with pubic hair stages and the relationship between the age at menarche and height, weight and BMI.

PATIENTS AND METHODS

In a cross-sectional design the presence of secondary sexual characteristics was studied. All participants of the 1997 nation-wide growth study aged $\geq$ 9 years completed a questionnaire on demographic variables (3909 boys and 3454 girls). This sample can be regarded as representative for the general population. In a subgroup ('puberty sample') we determined the stage of sexual maturation and age at menarche. The age distribution in the puberty sample showed an overrepresentation of children < 15 years of age (around 250 in each age group) compared to children > 15 years (100 – 150 per each group) (see Table 1).

In the analyses in this article age was used as covariate, so this skewed age distribution will not affect the results. The composition of the puberty sample was comparable with the sample of a national survey with regard to region and level of education.

Table 1: Puberty sample characteristics, 3909 boys and 3454 girls

Age (yrs)	PH boys	G boys	TV boys	PH girls	B girls	Men
9-12	73.2%	72.9%	66.5%	78.1%	80.5%	85.6%
12-15	54.4%	54.3%	51.3%	57.4%	58.5%	76.9%
15-18	49.9%	49.9%	47.6%	51.1%	52.1%	74.8%
18-21	42.5%	42.4%	40.4%	33.6%	33.9%	83.8%

Participating boys or girls per age group as percentage of age group in the national survey. PH: pubic hair; G: genital stage; TV: testicular volume; B: breast stage; Men: menarche

The measurements of height and weight were performed by trained staff. The pubertal stages were determined by visual inspection, using Tanner's criteria (15). In boys testicular volume was assessed using an orchidometer. To validate the accuracy of the measurement of testicular volume, the testicular volumes in 79 boys were measured by 2 observers. The Spearman correlation coefficient between the measurements of 2 observers was 0.82; the 95 % confidence interval for the difference between observers appeared to be 0.4 – 2.0 ml (1.2 $\pm$ 0.8). In midpuberty the interobserver differences were highest. Zachman and Prader reported a correlation coefficient of 0.83 and a mean difference in testicular volume between 2 observers of 2.9 ml (16). The age at menarche was determined by the status quo method, asking a girl whether she had had her first period at the moment of the survey.

Demographic variables were assessed by a questionnaire. The highest level of completed education of the parents was used as a measure for socio-economic status. The country was divided in 5 geographical regions, one of them containing the 4 largest cities (13).

Statistical methods

For menarche and stages of secondary sexual characteristics the reference curves were estimated by a generalised additive logistic model for each stage transition separately (17). This model describes the probability of each stage as a smooth function of age. The amount of smoothing was determined by cross-validation. LMS reference curves were derived for testicular volumes, where the measured volumes were considered as a continuous measure (18). To compare B or G stages and pubic hair stages in girls and boys we calculated *kappa* (κ) as measure of agreement (19).

Results

1. Reference curves for pubertal stages and testicular volume

In figures 1 a-f we present the reference curves for sexual development. The dotted lines represent the crude data. The 50th percentile ages can be read from the figures. The 10th and 90th percentile ages, that were published earlier as numerical data (13), can also be read from these graphics being the ages at which the curves cross the 10th and 90th percentile respectively.

Figure 1 also shows the intervals between the consecutive pubertal stages, with a general pattern of a shorter interval between the third and fourth stage compared to the interval between stage 2 and 3. In figure 1-g reference curves are presented for various testicular volumes.

The P_3 for B2 and G2 were 8.2 and 9.8 years respectively, the P_{97} values were 12.7 and 13.4 years respectively

2. Comparison with the 1965 and 1980 growth studies

In figure 2 a-d we show comparisons between the timing of pubertal stages in this and the previous growth studies from 1965 and 1980. The P_{50} values are shown for both boys and girls. For all stages a decreasing trend is seen between 1965 and 1980 with a stabilisation afterwards. In contrast, G2 in boys increased from 11 years in 1965 to 11.5 years in 1997, whereas the P_{50} of a testicular volume of 4 ml decreased from 12.0 years in 1965 to 11.5 years in 1997. In all studies the SD of the P_{50} ages is approximately 1.

3. Relationship between pubertal stages

In tables 2a and 2b the relationships between the P stage on the one hand and B or G stages on the other hand are shown in absolute numbers. In girls in B1 60/531 (11.3%) showed pubic hair development, while from the girls in P1 23.3% showed breast development. In boys, G1 was accompanied by the presence of pubic hair in 10,2%; boys in P1 had genital development in 24.9%.

Table 2a: Distribution of B and P stages in girls

N=2213	**B1**	**B2**	**B3**	**B4**	**B5**	**Total**
P1	471	124	19	--	--	614
P2	55	138	50	3	1	247
P3	5	50	118	36	6	215
P4	--	4	58	186	71	319
P5	--	--	17	156	495	668
P6	--	--	5	23	122	150
Total	531	316	267	404	695	2213

kappa = 0.59 ($p<0.001$)

Table 2b: Distribution of G and P stages in boys

N=2360	**G1**	**G2**	**G3**	**G4**	**G5**	**Total**
P1	529	151	21	2	1*	704
P2	59	222	53	4	--	338
P3	1	47	124	20	1	193
P4	--	2	56	178	32	268
P5	--	1	10	130	387	528
P6	--	--	1	15	313	329
Total	589	423	265	349	734	2360

kappa= 0.63 ($p<0.001$); * this boy was 17.1 years old, testicular volume 20 ml

The agreement between P and B or G stage was expressed as kappa (κ) and Spearman correlation. The κ's were 0.59 and 0.63 for girls and boys respectively indicating moderate to substantial agreement. The difference between the 2 κ's was significant ($p<0.05$). Spearman rank-order correlation was 0.91 both in boys and girls ($p < 0.001$).

Thus, in line with our hypothesis, in boys the gonadal and pubic hair development show a closer mutual agreement than in girls, although the difference in kappa is only small.

4. Menarcheal age in relation to auxological variables

In figure 3 a-f the probability to have menarche is depicted as function of age (X-axis) and weight, weight SDS, height, height SDS, BMI and BMI-SDS respectively (plotted on the Y-axis).

All figures show the expected increase in probability of menarche with increasing age and the additional effect of weight, height and SDS (expressed as nominal values or as SDS). They demonstrate that at a given age the heavier and taller girls have a higher probability to have menarche. However, the shape of the probability curves is different for the indices of weight (i.e. weight and BMI) and height. When weight or BMI exceeds a certain point, no or just a slight further increase in probability is observed anymore, as the curves have an almost vertical course from there. For weight this point is close to 62 kg (1 SDS), and for BMI it is approximately 20 kg/m^2 or 1 SDS.

In contrast, the figures on height show a continuing effect of height at a certain age. Some examples of the different ages at which there is 50 % probability to have menarche with various SD scores for weight, height or BMI are shown in table 3.

Table 3: Influence of different SD scores on the P_{50} age of chance of menarche

	SD score	Age P_{50} (yr)*
Weight	+2	12.1
	+1	12.7
	0	13.2
	-1	13.8
	-2	14.5
Height	+2	12.2
	+1	12.8
	0	13.3
	-1	13.7
	-2	14.0
BMI	+2	12.7
	+1	12.7
	0	13.1
	-1	13.8
	-2	14.7

* estimated form the curves in fig 3b, d and f

Discussion

This study provides up-to-date references for pubertal stages in the Dutch population, which can be used for clinical purposes. In the interpretation of the reference curves for the consecutive pubertal stages, one should be aware, however, that our data is derived from a cross-sectional study. The reliability of the data is high, due to the relatively large numbers of subjects. On the other hand no information is available about the tempo at which a child passes through the consecutive stages. Such information can only be obtained by a longitudinal study, like the longitudinal assessment of puberty in boys and girls performed by Marshall and Tanner (2, 3). In general, reference centiles based on cross-sectional data have a larger variance than those based on longitudinal data. For pubertal development curves, this implies that the progression of stages for individuals is generally faster than the intervals between P_{50}-stages obtained from cross-sectional references.

The second finding is that the positive secular change towards an earlier development of puberty between 1965 and 1980 has almost stabilised thereafter. Over the whole period between 1965 and 1997 the P_{50} age of onset of puberty (stage B2) in girls decreased from 11.0 years in 1965 to 10.7 years in this study. The median age at menarche decreased by 0.25 yrs in the same period, and by 0.5 years from 1955. In the last 17 years only a small decrease of about 1.5 months from 13.28 to 13.15 years was observed. A similar pattern of an apparent stabilisation of a previously decreasing trend was observed in Oslo schoolgirls, where menarcheal age has reached a stable level for several decades (20). However, in Norway the secular trend in body stature appears to have stopped as well (20). Maybe the stabilisation reflects a situation in which the environmental conditions have allowed the child to reach the optimal genetic potential given the actual environmental conditions (4).

The only exception to this trend of a slow positive secular trend between 1965 and 1980 followed by near stabilisation is the apparent increase of the median age at which boys reach G2 from 11.0 years in 1965 to around 11.5 years in 1997. This finding contrasts with a decrease of the median age at attaining a testicular volume of 4 ml from 12.0 years in 1965 to 11.5 years in 1997.The best way to control for the reliability of the observation of the increase in age at G2 would be to compare it with testicular development in the consecutive growth studies. However, testicular volume was not assessed in 1980.

The most likely explanation of this discrepancy is that the interpretation of the definition of stage G2 must have been different in 1965 in comparison to 1980 (1, 21) and 1997. In fact, the original definition of G2 as proposed by Marshall and Tanner (3) leaves much room for confusion, as it states that "The scrotum and testes have enlarged and there is a change in the texture of the scrotal skin. There is also some reddening of the scrotal skin ...." This description is not pertinent about the question which of the three criteria mentioned is most relevant, and about whether all criteria have to be met or at least one or two of them. In addition, it does not strictly describe the minimum volume that the testis should have before the genital stage may be labelled as G2. For example, there are good arguments that a testicular volume of 3 ml can already be considered as a sign of puberty (16, 22). In the present study the observers were taught to describe the genital stage as G2 if both enlargement of testicular volume and scrotum was observed and reddening of the scrotal skin was present. It appears likely that in 1965 the observers might have labelled the genital stage as G2 if at least one of the three criteria was present.

Based on these findings, but also on our experience in clinical trials (23), we believe that it is opportune to come to a redefinition of stage G2, to prevent more confusion in the future. We would prefer that the testicular volume, the criterion that is most easily measured, should be used as the only criterion. Furthermore, a volume of 3 ml appears a better indication of the onset of puberty than 4 ml (22, 24).

Little data is available on the accordance between P and G or M stages during puberty. Based on the theoretical view that for girls breast development is the initial event in pubertal development and testicular development for boys , these parameters should be used as markers in clinical practice. In girls P stage is a reflection of adrenal maturation and in boys of a combined adrenal and testicular maturation, so that a higher agreement would be expected in boys than in girls. In fact, we found a higher agreement between P and G or B stage (expressed as kappa) in boys than in girls, but both were significant. This suggests that pubertal development and pubic hair development frequently synchronise. With regard to the timing of both phenomena, we found that in general breast development starts somewhat earlier than pubic hair, in line with the findings of Marshall. However, pubic hair was seen before breast development in about a third of all girls in the English study (2), and in about 10 % in our study. In stages B3 and G3 the distribution of P stages is equally divided and in the higher B or G stages the P scores tend to shift to the right, especially in boys, with higher P than G or M stage.

The definition of precocious puberty and delayed puberty should be based on the normal occurrence of secondary sexual characteristics in the population, but there is no consensus whether -2 SDS or -2.5 SDS should be used as a cut-off. We chose to use the usual cut-off measure of -2 SD, which is close to the 3rd percentile, which can be read from the reference curves in figure 1.

The third percentile age for B2 (8.2 years) is close to the age of 8.0 years which is generally and internationally used as age limit for the definition of precocious puberty, and we would therefore propose to continue using this figure. For boys the P_3 of G2 stage is 9.8 years, whereas no reliable P_3 data for testicular volume of 4 ml can be presented. Thus, the current cut-off ages for precocious puberty, i.e. 8 years for girls and 9 years for boys, can be maintained in our country.

For delayed puberty, the P_{97} for B2 and G2 presented in the results section, as well as the P_{97} age for testicular volume of 4 ml (13.8 years), point to a cut-off age for delayed puberty of 13 years in girls and 14 years in boys.

As mentioned before, in the United States a decrease in the age at onset of puberty in girls was observed (6). However, in that study the sample was not representative for the general population, as the girls were examined when they visited a general practitioner. The girls were heavier and taller than in the national American growth survey, and in 15% of the girls rated B2 by visual inspection no breast tissue was found at palpation (7).

We have shown that, besides age, weight, height and BMI influence the chance of having menarche in the age range 11-15 years as well. Interestingly, the probability lines for weight and BMI show a vertical pattern in the range where the SDS exceeds approximately 1. Beyond such degree of (over)weight, weight or BMI does not affect the probability of having menarche anymore. The cut-off level for BMI data is consistent with the results published in our earlier report, showing that premenarcheal girls in all age ranges had mean BMI < 20 kg/m^2 (14). Our data are in contrast to those of Marshall, who stated that the occurrence of menarche was not related to the attainment of a particular height, weight or body composition, but mostly occurred after the peak of the adolescent growth spurt (8). However, the limited number of subjects in that study may have precluded the appearance of statistical significance in this respect. An interesting phenomenon is that height, in contrast to weight and BMI, exerts its influence on the probability of menarche over the full range.

It is generally assumed that the increase in socio-economic conditions and general health is the main contributing factor for the trend towards earlier maturation (4, 25, 26). In most industrialised countries the increase in public health and socio-economic conditions was accompanied by an increase in adult height and a decreasing age at attainment of pubertal events (27). The mechanisms through which these changes occur are unknown. On the physiological substrate for earlier pubertal development several hypotheses were discussed, for example the so-called critical weight hypothesis (9, 28). Recent studies on leptin have suggested that this protein could act as a link between fat tissue and the central activation of the hypothalamus (29-31). Another line of research concerns the possible influences of estrogen-like substances in the environment on the timing of puberty, for example phyto-estrogens present in soy-based feeding (32). However, no human data is available that show an influence of infant feeding, containing phytoestrogens, on sexual maturation (33). The stabilisation of the age at onset of puberty in a period where an increasing exposure to estrogen-like substances can be assumed, argues against a causal link.

We *conclude* that the secular change towards earlier puberty has been stabilised in the last 2 decades in The Netherlands. No change in the definition of precocious puberty is warranted. The occurrence of menarche is not only dependent on age, but also on height, weight and BMI. Beyond a weight or BMI of +1.0 SDS this dependency disappears. The agreement between the expression of gonadal maturation and pubic hair is slightly higher in boys than in girls.

Acknowledgement: The contribution of Inge Everhardus, studying the observer characteristics for testicular volumes, is greatly acknowledged.

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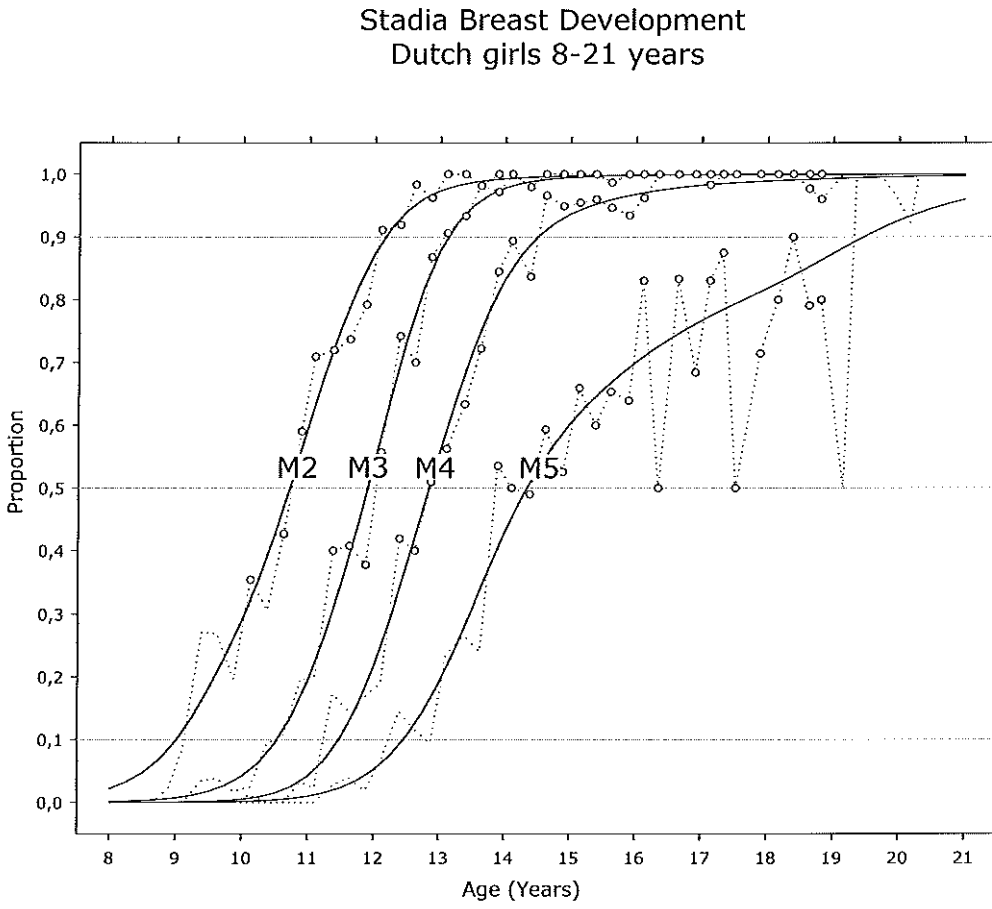
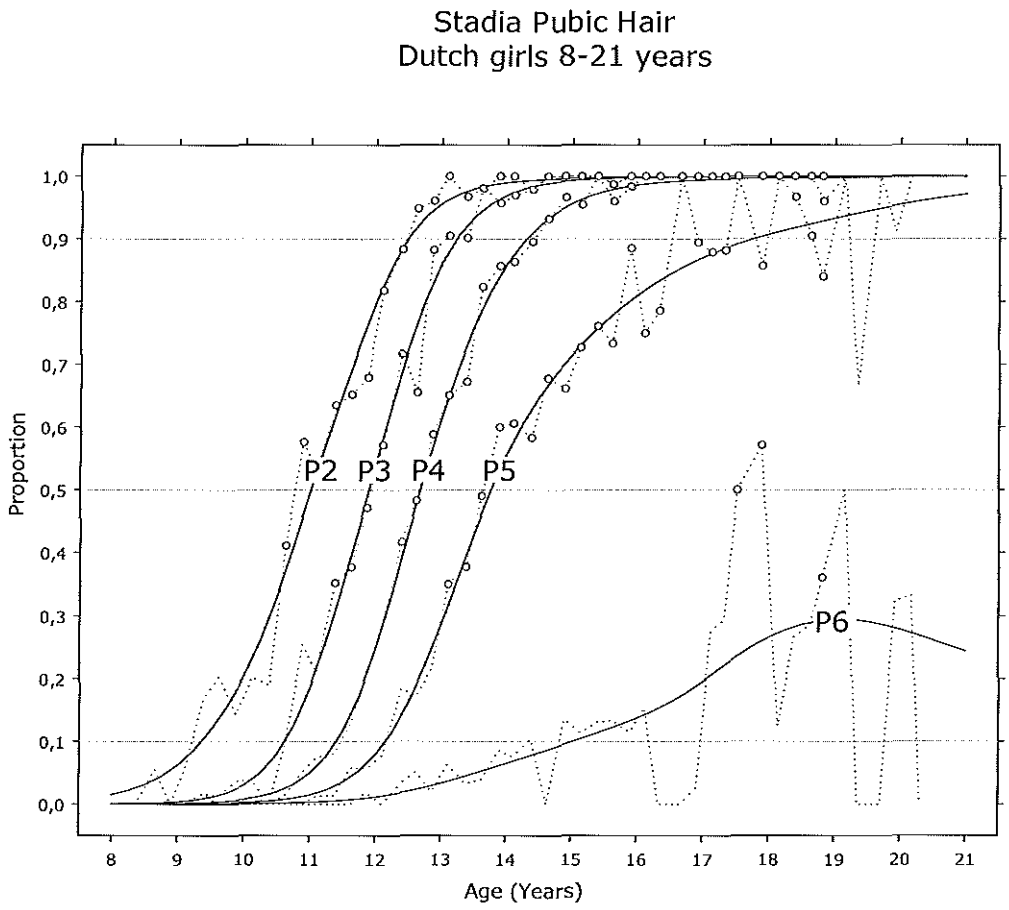
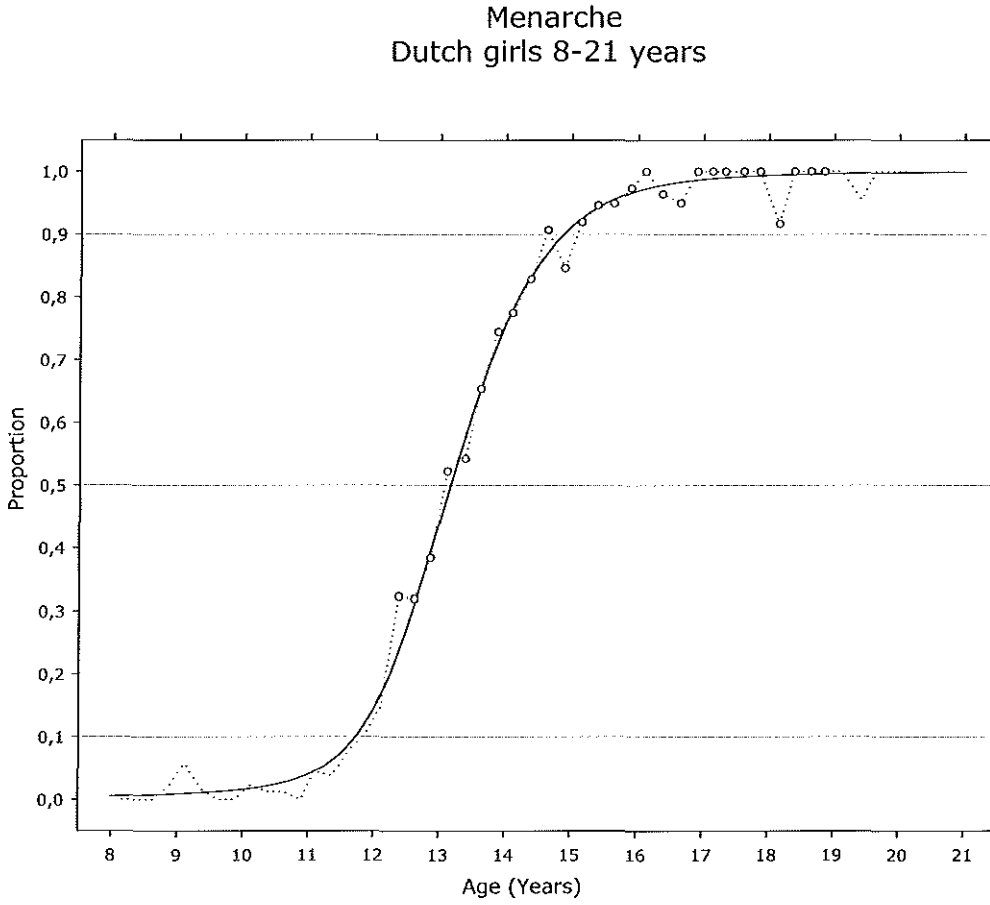
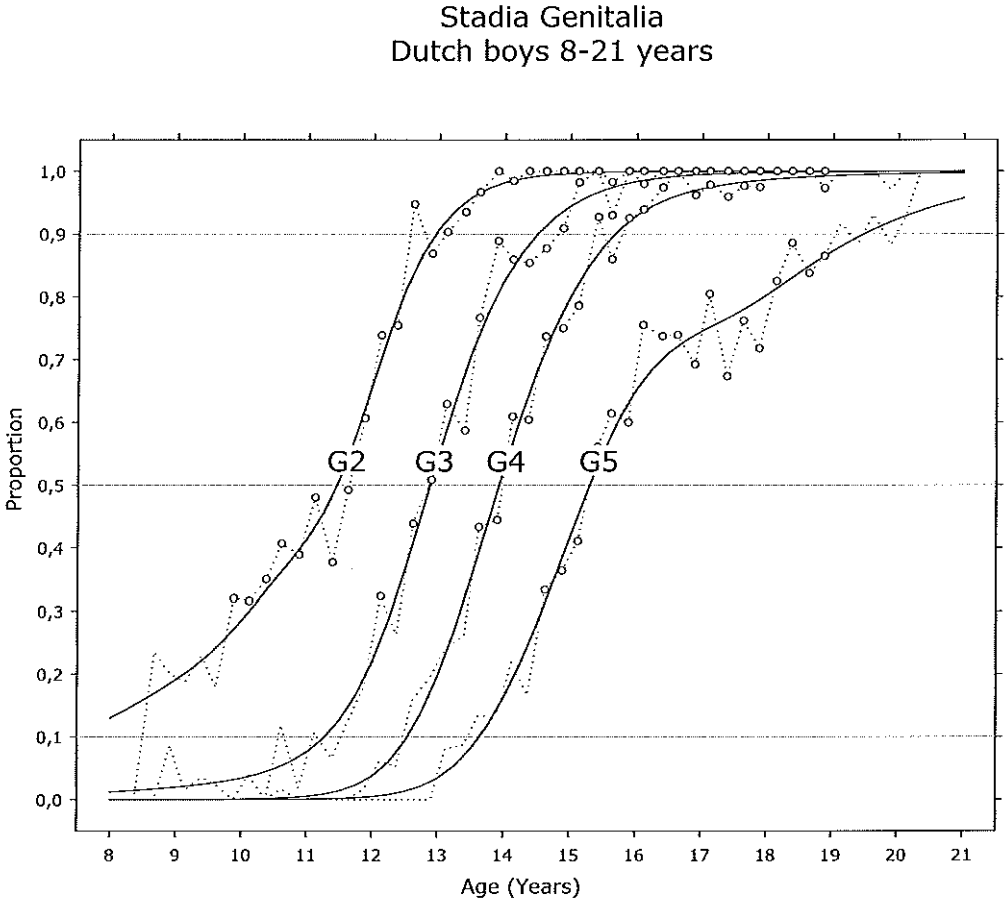
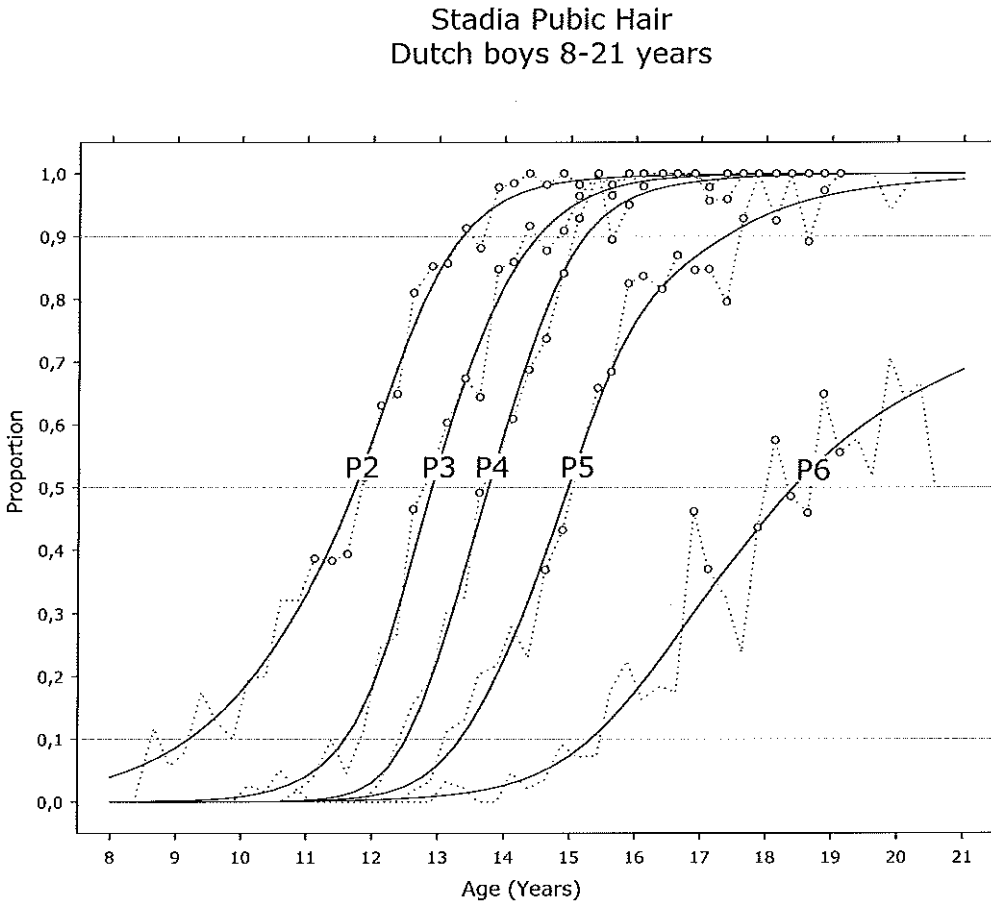
Figure 1:(pages 50 – 56): Reference curves for secondary sexual characteristics in The Netherlands 1997. **a**: breast stage in girls; **b**: pubic hair stage in girls; **c**: menarche; **d**: genital stage in boys; **e**: pubic hair stage in boys; **f**: mean testicular volume; **g**: specific testicular volumes in early and midpuberty.


Stadia Breast Development
Dutch girls 8-21 years
Proportion
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
M2
M3
M4
M5
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Age (Years)


Stadia Pubic Hair
Dutch girls 8-21 years
Proportion
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
P2
P3
P4
P5
P6
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Age (Years)


Menarche
Dutch girls 8-21 years
Proportion
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Age (Years)


Stadia Genitalia
Dutch boys 8-21 years
Proportion
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
G2
G3
G4
G5
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Age (Years)


Stadia Pubic Hair
Dutch boys 8-21 years
Proportion
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
P2
P3
P4
P5
P6
8
9
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12
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14
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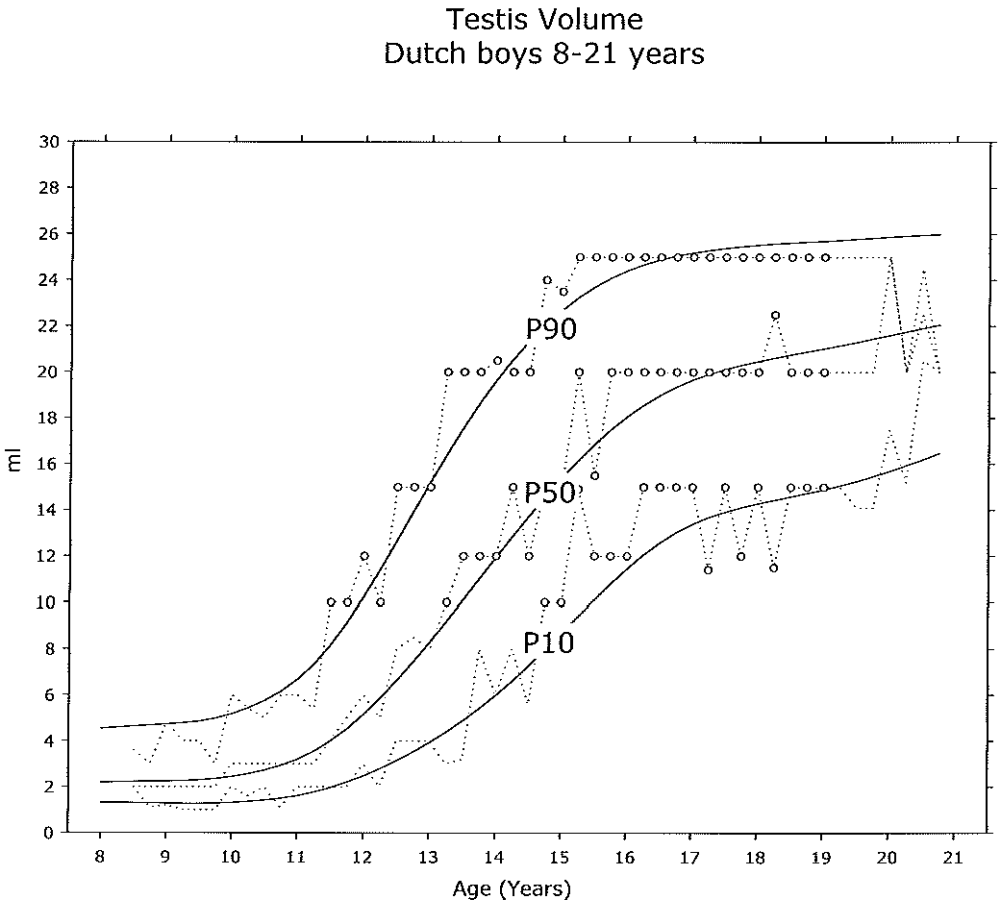
16
17
18
19
20
21
Age (Years)


Testis Volume
Dutch boys 8-21 years
ml
Age (Years)
P90
P50
P10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
8
9
10
11
12
13
14
15
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17
18
19
20
21

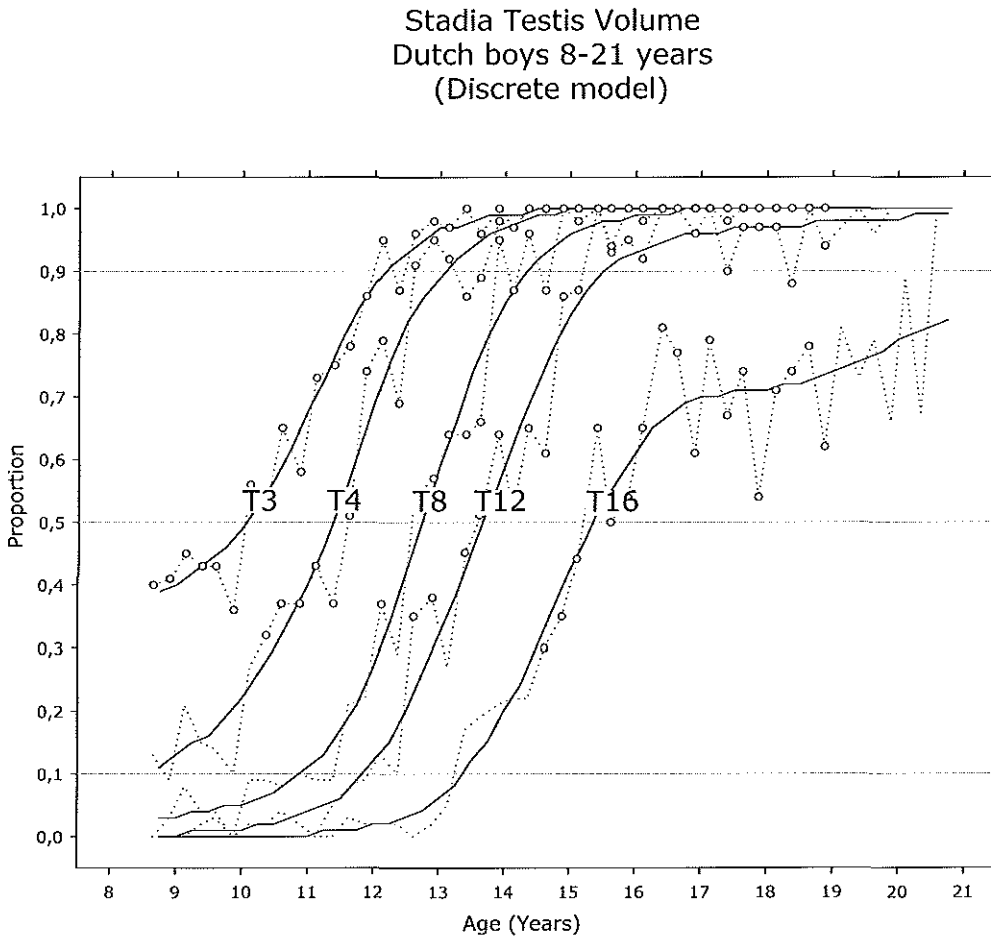
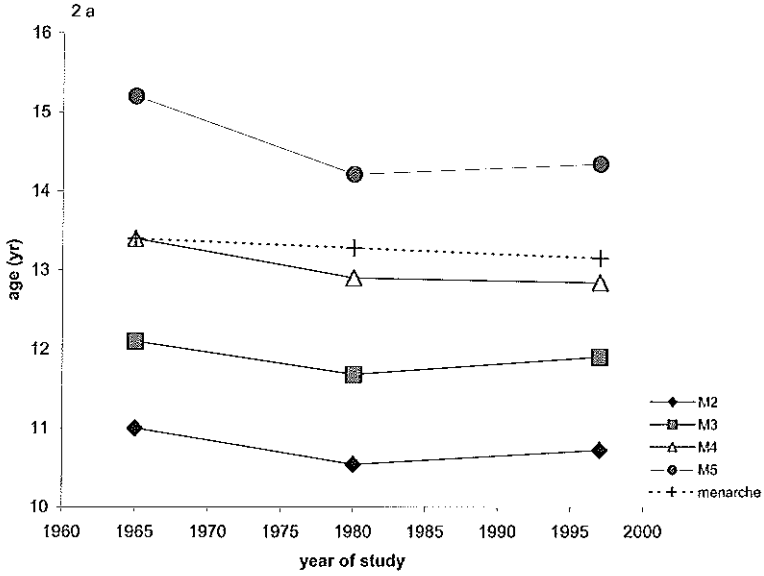
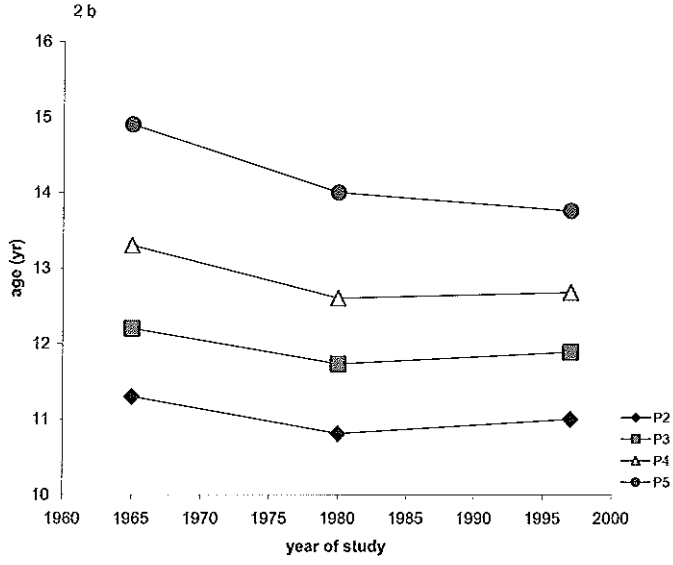


Figure 2: (next two pages): Sexual maturation in The Netherlands 1965 – 1997*; the P50 values of the different pubertal stages are given.
A: breast stage and menarche; **b**: pubic hair stage in girls; **c**: genital stage in boys and testicular volume 4 ml; **d**: pubic hair stage in boys. (* refer 1 and 12 and data from this study)


2 a
16
15
14
13
12
11
10
age (yr)
1960
1965
1970
1975
1980
1985
1990
1995
2000
year of study
M2
M3
M4
M5
menarche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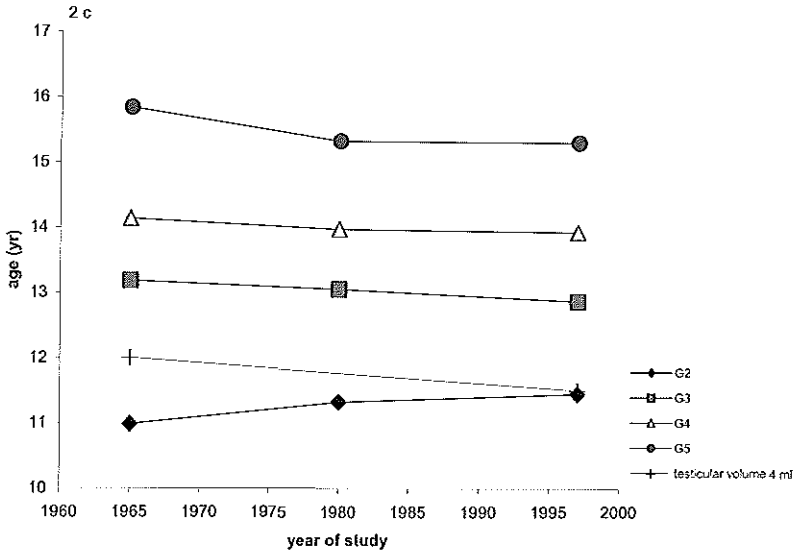
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age (yr)
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1965
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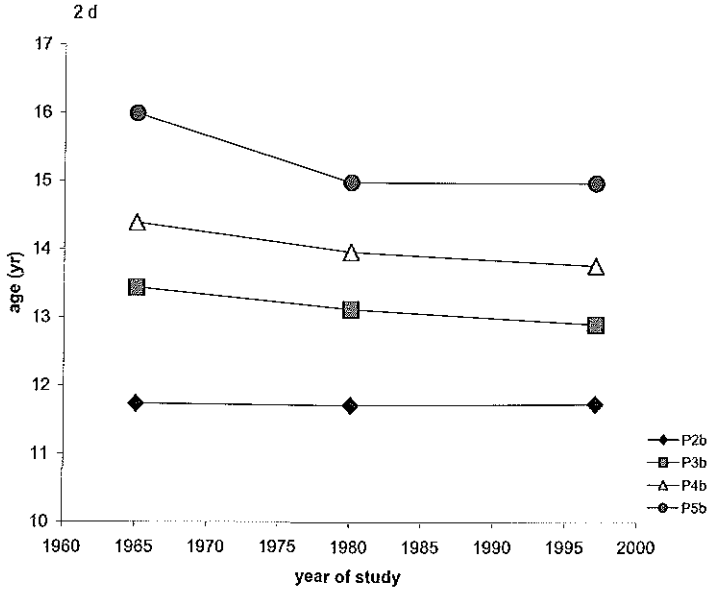
1995
2000
year of study
G2
G3
G4
G5
testicular volume 4 ml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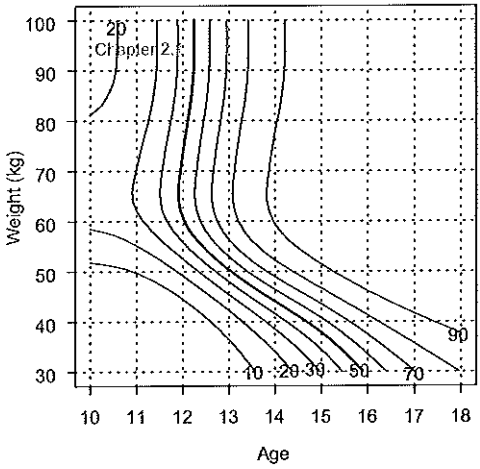
year of study
P2b
P3b
P4b
P5b


Weight (kg)
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80
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60
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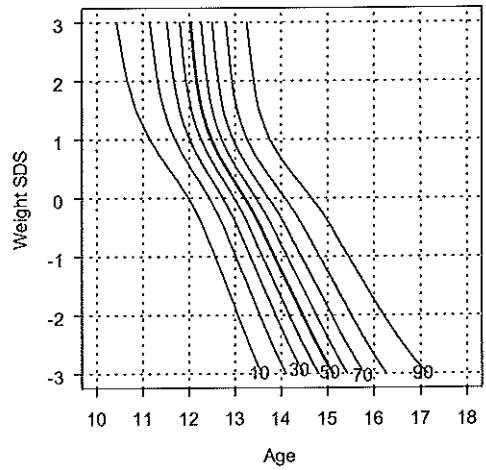
10 11 12 13 14 15 16 17 18
Age


Weight SDS
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1
0
-1
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10
30
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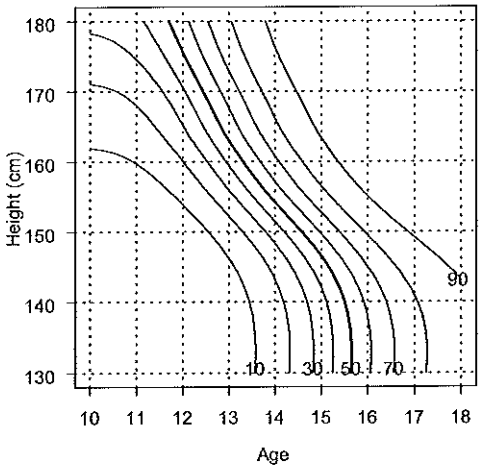
10 11 12 13 14 15 16 17 18
Age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170
160
150
140
130
10
30
50
70
90
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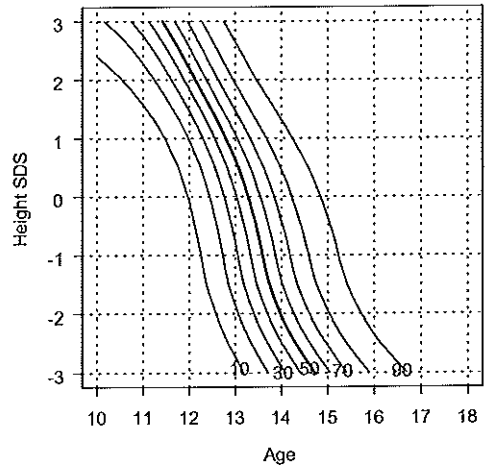
Age


Height SDS
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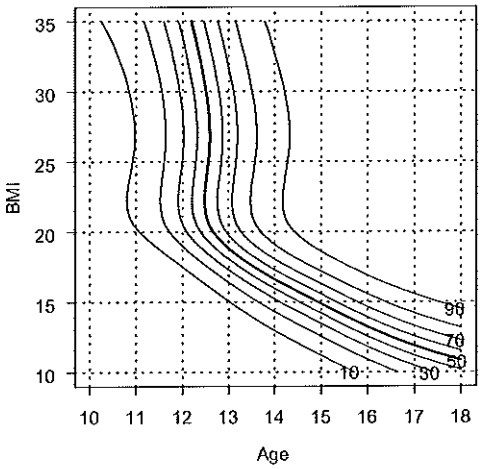
Age


BMI
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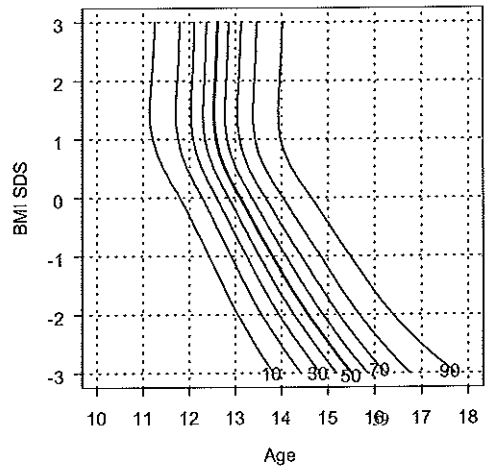
10 11 12 13 14 15 16 17 18
Age


BMI SDS
3
2
1
0
-1
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-3
10
30
50
70
99
10 11 12 13 14 15 16 17 18
Age

(Previous page): **Figure 3**: Probability on having menarche as a function of age and weight (**3a**), weight SDS (**3b**), height (**3c**), height SDS (**3d**), BMI (**3e**) and BMI SDS (**3f**).
The probability is expressed as a percentage. A vertical course of the lines means that at a certain age the parameter on the y-axis does not further contribute to increase the probability on menarche. A transverse course implies additional effect of the parameter of the y-axis on the probability to have menarche

◄——————

REFERENCES

1. Roede MJ, Wieringen JC van. Growth diagrams 1980. Netherlands third nation-wide survey. Tijdschr Soc Gezondheidszorg 1985;63 (suppl.):11-32.
2. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Childh 1969;44:291-303.
3. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child 1970;45(239):13-23.
4. Hauspie RC, Vercauteren M, Susanne C. Secular changes in growth and maturation: an update. Acta Paediatr Suppl 1997;423:20-7.
5. Lindgren G. Pubertal stages 1980 of Stockholm schoolchildren. Acta Paediatr 1996;85(11):1365-7.
6. Herman-Giddens ME, Slora EJ, Wasserman RC, et al. Secondary sexual characteristics and menses in young girls seen in office practice: A study from the pediatric research in office settings network. Pediatrics 1997;99:505-512.
7. Kaplowitz PB, Oberfield SE. Reexamination of the age limit for defining when puberty is precocious in girls in the United States: implications for evaluation and treatment. Drug and Therapeutics and Executive Committees of the Lawson Wilkins Pediatric Endocrine Society. Pediatrics 1999;104(4 Pt 1):936-41.
8. Marshall WA. The relationship of puberty to other maturity indicators and body composition in man. J Reprod Fertil 1978;52(2):437-43.
9. Frisch RE, Revelle R. Height and weight at menarche and a hypothesis of critical body weights and adolescent events. Science 1970;169(943):397-9.
10. Georgopoulos N, Markou K, Theodoropoulou A, et al. Growth and pubertal development in elite female rhythmic gymnasts. J Clin Endocrinol Metab 1999;84(12):4525-30.
11. Wijn JF de, Haas JH de. Groeidiagrammen van 1-25 jarigen in Nederland [Growth diagrams for ages 1-25 years in The Netherlands]. Leiden: Nederlands Instituut voor Praeventieve Geneeskunde; 1960.
12. Wieringen JC van, Wafelbakker F, Verbrugge HP, Haas JH de. Groeidiagrammen 1965 Nederland [Growth diagrams 1965 The Netherlands]. Leiden: Nederlands instituut voor praeventieve geneeskunde TNO; 1968.
13. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 2000;47(3):316-23.
14. Fredriks AM, van Buuren S, Wit JM, Verloove-Vanhorick SP. Body index measurements in 1996-7 compared with 1980. Arch Dis Child 2000;82(2):107-12.
15. Tanner JM. Growth and endocrinology of the adolescent. In: Gardner L, editor. Endocrine and genetic diseases of childhood. Philadelphia and London: Saunders; 1969.
16. Zachmann M, Prader A, Kind HP, Hafliger H, Budliger H. Testicular volume during adolescence. Cross-sectional and longitudinal studies. Helv Paediatr Acta 1974;29(1):61-72.
17. Hastie TJ, Tibshirani RJ. General additive models. London: Chapman and Hall; 1990.
18. Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. Stat Med 1992;11:1305-1319.
19. Altman DG. Practical statistics for medical research. London: Chapman & Hall; 1991.
20. Liestol K, Rosenberg M. Height, weight and menarcheal age of schoolgirls in Oslo--an update. Ann Hum Biol 1995;22(3):199-205.
21. Taranger J, Engstrom I, Lichtenstein H, Svennberg - Redegren I. VI. Somatic pubertal development. Acta Paediatr Scand Suppl 1976;258:121-35.
22. Biro FM, Lucky AW, Huster GA, Morrison JA. Pubertal staging in boys. J Pediatr 1995;127(1):100-2.

23. Rekers-Mombarg LTM, Kamp GA, Massa GG, Wit JM, Group DGHW. Influence of growth hormone treatment on pubertal timing and pubertal growth in children with idiopathic short stature. J Ped Endocrinol Metab 1999;12:611-622.
24. Largo RH, Prader A. Pubertal development in Swiss boys. Helv Paediatr Acta 1983;38(3):211-28.
25. Wyshak G, Frisch RE. Evidence for a secular trend in age of menarche. N Engl J Med 1982;306(17):1033-5.
26. Susanne C, Bodzsar EB. Patterns of secular change of growth and development. In: Bodzsar EB, Susanne C, editors. Secular growth changes in Europe. Budapest: Eötvös University Press; 1998. p. 5-27.
27. Bodzsar EB, Susanne C. Secular growth changes in Europe. Budapest: Eötvö University Press; 1998.
28. Frisch RE. Body fat, menarche, fitness and fertility. Hum Reprod 1987;2(6):521-33.
29. Flier JS. What's in a name? In search of leptin's physiologic role. J Clin Endocrinol Metab 1998;83:1407-1413.
30. Kiess W, Reich A, Meyer K, et al. A Role for Leptin in Sexual Maturation and Puberty? Horm Res 1999;51(Suppl S3):55-63.
31. Clayton PE, Trueman JA. Leptin and puberty. Arch Dis Child 2000;83(1):1-4.
32. Mazur W. Phytoestrogen content in foods. Baillieres Clin Endocrinol Metab 1998;12(4):729-42.
33. Klein KO. Isoflavones, soy-based infant formulas, and relevance to endocrine function. Nutr Rev 1998;56(7):193-204.

I INTRODUCTION

Chapter 1

General introduction

Chapter 2: Normal puberty

2.1 Pubertal development in The Netherlands 1965 - 1997

2.2 Trends in pubertal development in Europe

submitted

Chapter 2.2

Trends in pubertal development in Europe

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ABSTRACT

The secular changes in growth and maturation can be seen as indicators of socio economic and health status. In most European countries the age at onset of puberty and menarcheal age is still decreasing in the last decades. The duration of puberty seems to decrease as well although only few studies have sufficient data. The four Dutch nation-wide growth surveys are useful examples assessing the secular trend in pubertal development over the last 45 years. Genetic and environmental factors contribute to the secular changes. Environmental factors seem to be the most important ones. Recently, attention has been given to substances with oestrogen-like actions, present in nutrients. The possible role of these substances is discussed in this review.

INTRODUCTION

Secular changes in growth and development should be regarded as indicators of socio-economic and socio-hygienic condition and of the state of health of a population. The term "secular trend "is often used to describe a slowly continuing change in growth and development over successive generations living in the same territories. Since these changes can be positive, nil, or negative, the term ' secular changes " is preferred (1), but both terms will be used interchangeable in this text.

During the course of the past two centuries a striking increase of mean stature and an earlier sexual maturation has been observed in all countries in Europe (2). However, this did not occur in the same period in every European country. Both genetic and environmental factors result in population differences. The same environmental factors will not affect all children in the same manner, as their genetic sensitivities will be different (3). These secular changes towards a taller stature, heavier weight, and earlier maturation are predominantly linked to improvements in nutrition and health (3). Therefore, growth of a population can be phrased as " a mirror of conditions in society" (4). However, secular changes may slow-down and may become reversed as well. Negative trends have been observed in periods of socio-economic problems, e.g. during World War II. Observations of no change in tempo of growth might be explained by cessation of improvement of environmental conditions or that these already allowed the genetic potential for growing to be fully expressed.

In The Netherlands, in 1955, 1965, 1980, and recently in 1997, large cross-sectional nation-wide growth studies were performed (5-8). In these studies, next to growth data, reference curves for menarcheal age and the stages of secondary sex characteristics were estimated. In the next paragraphs we will compare these data with the secular changes in several European countries. Further, we will highlight potential mediators of the process of the secular trend.

SECULAR TREND IN THE ONSET OF PUBERTY

The onset of puberty is mediated by the increase in pulsatile LH release by the pituitary, resulting in the production of male or female sex steroids that initiate the secondary sexual characteristics. There are many hypotheses regarding the primary event that causes activation of the hypothalamic GnRH pulse generator. An interesting hypothesis is that leptin, produced by human adipocytes interacts via several neuropeptides as a permissive factor for the onset of puberty (9), thus connecting an aspect of body composition with the onset of puberty.

During the middle ages in Europe pubertal onset occurred around the age of 14 and became delayed until a markedly older age (17 to 18 years) just around the turn of the 19th century (10). In the last century the onset of puberty progressively shifted back towards younger ages in several countries of Europe, with a levelling off in the last decades. Table 1 shows a comparison of average ages at onset of puberty (breast stage 2 (B2) in girls and genital stage 2 (G2) in boys according to Tanner(11)) in various European studies.

Table 1: pubertal maturation in various European countries

Country (Yr of study)	Girls			Boys			Reference
	B2 (yr)	B5 (yr)	B2-B5 (yr)	G2 (yr)	G5 (yr)	G2-G5 (yr)	
Netherlands							
- 1965	11.0	15.2	4.2	11.0	15.85	4.9	6
- 1980	10.54	14.21	3.7	11.33	15.33	4.0	7
- 1997	10.72	14.34	3.6	11.45	15.30	3.8	8
Sweden							
- 1970	11.0	15.6	4.6	12.2	15.1	2.9	derived from:12
- 1980	10.8	14.8	4.0	11.6	15.1	3.5	12
England							
- 1960	11.2	15.3	4.1	11.6	14.9	3.3	derived from: 12
- 1975	10.8	14.0	3.2	-	-	-	derived from: 12
Switserland							
- 1970	10.9	14.0	3.1	11.2	14.7	3.5	derived from: 12

In The Netherlands, comparison of the results of the fourth nation wide growth study with those of the three previous studies shows that the mean height of Dutch children, adolescents, and adults, in 1980 already among the world's tallest, has further increased during the past 17 years (8) (Table 2).

Table 2 Final height, menarcheal age and change in menarcheal age per decade in Dutch girls

	1955	1965	1980	1997
Mean FH girls	163.0	166.3	168.3	170.6
Menarche (yr)	13.75	13.40	13.28	13.15
Observed shift per decade (yr)	-	- 0.35	-0.08	-0.08

Usually, a positive secular growth change is accompanied by an advance of sexual maturation. However, in the Dutch boys the median age at G2 tended to increase during the past 30 years. In girls, the age of onset of puberty (age at B2) declined from 1955 to 1980, but tended to occur slightly later in the most recent study (Table 1). Lindgren reported pubertal stages of Stockholm schoolchildren in 1980 and compared those with two earlier studies in the same area (table 1) (12).

The girls of the 1980 study were somewhat earlier in breast and pubic hair development than the girls investigated in 1970 and 1975. The boys studied in 1980 entered G2 earlier than boys studied in 1970. Thus, in the last decades the tendency of an earlier start of puberty levelled off or came to a halt in some European countries.

SECULAR TREND IN TEMPO OF PUBERTY AND AGE OF MENARCHE

Tempo of puberty

The duration of puberty can be defined in several ways, for example as the interval between B2 and menarche or B5 stage in girls and in boys between G2 and G5 stage. In this article we use the period between B2 and B5 for girls and for boys between G2 and G5. It should be noted that data from cross-sectional studies result in longer intervals than compared to data from longitudinal studies. From the different surveys it becomes clear that the secular trend towards earlier onset of puberty is accompanied by a decrease in duration of puberty. Not many studies do show all stages of sexual maturation from the onset of puberty to the last stage, thus we limited ourselves to studies providing complete data. In table 1 a summary of maturation data from 4 countries in the northern part of Europe is shown.

From table 1 it can be concluded that there is a secular trend towards a higher tempo of sexual maturation in girls. In boys a similar trend can be assumed but only the Dutch data support the assumption. There is scarce data on pubertal development in boys due to lack of a marked indicator such as menarche is in girls. Only a few studies used spermarche as indicator for male puberty (3). Some reports on peak height velocity (PHV) in boys are available but it is difficult to estimate these data from cross-sectional studies and a comparison to estimate a secular change can be assumed to be inaccurate (13). Factors that determine the tempo of puberty are not known. Clinical observations in children on growth hormone treatment and in several form of early pubertal development suggest a role of the GH-IGF axis (14, 15).

A shorter duration of puberty can, theoretically, cause less pubertal height gain, unless growth velocity is higher during that shorter period (16). The data on the secular increase in final height in the same periods as in which duration of puberty decreases, however, does show that either a higher stature at start of puberty or sufficient pubertal growth in a shorter period is obtained. It was reported that the largest part of the secular increase in adult height was established in childhood and due to increase in leg length (17)

Methodological aspects of the assessment of age at menarche

To compare the different studies on final height and age of menarche some methodological issues should be addressed. First the quality of the survey in terms of the composition of the study population, its representativeness for the whole population and a balanced distribution of all age groups (8). Then the assessment of the age of menarche. Several methods can be used: one is the *status quo* method where it is asked whether or not the girl had had her first period. Another method is to ask at what age menarche occurred. The latter *recall method* was compared with the status quo method and resulted in comparable age at menarche (18). However, in most studies the status quo method is preferred as it was shown that the longer the recall period the more inaccurate the estimation of menarcheal age was (19).

Changes in age of menarche

Many studies address the age of menarche as a marker for the timing of puberty. It is well known and a world-wide phenomenon that there is a difference between urban and rural regions in menarcheal age, with the urban girls having their menarche earlier than rural girls (20, 21). In fig 1 a summary is shown of results from several growth studies providing data on menarcheal age over time. The largest decrease in the age of menarche was observed until the end of the seventies and early eighties. Thereafter, in some countries there is a continuous small decrease in the age at menarche (The Netherlands, Germany, Bulgary), while an increase in the age at menarche has been observed in Italy and Croatia. Age at menarche has not changed recently in Belgium and Norway (13) .

The observation of a decreasing age of menarche should carefully be interpreted. It was shown that the decrease in number of late maturers was the most marked change observed (8, 13, 22). This phenomenon can contribute to a decrease in mean age and in the variability around the mean. One might speculate that the number of late maturers is a sensitive indicator of the effect of final changes in socio economic and health status. The cause of the reduction in number of late maturers can be the increasing awareness of delayed puberty and better treatment options available.

Although the age at menarche is going down it seems that the size at menarche, based on body weight underwent only minor changes (23). This may lead to the hypothesis that the earlier occurrence of menarche is the result of the optimalisation of growth so that the minimal body size (needed for menarche) is achieved at an earlier age. This can explain why the decreasing age of menarche is not accompanied by a decrease in final height such as happens in case of precocious puberty (24)

MEDIATORS IN SECULAR CHANGE IN TIMING AND PROGRESSION OF PUBERTY

The age at onset of puberty and menarche is determined by genetic and environmental factors (25). The relative contribution of each of these is difficult to assess. Probably the secular trend towards earlier occurrence of pubertal onset and menarche is mainly due to environmental factors (26) such as increasing socio economic conditions, better health care and prevention (13). Because of improvement in general living conditions, favouring the lower social groups in particular, growth and maturation differences diminish between social groups (26). The effect of racial mixing might contribute to the secular changes as well (27). However, the phenotypic effect of heterosis (the increase in size, strenght, etc.often found in a hybrid as compared with inbred plants or animals) remains a controversial issue (3). Thereby, gene-environment interactions do occur. Modifications under the effect of external agents will especially become visible during periods of more intense change. In table 3 some factors that may contribute to secular change are summarised.

Table 3 : Possible mediators explaining secular trend in timing and progression of puberty

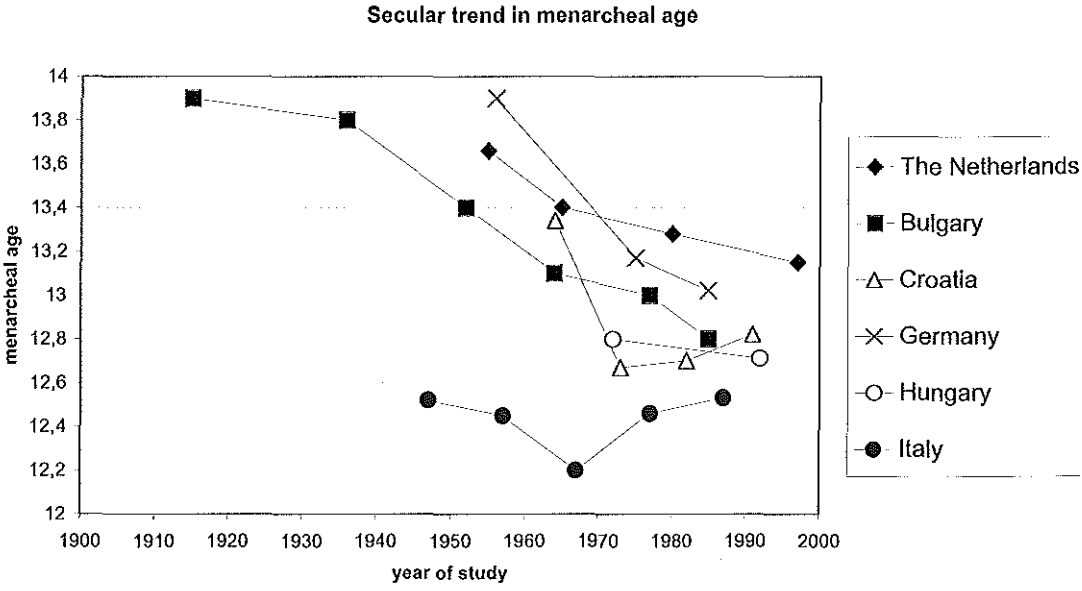
Genetic	
- Migration	- gene flows
	- phenotypic effect of heterosis[#]
Environmental	
- Improvement of	- socio-economic conditions
	- hygienic conditions
	- health service
- Growing urbanisation	
- Reduction of family size	
- Changes in nutrition	- increased intake of animal proteins
	- phyto- or xeno estrogen intake (?)
- Environmental pollutants (?)	

Heterosis : the increase in size, strenght, etc.often found in a hybrid as compared with inbred plants or animals (Webster's Dictionary)

The levelling off of the menarcheal age in some countries is either the effect of a ceasing improvement of environmental factors or of reaching the genetically determined limits of normality in menarcheal age (13).

Nutritional differences between rural and urban populations still exist in some European countries with more vegetables and fruit, meat and milk-based products consumed in the urban areas (3). In The Netherlands, the general wealth of the population over the past 42 years has increased considerably and at present virtually all children have easy access to food. With regard to the quality of food, a clear rise of consumption of animal protein and saturated fat was observed between 1936 and 1975 in The Netherlands and the present consumption of dairy products is one of the highest in the world (8). Recently, emphasis is placed on food-substances that have oestrogen related physiological effects such as phyto-oestrogens and lignans. These substances act as either agonists or antagonists of oestrogens. They are found in vegetables, fruits, seeds, and grains and are substantial constituents of our daily food (28). Rat studies have shown that feeding lignan-rich food like flaxseed during pregnancy had dose dependent hormonal effects in the offspring such as an earlier age and lighter weight at puberty (29). Although it is often stated that soy-based formulae (containing relatively high isoflavonoid levels) do not alter the timing of puberty, there are in fact no studies of sexual maturation in relation to the type of infant feeding (30). In women intake of some phyto-estrogens was shown to be related to a reduction in breast cancer risk (31).

Figure 1: Secular trend in menarcheal age. Data from Bodzsar & Susanne 1998 and 4 Dutch national growth surveys mentioned in the text.



On the other hand there is a theoretical, yet disputed, increase in breast cancer risk as an effect of an earlier start of oestrogen exposure in women with a history of early menarche (32, 33). However, no data is available about the net effect on breast cancer risk when women with early puberty would ingest more phyto-oestrogens.The early onset of puberty in many adopted children from developing countries to Europe (34, 35) raised the question whether exposure to low levels of oestrogenic chemicals in the environment, xeno estrogens, may lead to higher expression of oestrogen regulated genes in children that were used to vegetarian diets (Mengarda, unpublished data). A possible role of intra-uterine growth retardation can be hypothesised in these children as well (36). With regard to diet, however, data from the literature suggest that the composition of the diet is a less important determinator of pubertal events than is the attainment of a certain body size or fat mass at which pubertal onset is permitted (37-39)

Another potential explanation for the decreasing age of onset of puberty might be the increasing prevalence of obesity both in the United States and Europe which seems to be related to earlier onset of puberty, as the hypothesised minimal body mass is reached at an earlier chronological age (40-42).

CONCLUDING REMARKS

During the last century an earlier start of puberty has been observed in most European countries. In the last decades, however, this decrease slowed down or came to a halt as illustrated by the very recent data from The Netherlands (8).

The shift towards a younger age has also been observed for age at menarche and still continues in several European countries, albeit at a slower rate. Occasionally, during periods of socio-economic instability age at menarche was found to have shifted towards a later time. From several reports it became clear that the more recent decline of menarcheal age resulted mostly from a reduced frequency of late maturers.

Improved socio-economic conditions, improved health service and hygienic circumstances, changes in nutrition, and growing urbanisation are among the most important factors influencing these secular changes. The observation that the earlier maturation slowed down, stopped, or even reversed may indicate either that environmental conditions have ceased to improve, or that these have already allowed the full expression of genetic potential (13).

The striking decrease in age at onset of puberty and menarcheal age as reported from the United States, especially in African-American girls (33) has not been found in Europe. The magnitude of the decrease is in part due to the comparison with old references from Marshall and Tanner. The possible influences of substances that have oestrogen-related effects have been mentioned and require further study (33).

REFERENCES

1. Wieringen JC van. Secular growth changes. In: Falkner F, Tanner JM, editors. Human growth - a comprehensive treatise. 2 ed. New York: Plenum Press; 1986. p. 3-7-331.
2. Bodzsar EB, Susanne C. Secular growth changes in Europe. Budapest: Eötvö University Press; 1998.
3. Susanne C, Bodzsar EB. Patterns of secular change of growth and development. In: Bodzsar EB, Susanne C, editors. Secular growth changes in Europe. Budapest: Eötvös University Press; 1998. p. 5-27.
4. Tanner JM. Growth as a measure of the nutritional and hygienic status of a population. Horm Res 1992;38(Suppl 1):106-15.

5. Wijn JF de, Haas JH de. Groeidiagrammen van 1-25 jarigen in Nederland [Growth diagrams for ages 1-25 years in The Netherlands]. Leiden: Nederlands Instituut voor Praeventieve Geneeskunde; 1960.
6. Wieringen JC van, Wafelbakker F, Verbrugge HP, Haas JH de. Groeidiagrammen 1965 Nederland [Growth diagrams 1965 The Netherlands]. Leiden: Nederlands instituut voor praeventieve geneeskunde TNO; 1968.
7. Roede MJ, Wieringen JC van. Growth diagrams 1980. Netherlands third nation-wide survey. Tijdschr Soc Gezondheidszorg 1985;63 (suppl.):11-32.
8. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 2000;47(3):316-23.
9. Flier JS. What's in a name? In search of leptin's physiologic role. J Clin Endocrinol Metab 1998;83:1407-1413.
10. Backman G. Die beschleunigte Entwicklung der Jugend. Verfrühte Menarche, verspätete Menopause, verlängerte Lebensdauer. Act. Anat. 1948;4:421-480.
11. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height,weight,height velocity, and stages of puberty. Arch Dis Childh 1976;51:170-179.
12. Lindgren G. Pubertal stages 1980 of Stockholm schoolchildren. Acta Paediatr 1996;85(11):1365-7.
13. Hauspie RC, Vercauteren M, Susanne C. Secular changes in growth. Horm Res 1996;45(Suppl 2):8-17.
14. Rekers-Mombarg LTM, Kamp GA, Massa GG, Wit JM, Group DGHW. Influence of growth hormone treatment on pubertal timing and pubertal growth in children with idiopathic short stature. J Ped Endocrinol Metab 1999;12:611-622.
15. Fontoura M, Brauner R, Prevot C, Rappaport R. Precocious puberty in girls: early diagnosis of a slowly progressing variant. Arch Dis Child 1989;64(8):1170-6.
16. Bourguignon JP. Linear growth as a function of age at onset of puberty and sex steroid dosage: therapeutic implications. Endocrine Rev 1988;9:467-488.
17. Hauspie RC, Vercauteren M, Susanne C. Secular changes in growth and maturation: an update. Acta Paediatr Suppl 1997;423:20-7.
18. Henneberg M, Louw GJ. Average menarcheal age of higher socioeconomic status urban Cape coloured girls assessed by means of status quo and recall methods. Am J Phys Anthropol 1995;96(1):1-5.
19. Koo MM, Rohan TE. Accuracy of short-term recall of age at menarche. Ann Hum Biol 1997;24(1):61-4.
20. Indian Council of Medical Research. Indian average population. Growth and physical development of Indian infants and children. Technical report Series 1989(18).
21. Bielicky T, Hulanicka B. Secular trend in stature and age at menarche in Poland. In: Bodzsar EB, Susanne C, editors. Secular growth change in Europe. Budapest: Eötvös University Press; 1998. p. 263-280.
22. Bodzsar EB. Secular growth changes in Hungary. In: Bodzsar EB, Susanne C, editors. Secular growth changes in Europe. Budapest: Eötvös University Press; 1998. p. 175-206.
23. Foster DL, Nagatani S. Physiological perspectives on leptin as a regulator of reproduction: role in timing puberty. Biol Reprod 1999;60(2):205-15.
24. Sigurjonsdottir TJ, Hayles AB. Precocious puberty. A report of 96 cases. Am J Dis Child 1968;115(3):309-21.
25. Treloar SA, Martin NG. Age at menarche as a fitness trait: nonadditive genetic variance detected in a large twin sample [see comments]. Am J Hum Genet 1990;47(1):137-48.
26. Lindgren G. Secular growth changes in Sweden. In: Bodzsar EB, Susanne C, editors. Secular growth changes in Europe. Budapest: Eötvös University Press; 1998. p. 319-334.
27. Roche AF. Secular trends in human growth, maturation, and development. Monogr Soc Res Child Dev 1979;44(3-4):1-120.
28. Mazur W. Phytoestrogen content in foods. Baillieres Clin Endocrinol Metab 1998;12(4):729-42.
29. Tou JC, Chen J, Thompson LU. Flaxseed and its lignan precursor, secoisolariciresinol diglycoside, affect pregnancy outcome and reproductive development in rats. J Nutr 1998;128(11):1861-8.
30. Klein KO. Isoflavones, soy-based infant formulas, and relevance to endocrine function. Nutr Rev 1998;56(7):193-204.
31. Ingram D, Sanders K, Kolybaba M, Lopez D. Case-control study of phyto-oestrogens and breast cancer [see comments]. Lancet 1997;350(9083):990-4.

32. Rockhill B, Moorman PG, Newman B. Age at menarche, time to regular cycling, and breast cancer (North Carolina, United States). Cancer Causes Control 1998;9(4):447-53.
33. Herman-Giddens ME, Slora EJ, Wasserman RC, et al. Secondary sexual characteristics and menses in young girls seen in office practice: A study from the pediatric research in office settings network. Pediatrics. 1997;99:505-512.
34. Proos LA, Hofvander Y, Tuvemo T. Menarcheal age and growth pattern of Indian girls adopted in Sweden I menarcheal age. Acta Paediatr Scand 1991;80:852-858.
35. Oostdijk W, Yap YN, Doude van Troostwijk ML, et al. Final height and timing of puberty of adopted children in The Netherlands. Horm Res 1991;35(2):24.
36. Persson I, Ahlsson F, Ewald U, et al. Influence of perinatal factors on the onset of puberty in boys and girls: implications for interpretation of link with risk of long term diseases. Am J Epidemiol 1999;150(7):747-55.
37. Meyer F, Moisan J, Marcoux D, Bouchard C. Dietary and physical determinants of menarche [see comments]. Epidemiology 1990;1(5):377-81.
38. Maclure M, Travis LB, Willett W, MacMahon B. A prospective cohort study of nutrient intake and age at menarche. Am J Clin Nutr 1991;54(4):649-56.
39. Kiess W, Reich A, Meyer K, et al. A Role for Leptin in Sexual Maturation and Puberty? Horm Res 1999;51(Suppl S3):55-63.
40. Morrison JA, Barton B, Biro FM, Sprecher DL, Falkner F, Obarzanek E. Sexual maturation and obesity in 9- and 10-year-old black and white girls: the National Heart, Lung, and Blood Institute Growth and Health Study. J Pediatr 1994;124(6):889-95.
41. Kaplowitz PB, Oberfield SE. Reexamination of the age limit for defining when puberty is precocious in girls in the United States: implications for evaluation and treatment. Drug and Therapeutics and Executive Committees of the Lawson Wilkins Pediatric Endocrine Society. Pediatrics 1999;104(4 Pt 1):936-41.
42. Fredriks AM, van Buuren S, Wit JM, Verloove-Vanhorick SP. Body mass index measurements in 1996-7 compared with 1980. Arch Dis Child 2000;82(2):107-12.

Chapter 3:
Pituitary and gonadal responsiveness in 2 different Gonadotrophin Releasing Hormone (GnRH) stimulation tests in girls with precocious or early puberty
submitted

Chapter 4
Auxological and biochemical evaluation of pubertal suppression with the GnRH agonist Leuprolide acetate in early and precocious puberty

Chapter 5: Final height after CPP
5.1 Final height after Gonadotrophin Releasing Hormone agonist treatment for central precocious puberty: the Dutch experience
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Chapter 3

Pituitary and gonadal responsiveness in 2 different Gonadotrophin Releasing Hormone (GnRH) stimulation tests in girls with precocious or early puberty.

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ABSTRACT

To test the activity of the hypothalamo-pituitary-gonadal (HPG) axis in girls with clinical signs of puberty a stimulation test can be performed with native Gonadotropin Releasing Hormone (GnRH) or with GnRH agonists (GnRHa). It has been suggested that GnRHa testing with leuprolide acetate is a better tool than GnRH testing for the early diagnosis of pubertal disorders. We compared the response to native GnRH and GnRHa leuprolide acetate (0,5 mg. s.c.) in 65 girls with early or precocious puberty. The correlations between the gonadotropin peak values in both tests were 0.87 for LH and 0.80 for FSH. Using a cut off level of 10 IU/L for a LH peak as indicative for HPG activation, significantly more girls in Tanner stage 2 could be diagnosed by the GnRHa stimulation test compared to the test with native GnRH. The mean LH/FSH ratio was less than 1.0 after the GnRHa stimulation in girls in Tanner stage 2 and we suggest that this ratio has limited value in the decision with regard to initiating therapy. We conclude that GnRHa stimulation with leuprolide acetate demonstrates HPG axis activation in an early phase and can replace the standard iv GnRH stimulation test.

INTRODUCTION

Several strategies have been proposed to assess central activation of the pituitary in children with a history of pubertal development at a young age or in other disorders of puberty (1). Stimulation tests with Gonadotropin Releasing Hormone (GnRH) all use the increased reactivity of the pituitary to hypothalamic GnRH as indication of central puberty (2-4). The luteinizing hormone (LH) response to GnRH is biphasic: first there is depletion of stores of gonadotrophins in the pituitary, and secondly the newly synthesised pool is released (5). The LH response to intravenously administered native GnRH seems to represent the readily releasable pool of pituitary LH, while the newly synthesised pool of LH may cause the response to GnRH agonist (GnRHa) (6-8). To release LH and follicle stimulating hormone (FSH) sufficiently, pituitary maturation is required. Prior exposure to GnRH is necessary for sufficient amounts of quickly releasable LH. The other aspect of maturation is the ability of the cells to produce de novo gonadotropins (5). Agonists of GnRH are able to produce larger LH peaks in comparison to native GnRH (6, 7): in rats this peak is 50 - 100 times greater (8, 9). One might therefore expect that in children with recent onset of puberty GnRH agonists are more capable in demonstrating pituitary activation than native GnRH. This may allow a diagnosis of central precocious puberty (CPP) in an earlier phase.

Ibanez *et al.* demonstrated that pubertal LH peaks (> 8 IU/L) after GnRH agonist leuprolide acetate administration correlated with progression of puberty during a mean follow-up period of 12.9 months (6). The authors suggested that for the early diagnosis of pubertal disorders GnRHa testing with leuprolide acetate is a better tool than GnRH testing.

In the present study the usefulness of the GnRHa test is further investigated: we compared the response of LH, FSH and estradiol (E2) following administration of native GnRH or GnRH-agonist leuprolide acetate. Special emphasis is put on patients in which pubertal development has progressed only to Tanner stage 2, as in our experience the standard test with native GnRH was often found to be non-discriminatory. We hypothesise that the stimulation test with GnRH-a leuprolide acetate would be able to demonstrate hypothalamo-pituitary-gonadal (HPG) axis activation in an earlier phase of puberty than after stimulation with native GnRH. Furthermore, in case both tests are comparable, the GnRHa stimulation test is still less invasive and less time consuming and may therefore be cost-effective.

PATIENTS AND METHODS

In all girls with clinical signs of puberty, consecutively referred to our endocrinology unit between 1995 and summer 1999, a standard GnRH stimulation test with 100 micrograms GnRH intravenously (Relefact LH-RH, Hoechst AG, Frankfurt am Main) was performed. Blood samples for LH, FSH and E2 were taken at baseline and for LH and FSH at 30 and 60 minutes. After a week (range 5 - 10 days) a GnRHa stimulation test was performed with 500 micrograms leuprolide acetate (5 mg/ml preparation, 0.1 ml), given s.c. After 3 hours one single blood sample was taken and LH, FSH and E2 levels were measured. Laboratory assessments for LH and FSH were performed by monoclonal specific immuno radiometric assay (IRMA) (BioSource Europe SA, Nivelles, Belgium), sensitivity 0.2 and 0.1 mIU/ml respectively. E2 was assessed by coated tube radio-immuno assay (RIA; Orion Diagnostica, Espoo, Finland), sensitivity 20 pmol/L. We calculated the LH peak/FSH peak ratio as a variable as this ratio was described previously to distinguish prepubertal from pubertal children based on the sharp rise in LH in early pubertal girls (4, 10-12).

As the best available standard to indicate pubertal activity we used an a priori cut off peak of LH in IRMA of 10 IU/L in the standard GnRH stimulation test (13). In some children both tests were performed more than once. This was done when both tests resulted in a

prepubertal response while clinical appearance suggested puberty. Then, pubertal development was monitored for 3-6 months and the child was retested.

Values in the standard GnRH stimulation test and GnRHa test were non-parametrically tested with the Wilcoxon signed rank test for paired values. The Mann-Whitney test was used to compare between groups. For bivariate correlations we used Spearman rho, as the distribution of the parameters was not normal. As the data were paired we used the McNemar test to compare the fractions of pairs that were discordant in LH peak after either standard GnRH or GnRH-a leuprolide-acetate. A significance level of < 0.05 was indicative for statistical significance.

Patients were included based on their first clinical presentation, and divided in 2 subgroups: group 1: precocious puberty: pubertal development (according to Tanner $\geq$ B2 in girls before the age of 8 years); group 2: early puberty: pubertal development before the age of 10). In table 2 some clinical data on a subgroup of patients is presented. The description of further diagnostic tests is beyond the scope of this article.

It was decided to treat patients when auxological or psychosocial indications were present and when the peak value of LH in the GnRH test was > 10 IU/L. When the LH peak in the GnRHa stimulation test was > 10 IU/L progression of puberty was probable based on data in the literature (6), and in that case we regarded it unethical to postpone GnRH agonist treatment.

Results

Central activation: LH and FSH

Seventy-seven pairs of tests were performed in 65 girls (44 presenting with Tanner stage 2, 31 with Tanner stage 3 and 2 with Tanner stage 4). Fifty-six tests were performed in group 1 (22 with organic causes of precocious puberty) and 21 in group 2 (4 with organic causes of early puberty). No significant differences were present for mean LH, FSH and E2 values and LH/FSH ratio's in either test comparing children with or without organic reasons that could explain the early or precocious onset of puberty. No significant differences between the mean values of LH and FSH peaks with or without the repeated tests were observed. Therefore each test was used as independent test.

Peak values of LH and FSH obtained in the GnRH stimulation test significantly correlated with values obtained after GnRHa stimulation: Spearman rho was 0.87 for LH and 0.80 for FSH (both $p < 0.001$). In group 1 these correlations were 0.89 and 0.83, and in group 2: 0.83 and 0.76 respectively (all $p < 0.001$). In figure 1a mean LH and FSH peak values and in figure 1b the LH/FSH ratios are shown for Tanner stage 2 and 3.

Girls with Tanner stage 2 (n=44) were studied in more detail. Peak values for LH and FSH in both tests are plotted against each other in figure 2. In all children with prepubertal LH peaks in both tests the LH/FSH ratios were < 1. In Tanner stage 2 we found values of the LH/FSH ratio below and above 1.0, ranging from 0.15 to 10.3 in the GnRH test (median: 0.67) and from 0.11 to 8.9 in the GnRHa stimulation test (median: 0.61). The number of Tanner 2 girls with LH peak > 10 in the GnRH test (n=14) and LH/FSH ratio < 1.0 is two.

Table 1: Summary of results of 44 pairs of GnRH and GnRHa stimulation tests in girls in Tanner stage 2; the number of patients is given.

	LH peak in GnRH test > 10 IU/L	*LH peak in GnRH test < 10 IU/L*
LH peak in GnRHa test > 10 IU/L	13	8
LH peak in GnRHa test < 10 IU/L	1	22

Figure 1a: FSH- and LH -peak values in 2 different GnRH stimulation tests; * = p < 0.001

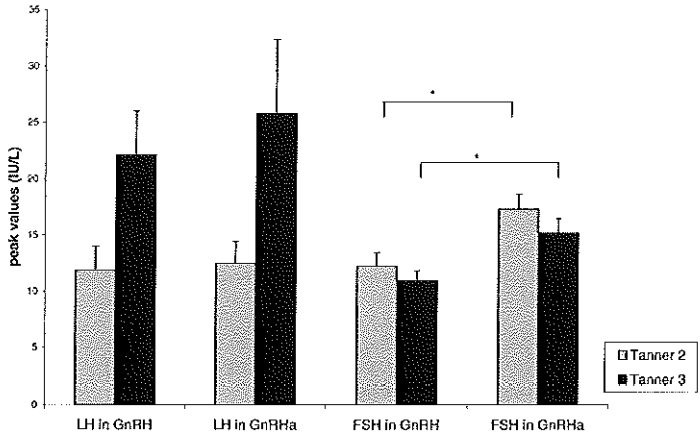
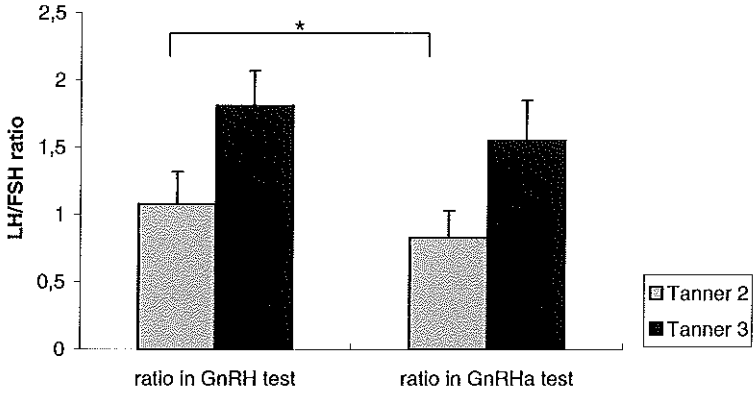
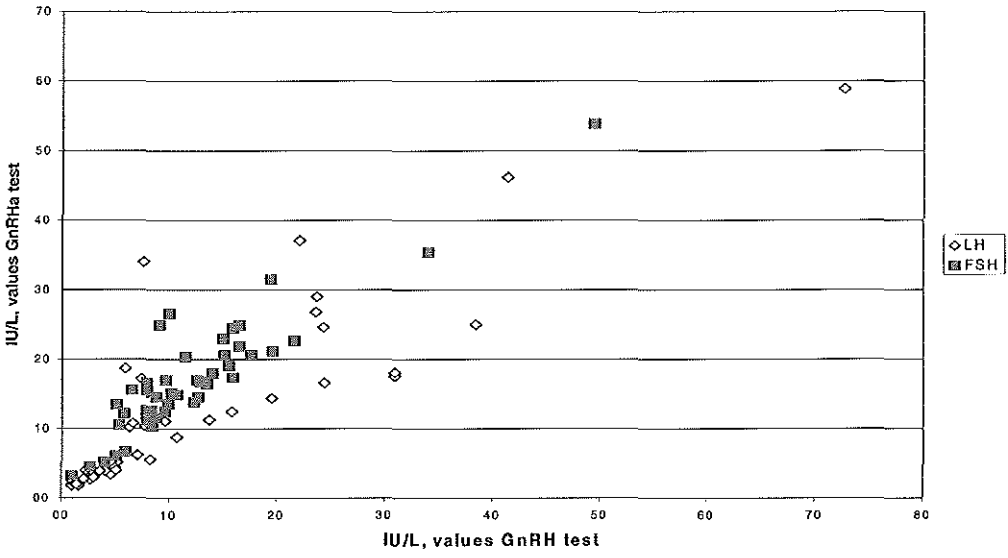


Figure 1 b: Peak LH / peak FSH ratio in girls after GnRH or GnRHa challenge according to Tanner stage; * p = 0.001;



When children were divided in 2 groups according to the LH peak in the GnRH stimulation test, the McNemar test for paired observations showed a significant difference ($p<0.02$) between the fractions that are discordant for results of the test (8/30 and 1/13 respectively) (table 1).

Figure 2: Scatterplot of peak LH and peak FS values in GnRH test vs GnRHa test; Spearman rho : 0.87 for LH and 0.80 for FSH (both $p < 0.001$).



The children with discordant responses in the 2 tests were studied in more detail. Those with a pubertal response in the GnRHa test and no pubertal response in the standard test (n=8) are summarised in table 2. We added clinical signs of HPG axis activation in this table. In these 8 girls, the patients with organic reasons for CPP (nrs 1, 2 and 6) all had LH peak values just above the cut off value. Mean E2 values in the 22 girls with 2 LH peaks < 10 IU/L ranged from < 20 to 86 pmol/L (mean: 31.7 pmol/L) in the GnRH stimulation test and from < 20 to 109 pmol/L (mean 43.8 pmol/L) in the GnRHa stimulation test.

Table 2: Individual data of 8 patients with LH > 10 IU/L in GnRHa test and LH < 10 IU/L in GnRH stimulation test.

Nr	Diagnosis	Age	GnRH test			GnRHa test			Clinical evidence*
			LH peak (IU/L)	**E2 (pmol/L)**	**LH/FSH ratio**	**LH peak (IU/L)**	**E2 (pmol/L)**	**LH/FSH ratio**	
1	Braintumor	3.5	6.3	80	0.72	10.2	123	0.88	A
2	MMC	9.7	6.6	23	0.62	10.8	111	0.72	A
3	ICPP	8.9	7.6	190	0.76	34.0	181	1.28	B,C
4	ICPP	7.7	5.9	57	0.75	18.7	103	1.13	B,C
5	ICPP	4.6	7.7	33	0.16	10.4	100	0.19	B
6	Cranio	7.0	9.6	28	0.74	11.1	44	0.66	C
7	ICPP	6.6	8.0	22	0.48	15.2	19	0.61	B
8	ICPP	8.8	7.4	70	0.81	17.2	160	0.69	A

*for presence of HPG axis activation: A: pubertal E2 levels > 50 pmol/L (ref: (26); B: advanced (≥ 2 yr) bone age; C: pubertal ultrasound uterus. ICPP = idiopathic central precocious puberty; MMC = meningo myelocele; cranio = craniopharyngeoma

Compared to these girls, the 8 patients presented in table 3 had significantly higher E2, LH peak and LH/FSH ratio values in both tests and higher FSH peak levels in the GnRHa test (all $p < 0.05$, for LH peaks: $p<0.001$). The only girl (age: 9 years) with a pubertal LH peak (10.7 IU/L) in the standard GnRH test but not in the GnRHa stimulation test (8.8 IU/L) had an advanced bone age, low E2 levels (< 20 pmol/L in both tests) and ratio's below 1 in both tests.

Table 3: Follow-up data of girls without pubertal LH peak in both stimulation tests

Category	**Number**
Gonadotropin independent PP	6[#]
Progression with central activation within 6 months	5
Slow progression without central activation within 6 months	7
Other	2
No data on progression available	2
Total	22

#: ovarian cysts (n=4), obesity (n=1), possibly McCune-Albright syndrome (n=1)

The LH/FSH ratio was < 1.0 in 4 of 14 girls with a LH peak > 10 IU/L in the GnRH stimulation test and in 8 out of 21 girls with a LH peak > 10 IU/L in the GnRHa stimulation test.

Gonadal activation

In girls in Tanner stage 2 E2 values were studied in both tests. Median basal value was 35.0 (range 6.60-190) pmol/L in the standard test. Mean (SD) E2 in the GnRHa test, 3 hours after GnRHa administration, was 72.1 (50.7) pmol/L.

Spearman correlations between LH peak and E2 serum levels in the group girls with Tanner stage 2 were 0.49 in the standard GnRH test and 0.66 in the GnRHa stimulation test (both $p< 0.01$). For girls in Tanner stage 3 these correlations were 0.40 ($p<0.05$) and 0.56 ($p<0.01$) respectively. In the group of girls with ' pubertal' LH peaks after GnRHa (n=21) only 3 had E2 levels below 50 pmol/L, indicating that already 3 hours after a GnRHa stimulus the E2 production is clearly activated.

No activation of HPG axis (LH peak < 10 IU/L) in either test was seen in 29 pairs of tests, referring to 22 children (table 3). Five of these children demonstrated HPG axis activation during follow-up within 6 months when the tests were repeated.

Discussion

The decision to start a dignostic procedure in children with precocious or early puberty is related to the age of the normal occurence of sexual characteristics in the population. In The Netherlands the age at onset of puberty in girls did not decrease dramatically over the last 20 years (15, 16). Therefore, the adaptation of the age limit for the definition of precocious puberty recommended in the USA is not applicable for the Dutch population (17). Then, one would be certain about further progression of puberty in the near future in order to prevent unnecessary treatment. In some children there is rapid progression of sexual development, while in others there is only slow progression without marked acceleration of bone maturation and without decrease in final height, as Palmert *et al.* showed (14). The children with slowly progressive puberty all had low LH peaks in a GnRH stimulation test. The authors state that slowly progressive puberty in girls does not warrant therapy and it was advised to monitor girls with FSH predominant response in GnRH stimulation test and moderate bone age advancement for at least 6 months before initiation of therapy. It would be helpful when a single assessment of gonadotropins in serum could discriminate between prepubertal and pubertal girls. Recently, a sensitive immunofluorometric assay (IFMA) for basal LH and FSH was used for the diagnosis of CPP, but this method was able to diagnose CPP in only 62.7 % of the girls and has a limited availability in clinical practice (18).

Thus, stimulation tests that challenge the HPG axis are still to be used to discriminate between prepubertal and pubertal girls. The classical test is performed with an iv. bolus of GnRH. Alternatively, agonists of GnRH could be applicable. The potent GnRH agonist leuprolide acetate was shown to be able to distinguish children with progression of puberty from those without (6). Apparently, the GnRH agonist is able to induce de novo synthesis of gonadotropins in the pituitary indicating the onset of puberty (19). Therefore, in our study we used the same GnRH agonist and used an even higher LH peak as cut-off to be sure that all children with a LH peak > 10 IU/L in the GnRHa stimulation test were indeed prone to progression of puberty. Comparing the two tests high correlations were found between the peak values of LH and FSH without differences between early or precocious puberty.

We put emphasis on the subgroup of patients with early pubertal development (Tanner stage 2). In clinical practice we have often seen that the standard GnRH stimulation test does not substantiate central HPG axis activation: Sometimes there is hardly any rise in LH, FSH levels are higher than LH levels and the LH/FSH ratio shows a prepubertal pattern, although

puberty progresses in the following months. We have shown that application of the GnRHa stimulation test results in a considerable reduction of the number of these 'prepubertal responders' (8/30) now demonstrating the HPG-axis activation. This phenomenon was observed despite the highly significant correlations of the LH and FSH peak values obtained in the standard GnRH test and after GnRHa stimulation. The lower potency of natural GnRH compared to leuprolide acetate may explain the prepubertal- like response (20). It is not clear from the literature why GnRH agonist administration stimulates the de novo gonadotropin synthesis. A possible explanation is the longer duration of stimulation, due to the subcutaneous route of administration and sustained delivery to the circulation (2, 20).

The data of Roger *et al.* in normal girls (5) suggest that the ratio LH/FSH is not consistently > 1 in healthy girls that have reached Tanner stage 2. We and others (21) showed the same for girls with early or precocious puberty. The use of the LH/FSH ratio with a cut-off of 1.0 to demonstrate pubertal onset in Tanner stage 2 might be of questionable value as we show ratio's ranging from values far below 1.0 up to 10 in both tests. Furthermore, in the GnRHa test, girls in Tanner stage 2 showed a mean value for the LH/FSH ratio below 1.0. Detailed analysis of the girls in Tanner stage 2 showed that the combination of a LH/FSH ratio > 1 and a pubertal LH peak was more frequently present in the GnRH stimulation test than in the GnRHa stimulation test. Thus, the usefulness of the LH/FSH ratio in distinguishing prepubertal from pubertal girls is debatable, and possibly limited to the GnRH stimulation test. This is a remarkable finding, indicating that GnRHa is able to induce an increase of both FSH and LH in recently started puberty. It is in line with the observations that FSH release after GnRHa stimulation is more gradual and has not the biphasic pattern as LH release. Therefore FSH peaks will be demonstrated on a later moment than LH peak (2, 22, 23).

In patients without pubertal LH peak in either test, the decision not to treat was justified by the clinical course. No rapid progression was observed in these patients. In this study, the decision to treat patients was not used as a validation for the test under investigation. We already applied the results of earlier findings on the use of the GnRHa stimulation test (6) in our clinical practice. Thus, we make no recommendations regarding the predictive value of the GnRHa stimulation test for progression of puberty, because the study design does not allow a statement on this.

We show that GnRHa induces E2 production even 3 hours after administration. An early rise in serum E2, described in adult females, was not observed by Rosenfield after nafarelin administration in girls with CPP (24). The normal response pattern of early pubertal ovaries to gonadotropin exposure is not known. One might speculate that the first LH pool could be responsible for the short term response. The maximum E2 level is probably not reached after 3 hours as it was shown that the time lag between LH and E2 was between 6 and 9 hours due to the time required for aromatization (25).

Based on our findings, we advise to test children with clinical development of secondary sexual characteristics with 500 microgram of leuprolide acetate s.c. and serum sampling after 3 hours for LH, FSH and E2. The test has to be repeated after 3-6 months in case HPG axis activation can not be demonstrated. The benefit of our advice for the patients is a less invasive procedure and results in an earlier diagnosis to start the appropriate treatment. Secondly, the test is less time consuming and less expensive.

In conclusion: the GnRHa stimulation test with leuprolide acetate demonstrates HPG axis activation in an early phase of puberty, therefore improving the diagnostic process in children with early or precocious puberty.

REFERENCES

1. Lanes R, Gunczler P, Osuna JA, et al. Effectiveness and limitations of the use of the Gonadotropin-Releasing Hormone agonist Leuprolide Acetate in the diagnosis of delayed puberty in males. Horm Res 1997;48:1-4.
2. Crowley JR.WF, Beitins IZ, Vale W, et al. The biologic activity of a potent analogue of Gonadotropin- Releasing Hormone in normal and hypogonadotropic men. NEJM 1980;302:1052-1057.
3. Eckert KL, Wilson DM, Bachrach LK, et al. A single-sample, subcutaneous gonadotropin-releasing hormone test for central precocious puberty. Pediatrics 1996;97:517-519.
4. Cavallo A, Richards GE, Busey S, Michaels SE. A simplified gonadotrophin-releasing hormone test for precocious puberty. Clin Endocrinol 1995;421:641-646.
5. Roger M, Lahlou N, Lindner D, Chaussain JL. Gonadotropin-Releasing Hormone testing in pediatrics. In: Ranke MB, editor. Functional endocrinologic diagnostics in children and adolescents. Mannheim: J&J Verlag; 1992. p. 229-247.
6. Ibanez L, Potau N, Zampolli M, et al. Use of Leuprolide acetate response patterns in the early diagnosis of pubertal disorders: comparison with the Gonadotropin Releasing Hormone test. J Clin Endocrinol Metab 1994;78:30-35.
7. Rosenfield RL, Perovic N, Ehrmann DA, Barnes RB. Acute hormonal responses to the Gonadotropin Releasing Hormone Agonist Leuprolide: dose-response studies and comparison to Nafarelin - A clinical research center study. J Clin Endocrinol Metab 1996;81:3408-3411.
8. Aubert L, Kreuter R, Sizonenko PC. Mechanisms of action of GnRH analogues in sexual maturation and function. In: Vickery BH, Lunenfeld B, editors. Precocious puberty, contraception and safety issues. Dordrecht: Kluwer Academic Publishers; 1990. p. 23-39.
9. Yeo T, Grossman A, Belchetz P, Besser GM. Response of luteinizing hormone from columns of dispersed rat pituitary cells to a highly potent analogue of luteinizing hormone releasing hormone. J Endocrinol 1981;91(1):33-41.
10. Partsch CJ, Hümmelink R, Lorenzen F, Sippell WG, Studiengruppe dD-D. Bedeutung und Charakteristika des LHRH-Testes in der Diagnostik der vorzeitigen Pubertatsentwicklung bei Madchen: Der stimulierte LH/FSH-Quotient differenziert zwischen zentraler Pubertas praecox und pramaturer Thelarche. Monatssch Kinderheilkd 1989;137:284-288.
11. Oerter KE, Uriarte MM, Rose SR, Barnes KM, Cutler Jr GB. Gonadotropin secretory dynamics during puberty in normal girls and boys. J Clin Endocrinol Metab 1990;71:1251-1258.
12. Dickerman Z, Prager-Lewis R, Laron Z. Response of plasma LH and FSH to synthetic LH-RH in children at various pubertal stages. Am J Dis Child 1976;130(6):634-8.
13. Cavallo A, Zhou XH. LHRH test in the assessment of puberty in normal children. Horm Res 1994;41:10-15.
14. Palmert MR, Malin HV, Boepple PA. Unsustained or slowly progressive puberty in young girls: initial presentation and long-term follow-up of 20 untreated patients. J Clin Endocrinol Metab 1999;84:415-423.
15. Roede MJ, Wieringen JC van. Growth diagrams 1980. Netherlands third nation-wide survey. Tijdschr Soc Gezondheidszorg 1985;63 (suppl.):11-32.
16. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955-1997 [In Process Citation]. Pediatr Res 2000;47(3):316-23.
17. Kaplowitz PB, Oberfield SE. Reexamination of the age limit for defining when puberty is precocious in girls in the United States: implications for evaluation and treatment. Drug and Therapeutics and Executive Committees of the Lawson Wilkins Pediatric Endocrine Society. Pediatrics 1999;104(4 Pt 1):936-41.
18. Brito VN, Batista MC, Borges MF, et al. Diagnostic value of fluorometric assays in the evaluation of precocious puberty. J Clin Endocrinol Metab 1999;84(10):3539-44.
19. Potau N, Ibanez L, Sentis M, Carrascosa A. Sexual dimorphism in the maturation of the pituitary-gonadal axis, assessed by GnRH agonist challenge. Eur J Endocrinol 1999;141(1):27-34.
20. Handelsman DJ, Swerdloff RS. Pharmacokinetics of gonadotropin-releasing hormone and its analogs. Endocr Rev 1986;7(1):95-105.
21. Pescovitz OH, Hench KD, Barnes KM, Loriaux DL, Cutler GB, Jr. Premature thelarche and central precocious puberty: the relationship between clinical presentation and the gonadotropin response to luteinizing hormone-releasing hormone. J Clin Endocrinol Metab 1988;67(3):474-9.
22. Bremner WJ, Paulsen CA. Two pools of Luteinizing Hormone in the human pituitary: evidence from constant administration of Luteinizing Hormone- Releasing Hormone. J Clin Endocrinol Metab 1974;39:811-815.
23. Kastin AJ, Schally AV, Gual C, Arimura A. Release of LH and FSH after administration of synthetic LH-releasing hormone. J Clin Endocrinol Metab 1972;34(4):753-6.

24. Rosenfield RL, Garibaldi LR, Moll GW, Watson AC, Burstein S. The rapid ovarian secretory response to pituitary stimulation by the Gonadotropin-Releasing Hormone Agonist Nafarelin in sexual precocity. J Clin Endocrinol Metab 1986;63:1386-1389.
25. Goji K. Twenty-four-hour concentration profiles of gonadotropin and estradiol (E2) in prepubertal and early pubertal girls: the diurnal rise of E2 is opposite the nocturnal rise of gonadotropin. J Clin Endocrinol Metab 1993;77(6):1629-35.
26. Mul D, de Muinck Keizer-Schrama SM, Oostdijk W, Drop SL. Auxological and Biochemical Evaluation of Pubertal Suppression with the GnRH Agonist Leuprolide Acetate in Early and Precocious Puberty. Horm Res 1999;51(6):270-276.

II EARLY AND PRECOCIOUS PUBERTY

Chapter 3:

Pituitary and gonadal responsiveness in 2 different Gonadotrophin Releasing Hormone (GnRH) stimulation tests in girls with precocious or early puberty

Chapter 4

Auxological and biochemical evaluation of pubertal suppression with the GnRH agonist Leuprolide acetate in early and precocious puberty

Horm Res 1999;51:270-276

Chapter 5: Final height after CPP

5.1 Final height after Gonadotrophin Releasing Hormone agonist treatment for central precocious puberty: the Dutch experience

5.2 Effect of Gonadotrophin Releasing Hormone agonist (GnRHa) treatment in boys with central precocious puberty: Final height results

Auxological and biochemical evaluation of pubertal suppression with the GnRH agonist leuprolide acetate in early and precocious puberty

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ABSTRACT

We studied the auxological effects of treatment with the GnRH agonist leuprolide acetate (Lucrin ®) at 3.75 mg/28 days in 38 children with early or precocious puberty. We present our newly developed scoring system, the Puberty Suppression Score (PSS), in which clinical and biochemical parameters determine whether suppression was effective. Leuprolide acetate suppressed pubertal development in the majority of cases. During treatment there was a significant correlation between the number of times that PSS was 10 and gain in predicted adult height (PAH) compared to initial prediction at the start of treatment. After 6 months of treatment, ineffective suppression measured by PSS was associated with the magnitude of gain in PAH. We conclude that a leuprolide acetate dosage of 3.75 mg every 28 days effectively suppresses puberty. PSS is helpful in monitoring the suppressive capacity of a GnRH agonist. We recommend to start with leuprolide acetate at 3.75 mg/28 days and to increase the injection frequency or dose in case PSS is 10 after 6 months of treatment.

Introduction

For years, gonadotrophin-releasing hormone (GnRH) agonists have been used as potent therapeutic suppressors of gonadotrophin release from the pituitary (1–6). In children with early or precocious puberty, the depot formulations of GnRH agonists have made this treatment more acceptable. With the use of depot formulations, a large difference in dose per kilogram body weight is observed in daily practice (7). To assess whether this dose is sufficient for a particular child, clinical, auxological and biochemical evaluation of the suppression of pubertal development is demanded. In the recent literature, a suppressed peak level of luteinizing hormone (LH) in a GnRH stimulation test is regarded to be the best parameter to establish pubertal suppression (7–9). Cook *et al.* (10) suggest that overnight monitoring of LH release is more sensitive than GnRH stimulation testing. These authors and others (11, 12) also stressed the importance of clinical regression of pubertal characteristics and decrease in skeletal maturation rate.

So far, no scoring system has been developed which evaluates pubertal suppression during treatment. Such a scoring system should include clinical, auxological and biochemical parameters and should be applicable in clinical practice. Besides evaluation of the ongoing treatment in the individual patient, it may be useful for comparison of different treatment modalities or formulations. In the present study, the pubertal suppression of 38 children with early or precocious puberty is evaluated by a newly developed scoring system, the Puberty Suppression Score (PSS). We evaluate the usefulness and validity of the PSS and discuss possible clinical applications.

Patients and methods

Thirty-eight patients (33 girls and 5 boys) with early or central precocious puberty (CPP) treated with the GnRH agonist leuprolide acetate were studied in a retrospective analysis. Thirty patients had true CPP (signs of puberty before the age of 8 in girls and before the age of 9 in boys). In 8 children, puberty was early (signs of puberty before the age of 10 in girls and before the age of 11 in boys). Leuprolide acetate (Lucrin ®; Abbot, Amstelveen, The Netherlands) was given subcutaneously, 3.75 mg every 28 days, but the interval between the first 3 injections was 14 days. Thirty-eight children received treatment for 18 months, 32 of them for 24 months. Height was measured by a Harpenden stadiometer in an outpatient clinic. Height and body mass index (BMI) were compared to Dutch standards (13), and height standard deviation scores (H-SDS) for chronological age (CA) or bone age (BA) were calculated. LH and follicle-stimulating hormone (FSH) levels were assessed by immunoradiometric assay, estradiol (E2) and testosterone (T) were assessed by radioimmuno assay. At diagnosis, a GnRH stimulation test with 100 µg LHRH intravenously was performed, and LH peak values > 10 IU/l were considered to indicate central activation of the hypothalamic-pituitary-gonadal axis (14). In 13 children, an additional GnRH stimulation test was performed with 500 µg leuprolide acetate subcutaneously and blood sampling 3 hours after injection (15). During treatment, LH peak levels >5 IU/l (7) in a GnRH stimulation test and basal E2 levels >50 pmol/l or T levels >1.0 nmol/l were considered to indicate effective suppression. BA was assessed by one observer with the method described by Greulich and Pyle (16). Prediction of adult height (PAH) was calculated with the Bayley-Pinneau tables for average girls or boys (17). Skeletal maturation was expressed as $\Delta BA/\Delta CA$.

To evaluate pubertal suppression by the GnRH agonist, the PSS was developed with 4 parameters: Tanner stage progression, growth, bone maturation and sex steroid level (table 1). These parameters were chosen because they reflect the major endocrine activity in CPP.

For each parameter, the score is 0 in case of effective suppression, while the score is 1 in case of ineffective suppression. The Tanner stage criterion reflects the clinical appearance of a child under treatment. In case of progression of Tanner B or G stage of more than 1 compared to the start of treatment, suppression is clearly not effective.

Table 1. Puberty Suppression Score

Parameter	Suppression effective	Score	Suppression not effective	score
Tanner stage	≤ 1 stage progression compared to start of treatment	0	> 1 stage progression compared to start of treatment	1
Growth	ΔH-SDS$_{ca}$ ≤ 0.25 / 6 months	0	ΔH-SDS$_{ca}$ > 0.25 / 6 months	1
ΔBA / ΔCA	≤ 1	0	> 1	1
Sex steroids	E2 ≤ 50 pmol/l; T ≤ 1.0 nmol/l	0	E2 > 50 pmol/l; T > 1.0 nmol/l	1
Total PSS		**0**		**1-4**

A progression of 1 stage was considered to be due to inter- or intra-observer variation. In a recent Dutch consensus statement (18), growth retardation or acceleration was defined as a change in height standard deviation score for CA (H-SDSCA) >0.25 SDS in 12 months. To assess accelerated growth during GnRH agonist treatment, we used this increase in H-SDSCA for a period of 6 months. When the increase in H-SDS exceeded 0.25 SD in 6 months, puberty was regarded to be ineffectively suppressed and the score was 1. Bone maturation is retarded during effective treatment (4), and therefore ΔBA/ΔCA is <1. In the PSS, a ratio of >1 is regarded to indicate ineffective suppression and the score is 1. Basal values of sex steroids were considered to be prepubertal when the T level was ≤1.0 nmol/l or the E2 level was ≤ 50 pmol/l. When these basal levels exceeded these limits, suppression was considered to be ineffective and the score was 1. In a child with effective suppression, the PSS is 0 for each parameter. In case of ineffective suppression the PSS ranges from 1 to 4, suggesting a sliding scale of ineffectiveness of suppression.

Statistical analysis was performed using the Student t-test for paired observations. Values are expressed as means (± SD). Simple and multiple regression analysis was used to assess correlations between the PSS and gain in PAH.

Results

Baseline parameters of the start of therapy are summarised in table 2. In 4 patients, LH peak values in a GnRH stimulation test with GnRH were below 10 IU/l (9.6, 6.6, 7.7 and 3.1 IU/L); in 3 of them, LH peak values > 10 IU/l (10.8, 34.0 and 27.9 IU/l, respectively) were shown in the GnRH stimulation test with leuprolide acetate. The fourth one, a boy, had a LH peak value of 9.6 IU/l, but his testes were bilaterally enlarged (10 ml) and his T level was 2.10 nmol/l. In a fifth patient with Tanner stages B4 P4, the E2 level was 188 pmol/l and no GnRH stimulation test was performed.

At baseline, the mean LH peak was 24.9 IU/l (SD: 18.0); after 3 months of treatment, the mean LH peak level had decreased to 1.5 (0.8) IU/l (range < 1.0–4.5 IU/l), $p < 0.001$ compared to baseline. The mean E2 level was 91.1 (65.1) pmol/l at diagnosis; after 6 months, all girls had an E2 level <50 pmol/l, except for 1 girl (89 pmol/l) who had suppressed E2 levels on subsequent visits. H-SDS for CA significantly decreased from 0.79 (1.47) at

start to 0.68 (1.36) ($p < 0.01$). PAH increased from 155.6 (7.19) cm to 161.4 (6.44) cm ($p < 0.001$) at 24 months of treatment.

Table 2. Baseline characteristics (mean ± SD) of the study population

	Girls (n=33)	boys (n=5)
CA, years	8.2 (1.9)	8.7 (2.4)
BA, years	10.3 (2.2)	12.2 (2.5)
Height, cm	134.8 (11.5)	140.5 (13.5)
$H\text{-}SDS_{CA}$	0.72 (1.44)	1.23 (1.76)
$H\text{-}SDS_{BA}$	-1.37 (0.97)	-1.41 (0.28)
Target H-SDS	-0.23 (1.02)	-0.24 (0.87)
PAH, cm	154.4 (6.7)	164.8 (2.9)
Basal LH level IU/L	1.7 (1.00)	2.6 (1.9)
Peak LH, IU/L	27.1 (18.8)	13.4 (2.4)
Basal E2, pmol/L	91.1 (65.1)	-
Basal T, nmol/L	-	3.25 (1.95)

Table 3. Auxological data during treatment

	treatment period (months)			
	0 - 6	6 - 12	12 - 18	18 - 24
ΔBA /ΔCA	0.75 (0.66)	0.56 (0.38)	0.42$^{\#}$ (0.34)	0.56 (0.31)
ΔH-SDS / 6 mo	-0.02 (0.20)	-0.06 (0.15)	-0.12 (0.13)	-0.10 (0.13)
HV (cm/yr)	5.9 (2.2)	5.3$^{\#}$ (1.8)	4.6‡ (1.6)	5.0* (1.7)

HV = height velocity; * $p = 0.05$ compared to 0-6 months; $^{\#}$ $p < 0.05$ compared to 0-6 months
‡ $p < 0.01$ compared to 0-6 months

The dose of leuprolide acetate decreased significantly ($p < 0.001$) from 111.3 (31.5) µg/kg (range: 59.6–202.7 µg/kg) between 0 and 6 months to 92.4 (24.1) µg/kg (range 46.5–167.4 µg/kg) between 18 and 24 months of treatment because of gradual increase in body weight. The BMI SDS significantly increased during treatment ($p < 0.01$) from 1.13 (1.11) to 1.39 (1.11). In table 3 height velocity, skeletal maturation and growth during treatment are shown. To evaluate pubertal suppression, the PSS was calculated after 6, 12, 18 and 24 months of treatment. The results are given in table 4. In 6 children, the PSS was 10 on more than one occasion during treatment, and 13 patients showed signs of ineffective suppression at any moment. In children with a PSS > 0, the data of the GnRH stimulation test at 3 months were reviewed. None of these children had LH peak values indicating insufficient suppression: mean LH peak value 1.48 (0.65) IU/l (range: 1.0–2.8 IU/l).

Because of signs of escape from treatment, the dose of leuprolide acetate was increased to 7.5 mg/28 days in 1 girl. Her PSS score was >0 in the period of escape because of accelerated skeletal maturation and an increased E2 level. In another child, at 22 months

of treatment, the frequency of injections was increased to 1x/3 weeks because of alterations in behaviour at the end of each 4-week interval. The PSS was not >0 during the interval studied. In none of these two girls progression of Tanner stage >1 was observed. In the remaining 11 children with a PSS >0, ineffective suppression escaped clinical attention and no dose adjustments were made.

Table 4. Number of patients with PSS > 0 (ineffective suppression) during treatment with leuprolide acetate

	treatment period (months)			
	6	12	18	24
Tanner stage progression > 1	0/38	0/38	0/38	0/32
H-SDS increase > 0.25 / 6 months	2/38	0/38	0/35	0/29
ΔBA/ΔCA >1	5/24	4/24	1/25	2/25
sex steroids (E2 > 50 pmol/l orT > 1.0 nmol/l)	2/32	1/38	1/29	2/22
Total PSS > 0	9	5	2	4

Table 5. Correlation coefficients of the correlation between BA at start (BA_0), age at start of treatment (Age_0) or PSS_{cum} and gain in PAH in simple and multiple regression analysis. Figures in parentheses denote p value

	Regression variable	Whole group	Girls alone
Simple regression, 18 months	PSS_{cum}	-0.49 (0.007)	-0.49 (0.01)
Simple regression, 24 months	PSS_{cum}	-0.37 (0.06)	-0.40 (0.05)
Multiple regression, 18 months	BA_0	0.09 (NS)	0.04 (NS)
	Age_0	-0.35 (NS)	-0.34 (NS)
	PSS_{cum}	-0.51 (0.01)	-0.55 (0.01)
Multiple regression, 24 months	BA_0	0.13 (NS)	0.28 (NS)
	Age_0	-0.38 (NS)	-0.34 (NS)
	PSS_{cum}	-0.37 (0.08)	-0.35 (NS)

The cumulative PSS during the treatment period (PSS_{cum}) was calculated by adding the PSS of each 6-month PSS, resulting in PSS_{cum18} or PSS_{cum24} for 18 or 24 months of treatment, respectively.

We studied the relationship between the PSS_{cum} and gain in PAH during treatment (table 5). First, we performed simple regression between PSS_{cum} and gain in PAH at 18 and 24 months of treatment; correlation coefficients are given in table 5, separately for the whole group and for girls alone. Simple regression for the individual parameters in PSS did not show any consistent correlation, while PSS_{cum} – including all these individual parameters together – significantly correlated with gain in PAH at 18 and 24 months of treatment. Second, multiple regression was performed for PSS, corrected for BA and CA at the start of treatment, as it is known that these parameters may influence the magnitude of gain in PAH [3]. PSS was significantly correlated with gain in PAH at 18 months (whole group and girls alone, $p = 0.01$).

At 24 months of treatment, none of the 3 regression variables (BA at start, CA at start or PSS_{cum}) significantly correlated with gain in PAH. Calculations were performed for the association between PSS_{cum} and gain in PAH with and without the PSS of the first 6 months of treatment. Gain in PAH was plotted against increasing PSS_{cum} values during 18 or 24 months of treatment (fig. 1a). After 6 months of treatment, it appeared that there was a significant difference in gain in PAH between children with $PSS_{cum} = 0$ and those with $PSS_{cum} > 0$ ($p < 0.05$).

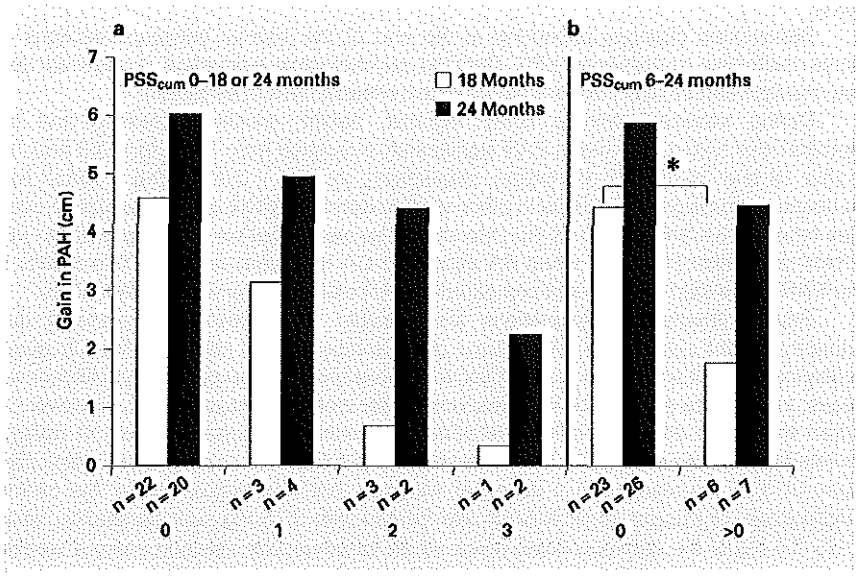

Figure 1. PAH gain during treatment with GnRH agonist: relationship to PSS_{cum18} or PSS_{cum24} (a) and without PSS of the first 6 months (b). * $p < 0.05$

Discussion

It was shown earlier that treatment of CPP with incomplete suppression of the pituitary-gonadal axis leads to disappointing final height results, due to the rapid bone maturation compared to treatment modalities with complete suppression (3, 5, 6, 17, 19–22). We therefore consider complete suppression superior to partial suppression to achieve the auxological goals of treatment of CPP. However, complete suppression leads to very low height velocities in some CPP patients, possibly due to a decrease in GH secretion as a result of the decreased prepubertal E2 levels (23–25).

In order to evaluate to what extent pubertal development can be suppressed by a GnRH agonist, the PSS was developed. This scoring system is based on generally accepted criteria for the evaluation of the effect of treatment and applicability in clinical practice. Although the cut-off values are arbitrarily chosen, we think that the criteria are appropriate to distinguish between effective and ineffective suppression. In this study, we showed that the combination of parameters correlates better with gain in PAH than any parameter alone. It might be questioned why we did not incorporate GnRH stimulation testing during treatment in

this scoring system. The first reason is that the applicability of GnRH testing in daily practice is limited because of the duration of testing and the intravenous route of administration of LHRH. Possibly, testing with leuprolide acetate subcutaneously (15) may be an alternative, but the value of this way of testing during treatment has to be established more extensively. A second reason is that GnRH testing is critically dependent on the moment of testing, while 3 out of 4 parameters in the PSS reflect effects of treatment over a 6-month period.
Thirdly, in this study we showed that it is possible to have a very well suppressed GnRH stimulation test while clinical suppression is clearly ineffective over a longer period. This limits the usefulness of the GnRH stimulation test as a marker of long-term suppression of puberty.

Behavioural changes appearing at the end of the interval between injections may indicate ineffective suppression (26). Detailed psychological evaluation is required before these changes can be used as parameters of the effectiveness of treatment.

During treatment with a GnRH agonist, height velocity decreased and bone maturation was halted. The mean PAH significantly increased from 155.6 to 158.9 and 161.4 cm after 18 and 24 months of therapy, respectively (both $p < 0.001$). The reported height gain based upon height predictions in other studies with the GnRH agonist leuprolide acetate varies between 3.4 and 5.5 cm (8, 9). One should be aware that we used the average tables for PAH calculation [17] even when BA exceeded CA by 1 year or more. This results in a lower PAH at the start of treatment compared to PAH calculated by accelerated tables.

Several theoretical explanations are possible for ineffective suppression during treatment: patient compliance, inadequate dose and/or way of administration. In general, compliance was not a problem, as the injections were given by the general practitioner every 28 days. Inadequate dosage could be responsible for ineffective suppression. However, in all children the mean dose per month exceeded the minimally needed dose (30 µg/kg) as calculated by Tanaka *et al.* ([27). In another study [7], it was also shown that in most children a dose of 3.75 mg resulted in a dose per kilogram that was largely above this minimally required dose. In Europe, most children are treated with 3.75 mg subcutaneously, while in the US 7.5 mg is the common dose, given as an intramuscular injection every 4 weeks. In our study, we increased the dose of leuprolide acetate in only 1 child to 7.5 mg/28 days resulting in better clinical suppression. A third reason for insufficient suppression could be the way of administration. In this study, leuprolide acetate was given subcutaneously, in other studies it was injected intramuscularly (8). No data are available on differences between subcutaneous and intramuscular administration. In our group, 1 child (2.6%) had a sterile abscess. Manasco *et al.* (28) described the occurrence of sterile abscesses in the subcutis during treatment with leuprolide acetate. The described percentages of this problem vary between 3–8% and 13% (7, 8,28).

Our PSS data in this study suggest that a few children treated with the GnRH agonist leuprolide acetate 3.75 mg/28 days have an ineffective suppression measured by the PSS. These patients would not easily be detected without this scoring system because of the subtle changes over a longer period measured with the PSS. The usefulness of the PSS was studied in simple and multiple regression models. In the simple regression model, total PSS during the treatment period was correlated with gain in PAH at 18 and 24 months of treatment. In multiple regression analysis, BA at the start of treatment and CA at start were entered as important covariables for gain in PAH during treatment. The regression coefficient for PSS_{cum} was the most important variable in the equation for the whole group. In girls alone, this was also the case for gain in PAH at 18 months of treatment. It appeared that the combination of parameters in the PSS had a stronger correlation with any gain in PAH than one of the parameters alone. This suggests that the individual parameters can in part be considered as independent, while they are apparently not physiologically unrelated. In the

first 6 months of treatment, the PSS was >0 in a considerable number of children. We therefore calculated the difference in gain in PAH between children with and without ineffective suppression from 6 to 18 or 24 months (fig. 1b) to exclude the possible bias of enhanced skeletal maturation in the first 6 months of treatment. It appeared that there was a significant difference between the groups, indicating that when the PSS is >0 after 6 months of treatment, adaptation of the dose regimen is required. In our group, no clear progression of puberty according to Tanner was detected in any patient during treatment. It could be argued therefore that this parameter has to be left out. We think, however, that because of the clinical relevance of this parameter for the work-up of any patient during treatment with a GnRH agonist it should be included. Especially when Tanner stage assessment is performed by one single observer, it can be useful.

With ineffective suppression, it is unlikely to reach the auxological goals of treatment. Besides, there is a risk of vaginal bleeding and ongoing breast development in girls leading to emotional disturbances. On the other hand, the use of a standard leuprolide acetate dose of 7.5 mg/28 days has obvious financial consequences. With the use of the PSS, children with ineffective treatment can be detected and dosage can be adjusted from 3.75 to 7.5 mg every 28 days or by increasing injection frequency to once in 3 weeks.

We conclude that thorough monitoring of the rate of suppression in children with CPP is essential. The PSS could serve as a useful tool to evaluate pubertal suppression during the treatment period. We advise to start treatment with leuprolide acetate 3.75 mg and to adapt the dose regimen in case of a PSS >0, especially after 6 months of treatment. Long-term studies have to be undertaken to assess the effect of ineffective suppression during treatment on final height gain.

References

1 Lee PA, Page JG, The Leuprolide Study Group: Effects of leuprolide in the treatment of central precocious puberty. J Pediatr 1989;114:321–324.

2 Boepple PA, Mansfield MJ, Crawford JD, Crigler JF, Blizzard RM, Crowley WF: Gonadotrophin-releasing hormone agonist treatment of central precocious puberty: An analysis of growth data in a developmental context. Acta Paediatr Scand 1990;367(suppl):38–43.

3 Oostdijk W, Rikken B, Schreuder S, Otten BJ, Odink RJH, Rouwé C, Jansen M, Gerver WJ, Waelkens JJ, Drop SLS: Final height in central precocious puberty after long-term treatment with a slow-release GnRH agonist. Arch Dis Child 1996;75:292–297.

4 Oostdijk W, Gevers EF, Drop SLS, Rikken B, Hümmelink R, Partsch CJ, Sippell WG: Growth and pubertal development during and after treatment with a slow-release gonadotro-pin-releasing hormone agonist in central precocious puberty. Horm Res 1991;36:121–125.

5 Oostdijk W, Drop SLS, Odink RJH, Hümmelink R, Partsch CJ, Sippell WG: Long-term results with a slow release gonadotrophin-releasing hormone agonist in central precocious puberty. Acta Paediatr Scand 1991;372(suppl): 39–45.

6 Partsch CJ, Hümmelink R, Peter M, Sippel WG, Oostdijk W, Odink RJH, Drop SLS, German-Dutch Precocious Puberty Study Group: Comparison of complete and incomplete suppression of pituitary-gonadal activity in girls with central precocious puberty: Influence on growth and predicted final height. J Clin Endocrinol Metab 1993;39:111–117.

7 Carel JC, Lahlou N, Guazzarotti L, Joubert-Collin M, Roger M, Colle M, The French Leuprorelin Trial Group, Chaussain JL: Treatment of central precocious puberty with depot leuprorelin. Eur J Endocrinol 1995;132:699–704.

8 Neely EK, Hintz RL, Parker B, Bachrach LK, Cohen P, Olney R, Wilson DM: Two-year results of treatment with depot leuprolide acetate for central precocious puberty. J Pediatr 1992;121:634–640.

9 Clemons RD, Kappy MS, Stuart TE, Perelman AH, Hoekstra FT: Long-term effectiveness of depot gonadotropin-releasing hormone analogue in the treatment of children with central precocious puberty. Am J Dis Child 1993;147: 653–657.

10 Cook JS, Doty KL, Conn PM, Hansen JR: Assessment of depot leuprolide acetate dose adequacy for central precocious puberty. J Clin Endocrinol Metab 1992;74:1206–1209.

11 Hümmelink R, Oostdijk W, Partsch CJ, Odink RJH, Drop SLS, Sippell WG: Growth, bone maturation and height prediction after three years of therapy with the slow release GnRH-agonist decapeptyl depot in children with central precocious puberty. Horm Metab Res 1992;24:122–126.
12 Brauner R, Malandry F, Fontoura M, Prevot C, Souberbielle JC, Rappaport R: Idiopathic central precocious puberty in girls as a model of the effects of plasma estradiol level on growth, skeletal maturation and plasma insulin-like factor I. Horm Res 1991;36:116–120.
13 Roede MJ, Wieringen JC van: Growth diagrams 1980. Netherlands third nation-wide survey. Tijdschr Soc Gezondheidszorg 1985; 73(suppl):11–32.
14 Cavallo A, Zhou XH: LHRH test in the assessment of puberty in normal children. Horm Res 1994;41:10–15.
15 Ibanez L, Potau N, Zampolli M, Virdis R, Gussinye M, Carrascosa A, Saenger P, Vicens-Calvet E: Use of leuprolide acetate response patterns in the early diagnosis of pubertal disor-ders: Comparison with the gonadotropin releasing hormone test. J Clin Endocrinol Metab 1994;78:30–35.
16 Greulich WW, Pyle SI: Radiographic Atlas of Skeletal Development of the Hand and Wrist, ed 2. Stanford, Stanford University Press, 1959.
17 Kauli R, Galatzer A, Kornreich L, Lazar L, Pertzelan A, Laron Z: Final height of girls with central precocious puberty, untreated versus treated with cyproteron acetete on GnRH analogue. A comparative study with re-evaluation of predictions by the Bayley-Pinneau method. Horm Res 1997;47:54–61.
18 Rikken B, Waelkens JJJ: Terminology and definitions, in De Muinck Keizer-Schrama SMPF, Boukes FS, Oostdijk W, Rikken B (eds): Diagnostiek kleine lichaamslengte bij kinde-ren. Alphen aan den Rijn, Van Zuiden Communications 1998.
19 Sorgo W, Kiraly E, Hamoki J, Heinze E, Teller WM, Bierich JR, Moeller H, Ranke MB, Butenandt O, Knorr D: The effects of cyproterone acetate on statural growth in children with precocious puberty. Acta Endocrinol (Copenh) 1987;115(1):44–56.
20 Schoevaart CE, Drop SLS, Otten BJ, Slijper FME, Degenhart HJ: Growth analysis to final height and psychosocial adjustment of treated and untreated patients with precocious puberty. Horm Res 1990;34:197–203.
21 Kauli R, Kornreich L, Laron Z: Pubertal development, growth and final height in girls with sexual precocity after therapy with the GnRH analogue D-Trp-6-LHRH. Horm Res 1990;33: 11–17.
22 Antoniazzi F, Cisternino M, Nizzoli G, Bozzola M, Corrias A, DeLuca F, De Sanctis C, Rigon F, Zamboni G, Bernasconi S: Final height in girls with central precocious puberty: Comparison of two different luteinizing hormone-releasing hormone agonist treatments. Acta Paediatr 1994;83:1052–1056.
23 Bourguignon JP: Linear growth as a function of age at onset of puberty and sex steroid dosage: Therapeutic implications. Endocr Rev 1988;9: 467–488.
24 Mansfield MJ, Rudlin CR, Crigler JF, Karol KA, Crawford JD, Boepple PA, Crowley WF: Changes in growth and serum growth hormone and plasma somatomedin-C levels during suppression of gonadal sex steroid secretion in girls with central precocious puberty. J Clin Endocrinol Metab 1988;66:3–9.
25 Ho KKY, O'Sullivan AJ, Weissberger AJ, Kelly JJ: Sex steroid regulation of growth hormone secretion and action. Horm Res 1996;45:67– 73.
26 Paterson WF, McNeill E, Reid S, Hollmann AS, Donaldson MDC: Efficacy of Zoladex LA (goserelin) in the treatment of girls with central precocious or early puberty. Arch Dis Child 1998;79:323–327.
27 Tanaka T, Hibi I, Kato K, Saito S, Shimizu N, Suwa S, Nakajima H, TAP-144-SR CPP Study Group: A dose finding study of a super long-acting luteinizing hormone-releasing hormone analog (Leuprolide acetate depot, TAP-144-SR) in the treatment of central precocious puberty. Endocrinol Jpn 1991;38:369–376.
28 Manasco PK, Pescovitz OH, Blizzard RM: Local reactions to depot leuprolide therapy for central precocious puberty (letter). J Pediatr 1993;123:334–335.

II EARLY AND PRECOCIOUS PUBERTY

Chapter 3:

Pituitary and gonadal responsiveness in 2 different Gonadotrophin Releasing Hormone (GnRH) stimulation tests in girls with precocious or early puberty

Chapter 4

Auxological and biochemical evaluation of pubertal suppression with the GnRH agonist Leuprolide acetate in early and precocious puberty

Chapter 5: Final height after CPP

5.1 Final height after Gonadotrophin Releasing Hormone agonist treatment for central precocious puberty: the Dutch experience
J Ped Endocrinol Metab 2000;13 (suppl 1):765-772

5.2 Effect of Gonadotrophin Releasing Hormone agonist (GnRHa) treatment in boys with central precocious puberty: Final height results

Chapter 5.1

Final height after Gonadotrophin Releasing Hormone agonist treatment for central precocious puberty: the Dutch experience.

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ABSTRACT

Final height (FH) data of 96 children (87 girls) treated with GnRH agonist for central precocious puberty were studied. In girls mean FH exceeded initial height prediction by 7.4 (5.7) cm ($p < 0.001$); FH was significantly lower than target height, but still in the genetic target range. When treatment started < 6 years of age height gain was significantly higher than when started > 8 years of age. Bone age (BA) and chronological age (CA) at start of treatment, as well as BA advance at cessation of treatment were the most important variables influencing height gain in multiple regression analysis. BA advance at start of treatment was most important in simple correlation. In girls, GnRHa treatment seems to restore FH into the target range. A younger age and advanced bone age at start of treatment are associated with more height gain from GnRHa treatment.

INTRODUCTION

When long-acting Gonadotrophin Releasing Hormone agonist (GnRHa) treatment became available for the treatment of children with central precocious puberty (CPP) in the mid-1980s, it was expected that long-term treatment would lead to substantial improvement of compromised final height (FH). It took many years to obtain FH data in children with CPP. Some authors reported on final height in relatively small samples or selected groups of patients, GnRH agonists were administered as daily injections or in nasal spray, or even after a period of treatment with cyproteron acetate.[1-10] Recently, results of larger study groups and of randomised trials have been described[11-14] revealing stronger evidence about the auxological effects of treatment. However, it is not yet clear which type of children will benefit from treatment with GnRH agonists.[15,16] In addition, questions about the appropriate chronological age (CA) or bone age (BA) to discontinue treatment remain unanswered.

The interpretation of analyses that focus on parameters at the start or discontinuation of treatment which interfere with the gain in adult height is difficult for at least three reasons. Firstly, as the parameters used are closely interdependent it is not possible to identify one or more independent factors determining effect. Secondly, in the methods of calculating predicted adult heigh (PAH), the current models are possibly less accurate in CPP.[3] Finally, it is necessary to allow for the effect of regression towards the mean: children with very advanced bone age at start of treatment have an *a priori* chance of ending up with FH in the normal range.[17] Furthermore, the question is how to define effect of treatment, as recent data on the natural course of CPP in untreated patients are not available. We present here results from a nation-wide analysis in The Netherlands of final height data in children with CPP uniformly treated with GnRH agonist triptorelin, providing data on the state of the art in The Netherlands.

PATIENTS & METHODS

In this retrospective multi-centre study the clinical records and X-rays for BA of children treated for CPP were re-examined. The children (n=36) from a former analysis by Oostdijk *et al.*[5] were included in this group. CPP was defined as the onset of symptoms of sexual development before the age of 8 in girls (Tanner stage 2) and before the age of 9 in boys (testicular volume $\geq$ 4 ml), pubertal response of LH in a GnRH stimulation test and pubertal levels of sexsteroids according to the individual hospital laboratories, or advanced BA: BA/CA ratio > 1.0. When available, perinatal data and family history were obtained, as well as the eventual adoption status and MRI findings.

A depot preparation of triptorelin (Decapeptyl CR®) was given IM every 28 days for at least 18 months in a dosage of 3.75 mg. Eight patients were treated with depot preparations after a period of intranasal buserelin or cyproterone acetate. Both idiopathic and organic forms of CPP were included. Height data were gathered at start of treatment with depot triptorelin, at the end of treatment and at final height. Final height was defined as BA of > 15 yr. in girls and >17 in boys, or a growth velocity of less than 1 cm/yr. measured over a 6-month period. Height data were expressed as height standard deviation scores (H-SDS) either for chronological age (H-SDS_{CA}) or for BA ($HSDS_{BA}$). Reference data and equations for calculating target heights (TH) and H-SD scores were derived from the 1980 growth study in The Netherlands.[18]

Suppression of puberty was monitored at 3 – 6 months' intervals and escapes from treatment (for example vaginal blood loss or clinical progression of puberty) were recorded. The decision to stop treatment was based on auxological or psychological factors. After treatment age of menarche was noted. One experienced observer performed bone age assessment with the method of Greulich and Pyle[19], and the average tables of Bayley and Pinneau[20] were used as suggested by Kauli[3] to calculate predicted adult height

Statistics included pairwise comparisons between PAH at start of treatment, PAH at the end of treatment, TH and final height (FH), and univariate correlations and multiple linear regression analysis (backward selection procedure).

Our objective was to identify pre-treatment or post-treatment factors influencing the main outcom parameter which is the gain in height between start of treatment and final height. Because of th objective we only used 5 variables in the multiple regression analysis that could have possib influence on decision making in clinical practice: chronological age at start and stop of treatmer bone age at start and stop of treatment and bone age advance (BA – CA) at the end of treatmer Because of the small number of boys multiple regression was not applied on them. A p-value of 0.05 was considered to indicate statistical significance.

Results

Ninety-six patients, 87 girls and 9 boys, were included in the analysis. Characteristics of the patien before treatment are shown in table 1. One or more signs of escape from suppression were note during reatment in ten girls: pubertal values in oestradiol levels were reported in five girls, four gir had temporary vaginal blood loss after the first 6 months of treatment, and in four girls clinical puber had progressed –in 1 girl due to non-compliance. In case of biochemical proven escape the dos regimen was adapted. No serious side effects were observed, some children complained abo increase in body weight.

Table 1: Patient characteristics before treatment (Rx); (mean ± SD).

	Girls (n=87)	**Boys (n=9)**
CA at start of puberty (yr.)	Median: 7.0 Range: 0.8 – 8.0	Median: 7.75 Range: 4.0 – 9.0
CA at start Rx (yr.)	7.7 (1.25)	8.3 (1.74)
BA at start Rx ('yr.')	10.4 (1.17)	10.9 (1.99)
BA advance (BA-CA) at start Rx (yr.)	2.8 (1.03)	2.7 (1.68)
H-SDS for CA at start Rx	1.54 (1.28)	1.57 (1.96)
H-SDS for BA at start Rx	-1.14 (0.94)	-0.96 (1.12)
Target height SDS	-0.05 (1.08)	-0.37 (0.79)
Peak LH after GnRH stimulation (IU/L)	31.7 (25.4)	21.6 (15.2)
Brain MRI or CT performed - Hamartoma - Neurofibromatosis - Tumour	61 2* 1 2	8 0 2 1
Number of adopted children	13	0
Type of CPP - Idiopathic - Organic	 76 11	 3 6
PAH at start Rx (cm) -average tables B&P -accelerated tables B&P	 155.3 (7.25) 160.6 (7.80)	 171.5 (4.14) 180.0 (8.40)

* both < 6 years of age at start puberty

BA = bone age; CA = chronological age; H-SDS = standard deviation score for height; LH = luteinizin hormone; GnRH = Gonadotrophin Releasing Hormone; CPP = central precocious puberty; PAH = predicte adult height; B&P = Bayley and Pinneau; MRI = magnetic resonance imaging; CT = computerised tomogram.

Mean treatment period was 3.4 (1.31) [mean (SD)] yr in girls and 3.8 (1.33) yr in boys. Bone age advance (BA-CA) was reduced to 1.3 yr in girls and 1.0 yr in boys at discontinuation. Mean period between cessation of treatment and the occurrence of menarche was 1.3 (0.8) yr, range: 0.1 to 4.3 yr. After treatment mean height increase was 9.7 (3.4) cm in girls and 12.9 (5.4) cm in boys. Mean FH-SDS was 0.63 SDS below mean TH-SDS in girls and 1.30 SD below TH in boys ($p < 0.001$ and < 0.01 respectively). Mean H-SDS for CA at start of treatment was 1.54 (1.28) in girls and 1.57 (1.96) in boys. It decreased to 0.70 (1.06) and 0.81 (1.37) in girls and boys respectively at discontinuation of treatment.

Mean final height was 162.5 (7.26) cm in girls and 170.8 (7.16) cm in boys. Mean height gain in girls was 7.4 (5.71) cm and – 0.6 (5.94) cm in boys. Height increase in the subgroup of adopted girls was 6.3 (3.87) cm. Figure 1 shows PAH at start and discontinuation of treatment, attained FH and TH for girls. Mean height gain between girls with start of puberty < 6 (n=21) and > 6 years of age (n = 64) was not significantly different: 9.3 and 6.7 cm respectively. In both age groups FH was significantly higher than pre-treatment PAH (both $p<0.001$) and FH-SDS was not significantly different between the two age groups. In boys there was a significant difference in FH-SDS between the older and younger boys ($p < 0.05$). In table 2 the girls are divided in 3 groups based on age at start of treatment and gain: the difference between FH-SDS and TH-SDS was calculated for each group.

Table 2: Predicted adult height at start (PAH1), final height (FH) and target height (TH) data of girls divided in 3 subgroups based on chronological age at start of treatment: group 1: < 6 years, group 2: 6-8 years, group 3: > 8 years (range: 8-9.8 years).

	Rx duration (yr)	PAH 1 (cm)	FH (cm)	Height gain (cm)	FH-SDS	TH-SDS	N
Group 1	6.2 (1.1)	151.4 (8.05)	163.1 (8.54)	11.7 (8.03)	-0.84 (1.38)	0.27 (1.02)[b]	8
Group 2	3.8 (0.8)	154.3 (6.04)	161.9 (6.31)	7.9 (5.54)	-1.03 (1.02)	-0.11 (1.09)[b]	37
Group 3	2.6 (0.7)	157.0 (7.75)	163.0 (7.90)	6.1 (4.97)[a]	-0.85 (1.27)	-0.06 (1.10)[c]	42
All girls	3.4 (1.31)	155.3 (7.24)	162.5 (7.26)	7.4 (5.71)	-0.93 (1.17)	-0.05 (1.08)[d]	87

a = $p < 0.05$ compared to group 1: b = $p < 0.01$ compared to FH-SDS in paired t-test; c = $p < 0.05$ compared to FH-SDS in paired t-test; d = $p < 0.001$ compared to FH-SDS in paired t-test (66 pairs)

Correlation and regression analysis

The difference between PAH at start of treatment and the attained FH (height gain) was considered to be the most important variable describing the effect of GnRHa treatment. Therefore this variable was analysed using simple correlations and multiple regression analysis as described above. Results of the simple correlations are given in table 3 for girls, in boys CA at discontinuation of treatment correlated significantly with height gain.

In the multiple regression analysis a model with BA at start and stop of treatment, CA at start of treatment and with BA advance at stop of treatment could explain 48.9 % of the variance. This model is described in table 4. In girls treated at CA > 8 years we studied the role of BA advancement at start of treatment. In table 5 the results are shown. No statistical significance was demonstrated between the values of gain between the groups, possibly due to the small number of girls with BA advance > 3 years. Non-parametric correlation did not reveal significance.

It can be derived from tables 2 and 3 that age at start of treatment was one of the most importa variables determining the effect of treatment. Figure 2a shows a correlation plot with height ga against age at start of treatment in girls, and figure 2b growth after treatment against BA discontinuation of treatment.

Table 3: Simple correlation with height gain as dependent variable (girls)

	Correlation (Pearson)	P value
BA advance at start Rx	0.63	< 0.001
CA at start	-0.39	< 0.001
Rx duration	0.38	< 0.001
H-SDS for CA at start	0.34	< 0.01
H-SDS for BA at start	-0.34	< 0.01
Ht at discontinuation of Rx	0.27	< 0.05
H-SDS for CA at discontinuation of Rx	0.23	< 0.05

Rx = treatment; BA = bone age; CA = chronological age; H-SDS = standard deviation score for height

Table 4: Total model of multiple regression analysis: dependent variable = gain; intercept = 12.5 (SE: 12.07); explained variance = 48.9 %)

Predictive factor	Slope (standard error)	P value
BA at start of Rx	5.55 (0.86)	< 0.001
CA at start of Rx	-4.89 (0.58)	< 0.001
BA advance at discontinuation of Rx	-2.89 (0.92)	< 0.001
BA at discontinuation of Rx	-1.77 (1.21)	0.15

BA = bone age; CA = chronological age; Rx = treatment

Table 5: Height gain related to BA advance at start of treatment in girls with CA at start of treatment > 8 years.

BA – CA (yr)	Height gain (mean (SD) in cm	Number of girls
< 2	4.5 (5.24)	13
2-3	6.7 (4.58)	22
> 3	7.4 (5.78)	6

BA = bone age; CA = chronological age

Legend

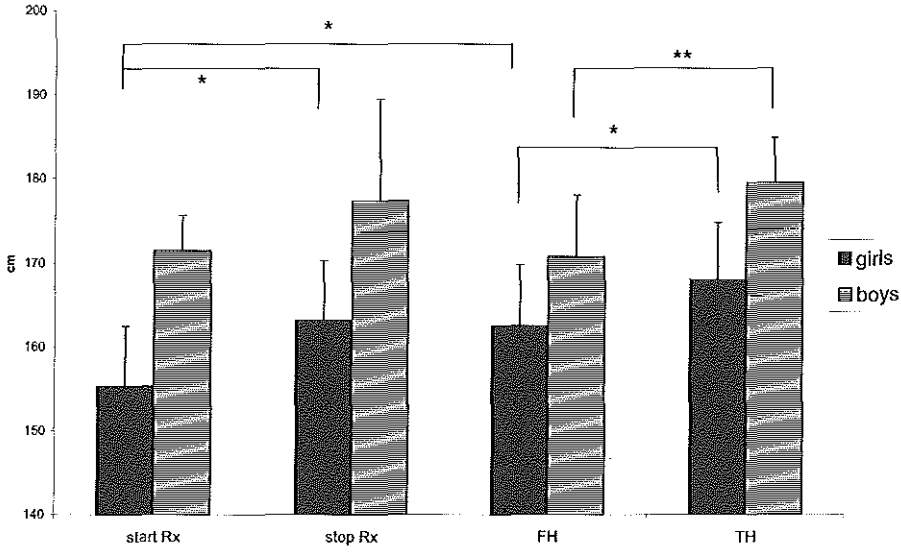
(Next page):

Figure 2. 2A: Scatterplot of age at start of treatment and height gain, r = -0.39, p < 0.001 (girls only) 2B: Scatterplot of BA at discontinuation of treatment and growth after discontinuation of treatment, in girls: r = -0.67, p < 0.001

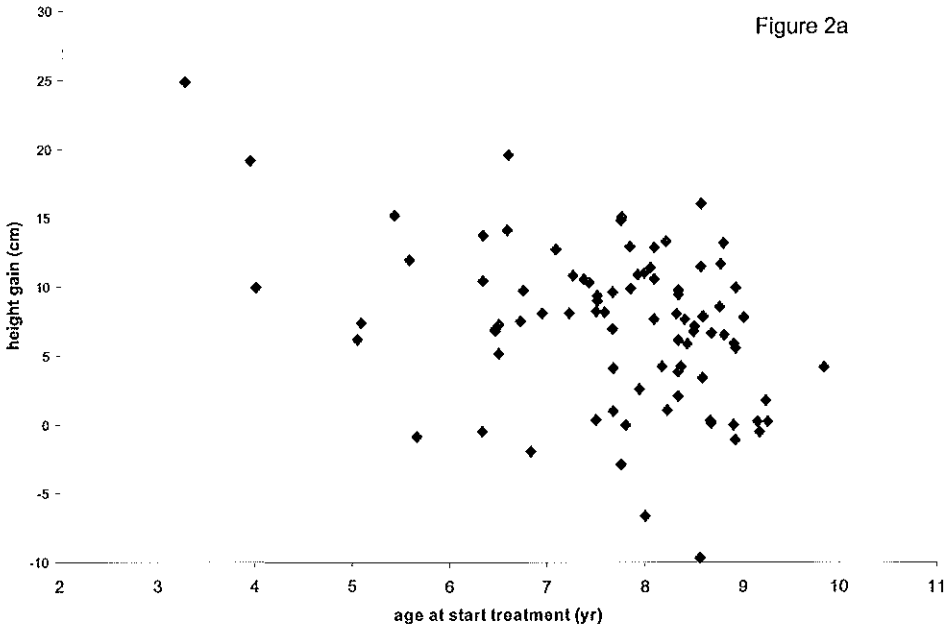
→

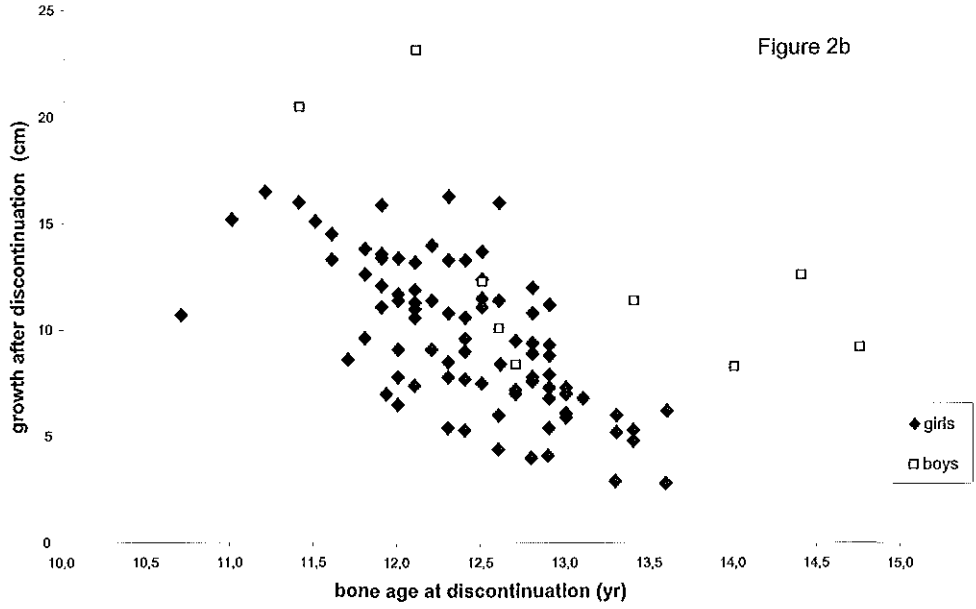
Figure 1. Predicted adult height (PAH) at start and stop of treatment, final height (FH) and target height (TH). * = p < 0.001, ** = p < 0.01

Figure 1:



Final height after GnRHa treatment





Discussion

In our study GnRHa treatment in children with CPP results in a final height that is significantly higher than pre-treatment PAH, but significantly lower than target height. Using multiple regression analysis, a model in which CA and BA at start of treatment have significant influence explains nearly 50% of variance in height gain. However, children with onset of puberty below 6 years of age did not gain significantly more than children > 6 year old at onset of puberty. Both groups gain compared to PAH at start of treatment. Height gain is observed even after treatment started after the age of 8 in girls.

Our study describes FH data in a large population of patients with CPP uniformly treated with GnRHa. Kletter and Kelch summarised data of several centres in different countries pertaining to 150 patients, however with substantial variations in treatment regimens.[4] The results of that study are frequently cited to support the statement that treatment in younger patients (< 6 years of age at onset of puberty) results in improvement of FH in contrast to children with onset from 6-8 years of age at onset of puberty.[21] Because of the use of different outcome parameters and the composition of their control group it is difficult to assess to what extent their results can be compared with ours. In the study of Carel *et al.* girls with onset of puberty between 6 and 8 years of age showed, as in our study, a positive height gain of 4.5 (5.3) cm, resulting in a FH which was significantly different from PAH at start.[11] Bouvattier *et al.* showed that girls in advanced puberty (age of onset between 8.4 and 10 yr) did not reach a final height significantly different from prediction at start.[14] From our data it can be concluded that there is more likely to be a positive effect of treatment when age at start of puberty is < 6 years of age. Cassio *et al.* showed that in girls with onset of puberty > 7.5 years old, GnRHa treatment does not improve auxological outcome.[13]

With respect to the age at start of treatment, which can have a variable delay after onset of puberty, we conclude that even when treatment starts > 8 years of age it is possible to improve FH compared to initial PAH, as was described previously.[12]

In our study, the gain in height, defined as the difference between PAH at start and FH was 7.4 (5.7) cm, other studies have reported 4.8 cm[11], 2.9[12] or 3.4 cm[5]. We used the average tables of BP at start of treatment, resulting in lower PAH at start of treatment if compared to accelerated tables. We believe that this method gives a better indication of the effect of GnRHa treatment, as Kauli showed that predictions with the average tables are more accurate in girls with CPP regardless of the advancement of bone age.[3] We used the same method in girls and boys, although this method has not been used previously or validated in boys.

Our results in girls show that FH-SDS, although in the genetic target range, is significantly lower than TH-SDS, which was also found by Kletter and coworkers[4], whereas Carel[11] reported a FH-SDS close to zero, therefore not different from the midparental height SDS. All the above-mentioned reports suggest a restoration of FH within the target range. The results for the boys suggest no substantial height gain, as reported previously.[11]

The best way to evaluate the effect of treatment of GnRH agonists would include the use of randomised trials with untreated controls. It is, however, considered unethical not to treat children with CPP. The use of a historical control group has its limitations as well because of the secular trend in age of puberty and adult height. One should also account for prediction errors in any method used. For example De Waal *et al.* demonstrated that the prediction error in different prediction methods in a sample of tall girls ≤ 11 years of age was 3.3 (2.5) cm.[22] In our opinion gain of height between the PAH at start and attained FH is the best way to analyse effect in studies without a control group. In the literature FH itself has also been used as measure of effect.[5,11,12]

For analysis of predictive factors for effect, we used variables in a multiple regression model in girls with probable clinical implications, e.g. BA at cessation of treatment. Chronological age and BA appeared to be important variables in this model as well as BA advancement at discontinuation. In other studies many variables were shown to influence height gain. In analysing these studies it appeared that the difference between BA and CA at start of treatment has significant influence on height gain in multiple regression analyses.[5,12] Taking together the results of simple and multiple

regression analysis one can conclude that the younger the girl, the more advanced bone age is at start of treatment, and the more BA equals CA at discontinuation, the more height gain from treatment with GnRH agonist can be expected. This conclusion is in line with the conclusion of Kaplowitz et al. that GnRHa treatment in children with slowly progressive puberty and less bone age advancement has not been proven to have a significant effect in improving FH.[21] No definite statement can be derived from our data as to when to discontinue treatment, while others did make recommendations.[11,12]

We suggest that children with CPP should be treated as early as possible and that children treated after the age of 8 years old can gain height especially when there is prominent BA advance at the moment of start of treatment, indicating progressive forms of CPP. In addition, apart from auxological considerations psychosocial aspects of precocious sexual development in children should be accounted for in the decision to treat or not to treat.

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References

1. Antoniazzi F, Cisternino M, Nizzoli G, Bozzola M, Corrias A, De Luca F, et al.: Final height in girls with central precocious puberty: comparison of two different luteinizing hormone-releasing hormone agonist treatments. Acta Paediatr 83:1052, 1994
2. Brauner R, Malandry F, Zantleifer D, Adan L: Adult height in girls with idiopathic true precocious puberty. J Clin Endocrinol Metab 79:415, 1994
3. Kauli R, Galatzer A, Kornreich L, Lazar L, Pertzelan A, Laron Z: Final height of girls with central precocious puberty, untreated versus treated with cyproteron acetate or GnRH analogue. A comparative study with re-evaluation of predictions by the Bayley-Pinneau method. Horm Res 47:54, 1997
4. Kletter GB, Kelch RP: Effects of Gonadotropin-Releasing Hormone analog therapy on adult stature in precocious puberty. J Clin Endocrinol Metab 79:331, 1994
5. Oostdijk W, Rikken B, Schreuder S, Otten BJ, Odink RJH, Rouwe C, Jansen M, Gerver WJ, Waelkens JJ, Drop SLS: Final height in central precocious puberty after long-term treatment with a slow-release GnRH agonist. Arch Dis Child 75:292, 1996
6. Paul D, Conte FA, Grumbach MM, Kaplan SL: Long term effect of Gonadotropin-Releasing Hormone agonist therapy on final and near-final height in 26 children with true precocious puberty treated at a median age of less than 5 years. J Clin Endocrinol Metab 80:546, 1995
7. Stasiowska B, Vannelli S, Benso L: Final height in sexually precocious girls after therapy with an intranasal analogue of Gonadotrophin-Releasing Hormone (Buserelin). Horm Res 42:81, 1994
8. Galluzzi F, Salti R, Pasquini E, La Cauza C: Adult height comparison between boys and girls with precocious puberty after long-term gonadotrophin-releasing hormone analogue therapy. Acta Paediatr 87:521, 1998
9. Kauli R, Kornreich L, Laron Z: Pubertal development, growth and final height in girls with sexual precocity after therapy with the GnRH analogue D-Trp-6-LHRH. Horm Res 33:11, 1990
10. Oerter KE, Manasco PK, Barnes KM, Jones J, Hill SC, Cutler GB: Adult height in precocious puberty after long-term treatment with deslorelin., in Grave GD, Cutler GB (eds): Sexual precocity: Etiology, diagnosis and management. New York, Raven Press Ltd., 1993, p 45
11. Carel JC, Roger M, Ispas S, Tondu F, Lahlou N, Blumberg J, Chaussain JL, French study group of Decapeptyl in precocious puberty: Final height after long-term treatment with Triptorelin slow release for central precocious puberty: Importance of statural growth after interruption of treatment. J Clin Endocrinol Metab 84:1973, 1999
12. Arrigo T, Cisternino M, Galluzzi F, Bertelloni S, Pasquino AM, Antoniazzi F, Borrelli P, Crisafulli G, Wasniewska M, De Luca F: Analysis of the factors affecting auxological response to GnRH agonist treatment and final height outcome in girls with idiopathic central precocious puberty. Eur J Endocrinol 141:140, 1999
13. Cassio A, Cacciari E, Balsamo A, Bal M, Tassinari D: Randomised trial of LHRH analogue treatment on final height in girls with onset of puberty aged 7.5-8.5 years. Arch Dis Child 81:329, 1999

14. Bouvattier C, Coste J, Rodrigue D, Teinturier C, Carel JC, Chaussain JL, Bougneres PF: Lack of effect of GnRH agonists on final height in girls with advanced puberty: a randomized long-term pilot study. J Clin Endocrinol Metab 84:3575, 1999
15. Palmert MR, Malin HV, Boepple PA: Unsustained or slowly progressive puberty in young girls: initial presentation and long-term follow-up of 20 untreated patients. J Clin Endocrinol Metab 84:415, 1999
16. Oerter Klein K: Editorial: precocious puberty: who has it? Who should be treated? J Clin Endocrinol Metab 84:411, 1999
17. Bland JM, Altman DG: Some examples of regression towards the mean. BMJ 309:780, 1994
18. Roede MJ, Wieringen JC van: Growth diagrams 1980. Netherlands third nation-wide survey. Tijdschrift voor Sociale Gezondheidszorg 63 (suppl.):11, 1985
19. Greulich WW, Pyle SI: Radiographic atlas of skeletal development of the hand and wrist (ed 2). Stanford, California, Stanford University Press, 1959
20. Bayley N, Pinneau SR: Tables for predicting adult height for skeletal age: revised for use with the Greulich-Pyle hand standards. J Ped 40:423, 1952
21. Kaplowitz PB, Oberfield SE: Reexamination of the age limit for defining when puberty is precocious in girls in the United States: implications for evaluation and treatment. Drug and Therapeutics and Executive Committees of the Lawson Wilkins Pediatric Endocrine Society. Pediatrics 104:936, 1999
22. de Waal WJ, Greyn-Fokker MH, Stijnen T, van Gurp EA, Toolens AM, de Munick Keizer-Schrama SM, Aarsen RS, Drop SL: Accuracy of final height prediction and effect of growth-reductive therapy in 362 constitutionally tall children. J Clin Endocrinol Metab 81:1206, 1996

II EARLY AND PRECOCIOUS PUBERTY

Chapter 3:

Pituitary and gonadal responsiveness in 2 different Gonadotrophin Releasing Hormone (GnRH) stimulation tests in girls with precocious or early puberty

Chapter 4

Auxological and biochemical evaluation of pubertal suppression with the GnRH agonist Leuprolide acetate in early and precocious puberty

Chapter 5: Final height after CPP

5.1 Final height after Gonadotrophin Releasing Hormone agonist treatment for central precocious puberty: the Dutch experience

5.2 Effect of Gonadotrophin Releasing Hormone agonist (GnRHa) treatment in boys with central precocious puberty: Final height results

submitted

Effect of Gonadotrophin Releasing Hormone agonist (GnRHa) treatment in boys with CPP: Final height results

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ABSTRACT

The small number of boys present in most studies on final height (FH) after Gonadotrophin Releasing Hormone agonist (GnRHa) treatment for central precocious puberty (CPP) offers uncertainty on the effects of treatment on final height in males. We therefore combined final height data from The Netherlands, Italy and France to study the effect of GnRHa treatment in a large group of 29 boys with CPP.

Mean chronological age at start of treatment was 7.9 (SD 2.1) years, BA was 11.0 (2.0) years. All boys were treated by the depot formulations of GnRH agonist triptorelin with established gonadal suppression for a mean treatment period of 4.6 (2.0) years.

Final height was 172.6 (6.7) cm. Final height SDS was –0.68 (1.20), not significantly different from the target height SDS of –0.27 (0.75). FH-SDS was significantly lower in the subgroup of 12 patients with organic CPP compared to patients with idiopathic CPP (-1.34 (1.06) vs. –0.21 (1.13) respectively, $p < 0.01$), but no difference in height gain was observed. Mean estimated height gain, defined as the difference between predicted and actual adult height was 5.8 (8.4) cm using the average tables of Bayley & Pinneau, and 0.3 (8.3) cm using the appropriate tables. Regional differences in height gain were observed between the different countries, reflecting different local practices in initiation of treatment.

We conclude that GnRH agonist treatment in boys results in a FH close to TH.

INTRODUCTION

In the studies published on the results of Gonadotrophin Releasing Hormone agonist (GnRHa) treatment for central precocious puberty (CPP), male patients are present just in small numbers (1-5). Comparisons between the effects of GnRHa treatment in boys and girls are scarce (6) also due to the low incidence of CPP in boys. The number of male patients with an organic substrate for the development of early puberty is relatively high compared to girls, and more often intracerebral pathology can be observed, further limiting the number of idiopathic CPP in boys (6, 7).

Furthermore, the relevance to compare boys and girls is limited. In boys, although the importance of oestrogen is acknowledged (31) the pubertal growth pattern is mainly driven by testosterone that is aromatized to oestrogens and by increased growth hormone levels, thus different from that in girls (8). Pubertal suppression in boys may thus have different effects on growth compared to girls. Consequently, no conclusions from girls' results of GnRHa treatment can be inferred to boys.

The small number of GnRHa treated boys offers uncertainty on the effects of treatment on final height (FH); uncertainty also persists on the issues when to start or stop GnRHa treatment in boys. Most studies were not able to thoroughly analyse the boys' data, suggesting that larger groups should be analysed.

A further issue in the analysis of FH in boys with CPP is the reliability of height predictions. In untreated girls with CPP the Bayley-Pinneau (BP) method overestimates FH by 4-6 cm (2). In tall boys a tendency to overestimate FH by the BP prediction method is present as well (9). In a small number of boys with CPP Zachmann *et al.* reported that the BP method was reasonably accurate (10). No final recommendation on the best method for height prediction can thus be given .

In the present study we collected data from 3 different countries on boys with CPP that had been treated with similar GnRHa treatment protocols and had reached final height (FH). We thereby provide the results on the largest population of GnRHa treated boys to date.

PATIENTS AND METHODS

Data from patient files were retrospectively collected in a standardised way. The patient data were gathered from The Netherlands, Italy and France and have been presented in part in earlier reports (2, 3, 5).

All patients had reached FH after a variable period of treatment with the GnRH agonist triptorelin (Decapeptyl®) as monthly IM depot (3.75 mg); some (n = 5) were treated previously for a short period by cyproterone-acetate or intranasal GnRHa before start of GnRHa depot treatment.

Pubertal development started before the age of 10 years with enlargement of testes ($\geq$ 4 ml) and elevated peak Luteinizing Hormone (LH) levels in a standard GnRH stimulation test, proving the central origin of the precocious development of secondary sexual characteristics.

In the analysis height data at start and discontinuation of treatment, as well as FH data were used. Final height was defined as a growth velocity < 1 cm per year in a 6 months period and/or a BA > 17 years. Because of the different backgrounds of the children height data were standardised by using standard deviation scores (SDS) for height. For the Dutch boys we used the 1980 Dutch references (11), for the Italian boys the Tanner references from 1965 (12), which are suitable for the Italian population, and for the French boys the Sempe references from 1979 (13), which are quite comparable with the Tanner 1965 references. Target heights were calculated by [($height_{mother}$ + $height_{father}$) / 2] + 6.5 in Italian

and French boys. In The Netherlands a formula is used that accounts for the secular trend: TH = [(height$_{mother}$ + height$_{father}$ +12) / 2] +3.

Bone age (BA) assessments using the Greulich and Pyle references (14) were not centralised in the French and Italian group. In the Dutch patients the BA assessment were performed by one observer. Predictions of adult height using the Bayley-Pinneau method were performed in two ways. First we used the method as initially described (15) using the appropriate tables (i.e. "accelerated" table when BA exceeds CA by more than a year or "average" table when BA is within a year of CA); second, as suggested by Kauli in girls with precocious puberty (16), we used the "average" table irrespective of BA.

Laboratory analyses were performed in the different hospitals using either RIA or IRMA. The diagnosis for CPP by GnRH stimulation test was made according to the local standards. Adequate gonadal suppression was assessed regularly by clinical and/or hormonal follow-up. GnRHa doses were adjusted to maintain complete suppression.

Statistics:

To describe the populations, the means (SD) were calculated. Comparisons between start and discontinuation of treatment and between start of treatment and FH were performed pair-wise with the appropriate test for distribution of the variables.

In order to assess the effect of treatment two different outcome variables were used: we compared the initial prediction with attained FH, which is called height gain; and secondly, we compared FH and TH, expressed as SD scores.

Correlations were studied between the 2 outcome variables and several baseline variables at start of treatment in order to elucidate the effect of any factor on outcome. We performed a multiple regression analysis with the 4 variables that had the highest correlation in univariate correlation analysis. P values < 0.05 were considered to indicate statistical significance.

RESULTS

a) *Descriptive*

Data of 29 boys were available for analysis: 9 from The Netherlands, 12 from Italy and 8 from France. Organic causes were present in 12 boys (e.g. neurofibromatosis (NF) n = 5, brain tumour, n = 4). All boys were treated for more than 1.5 years. Mean age at start of puberty was 6.7 (2.3) years. In table 1 the further patient characteristics at start of treatment are shown.

Mean treatment duration was 4.6 (2.0) yr. At the end of treatment BA was 13.7 (1.0) 'years'; the mean increase in BA was 0.57 (0.17) 'year' per year increase in CA. The changes in H-SDS for BA and for CA as well as FH-SDS and TH-SDS are shown in figure 1. Final height was 172.6 (6.7) cm; FH-SDS was –0.68 (1.20) and was not significantly different from TH-SDS (paired t-test, 27 pairs, p = 0.08)

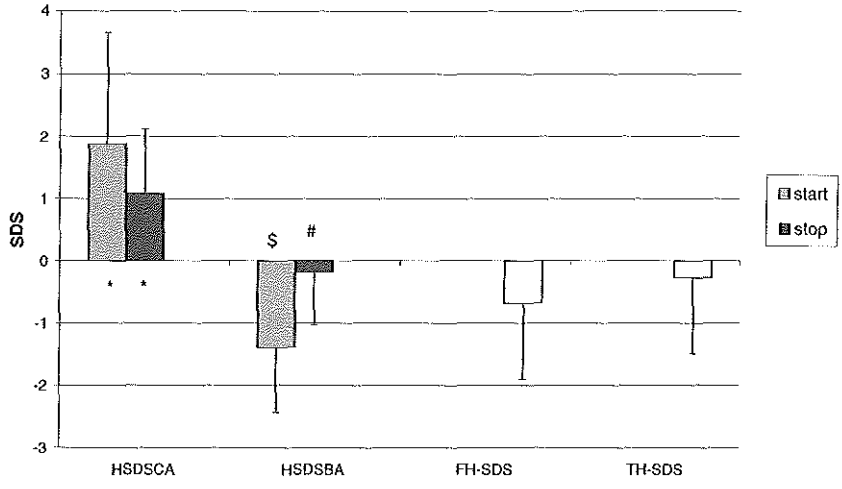
In figure 2 predicted adult height at start and stop of treatment, the attained FH and TH are shown in cm. Both FH predictions with the average tables and FH predictions with the appropriate BP tables are shown. For comparison, FH data of untreated boys with CPP reported by Thamdrup, Sigurjonsdottir *et al.* and Paul *et al.* are represented as well (4, 17, 18). Growth after treatment up to FH was 14.4 (7.7) cm (range: 4.3-43.7 cm), and correlated significantly with BA at discontinuation of treatment: Spearman correlation coefficient = -0.60 (p = 0.001).

Table 1: Clinical characteristics of the patients at start of GnRHa treatment.

	Mean	SD
Chronological age (CA) (yr)	7.9	2.1
Bone age (BA) ('yr')	11.0	2.0
H-SDS for CA	1.88	1.78
H-SDS for BA	-1.39	1.04
TH-SDS	-0.27	0.75
Predicted adult height (cm) according to *		
- Average tables (SDS)	-1.54	1.19
- Appropriate tables (SDS)	-0.70	1.37
- Average tables (cm)	166.8	8.5
- Appropriate tables (cm)	172.3	9.8

CA: chronological age; BA: bone age; H-SDS- height standard deviation score; TH: target height.
* see patients and methods for description of the use of BP tables.

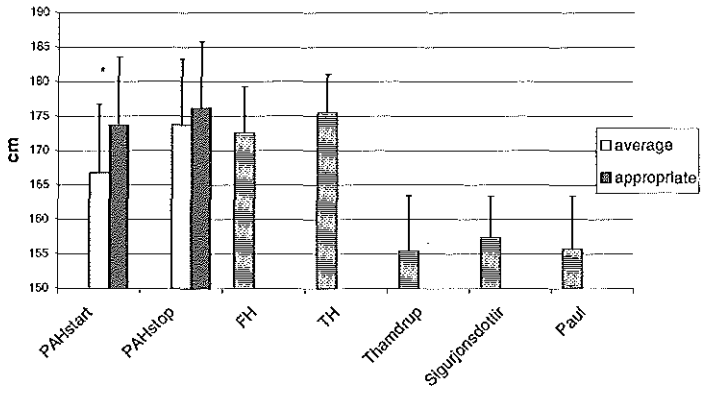
Figure 1: Height standard deviation scores (H-SDS) before and after treatment with GnRHa, final height SDS (FH-SDS) and target height SDS (TH-SDS) in 29 boys with CPP.



HSDSCA and HSDBA = HSDS corrected for chronological age and bone age respectively.
Significant difference compared to FH-SDS: # $p < 0.05$; \$ $p < 0.01$; * $p < 0.001$

Figure 2: Predicted adult height before (PAHstart) and at discontinuation of treatment (PAHstop) using average or appropriate tables, attained final height (FH) and target height (TH) in 29 boys treated with GnRHa

Figure 2



For comparison the FH of untreated boys in the studies of Thamdrup, Sigurjonsdottir and Paul (cited in the text) are shown likewise. * $p < 0.001$ compared to FH.

Final height was heigher than the initial height predictions. Mean height gain defined as the difference between predicted and actual final height was 5.8 (8.4) cm using the average table, and 0.3 (8.3) cm using the appropriate table.

b) *Comparisons between countries and subgroups*
In table 2 the data of boys are summarised for the individual countries [mean (SD)], showing significant differences in FH-SDS and height gain. The percentage of boys with organic CPP was high in the Dutch subgroup. No significant difference in duration of treatment was present between the countries. In boys with age of onset of puberty below 6 years of age (n=8) gain with average tables was significantly higher ($p<0.05$) than in the other boys, while FH-SDS was not significantly different.

FH in the 12 organic patients was 169.5 (5.1) cm, and FH-SDS was −1.34 (1.06), both significantly different ($p < 0.05$) from the FH outcome in idiopathic patients where FH was 174.7 (7.0) and FH-SDS was −0.21 (1.13). In organic patients FH-SDS was significantly lower than TH-SDS ($p<0.01$). There was neither a significant difference in TH-SDS, BA or BA-CA at start of treatment, nor in height gain between idiopathic and organic patients.

The subgroup of 5 patient with NF was compared to the remaining organic patients; FH-SDS and height gain (for both prediction methods) appeared to be significantly lower ($p < 0.01$ and $p < 0.05$ respectively) in the NF group.

Table 2: Patient characteristics differentiated per country.

	NL (9)	Italy (12)	France (8)
Age start puberty (yr)	7.1 (1.9)	6.2 (2.2)	7.1 (3.0)
Age start Rx (yr)	8.3 (1.7)	6.8 (2.2)#	9.2 (1.7)
BA start Rx (yr)	10.9 (2.0)	10.6 (2.3)	11.6 (1.7)
Duration of treatment (yr)	3.8 (1.3)	5.2 (2.5)	4.7 (1.8)
FH (cm)	170.8 (7.2)	173.7 (6.9)	172.9 (6.4)
FH-SDS	-1.67 (1.07)*#	-0.15 (1.02)	-0.36 (1.06)
TH-SDS	-0.37 (0.79)	0.02 (0.77)	-0.54 (0.63)
organic cause (%)	66.6	33.3	25
height gain (av) cm	-0.6 (5.9)@	12.7 (6.2)	2.7 (6.0)*
height gain (appr) cm	-7.3 (5.1)@#	6.8 (6.1)#	-0.9 (6.2)

# p<0.05 compared to France; * P<0.01 compared to Italy; @p< 0.001 compared to Italy; BA: bone age; H-SDS- height standard deviation score; TH: target height; FH: final height; Rx: GnRHa treatment; av : average table for heigh prediction; appr : appropriate table for height prediction

c) *Correlation and regression*

Height gain was correlated with several variables and the results are shown in table 3. The same procedure was performed for FH-SDS.

Table 3: Univariate correlations of height gain and FH-SDS

	Height gain		FH-SDS
	Average tables	**Appropriate tables**	
CA start symptoms	-0.60 (p<0.01)	-0.36 (NS, p=0.053)	-0.27 (NS)
CA at start Rx	-0.72 (p<0.01)	-0.49 (p<0.01)	-0.26 (NS)
BA at start Rx	-0.29 (NS)	-0.19 (NS)	-0.11 (NS)
BA advance start	0.50 (p<0.01)	0.45 (p<0.05)	0.20 (NS)
Duration of treatment	0.58 (p<0.01)	0.54 (p<0.01)	0.04 (NS)
TH SDS	0.28 (NS)	0.17 (NS)	0.28 (NS)

Correlation coefficients with P value are shown. CA: chronological age; BA: bone age; TH-SDS: target height standard deviation score; FH: final height; Rx: GnRHa treatment; NS: not significant.

Similar to other reports height gain was correlated with BA or BA advance at start of treatment. However, since BA is used to define height gain (by the BP method), this result should be interpreted with caution since the two variables were not obtained independently. Identical methodological considerations should be taken into account in the linear regression analysis that was performed with 4 variables: CA and BA at start of treatment, the difference between BA and CA, and duration of treatment, where height gain was the dependent variable. A model with a positive correlation coefficient for BA advance and a negative correlation coefficient for BA at start of treatment could explain nearly 60% of variation in height gain using average tables for height prediction (table 4).

Table 4: Factors associated with height gain; $r^2 = 0.60$; average tabes of BP were used

Predictive factor	Slope	95 % CI of slope	P
BA at start of treatment (yr)	- 2.68	- 3.81 / - 1.56	< 0.001
BA advance at start of treatment (yr)	+ 3.59	2.18 / 5.01	< 0.001

Discussion

In this study we show that FH-SDS was not significantly below TH-SDS in a relative large group of 29 boys after GnRHa treatment for CPP. The estimation of the mean height gain varied between 0.3 and 5.8 cm depending upon the prediction method.

Boys with advanced BA and early onset of CPP are expected to have the worse final outcome. We did not confirm this assumption suggesting that GnRHa treatment is effective.
In this retrospective study we observed regional differences in practices and effects of GnRHa treatment. The Italian boys had the highest estimated height gain, probably due to the relatively young age and the marked BA advance at start of GnRHa treatment. The differences in age at initiation of treatment reflects the use of more (or less) liberal criteria to start GnRHa treatment. In The Netherlands GnRHa treatment was used in boys entering puberty just before 10 years of age, whereas in Italy the mean age at start of treatment was lower. Another difference is the longer interval between onset of puberty and start of treatment in the French group. Combining data from several countries allowed the analysis of a wider spectrum of patients, since 'national data' reflect local practices and prescription habits.

The published studies on FH in GnRHa treated boys show variable results. In the study of Galluzi *et al.* (using appropriate tables) idiopathic CPP patients had a FH exceeding initial prediction by 7.2 cm (6). In the studies of Oostdijk *et al.* and Paul *et al.* FH-SDS was – 1.6 and -1.7 respectively (1, 4). In the recent study of Bertelloni *et al.* FH was close to TH (5). In an older study, where daily deslorelin was used, FH was 168.0 (8.3) cm, corresponding with -1.3 SDS (19). Patients from the studies by Oostdijk and Bertelloni are in part included in this paper. These relative discrepancies reflect the heterogeneity of the patients treated and the difficulties in assessing "height gain", based on predicted height at onset of puberty. Indeed, very little data on the validity of BP prediction methods in boys with CPP is available. With this in mind, the apparently normal predicted height (172.3 (9.8) cm) in our large series of boys with marked CPP using the appropriate BP tables strongly suggests that this method overestimates the true FH if boys were left untreated. This is in line with the historical reports showing poor FH outcome in untreated boys with CPP (4, 17, 18), see figure 2. It is also in accordance with the observation of a considerable overestimation of FH by the BP method in tall boys (9). Therefore, we also used the average tables as suggested in girls (16), although we could not validate this method in an untreated group of boys with CPP.

Performing a randomised trial with untreated controls in boys with CPP would resolve the issue of height gain, but is not ethical, given the psychosocial objectives of treatment. The comparison with historical controls suggests that the improvement of FH after GnRHa treatment is about 15 cm, although the secular change in height (8 cm between 1955 and 1997 for Dutch boys (20)) should be taken into account. Further, the difference in target height calculation should be taken into account. The correction of 3 centimetres for secular trend in the Dutch formula results in a higher TH of approximately 0.5 SDS compared to the TH resulting from the formula without this correction. Without the correction, TH in the Dutch

boys would have been about –0.9 SDS, explaining in part why the difference between TH-SDS and FH-SDS in this subgroup was considerably higher than in the boys from Italy and France. Another part of the expanation could be the differences in duration of treatment and in the percentage of boys with organic CPP.

Whether GnRHa treatment results in different effects in boys with organic and idiopathic forms of CPP has been debated as well. Our analysis shows that boys with organic CPP have a lower FH-SDS than boys with idiopathic CPP. However, 5 of the 12 boys with organic CPP had neurofibromatosis, a condition that may directly compromise FH (21-23). Accordingly, in NF patients height *gain* and FH-SDS were significantly lower compared to the remaining patients with organic CPP.

As in other studies in girls, the younger age at start of symptoms of CPP was associated with more height gain compared to initial height prediction (3, 24) and is known to be associated with poor spontaneous outcome (17, 18). Although this suggests that patients should be treated early, before irreversible BA maturation has occurred, strict criteria for CPP should be used.

We found no correlation of FH with initial patient characteristics, suggesting that even boys with initially a poor prognosis ended up in the range of the genetic height potential. In linear regression analysis an association of height gain with BA and BA advance at start of treatment was described. Interpreting these data would suggest that a higher BA results in a smaller height gain, especially when the difference with CA is small. Prospective studies should address this issue formally.

Several reports have shown that girls with non-progressive forms of CPP should not necessarily be treated at least for auxological goals (25). In boys the issue of progressive vs. non-progressive forms of CPP has not been clearly delineated as yet, probably because the assessment of a pubertal testicular volume and plasma testosterone are relatively robust criteria, unlike minimal breast development or plasma oestradiol.

Criteria used to decide on discontinuation of treatment have been debated and can influence post-treatment growth and final outcome. In our study post-treatment growth was highly correlated with BA at discontinuation of treatment ($r = -0.60$, $p<0.01$). Identical results were obtained in girls (2, 3). In normal children, peak HV occurs around a CA of 11.5 yr in girls and 13.5 yr in boys (26). In several studies in girls with CPP the average BA at interruption of treatment was > 12 yr (1, 2, 6, 24) while it is close to 13.5 years in boys ((6, 27) and this study). Therefore BA at discontinuation corresponds to early-mid puberty in boys and to late puberty in girls. Altogether, these differences may explain why boys grow an average of 15 cm after treatment while girls only grow 9-10 cm. Similarly, Galluzzi *et al.* have observed a more pronounced post-treatment growth-spurt in boys than in girls (6). Based on our study we cannot recommend a particular BA to discontinue treatment.

In boys with pseudo precocious-puberty anti-androgens and aromatase inhibitors have been used, sometimes in combination with GnRHa when central puberty develops. The analysis of the relative contribution of the anti-androgenic effect and anti-estrogenic effect is difficult in these cases. It was shown that Spironolactone and Testolactone were able to normalise the growth rate and rate of bone maturation (28). Further, observations of mutations in the estrogen-receptor gene (29) and of aromatase deficiencies (30) in males suggest that blocking the estrogenic effect would be enough to achieve the auxological goals in boys with CPP. However, blocking the androgenic effect in young boys with CPP is important as well.

We conclude that in boys with CPP GnRHa is effective in obtaining a FH not significantly different from TH.

REFERENCES

1. Oostdijk W, Rikken B, Schreuder S, et al. Final height in central precocious puberty after long-term treatment with a slow-release GnRH agonist. Arch Dis Childh 1996;75:292-297.
2. Carel JC, Roger M, Ispas S, et al. Final height after long-term treatment with Triptorelin slow release for central precocious puberty: Importance of statural growth after interruption of treatment. J Clin Endocrinol Metab 1999;84:1973-1978.
3. Mul D, Oostdijk W, Otten BJ, et al. Final height after Gonadotrophin Releasing Hormone agonist treatment for Central Precocious Puberty: The Dutch experience. J Ped Endocrinol Metab 2000;13(Suppl 1):765-772.
4. Paul D, Conte FA, Grumbach MM, Kaplan SL. Long term effect of Gonadotropin-Releasing Hormone agonist therapy on final and near-final height in 26 children with true precocious puberty treated at a median age of less than 5 years. J Clin Endocrinol Metab 1995;80:546-551.
5. Bertelloni S, Baroncelli GI, Ferdeghini M, Menchini-Fabris F, Saggese G. Final height, gonadal function and bone mineral density of adolescent males with central precocious puberty after therapy with gonadotropin-releasing hormone analogues. Eur J Pediatr 2000;159:369-374.
6. Galluzzi F, Salti R, Pasquini E, La Cauza C. Adult height comparison between boys and girls with precocious puberty after long-term gonadotrophin-releasing hormone analogue therapy. Acta Paediatr 1998;87:521-527.
7. Brunner HG, Otten BJ. Precocious puberty in boys [editorial; comment]. N Engl J Med 1999;341(23):1763-5.
8. Abbassi V. Growth and normal puberty. Pediatrics 1998;102(2 Pt 3):507-11.
9. Drop SLS, Waal W de, Muinck Keizer - Schrama SMPF de. Sex steroid treatment of constitutionally tall stature. Endocr Rev 1998;19:540-558.
10. Zachmann M, Sobradillo B, Frank M, Frisch H, Prader A. Bayley-Pinneau, Roche-Wainer-Thissen, and Tanner height predictions in normal children and in patients with various pathologic conditions. J Pediatr 1978;93:749-755.
11. Roede MJ, Wieringen JC van. Growth diagrams 1980. Netherlands third nation-wide survey. Tijdschr Soc Gezondheidszorg 1985;63 (suppl.):11-32.
12. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, and stages of puberty. Arch Dis Childh 1976;51:170-179.
13. Sempé M, Pédron G, Roy P. Auxologie, méthodes et séquences. Paris: Theraplix; 1979.
14. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. 2 ed. Stanford, California: Stanford University Press; 1959.
15. Bayley N, Pinneau SR. Tables for predicting adult height for skeletal age: revised for use with the Greulich-Pyle hand standards. J Ped 1952;40:423-41.
16. Kauli R, Galatzer A, Kornreich L, Lazar L, Pertzelan A, Laron Z. Final height of girls with central precocious puberty, untreated versus treated with cyproteron acetate or GnRH analogue. A comparative study with re-evaluation of predictions by the Bayley-Pinneau method. Horm Res 1997;47:54-61.
17. Thamdrup E. Precocious sexual development. Copenhagen: Munksgaard; 1961.
18. Sigurjonsdottir TJ, Hayles AB. Precocious puberty. A report of 96 cases. Am J Dis Child 1968;115(3):309-21.
19. Oerter KE, Manasco PK, Barnes KM, Jones J, Hill S, Cutler GB, Jr. Effects of luteinizing hormone-releasing hormone agonists on final height in luteinizing hormone-releasing hormone-dependent precocious puberty. Acta Paediatr Suppl 1993;388:62-8; discussion 69.
20. Leschek EW, Jones J, Barnes KM, Hill SC, Cutler GB, Jr. Six-year results of spironolactone and testolactone treatment of familial male-limited precocious puberty with addition of deslorelin after central puberty onset. J Clin Endocrinol Metab 1999;84(1):175-8.
21. Clementi M, Milani S, Mammi I, Boni S, Monciotti C, Tenconi R. Neurofibromatosis type 1 growth charts. Am J Med Genet 1999;87(4):317-23.
22. Carmi D, Shohat M, Metzker A, Dickerman Z. Growth, puberty, and endocrine functions in patients with sporadic or familial neurofibromatosis type 1: a longitudinal study. Pediatrics 1999;103(6 Pt 1):1257-62.
23. Virdis R, Sigorini M, Laiolo A, et al. Neurofibromatosis type I and precocious puberty. J Ped Endocrinol Metab 2000;13(Suppl1):841-844.
24. Arrigo T, Cisternino M, Galluzzi F, et al. Analysis of the factors affecting auxological response to GnRH agonist treatment and final height outcome in girls with idiopathic central precocious puberty. Eur J Endocrinol 1999;141(2):140-4.

25. Palmert MR, Malin HV, Boepple PA. Unsustained or slowly progressive puberty in young girls: initial presentation and long-term follow-up of 20 untreated patients. J Clin Endocrinol Metab 1999;84:415-423.
26. Liu YX, Wikland KA, Karlberg J. New reference for the age at childhood onset of growth and secular trend in the timing of puberty in Swedish. Acta Paediatr 2000;89(6):637-43.
27. Rizzo V, De Sanctis V, Corrias A, et al. Factors influencing final/near-final height in 12 boys with central precocious puberty treated with Gonadotrophin-Releasing Hormone agonists. J Ped Endocrinol Metab 2000;13(Suppl 1):781-786.
28. Feuillan P, Merke D, Leschek EW, Cutler GB, Jr. Use of aromatase inhibitors in precocious puberty. Endocr Relat Cancer 1999;6(2):303-6.
29. Smith EP, Boyd J, Frank GR, et al. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 1994;331(16):1056-61.
30. Bilezikian JP, Morishima A, Bell J, Grumbach MM. Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. N Engl J Med 1998;339(9):599-603.
31. Grumbach MM, Auchus RJ. Estrogen: consequences and implications of human mutations in synthesis and action. JCEM 1999;84: 4677-94.

III TREATMENT WITH GNRHA AND GH

Chapter 6: Study of the effect of treatment with GnRH-agonist (Decapeptyl) alone or in combination with biosynthetic human growth hormone (Genotropin) in adopted children with early puberty.

6.1 Gonadotrophin Releasing Hormone agonist (GnRHa) treatment with or without recombinant human growth hormone (GH) in adopted children with early puberty

submitted

6.2 Study of the effect of treatment with GnRH (GnRH)-agonist alone or in combination with recombinant human growth hormone in adopted children with early puberty: Psychological assessments before and after treatment

6.3 Motivation for treatment and psychological evaluation in adoptive families when a child presents with early puberty

Chapter 7

A randomized controlled trial of three years of growth hormone (GH) and GnRH agonist treatment in children with Idiopathic Short Stature and Intra-Uterine Growth Retardation.

Chapter 8

The effect of pubertal delay by GnRH agonist in Growth Hormone Deficient (GHD) children on final height

Gonadotrophin Releasing Hormone agonist (GnRHa) treatment with or without recombinant human growth hormone (GH) in adopted children with early puberty

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ABSTRACT

Early onset of puberty is frequently observed in adopted children. During treatment with Gonadotrophin Releasing Hormone agonist (GnRHa) a decrease in height velocity (HV) precludes height gain. We studied the effect of the addition of growth hormone (GH) to GnRHa treatment in a 3-year randomised trial in 30 adopted children with early puberty. Mean age (SD) at start of treatment was 9.6 (0.9) yr in girls and predicted adult height (PAH) using a segmented bone age (BA) assessment method was 148.0 (5.3) cm. HV decreased gradually in both groups with a higher HV in the group with GH addition (= group B). No significant difference between the rates of bone maturation (ΔBA / ΔCA) of both treatment groups was observed. After 3 years of treatment PAH increase was 5.7 (3.8) cm in group A (GnRHa alone) and 10.1 (3.8) cm in group B ($P < 0.01$). Insulin-like growth factor I (IGF-I) levels were higher in group B. HV decreased slowly in both groups during treatment, unlike stabilisation of IGF-I levels. We conclude that after 3 years of treatment the addition of GH to GnRHa results in a higher HV and a significant increase in PAH compared to GnRHa alone.

INTRODUCTION

In children adopted from developing countries the onset of puberty is often considerably earlier than in the countries the children originate from, resulting in compromised adult height(1-7). The occurrence of early puberty might be related to the improvement of nutritional status in early life (8, 9), possibly facilitated by an increase of leptin secretion acting as permissive factor for the onset of puberty by interacting with several neuropeptides (10).

In early or precocious puberty long-acting GnRHa treatment is currently considered the first choice of treatment. Data from recent literature suggest that adding growth hormone (GH) to GnRHa treatment is of benefit to improve final height (FH) (11). However, randomised prospective trials on the effect of combined treatment are scarce, especially in non-GH deficient patients (12).

In children adopted from Eastern Asia or South America the parental heights are generally not known, thus making the analysis of any growth promoting therapy difficult. These difficulties are of even more importance as bone age (BA) assessments can only be compared by western world standards. It is not known how these standards compare to foreign-born children and how to account for possible effects of catch up growth on skeletal maturation.

In this study we evaluate the effect of the addition of GH to GnRHa treatment in adopted children with early puberty. We compare initial height predictions at start of treatment with predictions after 3 years of treatment with an adapted bone age assessment.

PATIENTS & METHODS

Children in this study met the following inclusion criteria: (A) born in India, Sri Lanka, Colombia or South Korea; (B) pubertal development started between 7 and 10 years of age in girls with breast development and/or menarche; in boys, pubertal development with testicular enlargement ($\geq$ 4 mL) started between 8 and 11 years; (C) pubertal response of Luteinizing Hormone (LH) in a GnRH stimulation test (13); (D) bone age at start $\leq$ 12.5 yr in girls and $\leq$ 14 yr in boys; (E) Predicted adult height (PAH) below the third percentile for the Dutch population (14), which is the nation-wide accepted cut-off value for the diagnosis of short stature; (F) passing of a psychological screening test before start of the study; (G) written informed consent from parents or guardians; (H) no abnormalities in endocrine and biochemical screening.

Height was measured every 3 months by the same observer in the outpatient clinic using a Harpenden stadiometer. The mean of 4 measurements was used for analysis. Sitting height (SH) and weight were assessed at each visit as well. Pubertal staging was scored according to Tanner (15).

Bone age (BA) was assessed by a segmented Greulich & Pyle (GP) score. This method consists of the scoring of 7 regions of the hand and wrist (radius, ulna, metacarpals, proximal, medial and distal phalanges and carpals), assigning a GP bone age to each segment and dividing the summarised score by seven. This method combines the advantages of detailed scoring with the use of the most appropriate method of BA assessment in early puberty (16). For adult height prediction we used the average Bailey & Pinneau tables even when BA was more than 1 year advanced over chronological age (17).

Laboratory assessment at baseline included: a GnRH stimulation test with 100 µg GnRH and sampling after 30 and 60 minutes and an arginine GH stimulation test (0.5 g/kg body weight i.v. over 30 minutes) in order to exclude growth hormone deficiency. During the study GnRH stimulation tests were repeated after 12, 24 and 36 months to monitor pituitary suppression. Growth hormone, LH and FSH were assessed by time resolved

immunofluorimetric assay (Wallac, Turku, Finland); androstenedione by radioimmunoassay (DSL Sensheim (Germany) and DHEA sulphate by an in house RIA with tritiated DHEA-S. Estradiol and Testosterone levels were measured by RIA (Orion Espoo, Finland). IGF-I and IGFBP-3 were assessed and transformed to SD scores as described previously (18).
Ultrasound measurements of uterine and ovarian volumes were performed regularly, and ovarian volumes were compared with normative data for calculation of SD scores (19).

The children were randomised and treated for 3 years with either GnRH-agonist alone, *group A* (triptorelin (Decapeptyl®, Ferring and from 1998: Ipsen); dosage: 3.75 mg i.m. every 28 days) or in combination with human recombinant GH: *group B* (Genotropin®, (Pharmacia & Upjohn) 4 IU/ m^2 s.c. every day).

In the original design of the study a third arm was included as a non-treated control group, but this design was discontinued when the parents of the children that were randomised for no treatment refused further participation, as GnRHa treatment would be available elsewhere. Before randomisation, children and parents underwent extensive psychological evaluations aimed at assessing their motivation for treatment and current psychological status. The results of these evaluations will be published separately (chapters 6.2 and 6.3). The study was approved by the local Ethics Committees and by the Dutch National Board on the Ethics of Medical Research (KEMO).

Statistical analysis

Results are expressed as mean $\pm$ SD. The main outcome parameter of this study is height gain, defined as the difference between initial height prediction and height prediction at discontinuation of treatment. Differences between groups were analysed by Student's t-test. Comparisons between parameters within the treatment groups were performed pairwise. When appropriate non-parametric comparisons (Mann-Whitney) were used to compare group A and B. Univariate correlations were non-parametrically tested with Spearman rho in case of non-normal distribution.

Changes in variables during the study period between treated patients and controls were analysed by repeated measurement analysis. The changes during the course of treatment between groups and changes in the course of treatment for both groups together. A p value of <0.05 was considered to indicate statistical significance.

RESULTS

Auxology

Thirty patients were randomised for treatment, 27 girls and 3 boys. Children originated from Sri Lanka (10 girls, 2 boys), India (9 girls), Colombia (6 girls, 1 boy) and South Korea (2 girls). They had arrived in The Netherlands at a median age of 4.5 months (range 1.0 – 84). Fourteen girls were randomised for group B and the other girls as well as the three boys for group A. The children from each country were equally divided in group A and B.

Table 1: Patient characteristics at start of treatment (median and range).

	Group A (13 girls)	Group B (14 girls)	Boys (n=3)
Chronological age (yr)	9.8 (7.3 /10.7)	9.7 (7.6/10.6)	10.8 (10.6/11.6)
Age at arrival (months)	3.0 (1.0 / 84.0)	7.0 (1.0 / 51.0)	2.0 (1.5 /14.0)
Height (cm)	133.9 (116.8/148.1)	134.6 (126.6/145.2)	131.4 (129.6/135.1)
H-SDS for CA	-0.85 (-2.88 / 0.82)	-0.63 (-2.24/0.60)	-2.40 (-2.52/-2.33)
Bone age (yr)	11.0 (8.5 /12.1)	11.5 (10.0/12.6)	10.4 (9.1/11.9)
H-SDS for BA	-1.68 (-3.07/-0.74)	-2.32 (-3.12/-1.18)	-1.48 (-2.97/-1.35)
Predicted adult height (cm)	150.3 (140.7/159.2)	145.5 (141.1/155.2)	169.9 (158.1/170.3)
LH peak value (IU/L) in GnRH stimulation test	35.5 (1.0 /60.0)	22.7 (1.6/51.8)	26.0 (18.4/39.5)
GH peak (mU/L) in GH stimulation test	29.4 (9.0/106.7)	44.1 (15.8/126.6)	23.7 (21.3/26.6)

In table 1 patients characteristics at start of treatment are shown. The mean difference between carpal and phalangeal 'BA' appeared to be 0.5 yrs (range 0.1 – 1.1) at start of treatment. Height-SDS for BA (H-SDS_{BA}) for girls in group A stabilised after an initial increase, whereas group B showed a continuous increase (figure 1). The differences between the two groups did not reach statistical significance. The change in H-SDS_{BA} from start to 3 years of treatment was significantly different between group A and B: 0.39 (0.41) and 0.83 (0.38), respectively (p=0.01). Significance was also present during the course of treatment between group A and B in repeated measurements analysis ($p < 0.001$).

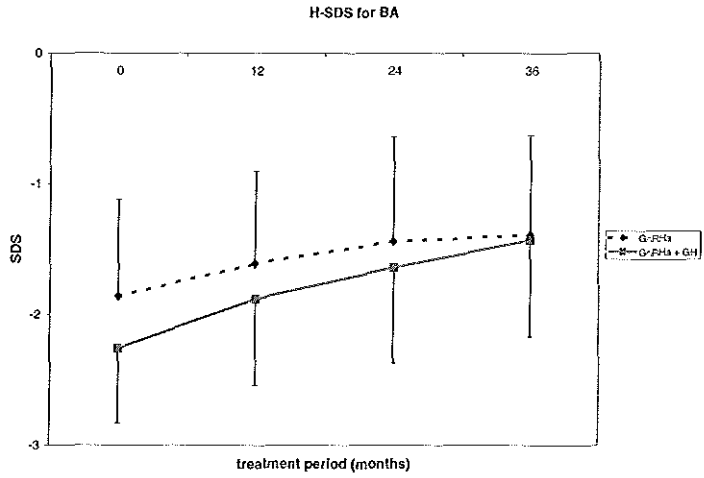


Figure 1. Growth during treatment: H-SDS for BA, significant differences between groups in course of treatment (p<0.001).

Sitting height SDS did not change significantly during treatment in group A and B and no significant differences were present between group A and B. Sitting height / height ratio SDS slowly increased during treatment with a significant difference in both groups between start and at 36 months ($p = 0.001$) but without significant differences between group A and B in the course of treatment.

Height velocity (HV) calculated over the consecutive 12-month periods was significantly higher in group B in the first year of treatment: 5.4 (1.15) and 6.7 (1.59) cm / yr in group A and B respectively ($p < 0.05$). HV gradually decreased to 3.6 (1.00) and 4.1 (1.67) cm /yr respectively in the last year (figure 2a). In table 2 the bone maturation, defined as ΔBA / ΔCA shows that BA maturation was < 1.0 during the whole treatment period and was not significantly different between the groups. BA at discontinuation of treatment was 12.3 (SD 0.9) yrs in group A and 13.0 (0.6) in group B.

Table 2: Bone age maturation in girls and boys (mean, SD)

ΔBA / ΔCA period (months)	Group A	Group B
0-12	0.64 (0.18)	0.64 (0.17)
12-24	0.54 (0.19)	0.46 (0.16)
24-36	0.54 (0.19)	0.42 (0.22)

The resulting parameter (PAH) is shown in figure 2b. In both group A and B the increase in PAH between start and 3 years of treatment was highly significant ($p < 0.001$), the change in PAH in time between group A and B was significant during the treatment period ($p< 0.001$).). In figure 2c the gain in PAH compared to initial prediction is shown at different moments. The gain in PAH was significantly different between group A and B at 36 months of treatment: 5.7 (3.8) and 10.1 (3.8) cm respectively ($p < 0.01$). In group B gain in height prediction was negatively correlated with BA at start of treatment ($rho = -0.60$, $p = 0.02$). In 13 girls actual height at discontinuation of treatment already exceeded initial height prognosis, 11 of them belonging to group B.

A temporary increase in Body Mass Index (BMI) SDS was observed in group A, while in the girls with combined treatment BMI-SDS increased from 0.58 (0.97) at start to 0.99 (0.81) at discontinuation of treatment ($p<0.05$). The change in BMI-SDS between start and

Legend to Figure 2: height velocity and changes in height prediction.
2a: Height velocity per treatment year. 2b: Absolute values of PAH; significant differences between groups in time ($p<0.001$); 2c: cumulative height gain compared to start of treatment; asterisks indicate differences between group A and B. *: $p<0.05$, **: $p<0.01$

Fig 2a

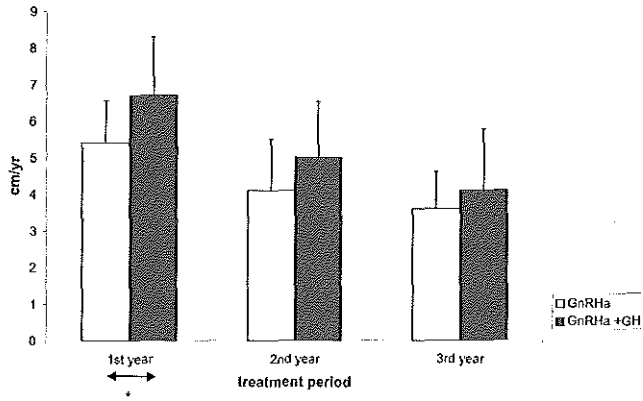


Fig 2 b

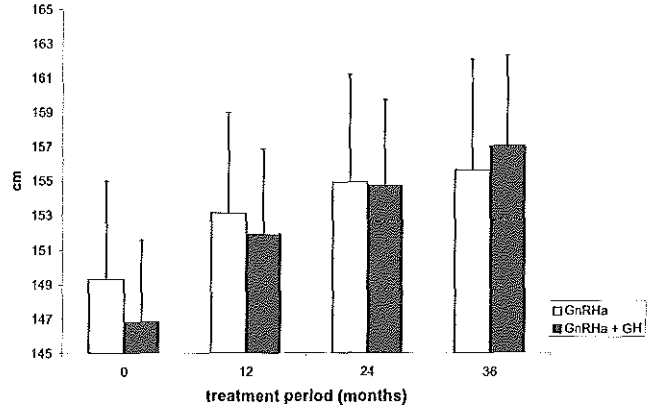
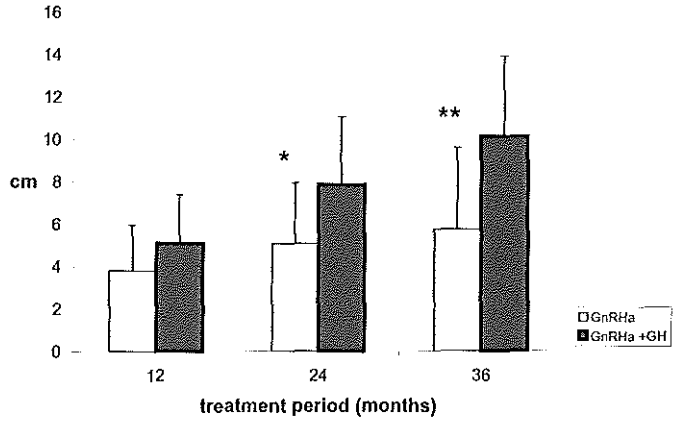


Fig 2c



discontinuation of treatment was not significantly different between group A and B. The number of girls with BMI-SDS > 2 did not change in group B. BMI-SDS was not different between the 2 groups during the treatment period.

Hormonal data

Suppression of puberty was monitored by a GnRH stimulation test. During treatment mean LH peaks for all patients were 0.95 (1.16), 1.18 (2.09) and 0.62 (0.43) at 12, 24 and 36 months respectively. Mean estradiol level in girls at start of treatment was 67.5 (45.6) pmol/L (range 39-203). Estradiol levels were suppressed during treatment and mean values did not exceed 40 pmol/L except for 1 girl in group B at 6 months of treatment with an E2 value of 50 pmol/L. No significant differences were seen between group A and B.

Serum androstenedione and DHEA-S levels in girls were not significantly different between group A and B during the course of treatment. When taken group A and B together, the difference between levels at start and at discontinuation of treatment were significantly different for both androstenedione and DHEA-S ($p < 0.001$, table 3)

Table 3 Main changes in serum DHEA-S and androstenedione levels in girls; see text for detailed description.

(mean; SD)	Group A	Group B
DHEA-S at start (µmol/L)	1.81 (1.20)	1.80 (0.85)
DHEA-S at 6 months (µmol/L)	2.04 (1.29)	2.26 (1.33)
DHEA-S at 36 months (µmol/L)	3.34 (1.80)*	3.32 (1.29)*
Androstenedione at start (nmol/L)	3.48 (2.21)	2.69 (1.46)
Androstenedione at 6 months (nmol/L)	1.85 (0.93)#	1.71 (0.89)#
Androstenedione at 36 months (nmol/L)	2.75 (1.72)	2.82 (1.45)

Paired t-test: * $p < 0.001$ compared to start # $p < 0.05$ compared to start

In girls, at start of treatment IGF-I SDS was 1.14 (1.02) when corrected for CA and 0.67 (0.90) when corrected for BA, both significantly higher than 0 ($p \leq 0.001$). IGF-I was higher in group B than in group A, both the serum levels (figure 3) and the SD scores for CA and BA. In the longitudinal analysis between groups, there were significant differences between group A and B for the IGF-I serum levels and for the SD scores (all $p < 0.01$). In group A IGF-I - SD_{BA} scores gradually decreased during treatment while the SD scores for group B stabilised at values significantly higher than in group A at 24, 30 and 36 months ($p < 0.05$).

No significant changes during the course of treatment, between groups or between group A and B during treatment were present for IGFBP-3 levels or IGFBP-3 SD scores. Mean IGFBP-3 SD scores for BA remained < 0 and showed only small variations within 1 SDS. The ratio IGF-I / IGFBP-3 remained stable in group B, whereas the values in group A were significantly lower in the second half of the treatment period.

In group B significant correlations were present between IGF-I-SDS_{BA} and HV at 0, 12 and 24 months of treatment, where IGF-I SDS_{BA} correlated with HV in the following year of treatment (Pearson correlation coefficients: -0.62, -0.63 and –0.67 respectively, $p < 0.05$ or < 0.001).

Figure 3: IGF-I and IGFBP-3 levels during treatment; 3a: absolute values of IGF-I in ng/ml; differences between group A and B: at 12 and 24 months: $p<0.05$ and at 36 months: $p<0.01$; the change between group A and B in time is significantly different ($p < 0.01$)
3b: absolute values of IGFBP-3 in mg/L. No significant changes between groups and/or in time

Fig 3a

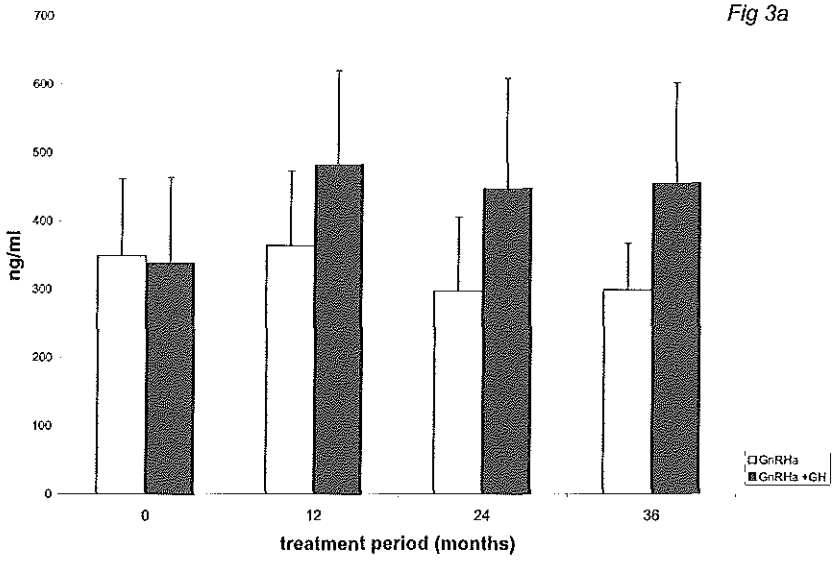
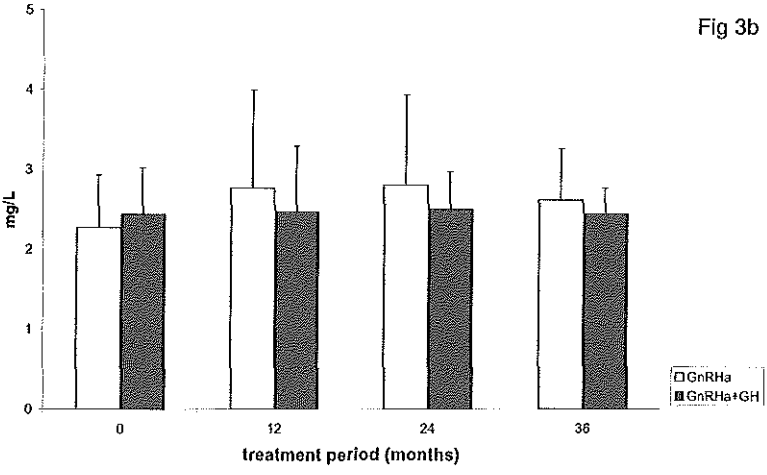


Fig 3b



Laboratory evaluation of renal and hepatic function did not reveal any abnormalities. In group B mean and maximal HbA1c levels remained equal or below initial values and did not exceed 6.6 % during the treatment period.

Ultrasound
Ovarian ultrasound measurements revealed a decrease in ovarian volumes, from 2.6 (1.1) ml at start to 1.5 (0.7) ml at 36 months ($p < 0.01$). Neither the absolute values nor the calculated SD scores were significantly different between group A and B in the course of treatment.

In both groups, the uterine volumes decreased, with higher values in group B, the difference reaching significance only at 24 months: 2.5 (1.83) ml and 4.0 (3.37) ml in group A and B respectively, ($p < 0.05$). During treatment, no significant difference was observed between the treatment groups.

Boys
In the 3 boys mean increase in PAH was 5.9 (1.46) cm. Height SDS for BA increased from –1.93 (0.90) to –0.89 (0.83) after 3 years of treatment. In this treatment period BA increased from 10.5 (1.4) to 12.1 (1.54) years and mean HV was 6.5 (0.71), 4.8 (0.27) and 4.3 (0.17) cm/yr in the first, second and third year respectively.

Side effects
No serious side effects were observed during treatment. One girl in group B had local erythema after the triptorelin injection. Treatment was continued after 15 months of triptorelin with leuprolide acetate in a comparable dosage given s.c. whereafter effective suppression was established. One girl in group A dropped out 15 months after start of treatment.

Discussion

In this randomised trial comparing the effect of GnRH agonist treatment alone or in combination with GH in adopted children with early puberty we show that the addition of GH to GnRHa treatment results in a significant increase in PAH after 3 years of treatment. Our results are in line with the data of Tuvemo et al. who also showed beneficial effects after 2 years of GH addition in adopted girls in a comparable study design using Buserelin nasal spray as the GnRH agonist (9).

The best way to perform our study would have been a design with an untreated control group. However, we did not succeed in performing such a trial as explained above. Thus, children treated with GnRH agonist alone served as the best possible controls and historical data of untreated adopted children will be gathered for final height analysis.

The importance of effective suppression of the hypothalamo-pituitary-gonadal axis to reach the auxological goals of GnRHa treatment is well-established (20). Effective suppression was ascertained in this study allowing proper analysis of the additive effect of GH.

The rationale for the addition of GH to GnRHa treatment is based on the clinical observation that after some period of GnRHa treatment height velocity decreases below even normal prepubertal levels (21, 22). In our study, HV decreased during treatment in both treatment groups, with a higher HV in group B at any time point although the difference was only small in the last 6 months of treatment. The IGF-I SDS corrected for BA also showed a gradual decrease during treatment. However, mean serum levels remained in the normal range in both groups, thus not providing a final explanation for the decrease in HV.

Walvoord, reviewing the current literature on the combined treatment concluded that the influence of GnRHa treatment on the GH-IGF-axis is not clear (23). Our data

demonstrate higher IGF-I levels and SD scores in the group with combined treatment, and both in group A and group B a decreasing trend over time is present. Thus, addition of GH is able to maintain IGF-I levels in a higher range during GnRHa treatment. These higher IGF-I levels seem to result in higher HV in group B. However, despite the stabilisation of IGF-I levels in the last year of treatment, HV shows a decline, suggesting that the amount of IGF-I alone is not sufficient to maintain the previous HV, neither on a rate appropriate for prepubertal girls nor appropriate for the the relative high SD scores for IGF-I. The explanation for reduced growth despite adequate GH and IGF-I levels is not clear. There might be a direct effect of severe sex steroid deficiency on skeletal growth or an involvement of local factors (22). In rat, the oestrogen withdrawal by GnRHa caused increased apoptosis, and no direct role of GnRHa itself could be demonstrated inhibiting growth (24). IGFBP-3 values did not change during treatment, in line with other reports in GnRHa treated children (25) but in contrast to an earlier report on IGFBP3 levels in children on combined treatment (26). The changes in the ratio between IGFBP-3 and IGF-I in group A may have resulted in higher binding of circulating IGF, thus limiting the free fraction. This may raise the question whether further GH dose increments during GnRHa treatment would result in higher HV, such as described in Turner syndrome (27).

From our study it can be concluded that the addition of GH to GnRHa results in higher IGF-I levels accompanied by higher HV in the first years of treatment. Thus GH is able to prevent the negative effects of GnRHa on the IGF-I or GH secretion. However, it is known that the results of GH testing do not adequately reflect growth characteristics during GnRHa, and one should account for the changes in BMI (22, 28). The absence of a difference in change in BMI-SDS between group A and B does not support the hypothesis that a decrease in GH dependent growth factors would lead to an increase in fat mass (29). On the other hand it should be realised that BMI is not the optimal parameter to assess body fat mass in children (30) and that the changes in BMI-SDS in our study were relatively small.

The difference between group A and B in height gain in our study is mainly explained by the higher height velocity during treatment in group B, occurring in the first 2 years of treatment, since the addition of GH had no significant effect on the rate of skeletal maturation. A similar observation was made by others (22, 26, 31). In the study of Tuvemo et al. the rate of maturation was considerably higher ($\Delta BA / \Delta CA = 1$) compared to our study, and height gain was considerably smaller. This may be due to the use of Buserelin as nasal spray that may have had a less suppressive effect on the hypothalamo-pituitary-gonadal axis when compared to suppression with depot preparations (32). However, in their study LH values in GnRH stimulation tests during treatment revealed suppressed pituitary activity with a comparable assay. These contradictory findings could be explained by the different methods of BA assesment and height prediction.

As pointed out by Carel *et al.* several methodological questions arise in the evaluation of the effect of treatment in CPP (33). The Greulich & Pyle (G&P) method is most often used in CPP, but the disadvantages of any method with regard to height prediction are well known (34). In 1997 Kauli et al. described that the use of average tables of BP prediction method in girls with CPP results in more reliable height predictions than when the accelerated tables are used as commonly done in children with CPP. As we described earlier, using this prediction method makes it difficult to compare our data with earlier reports, that may have overestimated initial height prediction (20). In our study we used an adapted G&P method for BA assessment, which is close to the original recommendations made by Greulich and Pyle (35): the "point scoring system" of BA results in smaller intra-observer variations than an "atlas matching" method such as the way the G&P method is used in daily practice (16).

The next issue is the reliability of western world standards for adopted children. Prakash studied skeletal maturity in well-off children in India and showed that the RUS

maturity score for girls reflected a parity with the British standards. The carpal scores ran from the 50th to the 25th percentile after 10 years in girls and after 11 years in boys (36). The influence of malnutrition or catch-up growth on skeletal maturation has been described (37, 38) but the effect of the transition to favourable circumstances is not known. One might hypothesise that catch-up growth of children coincides with an increased rate of skeletal maturation, resulting in advancement of BA compared to standards. Whether this is followed by a normal maturation rate after the period of catch-up growth is not known. In clinical practice we and others (2, 8) have seen that in some children, especially those who were adopted at an older age, catch-up growth after arrival in Europe passed into a pubertal growth spurt. The reported data on adopted children with early puberty led to the suggestion that increased growth rate during recovery from nutritional deprivation in a critical period could result in early onset of puberty. This was confirmed in an animal model where the role of increased IGF-I as an important mediator is suggested in the accompanying paper (8). Proos et al showed in Indian girls adopted in Sweden that there is a correlation between the rate of catch up growth and age of menarche in children that had arrived after 3 years of age (2). Catch up growth will not only increase IGF-I levels but leptin levels as well due to the increase in body fat mass. In girls with precocious puberty serum leptin levels were modestly increased compared to girls matched for pubertal stage and a negative correlation between leptin SDS and BMI was observed (39).

In girls adopted at a younger age another mechanism must be present. In those children catch up growth is present and is followed by a period of normal prepubertal growth. The mechanism for the early onset of puberty is not clear, and might be comparable with catch up growth in children after intrauterine growth retardation. There may be environmental factors in the pre- or postnatal period having long-term effects on the hypothalamo-pituitary action (40), for example on the hypothalamic control of LH release (41).

The hesitation observed around the decision to treat adopted children with early puberty and low predicted adult height may be influenced by the assumption that early puberty is normal for these children. However, the available data from the countries of origin show that the normal age of menarche is higher than the normal age of menarche in The Netherlands, thus indicating an effect of the transition to Europe (1, 6, 42, 43). In our view treatment is justified to limit the negative effects of early closure of the epiphyseal plates and offering the children an adult height as close as possible to their genetic potential. Our results imply that addition of GH could be considered not only in adopted children but also in non-adopted children without GH-deficiency with early puberty and treated with GnRHa. However, with respect to the high costs of GH treatment a balance should be found between cost and benefit. Further, it was described in CPP that height gain at FH was less than expected from the height prediction at the end of treatment, due to poor post-treatment growth (44).

We conclude that the addition of GH to GnRHa treatment in adopted girls with early puberty results in a significant increase in PAH after 3 years of treatment. Follow up until FH is required to assess the ultimate results.

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References

1. Proos LA, Hofvander Y, Tuvemo T. Menarcheal age and growth pattern of Indian girls adopted in Sweden I menarcheal age. Acta Paediatr Scand 1991;80:852-858.
2. Proos LA, Hofvander Y, Tuvemo T. Menarcheal age and growth pattern of Indian girls adopted in Sweden. II. Catch-up growth and final height. Indian J Ped 1991;58:105-114.
3. Virdis R, Street ME, Zampolli M, et al. Precocious puberty in girls adopted from developing countries. Arch Dis Childh 1998;78:15-154.
4. Marx M, Schoof E, Kapferer L, Dorr HG. Pubertas praecox vera bei einem indischen Mädchen nach Adoption durch deutsche Eltern. Monatsschr Kinderheilk 1998;146:957-960.
5. Oostdijk W, Yap YN, Doude van Troostwijk ML, et al. Final height and timing of puberty of adopted children in The Netherlands. Horm Res 1991;35(2):24.
6. Oostdijk W, Yap YN, Rekers-Mombarg LTM, Massa GG, Brand R, Drop SLS. The impact of early puberty on final height in foreign born, adopted children in The Netherlands. In: Central Precocious Puberty and gonadotropin releasing hormone agonist treatment. Thesis Erasmus University Rotterdam; 1996. p. 135-150.
7. Bureau JJ, Maurage C, Bremond M, Despert F, Rolland JC. [Children of foreign origin adopted in France. Analysis of 68 cases during 12 years at the University Hospital Center of Tours]. Arch Pediatr 1999;6(10):1053-8.
8. Bourguignon JP, Gerard A, Alvarez Gonzales ML, Fawe L, Franchimont P. Effects of changes in nutritional conditions on timing of puberty: clinical evidence from adopted children and experimental studies in the male rat. Horm Res 1992;38(suppl 1):97-105.
9. Tuvemo T, Gustafsson J, Proos LA. Growth hormone treatment during suppression of early puberty in adopted girls. Swedish Growth Hormone Advisory Group. Acta Paediatr 1999;88(9):928-32.
10. Hiteman SM, Pierroz DD, Flier JS. Leptin, nutrition, and reproduction: timing is everything [editorial; comment]. J Clin Endocrinol Metab 2000;85(2):804-7.
11. Kohn B, Julius JR, Blethen SL. Combined use of growth hormone and gonadotropin-releasing hormone analogues: the national cooperative growth study experience. Pediatrics 1999;104(4 Pt 2):1014-8.
12. Pasquino AM, Pucarelli I, Roggini M, Segni M. Adult height in short normal girls treated with gonadotropin-releasing hormone analogs and growth hormone. J Clin Endocrinol Metab 2000;85(2):619-22.
13. Partsch CJ, Hümmelink R, Lorenzen F, Sippell WG, Studiengruppe dD-D. Bedeutung und Charakteristika des LHRH-Testes in der Diagnostik der vorzeitigen Pubertatsentwicklung bei Madchen: Der stimulierte LH/FSH-Quotient differenziert zwischen zentraler Pubertas praecox und pramaturer Thelarche. Monatssch Kinderheilkd 1989;137:284-288.
14. Roede MJ, Wieringen JC van. Growth diagrams 1980. Netherlands third nation-wide survey. Tijdschr Soc Gezondheidszorg 1985;63 (suppl.):11-32.
15. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height,weight,height velocity, and stages of puberty. Arch Dis Childh 1976;51:170-179.
16. Bull RK, Edwards PD, Kemp PM, Hughes IA. Bone age assessment: a large scale comparison of the Greulich and Pyle, and Tanner and Whitehouse (TW2) methods. Arch Dis Childh 1999;81:172-173.
17. Kauli R, Galatzer A, Kornreich L, Lazar L, Pertzelan A, Laron Z. Final height of girls with central precocious puberty, untreated versus treated with cyproteron acetate or GnRH analogue. A comparative study with re-evaluation of predictions by the Bayley-Pinneau method. Horm Res 1997;47:54-61.
18. Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF- binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 1998;50(3):166-76.
19. Griffin IJ, Cole TJ, Duncan KA, Hollmann AS, Donaldson MDC. Pelvic ultrasound measurements in normal girls. Acta Paediatr 1995;84:536-543.
20. Mul D, de Muinck Keizer-Schrama SM, Oostdijk W, Drop SL. Auxological and Biochemical Evaluation of Pubertal Suppression with the GnRH Agonist Leuprolide Acetate in Early and Precocious Puberty. Horm Res 1999;51(6):270-276.
21. Oostdijk W, Drop SLS, Odink RJH, Hümmelink R, Partsch CJ, Sipell WG. Long-term results with a slow release gonadotrophin-releasing hormone agonist in central precocious puberty. Acta Paediatr Scand 1991;372(suppl):39-45.
22. Saggese G, Pasquino AM, Bertelloni S, et al. Effect of combined treatment with gonadotropin releasing hormone analogue and growth hormone in patients with central precocious puberty who had subnormal growth velocity and impaired height prognosis. Acta Paediatr 1995;84:299-304.

23. Walvoord EC, Pescovitz OH. Combined use of growth hormone and gonadotropin-releasing hormone analogues in precocious puberty: theoretic and practical considerations. Pediatrics 1999;104(4 Pt 2):1010-4.
24. Tomkinson A, Gevers EF, Wit JM, Reeve J, Noble BS. The role of estrogen in the control of rat osteocyte apoptosis. J Bone Miner Res 1998;13(8):1243-50.
25. Kanety H, Karasik A, Pariente C, Kauschansky A. Insulin-like growth factor-I and IGF binding protein-3 remain high after GnRH analogue therapy in girls with central precocious puberty. Clin Endocrinol (Oxf) 1996;45(1):7-12.
26. Tatò L, Saggese G, Cavallo L, et al. Use of combined Gn-RH agonist and hGH therapy for better attaining the goals in precocious puberty treatment. Horm Res 1995;44 (suppl.3):49-54.
27. Sas TC, de Muinck Keizer-Schrama SM, Stijnen T, et al. Normalization of height in girls with Turner syndrome after long-term growth hormone treatment: results of a randomized dose-response trial. J Clin Endocrinol Metab 1999;84(12):4607-12.
28. Kamp GA, Manasco PK, Barnes KM, et al. Low growth hormone levels are related to increased Body Mass Index and do not reflect impaired growth in Luteinizing Hormone-Releasing Hormone agonist-treated children with precoccious puberty. J Clin Endocrinol Metab 1991;72:301-307.
29. Boot AM, Muinck Keizer - Schrama SMPF de, Pols HAP, Krenning EP, Drop SLS. Bone mineral density and body composition before and during treatment with Gonadotropin-Releasing Hormone Agonist in children with central precocious and early puberty. J Clin Endocrinol Metab 1998;83:370-373.
30. Warner JT, Cowan FJ, Dunstan FD, Gregory JW. The validity of body mass index for the assessment of adiposity in children with disease states. Ann Hum Biol 1997;24(3):209-15.
31. Pasquino AM, Municchi G, Pucarelli I, Segni M, Mancini MA, Troiani S. Combined treatment with Gonadotropin-Releasing Hormone analog and growth hormone in central precocious puberty. J Clin Endocrinol Metab 1996;81:948-951.
32. Partsch CJ, Hümmelink R, Peter M, et al. Comparison of complete and incomplete suppression of pituitary- gonadal activity in girls with central precocious puberty: influence on growth and predicted final height. J Clin Endocrinol Metab 1993;39:111-117.
33. Carel J, Chaussain J. Gonadotropin Releasing Hormone Agonist Treatment for Central Precocious Puberty. Horm Res 1999;51(Suppl S3):64-69.
34. Hintz RL. Issues in final height prediction [comment]. Acta Paediatr Suppl 1996;417:51-2.
35. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. 2 ed. Stanford, California: Stanford University Press; 1959.
36. Prakash S, Cameron N. Skeletal maturity of well-off children in Chandigarh, North India. Ann Hum Biol 1981;8:175-180.
37. Briers PJ, Hoorweg J, Stanfield JP. The long-term effects of protein energy malnutrition in early childhood on bone age, bone cortical thickness and height. Acta Paediatr Scand 1975;64(6):853-8.
38. Maniar BM, Kapur PL, Seervai MH. Effet of malnutrition on bones of hand in children. Indian Pediatr 1974;11(3):213-226.
39. Palmert MR, Radovick S, Boepple PA. Leptin levels in children with central precocious puberty. J Clin Endocrinol Metab 1998;83(7):2260-5.
40. Cooper C, Kuh D, Egger P, Wadsworth M, Barker D. Childhood growth and age at menarche. Br J Obstet Gynaecol 1996;103(8):814-7.
41. Cresswell JL, Barker DJ, Osmond C, Egger P, Phillips DI, Fraser RB. Fetal growth, length of gestation, and polycystic ovaries in adult life [see comments]. Lancet 1997;350(9085):1131-5.
42. Pathmanathan G, Prakash S, Chhibber SR. Single occasion means of height and weight of SriLankan Tamil children from Jaffna. Ann Hum Biol 1990;17:107-113.
43. Indian Council of Medical Research. Indian average population. Growth and physical development of Indian infants and children. Technical report Series 1989(18).
44. Oostdijk W, Rikken B, Schreuder S, et al. Final height in central precocious puberty after long-term treatment with a slow-release GnRH agonist. Arch Dis Childh 1996;75:292-297.

III TREATMENT WITH GNRHA AND GH

Chapter 6: Study of the effect of treatment with GnRH-agonist (Decapeptyl) alone or in combination with biosynthetic human growth hormone (Genotropin) in adopted children with early puberty.

6.1 Gonadotrophin Releasing Hormone agonist (GnRHa) treatment with or without recombinant human growth hormone (GH) in adopted children with early puberty

6.2 Study of the effect of treatment with GnRH (GnRH)-agonist alone or in combination with recombinant human growth hormone in adopted children with early puberty: Psychological assessments before and after treatment *submitted*

6.3 Motivation for treatment and psychological evaluation in adoptive families when a child presents with early puberty

Chapter 7

A randomized controlled trial of three years of growth hormone (GH) and GnRH agonist treatment in children with Idiopathic Short Stature and Intra-Uterine Growth Retardation.

Chapter 8

The effect of pubertal delay by GnRH agonist in Growth Hormone Deficient (GHD) children on final height

Study of the effect of treatment with Gonadotrophin Releasing Hormone agonist alone or in combination with recombinant human growth hormone in adopted children with early puberty: Psychological assessments before and after treatment.

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ABSTRACT

Early puberty is frequently observed in adopted children. We treated 30 adopted children with early puberty and short stature in a randomised trial with either a Gonadotrophin Releasing Hormone agonist (GnRHa) alone or in combination with growth hormone (GH) for 3 years. In this trial the children and their parents underwent a psychological evaluation before start of treatment (T1) and at discontinuation (T2). At start of treatment the children did not have increased levels of behavioural or emotional problems as assessed by the Child Behaviour CheckList (CBCL). During treatment the CBCL scores did not increase. Self-perception of the children appeared to be normal, and after 3 years even a significant higher score for acceptance by peers was observed. At T1, in 80% of the children and in 17% in the parents an overestimation of future height was present. Lower family stress was observed at T1 and T2 compared to reference values. We discuss the findings with reference to the reported levels of behavioural and emotional problems in adopted children and psychosocial effects of precocious puberty. We conclude that in adopted children with early puberty the psychological evaluation did not reveal any consistent abnormality. The treatment with GnRHa with or without GH does not increase emotional and behavioural problems nor decrease their self-perception.

INTRODUCTION

Early puberty is frequently seen in foreign-born adopted children (1-4). No clear explanation is available to understand the occurrence of early puberty. It can be hypothesised that the improvement of nutritional status and socio-economic situation compared to the country of origin results in an accelerated maturation of neuro-endocrine structures, especially during a specific critical period after birth.

The effects of early puberty are well known: psychosocial distress and emotional problems (5-8) in addition to a decreased final height (7, 9, 10). Psychosocial evaluation in adopted children has revealed increased parent-reported emotional and behavioural problems compared to non-adopted peers (11, 12). It may be assumed therefore that adopted children with early puberty are specifically at risk for emotional and behavioural problems. These problems could be attributed to early pubertal development, short stature and the adoption status.

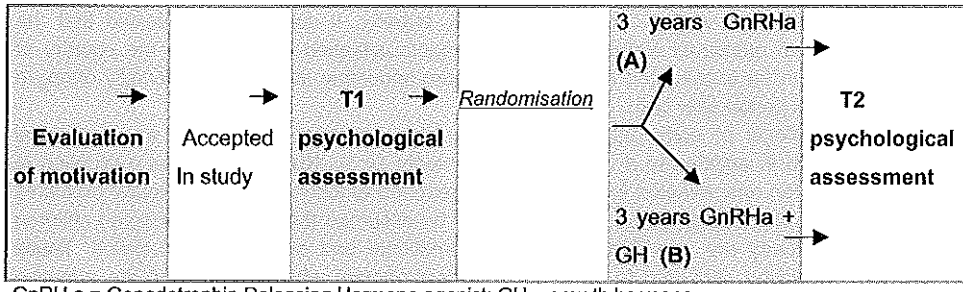
Currently the pubertal development in central precocious puberty or early puberty can be arrested effectively by Gonadotrophin-Releasing Hormone agonists (GnRHa) administration (13). We studied the effect of puberty-delaying treatment with GnRHa alone or in combination with recombinant human growth hormone (GH) to promote growth in adopted children with early puberty. The aim of the study was to evaluate the psychological effects of early puberty and short stature itself and of the treatment given. In this paper we describe the results of psychological assessments before treatment (T1) and after treatment (T2). The evaluation of motivation for treatment and the effects of treatment on growth and puberty will be reported separately.

PATIENTS AND METHODS

Children in this study originate from India, Sri Lanka, Colombia and Korea and were adopted by Dutch parents. The children developed early puberty, defined as pubertal development between 7 and 10 years of age in girls and between 8 and 11 years in boys. In girls, the onset of puberty was characterised by breast development and/or menarche, in boys by testicular enlargement ($\geq$ 4 ml). In both the physical signs were accompanied by a pubertal rise in gonadotropins in a GnRH stimulation test. To assess bone age (BA) an X-ray of the left hand was obtained. The predicted adult height (PAH) was calculated on the basis of height and BA. For inclusion in the study PAH had to be below the third percentile of Dutch children for sex (14).

The children in this study were treated for 3 years with either the GnRH-agonist triptorelin (Decapeptyl®) alone (*group A*) or in combination with recombinant human growth hormone (Genotropin®) (*group B*). A physician or nurse gave GnRH agonist every 28 days by an intramuscular (IM) injection; growth hormone was administered every day by subcutaneous (SC) injection at home by the patient him-/herself or one of the parents.
The study schedule is depicted in figure 1 .

Figure 1: Study schedule



GnRH a = Gonadotrophin Releasing Hormone agonist; GH = growth hormone

►Change in design

In the original study design a third arm with untreated children was scheduled as a control group. It was decided to leave this control group out of the study design after it appeared that the parents of all patients who were randomised in the untreated control group refused further participation in the study as GnRHa treatment could be obtained elsewhere. This article describes the results of the patients entering into the treatment protocol.

►T1 data collection

Before randomisation for group A or B, a psychological assessment (T1) was performed. The instruments used are shown in table 1.

Child Behaviour Checklist (CBCL): The CBCL is a standardised instrument to obtain parents' reports of competence and problem behaviour of their child (15). It consists of 20 competence items and 120 problem items. Competence ratings were scored on three competence scales: activities, social and school and also on a total competence score. The problem items that describe the child are summarised in a score for internalising (e.g. withdrawn, anxious /depressed), externalising (e.g. aggressive, delinquent) problems and a total problem score. The reliability and discriminative validity of the Dutch translation has been established (16). Reference values for adopted children in the Dutch population were used for comparison (17). The Teacher Report Form (TRF) is the CBCL version to be completed by the child's teacher. Reference values for the Dutch population are available (18).

Self-perception profile for children (SPPC): Harter developed this self-reported instrument for measuring self-esteem or perceived competence in children (19). Self-competence in different domains is measured (scholastic and athletic competence, social acceptance, physical appearance and behavioural conduct), as well as an independent assessment of global self-worth. A Dutch translation was validated by Van Dongen-Melman *et al.* (20). The SPPC has been used in several studies on short stature (21, 22). For this study we analysed data on social acceptance and physical appearance as children with early puberty might be less socially accepted and do not feel comfortable with their physical appearance.

Table 1: Psychological instruments at T1

Parents	*Child*
Standardised structured interview on signs of early puberty	Standardised structured interview on signs of early puberty
Standardised structured interview on expected aspects of treatment	Standardised structured interview on expected aspects of treatment
Silhouette Apperception Technique (SAT)	Silhouette Apperception Technique
Child Behaviour Checklist (CBCL) and Teacher Report Form (TRF)@	Self Perception Profile for children
Questionnaire on family stress (NVOS)	Wechsler Intelligence Scale for children (WISC)#

@: CBCL version to be completed by the teacher; #:short form: 'block design' and 'information' (39)

Silhouette Apperception Technique (SAT): This test describes the accuracy of estimation of current and future height and was developed to study expectations of treatment with recombinant growth hormone (23, 24)

NVOS: The "Nijmeegse Vragenlijst voor de Opvoedings Situatie" is a validated Dutch questionnaire evaluating family stress. It is to be completed independently by the father and the mother of a child. With the NVOS many aspects of family functioning and family stress are evaluated. We limited our analysis to 4 main items that we regarded to be of specific importance: first to what extent the family functioning is accepted or should be changed, the presence of problems (2nd) and whether the parents are able to deal with problems (3rd). For these items a higher score indicates increased family stress. A general assessment of family functioning is expressed in the 'satisfaction' item (4th): the higher the score, the more satisfied the parent is. The NVOS was used in earlier studies in children with precocious puberty and was validated for the Dutch population (25, 26).

Standardised structured interview on signs of early puberty and expected aspects of treatment: In the structured interviews issues on the duration of pubertal signs and the impact on the child as well as expected difficulties or problems with treatment or injections or with endurance were addressed.

▶Data collection during and after treatment

The T2 data collection was performed at the end of the 3-year treatment period. Except for the structured interviews we used identical instruments as were used in the T1 assessment. During treatment there was an evaluation of the treatment using a structured questionnaire for parents and the child, asking about endurance and burden of treatment and the perceived meaningfulness of the treatment.

Statistics

Comparisons from the observed data with known normative data were performed by one sample t-test. Non parametric (Wilcoxon signed rank) tests with paired data were used for the comparison between T1 and T2 values. Because of the small numbers of patients no extensive statistical methods were applied on the data.

The protocol was approved by the local medical ethical committees and by the Dutch National Board on Medical Research (KEMO). Written informed consent was obtained from all participants in this study.

RESULTS

After the assessment of motivation for treatment, 30 patients (27 girls) were eligible candidates for the study and entered into the treatment protocol, 12 from Sri Lanka, 9 from India, 7 from Colombia and 2 from South Korea.

We report results on the girls, unless stated otherwise. Mean age at start of treatment was 9.6 (SD: 0.90) years, mean height was 134.4 cm (7.0) cm and predicted adult height was 148.0 (5.3) cm (6 cm < P3). There was a wide range in age at adoption: from 1 to 84 months (median: 6 months). Thirteen girls were randomised to group A, 14 to group B. No significant differences were present between group A and group B at baseline.

During treatment, one girl dropped out from group A. The data collection was not complete for all parents as some of them refused participation in completing the questionnaires, mainly as they believed that there were no problems with the child to be reported or as they judged their family situation as being without problems.

The results of the height data are described separately (Mul *et al*; chapter 6.1). In summary, a mean increase in predicted adult height was observed in both groups, with a significant higher increase in group B compared to group A (10.1 vs. 5.7 cm).

1. Emotional and behavioural problems

We compared the CBCL scores from the study group with the known age matched norms for Dutch girls as well as with the findings in adopted girls in The Netherlands. Furthermore, scores on T1 and T2 were compared. Results are shown in table 2 (mean (SD)). There were no significant differences between the mean values at T1 and T2 when compared to either the norms of adopted or non-adopted children in The Netherlands. The decrease in total problem score and internalising and externalising scores did not reach statistical significance.

Table 2. CBCL results

	T1 N = 25	**T2 N = 23**	**Adopted children, (girls 10 and 11 yr, n = 235)**	**Dutch norm (girls 4-11 yr, n = 593)**
Total problem score	18.64 (17.49)	15.22 (13.01)	18.14 (17.12)	19.18 (14.82)
Internalising score	5.68 (5.36)	4.22 (4.98)	4.98 (5.49)	5.16 (5.02)
Externalising score	5.72 (5.98)	4.70 (5.16)	5.48 (6.35)	6.04 (5.57)

Total problem score in group A (GnRHa alone) was significantly higher than in group B (GnRHa + GH) at T1 assessment: 25.31 (19.98) and 11.42 (11.08) in group A and B respectively ($p<0.05$). All other measurements at T1 and T2, described in table 2, did not significantly differ between group A and group B.

In group B the total problem score was significantly lower than for the Dutch girls (T1: 11.42 (11.08), T2: 13.08 (8.30) vs.19.18 (14.82), n=12, both P < 0.05), but not compared to the adopted girls. No significant correlations were present between changes in the CBCL scores and the increase in predicted adult height during the study period. In 14 girls T1 and T2 scores of the TRF were available. No significant differences were observed between T1 and T2. Total problem score, internalising and externalising scores decreased according to the age-specific pattern.

2. Self-perception

The mean scores at T1 and T2 for girls for general self-worth scores were not significantly different from those of the Dutch references (20). Mean scores for physical appearance decreased from T1 to T2, and social acceptance values increased. The score for social acceptance at T2 was significantly higher than the reference population (p <0.01).

Table 3: HSPPC scores, n =23, mean (SD)

	T1 score (n=23)	T2 score (n=23)	Norm (girls)
General self-worth	3.34 (0.72)	3.25 (0.69	3.19
Physical appearance	3.12 (0.62)	2.81 (0.85)	3.03
Social acceptance	3.22 (0.71)	3.43 (0.49) **	3.02

** p < 0.01 compared to norm

There was no significant difference between the T1 or T2 scores for general self-worth, physical appearance and social acceptance between children with and without GH addition.
No significant correlation was present between the change in SPCC variables and the increase in predicted adult height.

3. Expectations of treatment

The Silhouette Apperception test before start of treatment showed that the parents did not expect an increase in height percentile from the estimated actual height to adult stature. The children however expected an increase from about the 30th percentile to about the 60th percentile at the time they would have reached adult stature (p<0.001).

At T2 assessment however, the expectations for future height were less optimistic in the children than at start of treatment, but the p value was still < 0.01 when compared to the estimation of present height. Data are summarised in table 3 for boys and girls.

When a score of ≥ P50 is assumed to indicate overestimation of height the percentage of children overestimating future height is 80 at T1 and 72 at T2; for parents the percentages are 17 and 15, respectively.

Table 4. Estimated actual and future height expressed in height percentiles in the study group for child and parents

	T1		T2	
	Child (n=30)	Parent (n=29)	Child (n=29)	Parent (n=26)
Actual height				
P3	12	14	5	11
P25	8	9	13	8
P50	4	6	8	4
P75	4	-	2	3
P97	2	-	1	-
Future height				
P3	1	10	1	9
P25	5	14	7	13
P50	10	4	6	2
P75	10	1	15	2
P97	4	-	-	-

4. Family stress

The NVOS scores from the mothers were used and studied for the whole group as a considerable number of fathers did not fill out the questionnaire. A summary of the main parameters is given in table 5.

Table 5: NVOS scores

	T1 (n=26)	T2 (n=22)	Norms Dutch
Acceptance	1.11 (0.18)♦	1.22 (0.33)*	1.40 (SD=0.49) n=234
Able to deal with problems	1.38 (0.33)♦	1.52 (0.31)#	1.71 (SD=0.56) n=234
Having problems	1.45 (0.41)♦	1.55 (0.39)♦	1.94 (SD=0.60) n=234
Satisfaction	4.09 (0.65)	3.80 (0.79)	3.86 (SD=0,58) n=167

♦ $p < 0.001$; * = $p < 0.05$; # = $p < 0.01$, all compared to norm

The 3 first mentioned items out of 4 listed in the table show lower values in the study group than in mothers from the general Dutch population. The higher the score on 'satisfaction' the higher the mother is satisfied about the rearing situation in the family. With respect to the studied NVOS items no significant differences were found between children with and without the addition of GH

5. Intelligence

The IQ levels for the whole group decreased significantly, but clinically not relevant from 100.2 (12.7) at T1 to 93.1 (10.5) at T2 (p=0.002). A comparable significant decrease was present in both groups. There were no significant differences between group A and group B at T1 or T2.

Table 6 Result of IQ subtests; 25 paired observations (mean SD)

	Verbal score	**Performal score**	**Total IQ**
Whole groupT1 Range:	9.08 (2.96) 3 - 19	11.48 (4.04) 6 – 26	100.2 (12.7) 77 – 138
Whole group T2 Range:	8.04 (1.92) 5 - 14	9.28 (3.08) 5 – 15	93.1 (10.5) 80 - 123
Difference T1-T2	NS	0.001	0.002

6. Structured interviews

Eighty % of the children reported that their classmates did not treat them differently from others, while in 13% (4 children) the child reported that she was bullied for any reason. With regard to the treatment, at T1 20% (6/22) of the children expected that it would be difficult to continue the monthly injections during the whole treatment period, while the parents expected only minor or no problems.

When the children (n=21) were asked to estimate pain from injections (scale: from much pain to no pain) the majority expected 'some pain', only 1 child expected 'pain' or 'much pain'. In parents (n=22) the distribution was shifted towards pain: 8 times: 'some pain' and 11 times 'pain' or 'much pain'.

The answers of parents to the question regarding the additive effect of GH administration indicated that they expected more gain from GH administration than the children did.

Discussion

When discussing and interpreting the results of this study we should acknowledge the limitations of the study with regard to sample size and the lack of an untreated control group. Furthermore, the several factors involved, for example the adoption status, early puberty and short stature make the interpretation even more difficult.

In contrast to our initial hypothesis, adopted children with early puberty and low predicted adult height did not differ markedly from adopted or non-adopted children with regard to emotional and behavioural problems or self-perception. Their parents did not exhibit higher scores on variables of family stress.

The results on the parents at T1 reveal that they are generally competent and realistic people, able to cope with the presence of early puberty in their adopted child. This is in line with the literature on families with adopted children or children born after reproductive techniques in which the quality of raising was shown to be better than in children born after normal conception (27). Explanatory factors for this quality could be the relatively high socio-economic status of adoption parents and the fact that they are selected on parenting capacities before adoption.

When the CBCL data are compared to normative data on Dutch adopted children we see that in our study population the children did not show more behavioural or emotional problems than their age and sex matched peers. The reason for this may be that most children in our study group had only a short period experiencing their secondary sexual maturation characteristics, e.g. breast development, not yet giving rise to shame or bullying. Due to a pubertal growth spurt the actual height can even be experienced positively by the child as it may bring the child 's height more close to its peers. Another reason might be the positive attitude of the parents to their children emphasising that being different from peers can be labelled positively. Moreover, many people estimate the early pubertal development as normal for a child adopted from developing countries. Thirdly, the children are strongly supported by their parents in coping with their early pubertal development. Similar factors could be used to explain the results from the HSPP social acceptance subscale demonstrating that adopted children feel themselves accepted by their peers, even now in the presence of signs of early puberty making them more different from their peers.

In the longitudinal analysis CBCL scores showed decrease in the total problem score and subscales for internalising and externalising problem scores. The decrease is comparable with the lower scores in older children in the reference population. Apparently, the treatment does not contribute to increase in problem behaviour or emotional problems as one might expect when accounting for the intensity of treatment. This can be explained by the positive attitude of parents and the fact that a lot of attention is paid to the children in the research setting. Furthermore, the relationship between parents and the adopted child was described by the parents as close (28). The major intervention of suppressing the pubertal development, causing regression or arrest of pubertal development may decrease problematic behaviour (5) or at least prevent a further increase.

It is known from the literature that early or precocious puberty leads to elevated CBCL scores on the internalising syndrome (withdrawal and anxious /depressed) up to 2 years after start of treatment (5). We did not confirm this observation which may be explained in part by the age at start of treatment that was relatively high compared to reports in children with precocious puberty. No association was found between idiopathic precocious puberty and long-term severe psychopathology. However, it was reported that precocious puberty in girls is associated with a long-term risk of minor psychopathology (6, 29, 30). With respect to psychosexual development, early pubertal development was associated with earlier, but not extremely advanced psychosexual development (6, 29). During treatment with GnRH agonist problematic behaviour and functioning decreased slightly, particularly in the girls showing regression of breast development (5). In our population in all patients regression of the signs of puberty was observed.

The most remarkable finding in the longitudinal assessment of HSPP was the relative increase in the score for social acceptance. Several factors could play a role: the regression of pubertal signs brings the child back into the age matched peers, children may feel that treatment makes them more 'normal' now and in the future and maybe the decreasing trend in emotional or behavioural problems improves acceptance in the peer group.

It is difficult to demonstrate quantitatively to what extent children suffer from the early puberty or short stature due to the lack of standardised instruments. Therefore the indication for growth promoting treatments such as performed in this trial is hard to sustain as was demonstrated in the case of children with short stature (31). On the other hand we have to deal with parent's and children's expectations of treatment. In the SAT we studied the perceived current height and the expected height after treatment. The development of the scores from the SAT show that the expectations of the children with regard to future height are still positive at the end of treatment, while those in the parents did not change in the treatment period. This points to the limited capacity of the children to predict and estimate figures in the future, while

they are able to estimate their own current height in comparison to their peers. Parents did not have unrealistic expectations of the effect of treatment, probably due to the thorough information procedure before the informed consent was given. On the other hand, the children expected to be considerably taller at the end of treatment than they were at the moment starting treatment. In children with short stature about 60% of the girls had unrealistic expectations of their future height (≥ p50), compared to about one-third in parents (32).

With respect to the family stress the presence of early puberty and expected short stature does not increase family stress to levels above normal. On the contrary, family stress is even significantly lower on the studied sub items. We show that the treatment does not give rise to family stress above the limits of normal. Probably, we have to do with stable families, already used and able to handle difficulties (33). Thereby, in general the socio-economic and educational level of parents of adopted children is relatively high. One can conclude that, as in children from IVF or KID procedures, the parents are competent in dealing with the problems of their children. On the other hand it was described in IVF parents, 1 year postpartum, that they reported lower self esteem and IVF mothers saw their children as more vulnerable and 'special' compared with controls (34). They often mentioned that their motivation was based on the idea of precluding future regret on not having tried every possible treatment option available. This might theoretically lead to some kind of overprotection with possible adverse effects on the child. In a quantitative analysis of motivation for treatment we concluded that the parents were in the fast majority of cases adequately motivated (Mul *et al*, chapter 6.3).

In our study population the delay between start of pubertal development and start of treatment was not as long as in the study of Xhrouet et al. which was interpreted as a result of a cultural taboo on sexuality and pubertal development. Our experience does not indicate such a taboo being present in our population. Probably, the early occurrence of puberty in adopted children is regarded as normal for this group of children.

The role of background variables for the adjustment of adopted children was described in the early seventies by Bohman (35) as relatively independent from each other. Verhulst *et al.* however found that several background parameters placed the child at increased risk for later maladjustment, but even in case of the presence of such variables the majority of adopted children function quite well (36). The results of the psychological assessments we performed suggest normal functioning of the children. Early puberty and/or short stature does not seem to influence this negatively; however, they still have to go through puberty and adolescence which is known to be a vulnerable period for some adopted children with respect to identity and relations to parents (12). Secondly, when puberty progresses in peers, the feeling of being short may be enforced by the magnitude of the growth spurt in Dutch boys and girls.

The results on IQ measurements in children with precocious puberty showed elevated IQ scores, with higher verbal than performal scores, and this was interpreted as a possible effect of sex steroids especially on the left hemisphere (5, 30, 37). The initial total IQ score in our group is not different from normal - comparable with the data of Xhrouet *et al.* (5) and a decrease of about 7 points is observed in the treatment period. Although significant, one might have doubts about the clinical relevance of this decrease. An interesting hypothesis on the decrease in verbal IQ scores is that withdrawal of sex steroid exposure to the brain brings the child back into a more age-appropriate IQ range. The lower verbal scores in our group, which was in contrast to what was known in CPP girls, could be explained by the adoption status of children, from which it is known, as in other children from foreign background, that verbal intelligence is lower than children born in their own country. In adopted children it was described that in primary school mathematics, which is part of verbal IQ, was problematic, especially in boys (38). The authors concluded that a deficient development of visual-spatial organisation and to a lesser extent the lower concentration was due to the lower prestations in mathematics rather than intelligence or fluency.

The observation that there are no consistent differences between group A and B suggests that in early puberty the addition of GH does not contribute to development in either a protective or negative direction and that pubertal arrest is the main intervention in children with early puberty.

We conclude that in adopted children with early puberty and predicted short stature the levels of emotional and behavioural problems, self-perception and family stress do not significantly differ from the population means. The treatment with either GnRHa alone or in combination with GH does neither increase emotional and behavioural problems or family stress nor decrease self-perception of the child.

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REFERENCES

1. Bourguignon JP, Gerard A, Alvarez Gonzales ML, Fawe L, Franchimont P. Effects of changes in nutritional conditions on timing of puberty: clinical evidence from adopted children and experimental studies in the male rat. Horm Res 1992;38 (suppl 1):97-105.
2. Virdis R, Street ME, Zampolli M, et al. Precocious puberty in girls adopted from developing countries. Arch Dis Childh 1998;78:15-154.
3. Proos LA, Hofvander Y, Tuvemo T. Menarcheal age and growth pattern of Indian girls adopted in Sweden I menarcheal age. Acta Paediatr Scand 1991;80:852-858.
4. Proos LA. Anthropometry in adolescence - Secular trends, adoption, ethnic and environmental differences. Horm Res 1993;39 (suppl 3):18-24.
5. Xhrouet-Heinrichs D, Lagrou K, Heinrichs C, et al. Longitudinal study of behavioral and affective patterns in girls with central precocious puberty during long-acting triptorelin therapy. Acta Paediatrica 1997;86:808-815.
6. Ehrhardt AA, Meyer-Bahlburg HFL. Psychosocial aspects of precocious puberty. Horm Res 1994;41 (suppl 2):30-35.
7. Kaplowitz PB, Oberfield SE. Reexamination of the age limit for defining when puberty is precocious in girls in the United States: implications for evaluation and treatment. Drug and Therapeutics and Executive Committees of the Lawson Wilkins Pediatric Endocrine Society. Pediatrics 1999;104(4 Pt 1):936-41.
8. Lagrou K, Xhrouet-Heinrichs D, Bourguignon JP. Deviations in growth and pubertal development and their treatment: How consistent are patient's and physician's outlooks? In: Eiholzer U, Haverkamp F, Voss L, editors. Growth, stature, and psychosocial well-being. Seattle: Hogrefe & Huber Publishers; 1999. p. 131-37.
9. Sigurjonsdottir TJ, Hayles AB. Precocious puberty. A report of 96 cases. Am J Dis Child 1968;115(3):309-21.
10. Thamdrup E. Precocious sexual development. Copenhagen: Munksgaard; 1961.
11. Verhulst FC, Althaus M, Versluis-den Bieman HJ. Problem behavior in international adoptees: I. An epidemiological study. J Am Acad Child Adolesc Psychiatry 1990;29(1):94-103.
12. Versluis-den Bieman HJ, Verhulst FC. Self-reported and parent reported problems in adolescent international adoptees. J Child Psychol Psychiatry 1995;36(8):1411-28.
13. Mul D, de Muinck Keizer-Schrama SM, Oostdijk W, Drop SL. Auxological and Biochemical Evaluation of Pubertal Suppression with the GnRH Agonist Leuprolide Acetate in Early and Precocious Puberty. Horm Res 1999;51(6):270-276.
14. Roede MJ, Wieringen JC van. Growth diagrams 1980. Netherlands third nation-wide survey. Tijdschr Soc Gezondheidszorg 1985;63 (suppl.):11-32.
15. Achenbach TM. Manual for the CBCL/4-18 and 1991 profile. Burlington VT: University of Vermont Department of Psychiatry; 1991.
16. Verhulst FC, Akkerhuis GW, Althaus M. Mental health in Dutch children: (I). A cross-cultural comparison. Acta Psychiatr Scand Suppl 1985;323:1-108.

17. Verhulst FC, Versluis-Den Bieman HJM. Comparison between adopted and non-adopted children (Dutch). In: Buitenlandse adoptiekinderen (Dutch). Assen/Maastricht: Van Gorcum; 1989. p. 27-48.
18. Verhulst FC, Ende J van der, Koot HM. Handleiding voor de Teacher's Report Form (TRF) [Users Guide for TRF, Dutch]. Rotterdam: Department of Child and Adolescent Psychiatry, Sophia Children's Hospital; 1997.
19. Harter S. The perceived competence scale for children. Child Dev 1982;53:87-97.
20. Van Dongen-Melman JEWM, Koot HM, Verhulst FC. Cross-cultural validation of Harter's self-perception profile for children in a Dutch sample. Educ Psychol Measurement 1993;57:739-753.
21. Zimet GD, Cutler M, Litvene M, Dahms W, Owens R, Cutler L. Psychological adjustment of children evaluated for short stature: a preliminary report. J Dev Behav Pediatr 1995;16:264-270.
22. Gilmour J, Skuse D. Peer and self-perception of children with short stature-The role of cognition. In: Eiholzer U, Haverkamp F, Voss L, editors. Growth, stature, and psychosocial well-being. Seattle: Hogrefe & Huber Publishers; 1999. p. 37-46.
23. Grew RS, Stabler B, Williams RW, Underwood LE. Facilitating patient understanding in the treatment of growth delay. Clin Pediatr (Phila) 1983;22(10):685-90.
24. Erling A, Wiklund I, Albertsson-Wikland K. Prepubertal children with short stature have a different perception of their well-being and stature than their parents. Quality of life research 1994;3:425-429.
25. Robbroeckx LMH, Van der Schot-de Jong LWA, Otten BJ. Family stress in parents of girls with early puberty. (Dutch). Kind en Adolescent 1990;4:180-192.
26. Wels PMA, Robbroeckx LMH. The assessment of family stress. In: First European Congress of Psychology; 1989; Amsterdam; 1989.
27. Golombok S, Cook R, Bish A, Murray C. Families created by the new reproductive technologies: quality of parenting and social and emotional development of the children. Child Dev 1995;66(2):285-98.
28. Tizard B. Intercountry adoption: a review of the evidence. J Child Psychol Psychiatry 1991;32:743-756.
29. Ehrhardt AA, Meyer-Bahlburg HF. Idiopathic precocious puberty in girls: long-term effects on adolescent behavior. Acta Endocrinol Suppl (Copenh) 1986;279:247-53.
30. Mazur T, Clopper RR. Pubertal disorders. Psychology and clinical management. Endocrinol Metab Clin North Am 1991;20(1):211-30.
31. Guyda HJ. Four decades of growth hormone therapy for short children: what have we achieved? J Clin Endocrinol Metab 1999;84(12):4307-16.
32. Huisman J. Medisch psychologische bijdragen aan nieuwe behandelingswijzen in de kindergeneeskunde [Dutch]. Amsterdam, thesis Free University; 1993.
33. Ferguson DM, Lynskey M, Horwood LJ. The adolescent outcomes of adoption: a 16-year longitudinal study. J Child Psychol Psychiatry 1995;36:597-615.
34. Gibson FL, Ungerer JA, Tennant CC, Saunders DM. Parental adjustment and attitudes to parenting after in vitro fertilization. Fertil Steril 2000;73(3):565-74.
35. Bohman M. A study of adopted children, their back-ground, environment and adjustment. Acta Paediatr Scand 1972;61:90-97.
36. Verhulst FC, Althaus M, Versluis-den Bieman HJM. Damaging backgrounds: later adjustment of international adoptees. J Am Acad Child Adolesc Psychiatry 1992;31:518-524.
37. Galatzer A, Beth-Halachmi N, Kauli R, Laron Z. Intellectual function of girls with precocious puberty. Pediatrics 1984;74(2):246-9.
38. Vries AK de, Bunjes LAC. [Zwakke rekenprestaties bij buitenlandse adoptiekinderen in de basisschool] Dutch. Kind en adolescent 1989;10(4):165-174.
39. Silverstein AB. Two- and four-subtest short forms of the Wechsler adult intelligence scale-revised. J Consult Clin Psychol 1982;50:415-418.

III TREATMENT WITH GNRHA AND GH

Chapter 6: Study of the effect of treatment with GnRH-agonist (Decapeptyl) alone or in combination with biosynthetic human growth hormone (Genotropin) in adopted children with early puberty.

6.1 Gonadotrophin Releasing Hormone agonist (GnRHa) treatment with or without recombinant human growth hormone (GH) in adopted children with early puberty

6.2 Study of the effect of treatment with GnRH (GnRH)-agonist alone or in combination with recombinant human growth hormone in adopted children with early puberty: Psychological assessments before and after treatment

6.3 Motivation for treatment and psychological evaluation in adoptive families when a child presents with early puberty
submitted

Chapter 7
A randomized controlled trial of three years of growth hormone (GH) and GnRH agonist treatment in children with Idiopathic Short Stature and Intra-Uterine Growth Retardation.

Chapter 8
The effect of pubertal delay by GnRH agonist in Growth Hormone Deficient (GHD) children on final height

Chapter 6.3

Motivation for treatment and psychological evaluation in adoptive families when a child presents with early puberty

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ABSTRACT

In internationally adopted children early puberty is frequently observed. In a clinical trial on hormonal treatment of early puberty the motivation of parents and child to participate was studied. The fast majority of parents and children were adequately motivated for treatment. No indication was found suggesting overmotivation in parents. Psychological assessment revealed no increase in emotional or behavioural problems in the children compared to reference values of adopted children in The Netherlands. We discuss the societal bias with regard to the role of adopted parents in clinical research and to growth promoting treatment in adopted children.
We conclude that there is no reason to view adoptive parents differently from parents of non-adopted children in the context of clinical research or medical treatment.

INTRODUCTION

In The Netherlands about 25.000 international adoptees are part of society (anonymous, in (1)). It was described by Oostdijk et al. that despite the catch-up growth after arrival final height in adopted children did not exceed that of the country of origin, due to early onset of puberty (2). In other European countries this phenomenon has been observed as well (3-7).

An early onset of puberty may lead to a decrease in final height by early closure of the epiphyseal growth plates of the long bones mediated by the sex steroids. Furthermore, precocious sexual development may cause adjustment problems (8, 9). In children with precocious puberty treatment with Gonadotrophin Releasing Hormone (GnRH) agonists has been shown to improve final height to some extent (10, 11).

To study the effect of the addition of growth hormone to GnRHa treatment to further improve final height we designed a study in adopted children with early puberty. In the process of approval the Dutch national board on the ethics of medical research (KEMO) demanded a psychological study to evaluate the motivation for treatment of parents and children and of the psychological development during treatment. This was remarkable as in other growth studies in non-adopted children such a requirement was never pointed out. One of the concerns was that adverse motives of the parents could harm the child, or at least that contrasting interests between parents and the child in participation or refusing participation would be present. This concern could be based on the fact that many adoptive parents are rather persistent in their demands for help. This attitude may be enforced when the child has a medical problem. Thus in the medical profession a biased view on adoptive parents may have developed.

Sharav points out the importance of a healthy motivation for adoption, which mainly consists of a deep wish to raise a child (12). The biological wish for a child has been given up (about 90% of parents of adopted children do not have biological children), and adoption is their conscious choice. This kind of motivation for adoption can be regarded as protective. The conscious choice of parents for adoption may lead to greater involvement in their children, even more than in mothers with biological children (13). In general, great involvement can also be viewed as a protective kind of motivation.

Hoksbergen has the opinion that motivation for adoption generally consists of external and internal motives (14). Parents with external motives prefer adoption to complete or extend their family or to help a child that is in distress. Those with internal motivation adopt a child primarily to satisfy their own needs. The latter may be harmful because of great hopes the parents nourish towards their adopted child, which can not always be answered by the child. Thus, too much involvement or strong internal motivation of the adoptive parents may be a burden for a child and could result in developmental problems. Combined with a longstanding and intensive treatment for early puberty serious problems may arise. From the point of view of the adopted child, it might be difficult to refuse a treatment proposed by the parents, based on feelings of loyalty, dependency and an unconscious feeling of gratitude. These feelings may also result in a difficulty to express anger and fear, which may contribute to an increase in behavioural problems.

The concept of motivation for treatment is not widely described in medicine, let alone in paediatrics. One study in adults addresses the factors affecting the decision to participate in a clinical trial (15). In that study the belief that the proposed treatment could improve or help to maintain the patients' condition was an important reason to participate in the trial. Many studies do discuss the ethics of participation of children in research trials mainly from the point of view of the right to consent (16). No literature is available on motivation for treatment in adopted children, whereas some studies were published on expectations of growth promoting treatment in non-adopted children (17, 18).

We studied the motivation for treatment and performed a psychological evaluation of parents and their adopted children who had volunteered for treatment of early puberty.

The aims of the study were to investigate whether parents and adopted children are adequately motivated for treatment of early puberty, to evaluate psychosocial development before start of treatment and to assess tolerance of treatment of early puberty.

PATIENTS AND METHODS

Children in this study originate from India, Sri Lanka, Colombia and Korea and were adopted by Dutch parents. The children developed early puberty, defined as pubertal development between 7 and 10 years of age in girls and between 8 and 11 years in boys. The onset of puberty was characterised in girls by breast development and/or menarche, in boys by testicular enlargement ($\geq$ 4 ml); in both the physical signs were accompanied by a pubertal rise in gonadotrophin hormones in a GnRH stimulation test. To assess bone age (BA) an X-ray of the left hand was obtained. The predicted adult height (PAH) was calculated on the basis of height and BA (19). For inclusion in the study PAH had to be below the third percentile of Dutch children for sex (20).

Detailed characteristics of the patients and the study design as well as the growth data are presented elsewhere (21). In brief: The children in this study were treated for 3 years with either the GnRH-agonist triptorelin (Decapeptyl®) alone (*group A*) or in combination with recombinant human growth hormone (Genotropin®) (*group B*). A physician or nurse administered GnRH agonist every 28 days by an intramuscular (im) injection; in group B the patient him-/herself or one of the parents administered growth hormone every day by a subcutaneous (sc) injection at home. An untreated control group was incorporated in the original study design, but this part of the design was abandoned when parents who randomised for no treatment all left the study.

The study was announced to adoptive families by means of the periodicals of adoption societies and we informed the parents orally during the procedure of informed consent

Many parents were interested in the study and phoned for information. Because of the strict inclusion criteria only a few children were eligible candidates for the study. Before randomisation and start of treatment a structured interview was performed with parents and child separately to assess motivation for treatment.

Instruments

Motivation study

An experienced psychologist (HJMV-dB) interviewed both parents and children. The interviews were recorded on videotape and scored by two independent raters. The general motivation score was composed of the ratings on four parameters:

(I) motivation for treatment, *(II)* expectations of treatment, *(III)* evaluation of psychological suffering from early puberty and short stature by the child and *(IV)* psychological strength of the child.

For the parents two more parameters were added: "expectations of treatment by the parents" and "psychological strength of the parents". The rating was performed in 2 steps: first, a qualitative judgement was given on an adequate versus inadequate range; subsequently, a quantitative score was applied. The cumulative score of parents and child ranged from 0-10 (table 1).

Motivation for treatment was assessed in parents by asking the ways they had used to reach our clinic and how they felt about the randomisation and treatment. The children were separately asked what they knew and felt about the treatment and how they felt about the randomisation procedure.

II *Expectations of treatment of the child* was assessed by asking the parents how they evaluated the psychological 'burden' of treatment for their child. The children were asked how easy or difficult they thought the treatment would be for a long period.
III *Psychological suffering of the child*: the parents were asked how much they thought their child suffered from early puberty, short stature and the different appearance. The children were asked the same questions.
IV *Psychological strength of the child* was estimated by rating the parents' answers on the question how they had been coping with difficulties in raising their adopted child and how the child had been coping with difficulties in life.
When the total score was lower than 6, the observers rated motivation as inadequate (either from the parents or the child). In that case, participation in the study was not allowed.

Table 1: Composition of the motivation score

Child	score	Parent	score
Motivation for treatment	1	motivation for treatment	1
Expectations of treatment	1	expectations of treatment	1
Evaluation of psychological suffering from early puberty and short stature by the child	1	evaluation of psychological suffering from early puberty and short stature by the child	1
Psychological strength of the child	1	psychological strength of the child	1
		expectations of treatment by the parents	1
		psychological strength of the parents	1
MOTIVATION SCORE	4	+	6
range 0-10 < 6 = inadequate, 9 = good enough, 10 = optimal			

T1 study
After the motivation study and before randomisation and treatment the psychological assessment (T1) was performed. This study was repeated after discontinuation of treatment.
The psychological assessment was performed to evaluate behavioural and emotional problems, self-perception, expectations of treatment and intelligence levels of adopted children. This psychological part of the study and the repeated assessment after 3 years of treatment is described in detail in a separate article (Mul *et al*, chapter 6.2). In this article we describe the psychological assessment of a slightly different study population. The difference between the groups is due to the fact that some parents did not agree to enter into the study protocol after they were randomised to the control group. These parents were included in this paper.

The following instruments were used: *Child Behaviour Checklist (CBCL)*: The CBCL is a standardised instrument to obtain parents' reports of competence and problem behaviour of their child (22). The problem items that describe the child are summarised in a score for internalising (e.g. withdrawn, anxious/depressed), externalising (e.g. aggressive, delinquent)

problems and a total problem score. Reference values for adopted children in the Dutch population were used for comparison (23).

Self-perception profile for children (SPPC): Harter developed this self-reported instrument for measuring self-esteem or perceived competence in children (24). Self-competence in different domains is measured, as well as an independent assessment of global self-worth. For this study we analysed data on social acceptance and physical appearance as children with early puberty might be less socially accepted and do not feel comfortable with their physical appearance.

Silhouette Apperception Technique (SAT): This test describes the accuracy of estimation of current and future height and was developed to study expectations of treatment with recombinant growth hormone (18, 25)

NVOS: The "Nijmeegse Vragenlijst voor de Opvoedings Situatie" is a validated Dutch questionnaire evaluating family. We limited our analysis to 4 main items that we regarded to be of specific importance: first to what extent the family functioning is accepted or should be changed, the presence of problems (2nd) and whether the parents are able to deal with problems (3rd). For these items a higher score indicates increased family stress. A general assessment of family functioning is expressed in the 'satisfaction' item (4th): the higher the score, the more satisfied the parent is.

During the 3-year course of treatment a questionnaire was sent three times, at 6, 18 and 30 months of treatment, to be completed by the parents (F1, F2, F3). The questionnaires contained items concerning psychosocial development of the child and their experience with medical treatment and injections in particular.

RESULTS

Before start of treatment: Motivation study

Thirty-five parent-child couples entered into the motivation study. Data of 5 couples were lost because of inadequate tape-recording, so 30 tapes were available for analysis and described in this article.

The interrater reliability of the 30 recordings was significant and high (r=0.95) for total scores of parents-child couples. Separately, the interrater reliability of the parents (6 parameters) or of the child (4 parameters) was also satisfactory and significant, r = 0.83 and 0.79 respectively. Agreement concerning the scores of the adopted children is lower but still satisfactory.

In total 29 adopted children and their parents were accepted for treatment, 1 was not accepted (Table 2) because of a score of 4 on the motivation parameters. This means that the parents were rated as inadequate on all but one parameter ('burden of the child') and the child scored inadequate on 1 parameter ('motivation for treatment').

Table 2: Results of motivation study

Group 1: optimal motivation	19
Group 2: motivation 'good enough'	10
Group 3: not accepted for treatment	1
Total	30

In the interview with the child it became clear that she was very afraid of being treated, especially with regard to the injections, but did not dare to tell her parents.

A score of 10 was considered to indicate optimal motivation (group 1), and a score of 9 was labelled as good enough motivation (group 2).

Of the 29 accepted couples 19 obtained a score of 10 and 10 a total score of 9. In group 2 more children than parents were inadequately motivated, they had significantly more unrealistic expectations of the effect of treatment (p=0.01). These children "could not think any problem to happen" and were significantly more inadequately motivated (p=0.05) compared to children in group 1.

In group 2, significantly more parents (p = 0.01) had unrealistic expectations concerning treatment as well, suggesting that they did not expect any problem to occur during treatment for their child or for themselves.

We compared the group with optimal motivation and the group with 'good enough' motivation using the instruments of T1 measurements. No significant difference was present between both groups in CBCL scores, SPPC scores, family stress or expectations of treatment with SAT.

Before treatment: psychological evaluation (T1)

The sample for the psychological evaluation consisted of 27 girls and 3 boys. To avoid a sex-effect the results in this section only concern the girls. The adopted girls in our sample did not significantly differ from adopted girls in the general Dutch population (23) with regard to behavioural and emotional problems, measured by CBCL. This holds for the internalising and externalising subscales as well for the total problem score. Compared to age-matched girls from the general population, the girls in our study had the same level of self-perception on all subscales of the Harter self perception profile. The expectations of the future height was overestimated by 80% of the children and 17% of the parents, when future height was defined as height > 50th percentile of the Dutch population. The mean IQ level measured by the WISC-subtest was 101.0, not different from the population mean of 100.0. The NVOS scores revealed that general satisfaction with the rearing situation was higher than in the normal population and that the family was significantly more able to deal with problems compared to the reference group.

During treatment

Thirty children entered into the treatment protocol, as some accepted couples did decide not to continue as they were allocated in the control group that was incorporated in the original study design. Three couples were admitted based on the psychologist's decision alone since independent rating of motivation could not be obtained due to loss of tape recordings.

At the F1 evaluation 6 months after start of treatment the parents reported that their children were doing well. In the children treated with GnRHa alone (group A) 96% of the children could stand the painful intramuscular injections well, 64% of the parents judged the treatment to be a little burden for their child and 36% a burden for themselves. The burden of treatment with GnRHa could be the painful and unpleasant injections, the stress before having an injection, the monthly visit to a physician and the loss of school time for the children because of these visits.

In group B where monthly GnRHa and daily GH injections were combined, 85% of the children tolerated the injections well, whereas the other 15 % found it annoying. The parents in this group reported the treatment to be a little burden for their child in 23%, and for themselves

in 15% of the cases. Forty-six percent of the children injected themselves with growth hormone.

Combined data in both groups revealed that 96% of the parents and 56% of the children thought the treatment was very advisable; and 68% of the parents reported a favourable psychological effect of treatment for their children. With regard to these last mentioned items no significant differences were present between group 1 and 2.

At the 6, 12 and 30 months assessments no significant differences were demonstrated between the group with optimal motivation and that with good enough motivation with regard to tolerance or experienced burden of GnRHa or GH treatment in parents and child. A non-significant tendency was observed that the judgements of parents in group 1 were more differentiated compared to group 2.

The results from F1, 2 and 3 suggest that the burden of treatment for the child due to GnRHa injections decreases over time: the percentage of problems with injections decreases from 64% at F1 to 38% at F3. The burden of treatment from the daily GH injections shows some increase from 23 to 31 % at F1 and F3 respectively.

Discussion

In contrast to the initial presumption we found in this study that the motivation of parents for treatment of their adopted child with early puberty is adequate in the fast majority of cases. Only one out of 30 patients was not accepted to enter into the treatment protocol due to a disagreement between the refusal of the child to participate and the desire of the parents to obtain treatment. The differentiation in 'optimal' and 'good enough' motivation did neither predict the level of behavioural or emotional problems or self-perception of the child during treatment, nor the way parent and child perceived treatment.

In clinical research in children, the process of decision-making is a balance between the interests of the child, the parents and the clinical researcher. Legally, it is the child that decides provided that he or she has sufficient understanding and intelligence to understand what is proposed. In that case the consent of the child and not of its parents is required. Thus " a reasoned refusal by a child to participate in research is likely to be taken as evidence for such understanding, and it would be unwise to rely on parental consent in such circumstances" (26). Only in case of severe disagreement between parents and child the child's well-being might be threatened.

As in many studies including therapeutic research in children, the distinction between motivation for participation in research and motivation for treatment is difficult to make. Van Stuijvenberg *et al.* studied participants of a randomised trial regarding febrile seizures. They found that the major factors in parents' approval to participate were the contribution to clinical science (in 51%) and benefit for their own child in 32% of the parents (27). It is likely that the way the researcher informs the parents influences parental motivation. Highly educated parents – and most adoptive parents in our study belong to this group- may on the contrary be less influenced by the researcher's information, as they might be able to address more specific questions to him or her.

One should assume a biased view towards adoptive parents, as only in this study, concerning adopted children, an evaluation of potential candidates was demanded. Such a prejudice towards adoptive parents that might be the basis for the demand of a motivation study is difficult to explain. In the introduction of this article we pointed to the biased view on adoptive parents possibly present in the medical profession. The *interaction* between the assertive, inspired and highly educated adoptive parents and the people that surround them may result in various feelings, maybe even resistance, in the latter. From a psychoanalytical point of view, one can think of unconscious hostile fantasies towards adoptive parents. These serve as a defence mechanism against feelings of guilt with regard to adopted

children that is felt in the interaction with adoptive parents. Alternatively, the good intentions of adoptive parents may be denied by unconscious feelings that adopted children should not get as much good as Dutch children. It is true that, as in other parents, adoptive parents have to deal with internal problems, and that these may concentrate around issues of defectiveness and restoration (28). However, in the literature no data have been published that report on adoptive parents who harm their child in dealing with these conflicts. Hoopes refers to literature from the nineteen sixties in a clinical sample in which adoptive mothers tended to overprotect their children as a result of feelings of inadequacy on childbearing (29). Our study shows that motivation in adopted parents is adequate. The evaluation of motivation for treatment could eventually be advisable, but should at least not be restricted to adoptive parents. Whether it is advisable at all for any trial with a major intervention in paediatric research might be questioned, as it might discard the parental autonomy and responsibility.

Questioning growth-promoting therapy in adopted children is a relevant issue. Many people reason that adopted children are short in their countries of origin and thus should not be subjected to any growth promoting treatment at all. However, this reasoning overlooks that the expected increase in final height, as a result of catch-up growth and improvement of socio-economic circumstances, may not be attained due to early puberty. A further reason for growth promoting treatment is to compensate for the possible growth inhibition of GnRHa treatment in children with early puberty (30).

The general picture derived from the psychological evaluation is described in a separate article (Mul et al, chapter 6.2). In summary it is described in that article that for the child no elevation of CBCL scores for emotional or behavioural problems, normal self-perception and acceptance by peers has been demonstrated. For parents it describes that no increased level of family stress and realistic expectations of treatment were shown. The latter may be due to the relative high socio-economic status in the group of adoptive parents (80 % in the highest category), that may contribute to the understanding of the information given before entering the study. Another possible bias to account for is that in The Netherlands parents go through an extensive selection procedure before being accepted as adoptive parents.

Once treated, children seem to get used to the monthly injections. Probably the recurrent and repeated character of the daily injections, especially when the effects of treatment are not clearly visible for a child, causes an increase in burden (31), while the effects of the monthly injections for suppression of puberty is visible.

We conclude that motivation for treatment in adoptive parents when their child enters in to puberty at an early age is adequate. The refusal of 1 out of 30 families to enter into the study can not be seen as a specific feature of the group of adoptive parents. They should be seen as normal parents.

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REFERENCES

1. Hoksbergen R, Walenkamp H. Adoptie: een levenslang dilemma [Dutch]. Houten: Bohn Stafleu Van Loghum; 2000.
2. Oostdijk W, Yap YN, Doude van Troostwijk ML, et al. Final height and timing of puberty of adopted children in The Netherlands. Horm Res 1991;35(2):24.
3. Proos LA, Hofvander Y, Tuvemo T. Menarcheal age and growth pattern of Indian girls adopted in Sweden I menarcheal age. Acta Paediatr Scand 1991;80:852-858.
4. de Monleon JV, Simonin G, Sarles J. Puberté précoce et enfants adoptés; une entité à connaitre. Le concours médical 1998;120(10):688-690.
5. Bureau JJ, Maurage C, Bremond M, Despert F, Rolland JC. [Children of foreign origin adopted in France. Analysis of 68 cases during 12 years at the University Hospital Center of Tours]. Arch Pediatr 1999;6(10):1053-8.
6. Marx M, Schoof E, Kapferer L, Dorr HG. Pubertas praecox vera bei einem indischen Mädchen nach Adoption durch deutsche Eltern. Monatsschr Kinderheilk 1998;146:957-960.
7. Virdis R, Street ME, Zampolli M, et al. Precocious puberty in girls adopted from developing countries. Arch Dis Childh 1998;78:15-154.
8. Schoevaart CE, Drop SLS, Otten BJ, Slijper FME, Degenhart HJ. Growth analysis to final height and psychosocial adjustment of treated and untreated patients with precocious puberty. Hormone Research 1990;34:197-203.
9. Xhrouet-Heinrichs D, Lagrou K, Heinrichs C, et al. Longitudinal study of behavioral and affective patterns in girls with central precocious puberty during long-acting triptorelin therapy. Acta Paediatr 1997;86:808-815.
10. Mul D, Oostdijk W, Otten BJ, et al. Final height after Gonadotrophin Releasing Hormone agonist treatment for Central Precocious Puberty: The Dutch experience. J Ped Endocrinol Metab 2000;13(Suppl 1):765-772.
11. Carel JC, Roger M, Ispas S, et al. Final height after long-term treatment with Triptorelin slow release for central precocious puberty: Importance of statural growth after interruption of treatment. J Clin Endocrinol Metab 1999;84:1973-1978.
12. Sharav D. Assessing adoptive parents using a combined individual and interaction procedure. In: Hibbs ED, editor. Adoption: International perspectives. Madison CT: Internat Univ Press; 1991. p. 73-89.
13. Golombok S, Cook R, Bish A, Murray C. Families created by the new reproductive technologies: quality of parenting and social and emotional development of the children. Child Dev 1995;66(2):285-98.
14. Hoksbergen RAC. Understanding and preventing "failing adoptions". In: Hibbs ED, editor. Adoption: International perspectives. Madison CT: Internat Univ Press; 1991. p. 265-76.
15. Elad P, Treves TA, Drory M, et al. Demented patients' participation in a clinical trial: factors affecting the caregivers' decision. Int J Geriatr Psychiatry 2000;15(4):325-30.
16. Foreman DM. The family rule: a framework for obtaining ethical consent for medical interventions from children. J Med Ethics 1999;25(6):491-6; discussion 497-500.
17. Huisman J. Medisch psychologische bijdragen aan nieuwe behandelingswijzen in de kindergeneeskunde [Dutch]. Amsterdam, thesis Free University; 1993.
18. Erling A, Wiklund I, Albertsson-Wikland K. Prepubertal children with short stature have a different perception of their well-being and stature than their parents. Quality of life research 1994;3:425-429.
19. Bayley N, Pinneau SR. Tables for predicting adult height for skeletal age: revised for use with the Greulich-Pyle hand standards. J Ped 1952;40:423-41.
20. Roede MJ, Wieringen JC van. Growth diagrams 1980. Netherlands third nation-wide survey. Tijdschr Soc Gezondheidszorg 1985;63 (suppl.):11-32.
21. Mul D, Oostdijk W, Waelkens JJJ, Drop SLS. The addition of growt hormone to GnRH agonist treatment is effective in adopted children with early puberty. Horm Res 2000;53(suppl 2):91.
22. Achenbach TM. Manual for the CBCL/4-18 and 1991 profile. Burlington VT: University of Vermont Department of Psychiatry; 1991.
23. Verhulst FC, Versluis-Den Bieman HJM. Comparison between adopted and non-adopted children (Dutch). In: Buitenlandse adoptiekinderen (Dutch). Assen/Maastricht: Van Gorcum; 1989. p. 27-48.
24. Harter S. The perceived competence scale for children. Child Dev 1982;53:87-97.
25. Grew RS, Stabler B, Williams RW, Underwood LE. Facilitating patient understanding in the treatment of growth delay. Clin Pediatr (Phila) 1983;22(10):685-90.

26. McIntosh N, Bates P, Brykczynska G, et al. Guidelines for the ethical conduct of medical research involving children. Royal College of Paediatrics, Child Health: Ethics Advisory Committee. Arch Dis Child 2000;82(2):177-82.
27. van Stuijvenberg M, Suur MH, de Vos S, et al. Informed consent, parental awareness, and reasons for participating in a randomised controlled study. Arch Dis Child 1998;79(2):120-5.
28. Brinich PM. Adoption from the inside out: a psychoanalytic perspective. In: Brodzinsky DM, Schechter MD, editors. The psychology of adoption. New York / Oxford: Oxford University Press; 1990. p. 42-61.
29. Hoopes JL. Adoption and identity formation. In: Brodzinsky DM, Schlechter MD, editors. The psychology of adoption. New York / Oxford: Oxford University Press; 1990. p. 144-166.
30. Proos LA, Hofvander Y, Tuvemo T. Menarcheal age and growth pattern of Indian girls adopted in Sweden. II. Catch-up growth and final height. Indian J Ped 1991;58:105-114.
31. Hindmarsh PC, Brook CG. Compliance with growth hormone treatment - is it a problem? Horm Res 1999;51(Suppl 3):104-8.

III TREATMENT WITH GNRHA AND GH

Chapter 6: Study of the effect of treatment with GnRH-agonist (Decapeptyl) alone or in combination with biosynthetic human growth hormone (Genotropin) in adopted children with early puberty.

6.1 Gonadotrophin Releasing Hormone agonist (GnRHa) treatment with or without recombinant human growth hormone (GH) in adopted children with early puberty

6.2 Study of the effect of treatment with GnRH (GnRH)-agonist alone or in combination with recombinant human growth hormone in adopted children with early puberty: Psychological assessments before and after treatment

6.3 Motivation for treatment and psychological evaluation in adoptive families when a child presents with early puberty :

Chapter 7

A randomized controlled trial of three years of growth hormone (GH) and GnRH agonist treatment in children with Idiopathic Short Stature and Intra-Uterine Growth Retardation.

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Chapter 8

The effect of pubertal delay by GnRH agonist in Growth Hormone Deficient (GHD) children on final height

A randomized controlled trial of three years Growth Hormone (GH) and GnRH agonist treatment in children with Idiopathic Short Stature and Intrauterine Growth Retardation

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ABSTRACT

We assessed the effectiveness and safety of three years combined growth hormone (GH) and Gonadotrophin Releasing Hormone agonists (GnRHa) treatment in a randomized controlled study in children with Idiopathic Short Stature (ISS) or intrauterine growth retardation (IUGR). Gonadal suppression, GH reserve and adrenal development were assessed by hormone measurements in both treated children and controls during the study period.

Thirty-six short children, 24 girls (16 ISS/ 8 IUGR) and 12 boys (8 ISS/4 IUGR) with a height SDS <-2 in early puberty (girls: B2-3 and boys: G2-3), were randomly assigned to treatment (n=18) with GH (Genotropin 4 IU/m^2/day) and GnRHa (Triptorelin, 3.75 mg/28 days) or no treatment (n=18). At start of the study mean (SD) age was 11.4 (0.56) or 12.2 (1.12) years whereas bone age was 10.7 (0.87) or 10.9 (0.63) years in girls and boys, respectively.

During three years of study height SDS for chronological age (CA) did not change in both treated children and controls, whereas a decreased rate of bone maturation after treatment was observed (mean (SD) 0.55 (0.21) 'year'/year versus 1.15 (0.37) 'year'/year in controls, $p<0.001$, girls and boys together). Height SDS for bone age and predicted adult height increased significantly after 3 years of treatment; compared to controls the predicted adult height gain was 8.0 cm in girls and 10.4 cm in boys. Further, the ratio between sitting height/height SDS decreased significantly in treated children, whereas body mass index was not influenced by treatment.

Puberty was effectively arrested in the treated children as was confirmed by physical examination and prepubertal testosterone and estradiol levels. GH dependent hormones including serum IGF-I, IGF-II, PICP, PIIINP, alkaline phosphatase and osteocalcin, were not different between treated children and controls during the study period. Thus, a GH dose of 4 IU/m^2 seems adequate for stabilization of the GH reserve and growth in these GnRHa treated children.

We conclude that 3 years treatment with GnRHa was effective in suppressing pubertal development and skeletal maturation, while the addition of GH preserved growth velocity during treatment. This resulted in a considerable gain in predicted adult height, without demonstrable side effects. Final height results will provide the definite answer on the effectiveness of this combined treatment.

INTRODUCTION

Since puberty initiates the process of epiphysial fusion that determines final height, the first signs of puberty are often perceived as an alarming signal in short children (1), and the question whether therapeutic options exist to improve final height is often asked. If initiated years before the onset of puberty, GH treatment may have beneficial effects on final height in children with Idiopathic Short Stature (ISS) and intrauterine growth retardation (IUGR) (2, 3). Once puberty has started, however, GH treatment in these short children has limited value; GH may stimulate a rapid progression through puberty, which is expected to reduce the gain in final height (4-6). The addition of Gonadotrophin Releasing Hormone agonists (GnRHa) to delay puberty has therefore been considered. GnRHa were initially used in children with central precocious puberty (CPP), with beneficial effects on adult height (7-9). Recent trials with GnRHa in short children showed an increment in adult height of 0.5 – 3.3 cm (10, 11). No controls were included except for one study with 4 years treatment with GnRHa versus placebo, showing a height gain of 7.6 cm compared to initial prediction in the GnRHa treated children and of 10.3 cm compared to placebo group. The study population, however, was very heterogeneous and therefore the results are difficult to interpret (12). More recent studies, using the combined treatment of GH and GnRHa in children with ISS or IUGR, report a gain in final height prediction between –0.5 to 10 cm (1, 13-17), but none of these studies used randomized controls.

To answer the question whether GH and GnRHa treatment may improve final height in pubertal children with ISS and IUGR we designed a randomized controlled study with 3 years treatment with combined GH and GnRH agonist treatment in 24 girls and 12 boys with ISS or IUGR. We verified the effectiveness of gonadal suppression by physical examinations and by measurements of sex steroids in all children. To assess the GH reserve during treatment we yearly measured IGF-I, IGF-II, IGFBP-3, and markers of collagen and bone metabolism, all GH dependent hormones (18). Adrenal hormones were measured to search for a possible effect of treatment on adrenal development. In this report we present data obtained during the first 3 years of the study.

PATIENTS & METHODS

Forty children were randomized for either combined treatment with growth hormone (GH) and Gonadotrophin Releasing Hormone agonist (GnRHa) or no treatment. GH, (Genotropin®, Pharmacia & UpjohnAB (Stockholm, Sweden)) was given in a dose of 4 IU/m^2/day sc, which is equivalent to 0.14 IU (0.05 mg) /kg body weight/day. GnRHa (triptorelin (Decapeptyl® Ferring); since Decapeptyl® was withdrawn by Ferring in 1998 we used the same preparation from Ipsen) was given in a dose of 3.75 mg i.m. every 28 days. The randomization was performed separately in children with a known history of intrauterine growth retardation (IUGR), defined as a birth length <-2 SDS (19). Directly after randomization, two patients randomized for treatment refused treatment and 2 controls refused follow-up. Additionally, one ISS boy and one IUGR girls, both controls, became drop-outs. We report 3 year-data of 18 treated (12 ISS, 6 IUGR /6 boys, 12 girls) and 16 controls (11 ISS, 5 IUGR / 5 boys, 11 girls).

The protocol was reviewed and approved by the medical ethics committees at the four participating centers (Catharina Hospital Eindhoven (n=12), Wilhelmina Children's Hospital Utrecht (n=12), Free University Hospital Amsterdam (n=10), and Sophia Children's Hospital Rotterdam (n=2), and the parents of all children gave written consent for the study. When appropriate, the consent of the children was also obtained.

Inclusion criteria were G2 or G3 in boys (testicular volume of $\geq$ 4 and $\leq$10 ml) and B2 or B3 in girls, an actual height < -2.0 SDS (20) or between –1.0 and –2.0 SDS with a

predicted adult height < -2.0 SDS (according to Bayley & Pinneau (21)), and a chronological age and bone age less than 12 and 13 years in girls and boys, respectively. Furthermore, a maximum serum GH level > 10 ug/l (1ug = 2IU, The First International Reference Preparation of hGH, MRC London, code 66/217 was used as standard) after provocation (exercise, arginine, clonidine, L-dopa or glucagon), and a normal ratio of sitting height/subischial leg length (between P3 and P97) (22) were established. At time of inclusion, screening blood tests and urinanalysis were normal, and none had evidence of malnutrition or hormonal or systemic disease.

All children were evaluated at baseline. Then, the children of the treatment group were followed every 3 months during treatment and at least once a year thereafter. Children in the control group were followed on a yearly basis. Evaluations included measurements of height (mean of 4 measurements performed by the same observer (LV) at the same hour of day on a Harpenden stadiometer), sitting height (mean of two measurements (LV)), and weight. Pubertal staging was assessed by one investigator in all children at all visits (GAK), according to the method of Tanner. The Prader orchidometer was used to determine testicular size in boys.

Height was expressed as SDS for chronological age (CA) and for bone age (BA) according to Dutch references (20). Body Mass Index (BMI) was calculated (weight/(height^2)) and expressed as SDS (23). Sitting height and sitting height/height were also expressed as SDS (22). Target height was calculated [father's height + mother's height + or – 12 cm for boys and girls, respectively) / 2] + 3 cm (for the secular trend) and expressed as SDS (20). Bone age radiographs were measured yearly in all children and were determined according to the method of Greulich and Pyle by one independent investigator (24). To evaluate the effect of treatment we used the gain in predicted adult height (PAH), defined as the difference between the height prediction at start and after 3 years of treatment or follow-up. In girls, a yearly ultrasound of uterus and ovaries was performed. We measured the volumes and examined the occurrence of ovarian cysts.

Hormone analysis

Laboratory tests at baseline and at yearly visits included full blood count, serum FT4, TSH, LH, FSH, estradiol in girls and testosterone in boys, blood HbA1c, fasting blood glucose, serum fasting insulin, IGF-I, IGF-II, IGFBP-3, leptin, DHEA, DHEAS, androstenedione, PICP, PIIINP, alkaline phosphatase and osteocalcin. Plasma levels of IGF-I, IGF-II and IGFBP-3 were determined in one assay in the endocrine laboratory of the Wilhelmina Children's Hospital, Utrecht on samples that had been stored at -20°C for a maximum of 4 years. These assays have been described previously (25). The levels of IGF-I, IGF-II and IGFBP-3 were expressed as ng/ml and compared with references based on measurements in 906 healthy individuals. Smoothed references for three plasma parameters and three ratios were constructed using the LMS method (26). This method allows to find the best transformation of data which lack a normal distribution (as do all these parameters) yielding a smoothed and statistically valid function. Serum leptin (ng/ml) was measured by RIA (Linco Research inc., St Charles, Missouri, USA). PICP (normal range 200-1000 µg/l (mean ± 2SD), decreases with age from 2-16 years) and PIIINP (normal range 5-18 µg/l (mean ± 2SD), decreases with age from 2-16 years) were measured with RIA kits (Orion Diagnostics, Finland). Serum alkaline phosphatase (IU/l) was measured with the VITROS analyser and osteocalcin (normal range 1.8-6.6 µg/l (mean ± 2SD)) with a RIA kit (DiaSorin, Stillwater, USA). Serum LH (IU/l) and FSH (IU/l) were performed with a solid phase, time resolved immunofluorometric assay ((IFMA), Wallac, Turku, Finland). Serum testosterone (nmol/L) (in boys), DHEA (nmol/L) and androstenedione (nmol/L) were measured with solid phase RIA's (DPC, Los Angeles, CA, USA). Serum estradiol (pmol/L) (in girls) was also measured with a

sensitive solid phase RIA (Orion, ESPO, Finland) and DHEA-S with an in house developed RIA (Leiden, Department of Clinical Chemistry (MF)). All samples were measured in one assay on samples that had been stored at -20°C for a maximum of 4 years.

Statistics

Results are expressed as mean ± SEM or SD. The statistical analysis comprised a paired t-test for comparisons between data at baseline and after three years, and when appropriate for non-normal distribution we used the Wilcoxon signed rank test. Differences between the treated children and controls were tested by Student t-test or Mann Whitney tests.

Correlation analysis was performed appropriate for the distribution of the variable. Changes in variables during the study period between treated patients and controls were analyzed by repeated measurement analysis. Reported are the changes in time between groups and changes in time for both groups together.

Results

Auxology

Table 1 shows the auxological data at baseline and after 3 years of study for ISS girls, ISS boys, IUGR girls and IUGR boys separately. There was no significantly different pattern between these four subgroups in changes in bone age (BA), height velocity (HV), height SDS for chronological age (H-SDS CA), height SDS for BA (H-SDS BA), BMI-SDS, or in predicted adult height (PAH) in cm or SDS. Combined auxological data of treated and control children are shown in figure 1-3 and in table 2.

Table 2. Changes (mean ± SEM) in predicted adult height in cm between start and after 3 years of study.

	N	Control	GH + GnRHa
Girls	23	2.4 (1.69)	10.4 (1.24)
Boys	11	-3.9 (2.09)	6.5 (0.87)
IUGR girls	7	0.3 (5.87)	11.1 (1.72)
ISS girls	16	3.1 (1.27)	10.1 (1.68)
IUGR boys	4	-3.6 (2.40)	6.5 (0.30)
ISS boys	7	-4.1 (4.50)	6.6 (1.58)

Bone age advanced significantly less in GH+GnRHa treated children compared to controls (0.55 (0.05) 'year'/year versus 1.15 (0.09) 'year'/year, $p<0.001$, all girls and boys together). In subgroups, a significant difference in BA was seen in treated compared to control ISS boys after 3 years of study (table 1). Height velocity (HV) decreased significantly ($p<0.001$) in the GH+GnRHa treated children from 7.0 (0.32) cm/year during the first year of treatment to 5.4 (0.24) and 4.9 (0.30) cm/year during the second and third year, respectively. In the control group HV was 7.4 (0.51), 7.0 (0.53) and 4.7 (0.4) cm/year during the first, second and third year of study, respectively (third year HV compared to the first year HV: $p < 0.05$). Height SDS for CA did not change during 3 years of study in all groups (see figure 1A for GH+GnRHa versus controls, and table 1 for subgroups).

Table 1. Auxological data at baseline and after 3 years of study for ISS girls, ISS boys, IUGR girls and IUGR boys

	Start				After 3 years							
	ISS girls	ISS boys	IUGR girls	IUGR boys	ISS girls		ISS boys		IUGR girls		IUGR boys	
N=	16	8	8	4	Co: 8	Rx: 8	Co: 3	Rx: 4	Co: 3	Rx: 4	Co: 2	Rx: 2
CA	11.5 (0.1)	12.2 (0.5)	11.3 (0.3)	12.2 (0.2)	14.4 (0.23)	14.6 (0.14)	16.4 (0.77)	14.5 (0.38)	14.8 (0.22)	14.0 (0.48)	14.9 (0.30)	15.6 (0.02)
BA	10.9 (0.2)	10.9 (0.2)	10.2 (0.4)	11.0 (0.4)	13.7(0.4)	12,7(0.2)	14.8(0.7)	13.1(0.1)♦	13.3(0.8)	12.3(0.1)	15.4(0.4)	12.9(0.1)
H-SDS CA	-2.01 (0.14)	-2.66 (0.20)	-2.29 (0.14)	-2.76 (0.19)	-2.22(0.19)	-1.97(0.31)	-2.02(0.11)	-2.00(0.36)	-2.20(0.18)	-1.68(0.21)	-2.02(0.91)	-2.99(0.03)
H-SDS BA	-1.63 (0.14)	-1.83 (0.34)	-1.30 (0.41)	-2.07 (0.37)	-1.78(0.30)	-0.86(0.31)♣	-1.04(0.42)	-0.98(0.65)	-1.25(0.61)	-0.57(0.39)	-2.46(0.32)	-0.590.10)
BMI-SDS	-0.39 (0.23)	-0.24 (0.36)	-0.64 (0.22)	-0.34 (0.77)	-0.36(0.4)	0.18(0.25)	-0.35(0.44)	0.19(0.82)	-0.57(0.45)	-0.06(0.36)	0.99(0.96)	-1.75(1.50)
PAH (cm)	151.8 (1.0)	168.4 (2.6)	154.3 (3.3)	165.9 (3.2)	155.4(1.9)♣	161.1(2.4)♣	175.9(6.3)	171.3(5.6)	158.6(3.5)	163.3(3.0)	159.4(4.2)	175.4(1.5)
PAH SDS	-2.65 (0.16)	-2.02 (0.39)	-2.24 (0.53)	-2.40 (0.48)	-2.06(0.30)♣	-1.14(0.38)♣	-0.92(0.94)	-1.59(0.83)	-1.55(0.57)	-0.79(0.49)	-3.38(0.62)	-0.99(0.22)
Birth weight (gr)	3084 (121)	2734 (301)	2338 (177)*	2341 (513)								
TH-SDS	-0.81 (0.20)	-0.82 (1.27)	-0.08 (0.39)	-0.93 (0.69)								

** p < 0.01 compared to girls with ISS*

♦ p < 0.05 between control and treatment group

♣ p < 0.05 compared to start

Table 3 Hormonal data (Mean ± SEM) at start of study and after 3 years for control and GH+GnRHa treated children.

	Start		After 3 years		Difference in time, between groups	Difference in time, whole group
	control	Rx	Control	Rx		
Leptin(ng/ml)	3.8 ± 0.5	4.2 ± 2.5	6.1 ± 1.1	7.7 ± 1.1	NS	P<0.001
DHEA (nmol/L)	4.9 ± 0.7	7.3 ± 1.2	10.3 ± 3.7	13.1 ± 5.9	NS	P<0.001
DHEA-S (µmol/L	1.7 ± 0.3	1.7 ± 0.3	2.7 ± 1.3	3.2 ± 0.3	NS	P<0.001
Androstenedione (nmol/L)	1.5 ± 0.1	2.2 ± 0.4	2.9 ± 0.7	2.8 ± 0.3	P = 0.01	P<0.001
PICP (µg/L)	275.4 ± 22.0	321.5 ± 30.6	251.3 ± 40.5	272.2 ± 14.7	NS	P<0.01
PIIINP(µg/L)	8.8 ± 0.8	9.0 ± 0.7	8.1 ± 0.8	10.0 ± 0.8	NS	P = 0.01
Osteocalcin (µg/L)	5.8 ± 0.7	5.8 ± 0.6	7.9 ± 1.2	8.1 ± 0.7	NS	P<0.01
Alkaline Phosphatase (IU/L)	250.3 ± 17.0	210.2 ± 10.4	179.5 ± 23.6	183.4 ± 21.9	NS	P<0.01

Height SDS for BA improved significantly in GH+GnRHa treated children compared to a decrease in controls ($p<0.001$, n = 30, figure 1B). In subgroups, in ISS treated girls height SDS for BA was significantly different between start of study and after 3 years (table 1). The changes in predicted adult height (PAH) in cm or SDS were significantly higher in GH+GnRHa treated children compared to controls ($p<0.001$); the change in PAH SDS is shown in figure 1C. The absolute values for changes in PAH (cm) between start of study and after 3 years are summarized in table 2.

There was no significant difference between ISS and IUGR children or between boys and girls in change in predicted adult height. In treated children, age, BA, height SDS for CA, height SDS for BA, BMISDS, or pubertal stage at start of treatment, IGF-I, IGF-II, IGFBP-3 at start of treatment, birth weight or target height SDS did not significantly correlate with the change in PAH after 3 years of treatment (univariate non-parametric correlations).

Body mass index SDS (BMISDS) did not change during the study period, in both groups. There was a significant change in sitting height SDS (SHSDS, $p=0.05$) and in the ratio sitting height/height SDS (SH_{Ht}, $p=0.016$) in treated children compared to controls. After 3 years of study, SH_{Ht} changed –0.33 (0.77) SDS in the treated group (having relatively longer legs after 3 years of treatment) compared to +0.43 (0.83) SDS in controls (figure 2).

Puberty

At start of the study period no significant differences were present between treated children and controls with regard to Tanner stage. Pubertal development was effectively arrested in the children treated with GH and GnRH agonist, while puberty progressed in the control group. Figure 3 shows the Tanner breast stage in relation to estradiol levels in girls and the mean testicular volume in relation to testosterone levels in boys. Ultrasound evaluation of uterus volume and ovarian volumes in girls showed reduction in ovarian volumes in the treated girls. We did not observe any abnormalities in the ovaries (indicative for the development of polycystic ovaries) or uterus by ultrasound in the children on combined treatment.

Hormonal data

Serum FT4, TSH, blood HbA1c, fasting blood glucose, and serum fasting insulin remained within the normal range in all children during the study period. Figure 4 shows the serum IGF-I, IGF-II, and IGFBP-3 levels (ng/ml) for treated children and controls. Serum IGF-I and IGFBP-3 levels were approximately 0 SDS, while serum IGF-II levels were around –1 SDS. Serum IGF-I (ng/ml or SDS), or IGF-II (ng/ml or SDS) were not different at start of study or during three years of follow up between treated children and controls, while IGFBP-3 (ng/ml or SDS) levels in controls decreased significantly in comparison to treated children during 3 years of follow-up ($p=0.01$). With the exception of androstenedione, DHEA, DHEAS, PICP, PIIINP, osteoalcin and alkaline phosphatase levels were also not different

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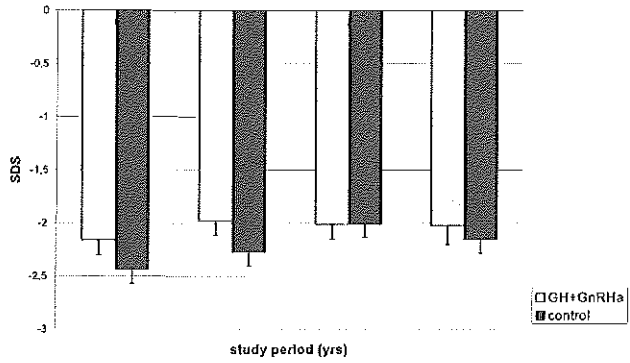
Figure 1: Height SDS for CA (fig **1a**) and for BA (fig **1b**) during the study period. H-SDS for CA does not change in time; HSDS for BA data show significant differences in time and between groups ($p<0.001$) Fig **1c** shows the increase in PAH-SDS compared to the initial height prediction. * = $p < 0.05$; *** = $p<0.001$

1A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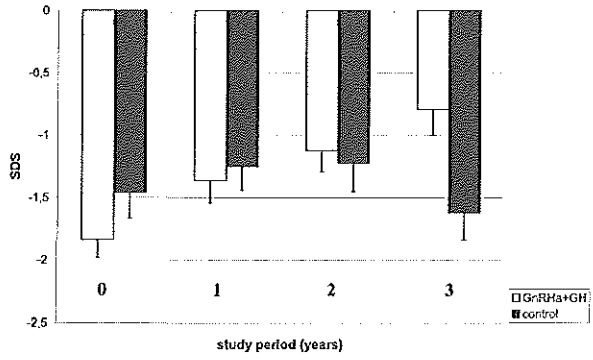
-0,5
-1
-1,5
-2
-2,5
-3
SDS
GH+GnRHa
control
study period (yrs)

1B


0
-0,5
-1
-1,5
-2
-2,5
SDS
0
1
2
3
GnRHa+GH
control
study period (years)

1C

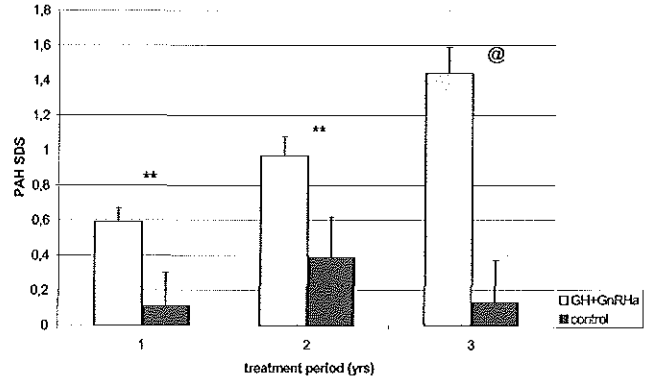
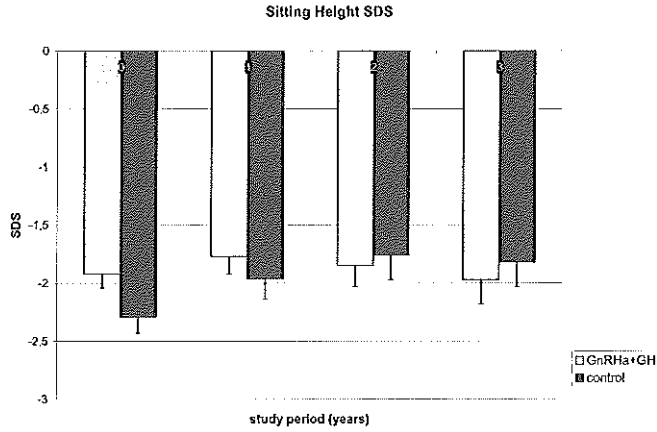

1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
PAH SDS
**
**
@
1
2
3
GH+GnRHa
control
treatment period (yrs)

Figure 2: Proportional growth during the study period, expressed as SDS scores. **2a**: Sitting Height SDS: significant difference in time and between groups ($p<0.01$), **2b** Sitting Height /Height SDS: significant difference between groups ($p < 0.05$).



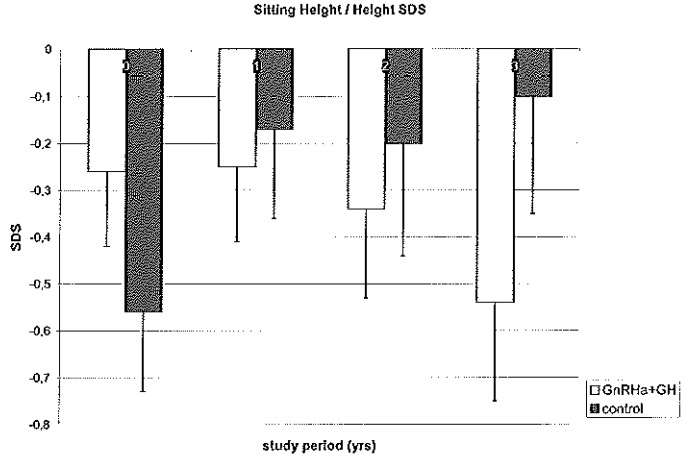
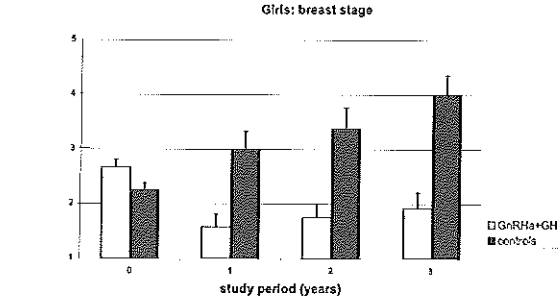
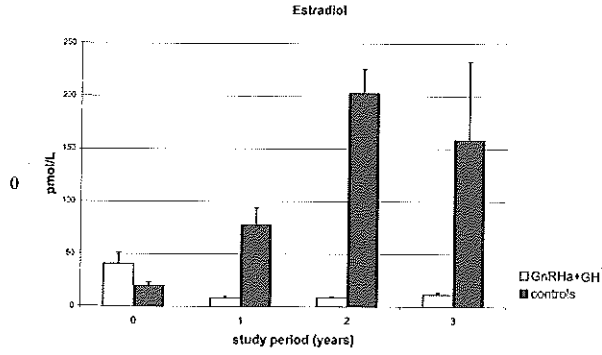
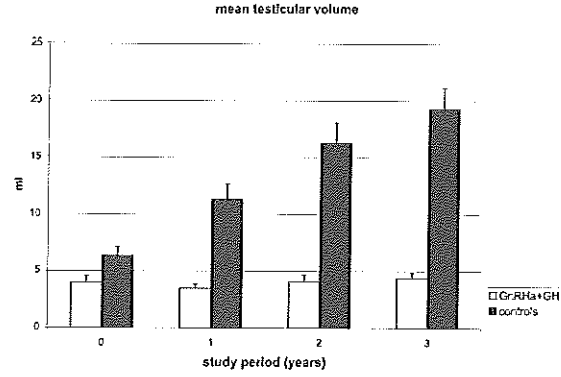
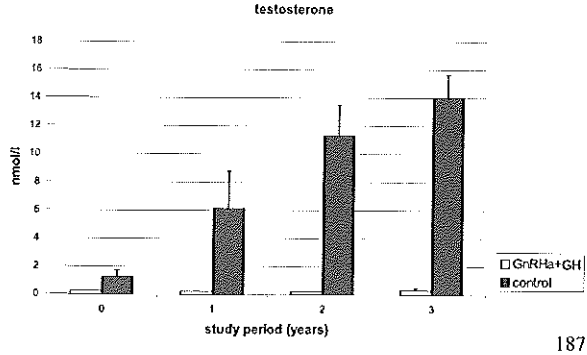


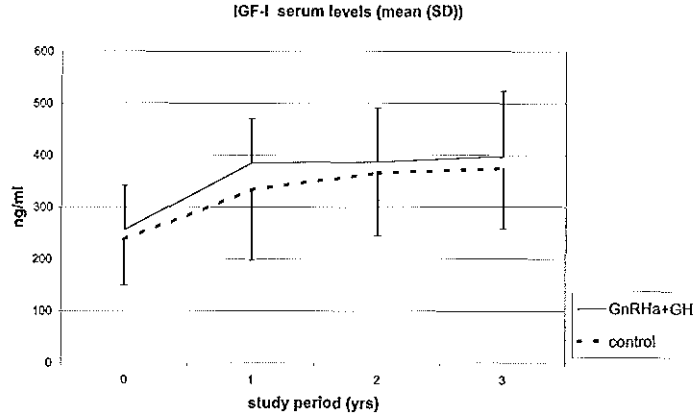
Figure 3: Suppression of puberty during the study period.
3a: Tanner breast stage in girls;
3b: serum estradiol levels in girls ;
3c: mean testicular volume in boys and
3d: serum testosterone levels in boys. Statistical differences are not shown in this panel.










IGF-I serum levels (mean (SD))
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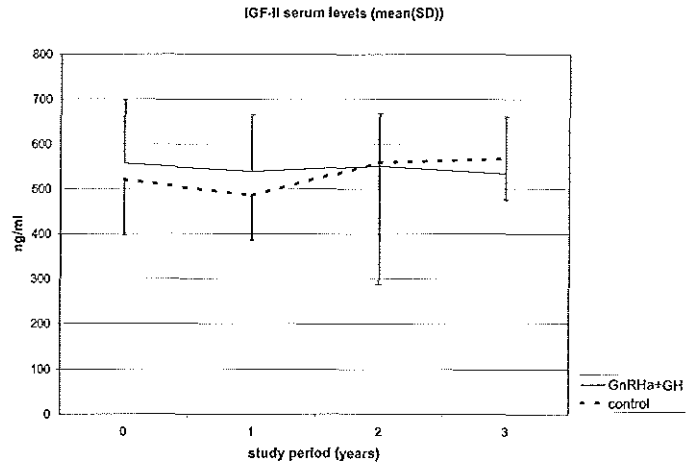
500
400
300
200
100
0
ng/ml
0
1
2
3
study period (yrs)
GnRHa+GH
control


IGF-II serum levels (mean(SD))
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700
600
500
400
300
200
100
0
ng/ml
0
1
2
3
study period (years)
GnRHa+GH
control

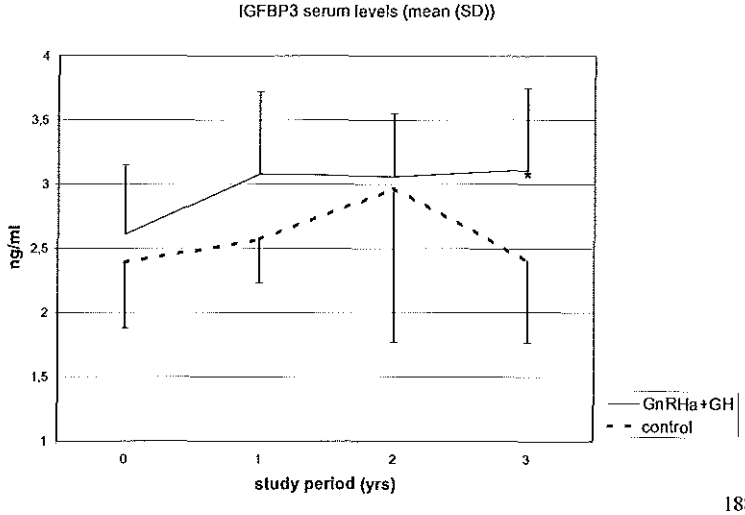
IGFBP3 serum levels (mean (SD))
4
3,5
3
2,5
2
1,5
1
ng/ml
0
1
2
3
study period (yrs)
GnRHa+GH
control

Figure 4: IGF-I, IGF-II and IGFBP3 levels during the study period (mean ± SD). **4a**: IGF-I levels (ng/ml) increase significantly in both groups during in time ($p<0.001$); **4b**: IGF-II levels (ng/ml); **4c** IGFBP3 levels (ng/ml), showing a significant difference in time ($p< 0.001$) and between groups ($p=0.01$).

between treated and control children during three years of follow-up. All hormone levels increased significantly with age in both treated and control children (table 3).

Serum leptin levels did not significantly differ between controls and treated children. Pearson correlations between BMI-SDS and serum leptin levels showed no consistent patttern: significant correlations were found at start and at 2 years in girls ($p < 0.05$) and at 1 and at 3 years in boys ($p<0.01$). Obesity defined as BMI-SDS > 2 was only present in 1 boy in the GH+GnRHa group at 1 time point.

Discussion

Combined treatment with GH and GnRH agonist in short children with ISS or IUGR in a randomized controlled trial resulted in an unchanged height SDS for chronological age and a decreased rate of bone maturation. Thus, height SDS for bone age and predicted adult height increased significantly after 3 years of treatment. Compared to controls the predicted height gain was 8.0 cm in girls and 10.4 cm in boys. Further, body proportions changed in favor of relatively longer legs in treated children, and body mass index was not influenced by treatment.

Previous studies using the combined treatment of GH and GnRH agonist show considerable variability in gain of predicted adult height, ranging from –0.7 to 10.5 cm (1, 13, 14, 16, 17, 27). Results of two of these studies seem comparable to our study (16, 27): The gain in predicted adult height of 9.3 cm in a group of 7 girls reported by Saggese and coworkers was reduced to 6.3 cm after comparison with a historical control group (27). The same pattern was observed in our study in 23 girls, with a reduction of 10.4 to 8.0 cm when compared to randomized controls. Pasquino *et al.* also reported a gain of 10.5 cm in girls, but no controls were included (16). A recent randomized controlled study of the effect of the addition of GnRHa to GH treatment in 7 GH deficient individuals showed a gain in near final height of 1.4 SDS or 9 cm (28). After comparison with randomized controls the gain in predicted adult height in our 11 boys increased from 6.5 to 10.4 cm. The decrease in height prediction in non-treated short boys shows that this prediction method is overestimating final height in boys. This finding is in line with the overestimation of final height in untreated ISS boys, as we suggested earlier (29). Contrasting our expectations based on findings from previous reports, a relative young bone age or an early pubertal stage were not predictive for a relative large gain in adult height. This finding may have clinical applications for future treatment of older children in whom puberty has progressed substantially. Thus, after 3 years of study our results are encouraging. However, the gain in final height will provide the definite answer on the effectiveness if this combined treatment. The final height gain may be probably less than 8 to 10 cm, as we have learned from experience in previous studies in CPP (7).

The sitting height SDS and the ratio of sitting height/height SDS decreased significantly in both treated girls and treated boys during the study period. This indicates that delaying puberty in our study has resulted in relatively longer legs. This result is in accordance with the observation in hypogonadotropic hypogonadism, who provide a natural experiment of pubertal delay. These men also have relatively longer legs compared to their

trunk at final height (30). At final height some change in body proportions may also be present in treated children, but we expect that the trunk/leg ratio will remain within the normal range. Our data on body proportions are in contrast with the findings at near final height in a recent study on GH deficient children where the addition of GnRHa resulted in no change in body proportions (28).

The clinical observation that treatment with GnRHa may lead to obesity could not be confirmed by changes in BMISDS or leptin levels in our study (31). Moreover, we could not identify a consistent pattern between changes in BMISDS and changes in leptin. It has to be emphasized that a BMI-SDS change is not a good marker of change in fat mass, particularly during puberty. Another explanation for the absence of obesity in our treated children may be the result of the combined treatment of GnRHa and GH we used. The probable effect of GnRHa on induction of fat mass which is usually reflected by increased leptin levels (32), may have been counterbalanced by the anabolic effect of GH on muscle mass in our treated children.

The therapeutic efficacy in terms of suppressing puberty and the good compliance to treatment were shown by the absence of progression of puberty at clinical assessments. This was further confirmed at the biochemical level by prepubertal levels of serum estradiol in girls and serum testosterone in boys during treatment. The development of polycystic ovaries, a previously suggested side effect of GnRHa treatment (Hindmarsh, personal communication), was not observed in the ultrasound studies in our treated children.

The decreased growth rate after GnRHa treatment without GH, as was observed in previous studies, may be explained by a reduction of pituitary GH secretion after withdrawal of sex steroids (33). The unchanged height SDS for chronological age after treatment in our study suggests that the addition of GH treatment seems to counteract this mechanism. Therefore, the combined treatment resulted in preservation of growth during treatment while a delay in bone maturation was accomplished. Assessments of IGF-I, IGF-II, IGFBP-3, PICP, PIIINP, alkaline phosphatase and osteocalcin, suggest that a GH dose of 4 IU/m^2 /day is needed for replacing the GH secretion in puberty in these GnRHa treated children. The assumption that adrenal development is not altered by GnRHa treatment was confirmed by the measurements of DHEA and DHEAS. All androgens increased with age in both groups. Androstenedione levels, however, increased significantly less after 3 years of study in treated children compared to controls.

We conclude that 3 years treatment with GnRHa was effective in suppressing pubertal development, while the addition of GH preserved the growth potential during treatment. This resulted in a considerable gain in predicted adult height, without demonstrable side effects. Final height results will provide the definite answer on the effectiveness of this treatment regimen.

Acknowledgement

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REFERENCES

1. Balducci R, Toscano V, Mangiantini A, Municchi G, Vaccaro F, Picone S, et al. Adult height in short normal adolescent girls treated with Gonadotropin-Releasing Hormone analog and growth hormone. J Clin Endocrinol Metab 1995;80:3596-3600.
2. Hintz RL, Attie KM, Baptista J, Roche A. Effect of growth hormone treatment on adult height of children with idiopathic short stature. Genentech Collaborative Group. N Engl J Med 1999;340(7):502-7.
3. Sas T, de Waal W, Mulder P, Houdijk M, Jansen M, Reeser M, et al. Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. J Clin Endocrinol Metab 1999;84(9):3064-70.
4. Hindmarsh PC, Brook CG. Final height of short normal children treated with growth hormone. Lancet 1996;348(9019):13-6.
5. Stanhope R, Albanese A, Hindmarsh P, Brook CG. The effects of growth hormone therapy on spontaneous sexual development. Horm Res 1992;38(Suppl 1):9-13.
6. Rekers-Mombarg LTM, Kamp GA, Massa GG, Wit JM, Group DGHW. Influence of growth hormone treatment on pubertal timing and pubertal growth in children with idiopathic short stature. J Ped Endocrinol Metab 1999;12:611-622.
7. Oostdijk W, Rikken B, Schreuder S, Otten BJ, Odink RJH, Rouwe C, et al. Final height in central precocious puberty after long-term treatment with a slow-release GnRH agonist. Arch Dis Child 1996;75:292-297.
8. Pasquino AM, Pucarelli I, Segni M, Matrunola M, Cerrone F. Adult height in girls with central precocious puberty treated with Gonadotropin-Releasing Hormone analogues and growth hormone. J Clin Endocrinol Metab 1999;84:449-452.
9. Carel JC, Roger M, Ispas S, Tondu F, Lahlou N, Blumberg J, et al. Final height after long-term treatment with Triptorelin slow release for central precocious puberty: Importance of statural growth after interruption of treatment. J Clin Endocrinol Metab 1999;84:1973-1978.
10. Lindner D, Job JC, Chaussain JL. Failure to improve height prediction in short stature pubertal adolescent by inhibiting puberty with luteinizing hormone-releasing hormone analogue. J Pediatr 1993;152:393-396.
11. Carel JC, Hay F, Coutant R, Rodrigue D, Chaussain JL. Gonadotropin-releasing hormone agonist treatment of girls with constitutional short stature and normal pubertal development. J Clin Endocrinol Metab 1996;81(9):3318-22.
12. Municchi G, Rose SR, Pescovitz OH, Barnes KM, Cassorla FG, Cutler Jr GB. Effects of Deslorelin-induced pubertal delay on the growth of adolescents with short stature and normally timed puberty: preliminary results. J Clin Endocrinol Metab1993;77:1334-1339.
13. Job JC, Toublanc JE, Landier F. Growth of short normal children in puberty treated for 3 years with growth hormone alone or in association with Gonadotropin-releasing Hormone agonist. Horm Res 1994;41:177-184.
14. Lanes R, Gunczler P. Final height after combined growth hormone and gonadotrophin-releasing hormone analogue therapy in short healthy children entering into normally timed puberty. Clin Endocrinol 1998;49:197-202.
15. Tanaka T, Satoh M, Yasunaga T, Horikawa R, Tanae A, Hibi I. GH and GnRH analog treatment in children who enter puberty at short stature. J Clin Endocrinol Metab 1997;10:623-628.
16. Pasquino AM, Pucarelli I, Roggini M, Segni M. Adult height in short normal girls treated with gonadotropin-releasing hormone analogs and growth hormone . J Clin Endocrinol Metab 2000;85(2):619-22.
17. Saggese G, Cesaretti G, Barsanti S, Rossi A. Combination treatment with growth hormone and gonadotropin-releasing hormone analogs in short normal girls. J Pediatr 1995;126(3):468-73.
18. Kamp GA, Zwinderman AH , van Doorn J, Hackeng W, Frölich M, Schönau E, and Wit JM. Biochemical markers of growth hormone (GH) sensitivity in children with idiopathic short stature: individual capacity of IGF-I generation after high dose GH treatment determines the growth response to GH. Thesis GA Kamp, Leiden 2000.
19. Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 1969;74(6):901-10.
20. Roede MJ, Wieringen JC van. Growth diagrams 1980. Netherlands third nation-wide survey. Tijdschr Soc Gezondheidszorg 1985;63 (suppl.):11-32.
21. Bayley N, Pinneau SR. Tables for predicting adult height for skeletal age: revised for use with the Greulich-Pyle hand standards. J Peds 1952;40:423-41.

22. Gerver WJM, Bruin R de. Paediatric Morphometrics; a reference manual. Utrecht: Bunge; 1996.
23. Cole TJ, Roede MJ. Centiles of body mass index for Dutch children aged 0-20 years in 1980--a baseline to assess recent trends in obesity. Ann Hum Biol 1999;26(4):303-8.
24. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. 2 ed. Stanford, California: Stanford University Press; 1959.
25. Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF- binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 1998;50(3):166-76.
26. Cole TJ. Using the LMS method to measure skewness in the NCHS and Dutch National height standards. Ann Hum Biol 1989;16(5):407-19.
27. Saggese G, Pasquino AM, Bertelloni S, Baroncelli GI, Battini R, Pucarelli I, et al. Effect of combined treatment with gonadotropin releasing hormone analogue and growth hormone in patients with central precocious puberty who had subnormal growth velocity and impaired height prognosis. Acta Paediatrica 1995;84:299-304.
28. Mericq MV, Eggers M, Avila A, Cutler GB, Jr., Cassorla F. Near final height in pubertal growth hormone (GH)-deficient patients treated with GH alone or in combination with luteinizing hormone-releasing hormone analog: results of a prospective, randomized trial. J Clin Endocrinol Metab 2000;85(2):569-73.
29. Wit JM, Kamp GA, Rikken B. Spontaneous growth and response to growth hormone treatment in children with growth hormone deficiency and idiopathic short stature. Pediatr Res 1996;39(2):295-302.
30. Burns EC, Tanner JM, Preece MA, Cameron N. Final height and pubertal development in 55 children with idiopathic growth hormone deficiency, treated for between 2 and 15 years with human growth hormone. Eur J Pediatr 1981;137(2):155-64.
31. Palmert MR, Mansfield MJ, Crowley WF, Jr., Crigler JF, Jr., Crawford JD, Boepple PA. Is obesity an outcome of gonadotropin-releasing hormone agonist administration? Analysis of growth and body composition in 110 patients with central precocious puberty. J Clin Endocrinol Metab 1999;84(12):4480-8.
32. Rogol AD. Editorial: leptin and puberty. J Clin Endocrinol Metab 1998;83:1089-1090.
33. Walvoord EC, Pescovitz OH. Combined use of growth hormone and gonadotropin-releasing hormone analogues in precocious puberty: theoretic and practical considerations. Pediatrics 1999;104(4 Pt 2):1010-4.

III TREATMENT WITH GNRHA AND GH

Chapter 6: Study of the effect of treatment with GnRH-agonist (Decapeptyl) alone or in combination with biosynthetic human growth hormone (Genotropin) in adopted children with early puberty.

6.1 Gonadotrophin Releasing Hormone agonist (GnRHa) treatment with or without recombinant human growth hormone (GH) in adopted children with early puberty

6.2 Study of the effect of treatment with GnRH (GnRH)-agonist alone or in combination with recombinant human growth hormone in adopted children with early puberty: Psychological assessments before and after treatment

6.3 Motivation for treatment and psychological evaluation in adoptive families when a child presents with early puberty :

Chapter 7

A randomized controlled trial of three years of growth hormone (GH) and GnRH agonist treatment in children with Idiopathic Short Stature and Intra-Uterine Growth Retardation.

Chapter 8

The effect of pubertal delay by GnRH agonist in Growth Hormone Deficient (GHD) children on final height

submitted

The effect of pubertal delay by GnRH agonist in Growth Hormone Deficient (GHD) children on final height

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On behalf of the Dutch Advisory Group on Growth Hormone

Introduction

Several strategies can be applied to optimize GH treatment in growth hormone deficient (GHD) children during puberty (1). In a recent issue of this journal Mericq and coworkers reported their results of a prospective, randomized trial on the effect of growth hormone (GH) alone or in combination with Gonadotrophin Releasing Hormone agonist (GnRHa) in pubertal GHD patients (2). They concluded that delaying puberty by GnRHa led to a near final height SD score (SDS) of -1.3 +/- 0.5 compared with -2.7 +/- 0.3 in the group treated with GH alone.

However, in this study the patients had been untreated up to the age of 12-18.5 years, which is not the usual situation in western countries. In addition, in several cases the growth retardation was less severe than would be expected in classical GHD patients without treatment.

Although a randomized controlled trial in a large and representative sample is certainly the best design to study the efficacy of a therapeutical regimen, a patient series with matched controls is a good second. We report on a retrospective analysis of the effect of the addition of GnRHa to GH treatment in GHD children, treated at a younger age and smaller stature with GH, in which GnRHa was added. Matched controls with GH treatment were used for comparison. The children were younger as well as shorter for age compared to the subjects in the randomised trial (2).

Patients and methods

From the nation-wide database of GH treated children (Dutch Growth Foundation/ Advisory Group on Growth Hormone) we selected 21 patients with GHD who had been treated with the combination of GH and GnRHa (Triptorelin or Leuprolide acetate, 3.75 mg per month), after a variable period of GH therapy alone. All patients had reached final height (FH). The GnRHa was given soon after entering into puberty at a relatively early age and/or low height for age.

Thereafter the database was screened for matched controls who were only treated with GH, consecutively taking as matching criteria: age at start of puberty, H-SDS and age at start of GH treatment, GH dosage, sex, BMI-SDS at start of treatment and type of GHD. These criteria were chosen, because they best predict the growth response to GH (3). For 10 patients on the combination therapy (group A) suitable matched controls were found (group B). For the remaining 11 patients (group C) no suitable controls were available.

Results

Selected clinical data are shown in the table [mean (SEM)]. In group A the mean age at start of GnRHa treatment was 11.9 (SE 0.5) yrs and mean treatment period was 3.0 yrs (range:1.2 - 5.6 yr).

Discussion

We conclude that patients who are at risk of not attaining their genetic growth potential by the onset of puberty when height has not sufficiently caught up, can reach a final height close to their genetic target by the addition of GnRHa to GH substitution therapy. This effect was seen in both groups A and C. In contrast, continuing GH therapy alone, as in group B, leads under these circumstances to a final height of 1 SDS below target.

The efficacy of the combination therapy is also shown by other relevant parameters such as the greater mean (SD) change in height SDS from the onset of puberty, and to a lesser extent the change in height SDS since the onset of GH treatment.

Table: Summary of patient data

	Group		
	A	B	C
Number (M/F)	2/8	7/3	3/8
Diagnosis			
- multiple / isolated GHD	7 / 3	8 / 2	6 / 5
- idiopathic / organic GHD	6 / 4	7 / 3	5 / 6
GH peak (mU/L)	10.9 (1.4)	8.5 (2.1)	7.6 (1.5)
MPH-SDS	-1.44 (0.40)	-0.93 (0.31)	-1.68 (0.30)
Age start GH (yr)	7.6 (1.0)	7.6 (1.2)	10.2 (1.4)
H-SDS start GH	-3.08 (0.58)	-3.22 (0.61)	-4.23 (0.48)
Age start puberty (yr)	10.7 (0.6)	11.5 (0.5)	11.5 (0.8)
H-SDS start puberty	-2.17 (0.39)	-1.78 (0.39)	-3.39 (0.58)
GH dosage during puberty[#]	16.0 (0.9)	14.7 (1.4)	17.0 (1.2)
FH-SDS	-1.31 (0.39)	-1.77 (0.34)	-1.66 (0.16)
FH-SDS – MPH-SDS	0.13 (0.37)*	-0.85 (0.36)	0.04 (0.31)
FH-SDS – H-SDS start puberty	0.86 (0.48)	0.01 (0.36)	1.73 (0.58)

GH: growth hormone; MPH: mid parental height; SDS: standard deviation score; FH: final height; [#]GH dosage: average in pubertal period (IU/m^2/week); * p=0.05 compared to group B; in other variables no signif. differ between group A and B

If the difference between FH SDS and MPH-SDS is taken as the most reliable outcome parameter, the effect of GnRHa addition on final height can be estimated at 1 SDS (approximately 6-7 cm).

The literature on the final effect of the addition of GnRHa to GH in GHD children is limited. Adan *et al.* reported that the combined treatment resulted in a normal adult height, albeit somewhat below target height (4). Hibi *et al.* reported in 24 GHD children treated with GH and cyproterone-acetate (CPA) and/or medroxyprogesterone acetate (MPA) for 4.4 years that FH-SDS was about 1 SDS higher than in a group children on GH alone. The final height SDS was –2.2 in boys and -1.9 in girls, probably somewhat lower than in the more recent studies due to less effective gonadal suppression by CPA or MPA (5).

In summary, in spite of the uncertainty about the representativeness of the patient sample studied by Mericq *et al.* (2), their data and those of other investigators discussed in their paper, as well as our retrospective analysis, all point in one direction: adding GnRHa in early puberty to GHD patients who have been treated with GH, or in whom GH and GnRHa are started simultaneously, enables the patients to reach their genetic target, while final height of patients on GH alone is about 1 SDS below MPH-SDS. This result is of similar size as the efficacy of GnRHa in idiopathic short stature (6).

REFERENCES

1. Saggese G, Federico G, Barsanti S. Management of puberty in growth hormone deficient children. J Pediatr Endocrinol Metab 1999;12(Suppl 1):329-34.
2. Mericq MV, Eggers M, Avila A, Cutler GB, Jr., Cassorla F. Near final height in pubertal growth hormone (GH)-deficient patients treated with GH alone or in combination with luteinizing hormone-releasing hormone analog: results of a prospective, randomized trial. J Clin Endocrinol Metab 2000;85(2):569-73.
3. Ranke MB, Lindberg A, Chatelain P, et al. Derivation and validation of a mathematical model for predicting the response to exogenous recombinant human growth hormone (GH) in prepubertal children with idiopathic GH deficiency. KIGS International Board. Kabi Pharmacia International Growth Study [see comments]. J Clin Endocrinol Metab 1999;84(4):1174-83.
4. Adan L, Souberbielle JC, Zucker JM, Pierre-Kahn A, Kalifa C, Brauner R. Adult height in 24 patients treated for growth hormone deficiency and early puberty. Journal of Clinical Endocrinology and Metabolism 1997;82:229-233.
5. Hibi I, Tanaka T, Tanae A, et al. The influence of gonadal function and the effect of gonadal suppression treatment on final height in growth hormone (GH)-treated GH-deficient children. J Clin Endocrinol Metab 1989;69(2):221-6.
6. Pasquino AM, Pucarelli I, Roggini M, Segni M. Adult height in short normal girls treated with gonadotropin-releasing hormone analogs and growth hormone. J Clin Endocrinol Metab 2000;85(2):619-22.

Chapter 9

Growth hormone in short children: beyond medicine?

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Chapter 9

Growth Hormone in Short Children: Beyond Medicine?

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ABSTRACT

The indications for growth hormone (GH) treatment in non-GH deficient short children are in debate. Some argue that it does not belong to the medical domain. We describe three different approaches to this issue. We argue that a disease oriented or client oriented approach are not sufficient. Both lead to withdrawal of medical interventions or to undesired application. An approach that focuses on suffering as an indication for treatment of short stature is the most appropriate. The challenge is to develop proper tools to evaluate suffering and the efficacy of GH treatment in these children in order to relieve or prevent suffering.

INTRODUCTION

Height seems to be extremely important; studies show that height has a significant impact on salaries of men (1), people with short stature are liable to societal bias (2), and famous models are tall (3). Taking into account the association of power, status and height, it is no wonder parents like to have tall children or at the least are concerned if their child seems to have growth problems.

Synthetic growth hormone (GH) is used in the treatment for short children with growth hormone deficiency (GHD). Growth hormone treatment (GH treatment) is also advocated for those with non-growth hormone deficient short stature, e.g. idiopathic short stature (ISS), intrauterine growth retardation (IUGR), Turner syndrome, and during GnRHa treatment for early and precocious puberty. The use of GH in children with short stature has led to serious discussions amongst the medical discipline as well as amongst philosophers and ethicists (4, 5, 6). GH treatment in idiopathic short stature, for example, may be seen as an enhancement, a treatment done for the sake of appearance just like breast enlargement for women or eye surgery for children with Down syndrome. This raises questions such as 'should GH treatment be covered by health care insurance' and 'how should the danger of medicalization and stigmatisation be valued'?

We will focus on the question, whether or not the use of GH in short children belongs to the medical realm. Three different lines of reasoning will be sketched. The first line contains that GH treatment should only be administered to those with a disease or disability (if treatment is proven to be efficacious). The second line of reasoning holds that medicine should focus on complete physical, mental and social wellbeing. This argumentation will imply that almost any wish for a medical intervention belongs into the medical realm. In this article we will analyse and apply both lines of reasoning and suggest a third that rests on the notion of prevention and elevation of suffering.

THE PROPER GOAL OF MEDICINE: THE DISEASE APPROACH

Some define health as the absence of disease and argue that the proper goal of medicine is the prevention and the cure of diseases and disabilities. This view on the proper aim of medicine clearly involves a distinction between disease and health, treatment and enhancement.

According to Daniels the general function of health care is "to maintain, restore and compensate for the loss of functioning that is normal for a member of our species" (7). Normal functioning of an organism may be disrupted or lost by diseases and disabilities. Disease and disability are seen as "departures from species-typical normal functional organization or functioning" (7). Daniels' view does not imply that health care should restore a situation in which individuals are equal: he emphasises that individuals differ with respect to natural endowments. There is human variation with respect to skills and talents such as creativity, intelligence and sports. Health care should not make people *equal* competitors but *normal* competitors. Health care should restore normal functioning in order to provide people with an equal opportunity to, for example, schools and jobs.

Daniels refers to the biostatistical theory of Boorse (8). His theory holds that health as the absence of disease is a value-free theoretical notion. "Health is normal functioning, where the normality is statistical and the functions biological." A function is in Boorse's view a contribution to a goal. Eventually the goals are individual survival and reproduction. The goals of an organism can be determined by averaging a large sample of that kind of organism (differentiated to age and gender). On the basis of this study a species design can be discerned. This species design serves as the basis for health judgements: the physician

can establish whether someone is healthy by determining whether the functioning of the body matches with the design of the species.

Reasoning along this line leads to the following conclusions. According to Daniels GH for idiopathic short children would be an enhancement rather than a treatment, and therefore not acceptable (7). If Sue has short stature due to GHD and Mary due to familial short stature, Sue would receive treatment while Mary would not. For Sue has a subnormal functioning organ while Mary seems not to suffer a disease from a biostatistical perspective. In the case of IUGR there seems to be no clear answer. The etiological factors in individual children are often unknown. Although some cases will be undisputed (e.g. the Silver-Russell syndrome (9)) it is difficult to determine the cause of growth retardation in the majority of cases. These practical difficulties are absent in the case of Turner syndrome. A clear chromosomal disorder can be found with growth retardation as one of the clinical symptoms. Moreover, recent studies have proven that growth hormone treatment is efficacious (10, 11). From Daniels' line of reasoning the administration of GH treatment therefore seems to be justified in the case of Turner syndrome.

In Sweden and The Netherlands studies are conducted on the effect of the addition of GH to GnRHa in adopted children on growth (12, 13). These children are studied because they have an early puberty, their predicted adult height is short and during treatment with GnRHa decrease in height velocity may be observed. Suppose clinical trials show that additional GH treatment results in a considerable increase in height compared to GnRH agonist alone. According to the disease-oriented model of Daniels, GH should not be prescribed. If these children after GnRHa treatment do not differ from children in their native country with respect to the normal functioning ability and the mean height, one should abstain from GH-treatment. The short stature is, in this view, part and parcel of the ethnic background of the adopted children. From a bio-statistical perspective nothing is wrong with these children. Some would argue that early puberty in these children is a disorder and the possible decreasing height velocity during its treatment needs intervention. However, the early onset of puberty seems to be related to the transition to the western world countries and does not belong to the normal functioning in the countries of origin. It is therefore doubtful whether the administration of GnRH agonist, (with or without addition of GH) would pass Daniels' disease-oriented model.

Daniels' line of reasoning has serious practical and moral problems. The case of Sue and Mary illustrates one of the problems of his treatment-enhancement distinction. The short stature of Sue can be ascribed to a subnormal functioning organism but in our view the cause of her short stature is not an ethically relevant characteristic. When it does not interrupt her total functioning, the subnormal functioning does not really matter.

The above-mentioned criticism concerns Boorse's concept of normal functioning ability. Some phenomena would be categorised by this concept as healthy or normal while the medical literature defines these as a disease that can be prevented, e.g. arteriosclerosis or dental caries (14). The biostatistical concept would categorise phenomena as normal that are categorised as diseases in medical textbooks. Boorse therefore extends his definition of disease: "a disease is a type of internal state that is a limitation on functional ability caused by environmental agents". The extended definition and these examples, however, illustrate that a cultural context should be understood in order to determine what is normal. In order to determine what normal or good functioning means, information is needed about the circumstances in which functioning takes place. A biostatistical concept will not do because biology gives too little guidance of what is normal or abnormal.

THE PROPER GOAL OF MEDICINE: THE CLIENT APPROACH

The second line of reasoning would define health in a broader sense as "a state of complete physical, mental, and social well-being", which is the well-known definition of the World Health Organisation. In this view the proper goal of medicine is to contribute to the physical, mental and social wellbeing of people. Provided people pay for it themselves nothing is wrong with medical interventions to change appearances i.e. short stature. Medicine may respect all these diverse wishes and desires, and its task may be making everyone happy. As long as the subjects are properly informed and are aware of both benefits and risks, the choice of parents and their children should be respected. This line of reasoning would imply that GH treatment should be given to children with a disease or disability such as GHD and to children with idiopathic short stature as well as to internationally adopted children (with or without treatment for early puberty). Moreover, if parents request GH treatment for their children in order to give them a better chance of being a college athlete, these parental requests should be respected.

This line of reasoning could imply that all differences between individuals should belong to the medical realm; differences between height, weight and skin colour. Too short, too fat, too black, too old, it has no end. Some would see no objections, provided that people pay the costs themselves. There may, however, be serious problems. If medicine would respect all these wishes this could lead to medicalisation and stigmatisation. Zola introduced the concept of medicalisation to criticise the increase of medical control over private and social life (15). Medicalisation is the process in which more and more phenomena in human life are governed by a medical perspective. As Verweij stated one should analyse the rather vague intuitions that underlie this concept and make explicit what is wrong with medicalisation (16).

One of the dangers of medicalisation is that it could turn healthy individuals into patients. Children who are under medical treatment may have more problems than non-referred children (17, 18). Treatment itself may give rise to feelings of inferiority or unacceptability. The child may become self-conscious or worried about her/his height (19). More studies are needed to determine the effects of the treatment on the psychosocial development. With respect to these negative effects, parental requests should be restricted. Secondly, the unrestricted use of medicine for the sake of appearance may reinforce existing social norms of privileged groups (20). Medicine would support the negative attitudes towards stigmatised individuals: persons who are unable to conform to standards which society calls 'normal'. Height prejudice, so-called 'heightism' may and should be countered by restricting extreme and ill-motivated requests (21).

THE PROPER GOAL OF MEDICINE: THE SUFFERING APPROACH

There is a third perspective that may more or less circumvent the problems linked with the above-mentioned approaches. According to this line of reasoning suffering is the real issue and the medical intervention may relieve or mitigate it. This view attaches great importance to subjective suffering: suffering as experienced by the individual. Not all existential and psychological suffering due to such diverse circumstances can or should be remedied by medicine. The suffering approach is based on the assumption that the suffering of the individual subject may (to a certain extent) be understandable to others, i.e. the doctor. Suffering is a condition that can not be objectified but the human condition makes it possible to understand that some conditions involve suffering. Maybe it is not possible to understand how someone experiences it, but it may be appreciated that somebody is hurt and is suffering. In medical practice physicians have to form a notion of the degree of the patient's

suffering. In complex cases like euthanasia and assisted suicide the physician is even required to determine whether the person involved is really suffering unbearably.

This approach implies firstly that not only the parents, but also their child and the physician try to determine whether the treatment may prevent or relieve the child's (present and future) suffering. Although most children will be too young to decide for themselves, their perspective should be included as well. Secondly, gain in stature is not a sufficient criterion to decide whether or not to start with GH treatment. Efficacy is an important but not sufficient aspect. Essential is whether or not the subject suffers and the treatment will prevent and relieve the suffering. Several studies emphasise the adverse effects of short stature on psychosocial wellbeing and educational attainment (2, 22, 23). Studies, however, also dispute the assumption that short stature is associated with psychosocial maladjustment and a reduced quality of life (18, 24, 25). The advantages of shorter statute have even been emphasised (26). The results of these latter studies seem to imply that not all categories of people with short stature may benefit from GH-treatment. Whether or not some categories (or individuals within a category) may derive more benefit from adequate coping techniques should be investigated thoroughly. Finally, children and adults with an average height will not be confronted with problems due to their height. A 'taller' stature will more likely create advantages than cause suffering. From the suffering perspective there are good reasons to withhold GH to these children whether or not the parents pay for it themselves.

If we try to apply the suffering criterion to the above-mentioned categories, the following conclusions can be drawn. In the case of growth hormone deficiency GH treatment would be accepted for it was described that adults with GHD were satisfied with their height after GH-treatment (mean height close to P3) (24). Moreover patients with GH deficiency suffer from other physical and psychological problems (e.g. lack of energy); GH treatment may mitigate these problems. Children with IUGR could benefit from GH treatment; psychosocial problems may be relieved by GH treatment (26). More studies to prove the benefits of GH-treatment, however, are necessary before conclusive, more reliable answers can be given. In Turner syndrome, where a reduction in quality of life in adults was demonstrated (24), GH treatment is effective and improves body image and results in greater self-esteem (28). With respect to ISS the cause of short stature is unknown. From the suffering perspective this is not relevant. At this moment there is little knowledge about whether GH treatment improves their psychological and social wellbeing. According to Van Busschbach *et al*, quality of life was not affected for a group of normal short adults (mean height close to the P3), while short adults with a medical history of shortness appeared to be negatively affected in their social functioning. But even here the impact of short stature on the quality of life was small. The complaints of patients with ISS may be the result of unsuccessful coping strategies. Thus the offer of GH treatment should take place in a research setting with adequate selection criteria and evaluation of psychosocial wellbeing.

Our last example concerns the use of GH to internationally adopted children during GnRHa treatment. From a suffering-perspective it matters that early puberty may have a negative influence on the emotional and cognitive development of these children (self-image, development of identity etc.). Medical intervention may be beneficial to these children. It seems reasonable, therefore, to offer it to them. There is, however, no unanimity about the negative effects of early puberty (29). With respect to short stature the adopted children possibly reach an adult height that is considerably lower than children in the country in which they grow up due to an early start of puberty and this may cause serious problems. More research is needed to determine how serious these problems are and whether GH-treatment can solve these problems effectively.

At this moment discussion about quality of life and degree of suffering is still taking place. Medical practice should be based on good (clinical and psychological) research.

Empirical science is therefore needed to determine whether suffering due to short stature is the case and whether GH treatment is the most appropriate intervention (6, 30). In case of demonstrating that GH treatment is not such an appropriate intervention one should decide not to treat these children with GH, thus reducing high costs and preventing unnecessary treatment. From a suffering perspective a clinical trial may therefore be justified on condition that psychosocial wellbeing is evaluated extensively and for a long time.

Conclusion

Different lines of reasoning can deal with the justification of GH-treatment. Some try to solve the issue of short stature by applying notions of disease or disability, others state that the proper goal of medicine is to contribute to physical, mental and social wellbeing of people. We have pointed to the weaknesses of both approaches and argued that a suffering-perspective is more appropriate, i.e. it focus on a better personal and social functioning and may minimise the dangers of medicalisation and stigmatisation. According to this approach the suffering of a person from his or her short stature is essential. Empirical science is needed to evaluate suffering and to evaluate whether GH treatment is a proper means to prevent or relieve serious suffering.

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References

1. Gortmaker SL et al. Social and Economic Consequences of Overweight in Adolescence and Young Adulthood. New Eng J Med 1993; 329: 1008-1012
2. Underwood LE, The social cost of being short: societal perceptions and biases. Acta Paediatr Scand Suppl 1991; 377: 3-8
3. Etcoff N. Survival of the Fittest. The Science of Beauty. Doubleday, New York, 1999, p. 173
4. Beaufort I de. Small children: Big Problems? In: I. de Beaufort, M. Hilhorst, S. Holm (eds.), In the Eye of the Beholder. Ethics and Medical Change of Appearance, Scandinavian University Press, Oslo, Copenhagen, Stockholm, Oxford, Boston, 1996: 9-14
5. Root AW. Editorial: Does Growth Hormone Have a Role in the Management of Children with Nongrowth Hormone Deficient Short Stature and Intrauterine Growth Retardation? J Clin Endocrinol Metab 1998; 83 (4): 1067-1069
6. Guyda HJ. Four decades of growth hormone therapy for short children: what have we achieved? J Clin Endocrinol Metab 1999; 84 (12): 4307-4316
7. Daniels N. The Genome Project, Individual Differences, and Just Health Care. In: T.F. Murphy, M.A. Lappé, Justice and the Human Genome Project. University of California Press, Berkeley, 1994, p. 123/4
8. Boorse C. Health as a Theoretical Concept. Philosophy of Science 1977; 44: 542-573
9. Marks LJ, Bergeson PS. The Silver-Russel syndrome: a case with sexual ambiguity, and a review of literature. Am J Dis Child 1977; 131(4): 447-451
10. Rosenfeld RG, Attie KM, Frane J et al. Growth hormone therapy of Turner's syndrome: beneficial effect on adult height. J Pediatr 1998; 132: 319-324
11. Sas TC, de Muinck Keizer-Schrama S.M.P.F, Stijnen T, Jansen M, Otten BJ, Hoorweg-Nijman JJ et.al. Normalization of height in girls with Turner syndrome after long-term growth hormone treatment: results of a randomized dose-response trial. J Clin Endocrinol Metab 1999; 84 (12): 4607-4612
12. Tuvemo T, Gustafsson J, Proos LA. Growth hormone treatment during suppression of early puberty in adopted girls. Swedish Growth Hormone Advisory Group. Acta Paediatr 1999; 88: 928-932

13. Mul D, Oostdijk W, Waelkens JJ, Drop S. Adopted children with early puberty: 2-year results of treatment with GnRH agonist alone or in combination with growth hormone. Horm Res 1999; 51 (suppl 2): 74
14. Have HAMJ ten, Meulen RHJ ter, Leeuwen E van. Medische ethiek. Bohn Stafleu Van Loghum, Houten/Diegem, 1998: 152
15. Zola IK. Medicine as an Institution of Social Control. Sociol Rev 1972; 20: 487-504
16. Verweij M. Medicalization as a moral problem for preventive medicine. Bioethics 1999; 13 (2): 89-113
17. Verhaar EJ, Bosch JD, Maidman PE. Klein zijn: een groot probleem? Kind en Adolescent 1991; 12 (4): 231
18. Rikken B, Busschbach J van, Cessie S te, Manten W, Spermon T, Grobbee R. Impaired social status of growth hormone deficient adults as compared to controls with short or normal stature. Clin Endocrinol 1995; 43: 210
19. Diekema DS. Is taller really better? Growth hormone therapy in short children. Perspect Biol Med 1990; 34 (1): 109-123
20. Little MO, Suspect Norms of Appearance and the Ethics of Complicity. In: I. de Beaufort, M. Hilhorst, S. Holm (eds.), In the Eye of the Beholder. Ethics and Medical Change of Appearance, Scandinavian University Press, Oslo, Copenhagen, Stockholm, Oxford, Boston, 1996, p. 151-167
21. Saenger P. Growth hormone and idiopathic short stature – it is too soon to call it a mis-trial. Eur J Endocrinol 1998; 138: 258
22. Law CM, The disability of short stature. Arch Dis Child 1987; 62: 855-859
23. Siegel PT, Clopper R, Stabler B. Psychological impact of significantly short stature. Acta Paediatr Scand Suppl 1991; 377: 14-18
24. Busschbach J van, Rikken B, Grobbee DE, Charro FT de, Wit JM. Quality of Life in Short Adults. Horm Res 1998; 49: 32-38
25. Downie AB, Mulligan J, Stratford RJ, Betts PR, Voss LD. Are short normal children at a disadvantage? The Wessex growth study. BMJ 1997;314: 97-100
26. Samaras TT, Elrick H, Storms LH. Height, health and growth hormone. Acta Paediatr 1999; 88: 602-609
27. Van der Reijden-Lakeman I. Growing Pains? Psychological evaluation of children with short stature after intrauterine growth retardation, before and after two years of growth hormone treatment. Dissertation, Erasmus University Rotterdam, 1996
28. Oberfield SE. Growth Hormone Use in Normal, Short Children – A Plea for Reason. New Engl J Med 1999; 340: 557-559
29. Kaplowitz PB, Oberfield SE, and the Drug and Therapeutics and Executive Committees of the LawsonWilkins Pediatric Endocrine Society. Reexamination of the Age Limit for Defining When Puberty Is Precocious in Girls in the United States: Implications for Evaluation and Treatment. Pediatrics 1999; 104(4):936-941
30. Haverkamp F, Ranke MB. The Ethical Dilemma of Growth Hormone Treatment of Short Stature: A Scientific Theoretical Approach. Horm Res 1999; 51: 301-304

Chapter 10
General discussion and recommendations

General discussion

General discussion and recommendations

This thesis deals with several aspects of the treatment of early puberty in children, either adopted or non-adopted. In this section the results of the various studies will be integrated and put into the perspective of current knowledge. Recommendations for clinical practice will be made at the end of this section.

I NORMAL PUBERTY

In chapters 2.1 and 2.2 trends in normal pubertal development have been described extensively. We reviewed the European situation and concluded that in most countries the age of onset of puberty and the age of menarche still decrease. The role of environmental factors is discussed. In some countries a stabilisation in the decreasing trend is observed. Since 1980, in The Netherlands no further decrease was observed in the age of occurrence of the first signs of puberty in girls and boys. The mean age at onset of puberty in girls is 10.7 years and 11.5 years in boys. In boys, the observed age of G2 stage was higher than in 1980, raising questions on the description of this stage. Only a slight decrease in age of menarche was observed, from 13.28 to 13.15 years between 1980 and 1997.

The observed relationship of menarcheal age and weight and height suggests that in countries with increasing height and weight menarche will occur earlier. Indeed, in the USA the age of onset of puberty is remarkably reduced, in parallel with increased obesity observed in children (1, 2). However, despite the ongoing secular change of increasing adult height as well as the increase in the number of obese children (3) no dramatic decrease was observed in the age of menarche nor in the age of onset of puberty in the Netherlands.

We *conclude* that in The Netherlands the decreasing trend in age of pubertal development has been stabilised between 1980 and 1997 and that only a slight decrease in the age of menarche was observed. There is no need to change the age limit for the definition of precocious puberty in The Netherlands.

II GONADOTROPHIN-RELEASING HORMONE AGONIST TREATMENT

Diagnosis and follow-up

In chapter 3 we analysed the gonadotrophin response to the GnRH or GnRHa stimulation test in children with clinical development of puberty.

We *concluded* that the GnRHa test was able to demonstrate HPG axis activation in children with early puberty more precisely than the GnRH stimulation test. Once HPG axis activation is demonstrated by GnRHa stimulation we expect further progression of puberty, as was also demonstrated by Ibanez *et al.* (18). The absence of pubertal LH peaks in a GnRH or GnRHa stimulation test was shown to predict a slow course of puberty (12). For the decision whether to treat patients with GnRHa HPG axis activation has to be demonstrated. We *conclude* that HPG axis activation is more efficiently demonstrated by the GnRHa stimulation test. As HPG axis activation indicates the onset of puberty, this test may be the decisive factor in the decision whether to treat children with signs of pubertal development with GnRHa (see section VI).

In order to reach the optimal auxological goals of treatment careful follow-up is demanded during treatment with GnRHa. We showed that in the Puberty Suppression Score (PSS), incorporating auxological and biochemical variables, signs of incomplete suppression during treatment correlate with less height gain during treatment compared to children who were effectively suppressed. With leuprolide-acetate in a dosage of 3.75 mg per 28 days efficient gonadal suppression was achieved with only a limited number of side effects or escapes. Triptorelin showed comparable efficacy and tolerability in the same dosage. In

several reports up to FH long-term safety of triptorelin has been demonstrated by normal resumption of puberty after discontinuation of treatment (chapter 5.1 and ref (4, 5)). After GnRHa treatment for precocious puberty normal pregnancies were reported (5, 19).

We *conclude* that the PSS can be used to monitor children under treatment with GnRHa for early or precocious puberty.

Precocious puberty

After long-term follow-up until final height a general conclusion can be drawn about the efficacy of GnRHa treatment for central precocious puberty . As described in the introduction, several studies reporting final height show a height gain of about 5 cm compared to the prediction at start of treatment. In most countries this is comparable with about 1 SDS.

It is also relevant to compare the achieved FH with the genetic potential expressed by the midparental height or target height. In some studies the results of GnRHa treatment show that FH is in the target range (4, 5). In other studies FH is significantly lower than target height (6, 7). It should be recognised that in Thamdrup's series of *untreated* children with CPP FH was 16 cm below midparental height (MPH) in girls and about 11 cm below MPH in boys (8).

In table 1 we present the data of the 6 largest studies, including our study, reporting on FH after GnRHa treatment in girls with CPP. In 4 of them a significant difference is present between TH and FH, either in cm or in SDS.

Table 1: Gain in FH in girls after GnRHa treatment for precocious puberty

First author	N =	CA_{start} (yr)	BA_{start} (yr)	Rx (yr)	FH minus PAH_{start} (cm)	FH minus-TH (in SDS)	Refer.
Mul[a]	87	7.7	10.4	3.4	7.4	-0.88[b] ■	(9)
Arrigo	71	7.0	9.8	4.0	2.9	-0.48 [c] ■	(7)
Carel	58	7.5	10.1	3.7	4.8	+0.15 [d]	(4)
Heger	50	6.2	9.3	4.4	5.9	-0.6	(5)
Oostdijk	31	7.7	10.8	3.4	3.4	-1.02[e] ■	(6)
Galluzzi	22	7.3	10.3	4.0	3.2	-0.84[f] ■	(10)

■ significant difference between FH and TH either in cm or in SDS

[a] see chapter 5.1 of this thesis; [b] $p < 0.001$;

[c] TH-SDS was not shown in the paper; calculated from raw data: TH –SDS = -0.12. When expressed in cm FH was significantly < TH;

[d] TH-SDS was not shown in article; calculated from raw data: TH-SDS = -0.55. When expressed in cm FH was not significantly different from TH;

[e] The difference between FH and TH expressed in cm: $p < 0.01$; [f] $p < 0.01$

Several factors may have contributed to differences in the outcome parameters shown in table 1, e.g. the method of adult height prediction, the different BA or CA at start of treatment, treatment duration and the difference between BA and CA. In univariate and multivariate analyses different factors appeared to be correlated with one of both outcome parameters, i.e. gain in height between initial prediction and final height or final height itself. Height gain was negatively correlated with BA at discontinuation of treatment (4) and positively correlated with BA advance at start of treatment (5, 7). Final height was positively correlated with target height (4, 6, 7) and treatment duration (6, 7).

In our study we found height gain to be positively correlated with BA at start of treatment and negatively correlated with CA and with BA advance at start of treatment. In an univariate analysis BA advance and treatment duration correlated positively with height gain, whereas CA at start of treatment correlated negatively.

The methodological problems in the analysis of FH data concern mainly the dependence of height predictions on current BA and height, as well as the a priori relationship that can be expected between TH and FH (4). Furthermore, the assessment of BA needs close attention. In our studies we used a segmented BA scoring, accounting for possible differences in the maturation of carpal or phalangeal bones.

The selection of children suitable for treatment, e.g. who will benefit from GnRHa treatment, is of major importance (11). In this paragraph we limit the discussion to the auxological goals of treatment: improvement of final height; the psychological aspects will be discussed later in this chapter. In the literature a differentiation has been described between children with rapid progression of puberty and children with slow progressive puberty. The children with slow progressive forms of puberty, not warranting GnRHa treatment for auxological reasons, were defined either by absence of well-defined biochemical evidence of activation of the hypothalamo-pituitary axis (12) or by low estrogenic activity and limited advance of BA over CA (13).

We consider a pubertal LH peak in a GnRH or GnRHa stimulation test as the most decisive parameter to assess onset of puberty, whereafter further progression of puberty is expected. No GnRHa treatment should be initiated when no or insufficient evidence for activation of the hypothalamo-pituitary axis is demonstrated.

The overall picture emerging from the literature and from our studies reported in this thesis is that young girls with progressive forms of CPP, thus with a considerable BA advance at start of treatment, will benefit most especially when treated till an appropriate BA for CA has been reached. The higher BA at discontinuation of treatment the worse FH will be. However, these statements come from retrospective analyses and were never addressed in formal prospective studies.

In the literature several authors make recommendations with regard to the timing of discontinuation of treatment in girls, e.g. that BA should not exceed 11 years (4) or 12.0 – 12.5 years (6, 7). The data from our study in chapter 5.1 do not allow to indicate a particular bone age to discontinue treatment. However, in our study of FH in girls *without* a fixed BA for discontinuation, we demonstrated that FH was nearly equal to the predicted adult height at discontinuation of treatment (9). The same observation was made by Heger et al (5), suggesting a limited role of BA as a single parameter for the decision to discontinue treatment. Besides, it should be noted that discontinuation of treatment is often dictated by psychosocial reasons.

In boys with CPP we showed that FH after GnRHa treatment was close to TH. However, in boys with organic CPP FH-SDS was significantly lower than TH-SDS. A mean height gain of 5.4 (SD 8.4) cm was obtained compared to initial height prediction at start of treatment (chapter 5.2). Boys with an organic cause for CPP ended up at a significantly lower FH-SDS compared to boys with idiopathic CPP. Bone age at start of treatment correlated negatively with height gain and BA advance at start of treatment correlated positively with height gain.

We *conclude* that GnRHa treatment in both girls and boys with CPP can restore FH in the normal range. A well-defined activation of the hypothalamo-pituitary axis, indicating the onset of puberty, has to be demonstrated in order to select children who will benefit from GnRHa treatment. No recommendations can be given as to which BA is optimal to discontinue treatment.

Children with short stature and early puberty

Some argue that manipulation of the timing of puberty in the relative normal age period for puberty is unlikely to affect final height to any great extent (14). Probably this is true when maturation occurs within a rather normal age range. Whether this holds for early maturers outside the normal range and for children with precocious puberty was subject of our studies. In this thesis 2 studies address the issue of pubertal arrest in short children with early puberty. The results in adopted children treated with GnRHa alone showed that during three years of treatment an improvement in mean predicted adult height of 5.7 cm is obtained (see chapter 6.1). In another group of children with either early or precocious puberty who were observed during 18 months of treatment gain in predicted height occurred as well (see chapter 4), although no differentiation was made between early and precocious puberty.

Only few studies have been reported on GnRHa treatment in children with early or normal puberty. A preliminary study by Municchi *et al.* showed an improvement of 5.9 cm in height prediction after 4 years of treatment and of 6.4 cm compared to placebo treated patients (15). In contrast, Carel *et al.* treated 31 girls for 2 years without a valuable improvement of near final height (16). A remarkable finding in the latter study was the rapid progression of BA after discontinuation of treatment, causing a substantial decrease in height prediction. This phenomenon was also seen in children with precocious puberty after GnRHa treatment (6).

In the above-mentioned study of Carel BA at discontinuation of treatment was 11.8 years (SD 0.6). In our study in adopted girls on GnRHa alone, BA at discontinuation of treatment was 12.3 (0.9) years, thus offering uncertainty about the final height gain.

At this moment we do not know whether in children with early puberty the same pattern of growth after discontinuation of GnRHa treatment will be observed as was seen after GnRHa treatment for CPP. In precocious puberty height gain can be estimated to be approximately 60 - 80% of the gain obtained at discontinuation of treatment (4, 6, 7, 10). In our report (chapter 5.1) FH was only slightly lower than predicted adult height at discontinuation of treatment, which was similarly observed by Heger *et al* (5).

We *conclude* that in short children with early puberty pubertal suppression is effective in short-term studies. Final height data can be compromised by a limited post-treatment growth and should be awaited before an indication for GnRHa treatment can be settled.

Growth hormone deficient children

The addition of GnRHa in children already treated with GH for growth hormone deficiency (GHD) presenting with early puberty at short stature and/or delayed bone age, resulted in an improvement in FH compared to matched controls without gonadal suppression.

We performed a retrospective analysis and found a mean FH-SDS of –1.31 (0.39) in children with GnRHa addition whereas the group without gonadal suppression ended up at a mean FH-SDS of –1.77 (0.34). We choose to compare the difference between FH and TH as effect parameter. In the group with both GH and GnRHa the outcome was significantly better than in the group without gonadal suppression. These data are in accordance with a recent prospective study of Mericq et al. (17) showing a difference of about 1.4 SDS in favour of the children co-treated with GnRHa. However, in that study only a few patients could be regarded as 'classical' GHD patients.

We *conclude* that adding GnRHa to GH in GHD patients with marked short stature who enter puberty at an early (bone) age enables them to reach a FH in their genetic target. Criteria for intervention with GnRHa such as age, bone age and height at onset of puberty need to be specified in the future.

III COMBINED TREATMENT WITH GNRHA AND GROWTH HORMONE

The observation of impaired growth during GnRHa treatment in CPP stimulated several investigators to start GH treatment in combination with GnRHa in those children with decreased HV (20-22). In general, they concluded that GH increased HV to normal values without accelerating bone maturation, resulting in an increase in predicted adult height. A recent report on FH in children with CPP after combined treatment showed a height gain of 7.9 cm at FH, when GH was added after a decreasing height velocity (23). No additional final height data after combination treatment in precocious puberty is available.

In children with *short stature* and in *adopted* children the addition of GH resulted in increased PAH as well (24-26). Final height results show a variable pattern ranging from a mean height gain of 10 cm compared to initial prediction (27) to a final height not different from controls or initial prediction (28, 29). Comparing our studies reported in chapters 6.1 and 7 with the studies reported in the literature our results, with a considerable height gain of 6 to 10 cm during treatment are encouraging.

An important issue to address here is the accuracy of the prediction method. A lower initial prediction due to the use of the average tables of Bayley and Pinneau may result in a larger difference with the attained final height. This issue is of main interest when final height data of different studies are being compared.

The results of the studies *combining* GnRHa and GH treatment suggest the importance of maintaining adequate height velocity during GnRHa treatment. In normal children prepubertal growth is characterised by *intensity* (mean HV) and *duration*; the intensity of prepubertal growth has most influence on FH, in boys more than in girls (30). Considering growth during GnRHa treatment (with or without GH) as prepubertal growth, elevation of HV should increase FH. A prerequisite to gain height is that GH does not accelerate BA maturation. We showed that BA maturation was neither accelerated in adopted children nor in children born after IUGR or in ISS children during combined treatment with GH and GnRHa.

In both studies on combined treatment BMI remained within the normal range. Recent reports in the literature suggest a major role of pretreatment BMI in children who increase markedly in BMI during GnRHa treatment (31). Theoretically, the lipolytic effect of GH may prevent adiposity to occur. Concerning body proportions, relatively longer legs were observed in ISS/IUGR children during combined treatment, whereas in the adopted children an opposite trend was observed both in children with and without GH addition. No explanation can be given to resolve these contrasting findings.

In both studies on the combined treatment of GnRHa and GH we observed a gradual decrease in HV not only in the group on GnRHa alone, but also in the group with combined treatment. No readily apparent explanation is present for the decrease in HV during GnRHa treatment. In both studies the gradual decrease was also visible in the group with GH. GH dependent growth factors IGF-I and IGF-II levels remained in the normal range. IGFBP-3 levels were significantly lower in untreated ISS/IUGR children (chapter 7). The bone parameters studied in chapter 7 (e.g. PICP, PIIINP, osteocalcin) were not significantly different between children on combined treatment and untreated controls. The decrease in height velocity in combination with equal levels of GH dependent growth factors suggest a gradually increasing insensitivity of tissues for growth factors; alternatively, when reaching a certain bone maturation or height percentile (e.g. target heights SDS) the response to GH may decrease as observed in GHD children during GH treatment.

We *conclude* that the addition of growth hormone to GnRHa treatment results in an improvement of predicted adult height compared to either GnRHa alone or controls.

Further research should elucidate whether a higher GH dose or a stepwise increment in GH dose would be beneficial.

IV PSYCHOLOGY AND MOTIVATION

In deciding whether or not to treat a particular child with early or precocious puberty auxological motives alone are not decisive for initiating GnRHa treatment. In case there is a discrepancy between pubertal and psychosocial development GnRHa therapy may be indicated to prevent progression of puberty and the occurrence of menarche. Children may feel embarrassed or ashamed in comparison with their peers and subject to teasing. GnRHa treatment may also be indicated in early pubertal development in mentally retarded children where the parents think it to be wise to postpone menarche for a couple of years.

In adopted children it was suggested that early puberty would increase psychological distress. Our *conclusion* is that in our group of adopted children, using standardised instruments, there is no evidence of psychological problems before, during or after treatment. When standardised instruments do not demonstrate increased problem behaviour while there is a clinical suspicion, one can doubt the seriousness of the psychological problems in early puberty (32). Alternatively, refined instruments should be designed to assess the specific emotional suffering that can be observed clinically.

We extensively described the background of the study regarding the motivation in adoptive parents and the remarkable demand for the evaluation of motivation specifically in this group. One of the concerns was that adverse motives of the parents could harm the child, or that conflicting interests between parents and their child in participation or refusing participation in a clinical trial would be present.

We *concluded* in our study that an adequate parental motivation was present. Therefore, adoptive parents should be approached just as other parents in clinical research.

Further psychological evaluation of the children with IUGR or ISS before and during treatment with GnRHa and GH described in chapter 7 is in progress. These data will provide a picture of the psychosocial aspects of ISS/IUGR and will be compared with normative data.

V ETHICAL ASPECTS

The use of growth promoting treatment, either by GH alone or by the combination of GnRHa and GH in adopted children does not deserve an isolated approach per se. However, specific considerations in these children deserve specific attention. First the role of catch-up growth providing the opportunity to reach a higher adult height than would probably have been obtained in the country of origin. Second, the relatively frequent occurrence of early onset of puberty, resulting in a compromised adult height. Third, early puberty is presumed to cause or increase psychological distress. However, we found no evidence for such a distress present in our patient group. Another reason not to isolate growth-promoting treatment in adopted children is our conclusion that adoptive parents have normal motivation for treatment, probably indistinguishable from parents of non-adopted children.

In this thesis we addressed the ethical issue from the suffering point of view, reasoning that when suffering is present in children growth promoting treatment should be considered (33). The studies described in this thesis do not specifically show suffering in adopted children with early puberty in the age group we studied. The increase in predicted adult height was accompanied by an increase in social acceptance. However, whether these increases relieve suffering cannot be concluded. Final height data should be awaited before a final conclusion can be drawn whether this intervention can relieve or prevent suffering. However, additional instruments are probably necessary to establish an ethical basis for treatment.

The use of growth hormone in short children has been discussed many times and especially the use in non GH-deficient children has received wide attention. The American Academy of Pediatrics, Committee on Drugs and Committee on Bioethics highlighted several

ethical issues: the agreement on the appropriate goals of GH treatment, the lack of an established risk-benefit ratio for GH therapy and how to define treatment "success" (34). In their conclusions the committee states that the benefits of GH therapy will remain somewhat elusive, as the individual child can escape from the stigma of being very short, but a group of short healthy children will always exist. The question remains whether the child with gain in adult height actually experiences a psychosocial benefit. In line with this, Haverkamp provided an analysis of the ethical dilemma pertaining to GH treatment in short children, emphasising the need for empirical evidence of suffering for recognition of the ethical need to improve this situation (35).

Until final height data are available from our studies on the combined treatment with GnRHa and GH we do not advocate standard GH treatment in these children. In the meantime, the current degree of suffering and the expected gain in FH has to be carefully balanced in the decision whether or not to treat adopted children with GnRHa alone or in combination with GH.

VI INDICATIONS AND RECOMMENDATIONS

AUXOLOGY

The age definition for the diagnosis of precocious puberty should be maintained at 8 years for girls and at 9 years for boys. Children with central precocious puberty should be treated with GnRHa as soon as possible, after it is shown that puberty has a progressive course. Puberty can be assumed to be progressive by a pubertal LH response > 10 IU/L in a GnRHa stimulation test combined with either an advanced BA or other signs of sex steroid action. In case of absence of signs of sex steroid action or a minimal presence of breast development GnRHa testing should be postponed. Clinical observation for 3-6 months is warranted in these cases.

In children with early, not precocious, puberty there is uncertainty about gain in FH after GnRHa treatment. This implicates that in this group no indication for GnRHa can be established just for the sake of height gain. Further considerations regard the predicted adult height and the psychological situation, including the suffering aspect. For prediction of adult height the use of the average BP tables is recommended.

In general, GnRHa treatment in girls > 10 yrs and boys > 11 yrs is not recommended. Specific conditions may warrant GnRHa treatment for example in children with mental retardation or in children with GHD. In GHD patients pubertal suppression can improve FH outcome when puberty starts at an early age, with delayed BA and at a marked short stature. In the *figure* we summarise our recommendations.

During GnRHa treatment in CPP gonadal suppression needs to be confirmed by the puberty suppression score and/or a GnRH stimulation test. No recommendation can be given on the moment of discontinuation of treatment. This should be determined by psychological and auxological parameters, for example the conformity with peers, BA and height velocity during treatment.

Before FH results of the combined treatment will become available, the addition of growth hormone to GnRHa treatment is not advocated as yet. Addition of growth hormone may be provided in a setting of a clinical study in children with a marked decrease in height prediction (before or during GnRHa treatment) or in height velocity during GnRHa treatment. We recommend that patients treated with GnRHa will be monitored until FH is reached and afterwards, not only to evaluate growth, but also to assess the development of gonadal and reproductive function.

PSYCHOLOGY

The psychological aspects and concerns of early development of puberty should be considered in the work-up of both adopted and non-adopted children (11). These include the emotional impact of the appearance of secondary sexual characteristics, the fear to have menarche, aspects of teasing and embarrassment and the expected future height. Specific questionnaires addressing these items should be developed in order to assess eventual suffering and the effects of interventions with GnRHa or other gonadal suppressants. Adoptive families were shown to be adequately motivated for treatment and should not be treated differently when they ask for any treatment or participation in a clinical trial.

ETHICS

For establishing indications for growth promoting treatment around puberty in adopted and non-adopted children, either by GH or by the combination of GH and GnRHa the suffering criterion needs careful consideration. Especially in non-GHD patients the use of GH deserves a specific attention in the context of cost and gain (33).

VII FURTHER RESEARCH

First of all, future research includes the analysis of final height data of the present studies. When a beneficial effect has been established in the final analysis, it is interesting to know whether adaptation of the GH regimen might further improve auxological outcome during GnRHa treatment. Maybe a short period with a GH dose higher than used in our studies (4 IU/m^2) might be beneficial. An alternative option is to treat patients with addition of growth hormone only when height velocity becomes impaired by GnRHa treatment.

The development of prediction models for the effect of treatment with GnRHa for CPP, as used in children with GHD, might improve the assessment of the patients suitable for treatment.

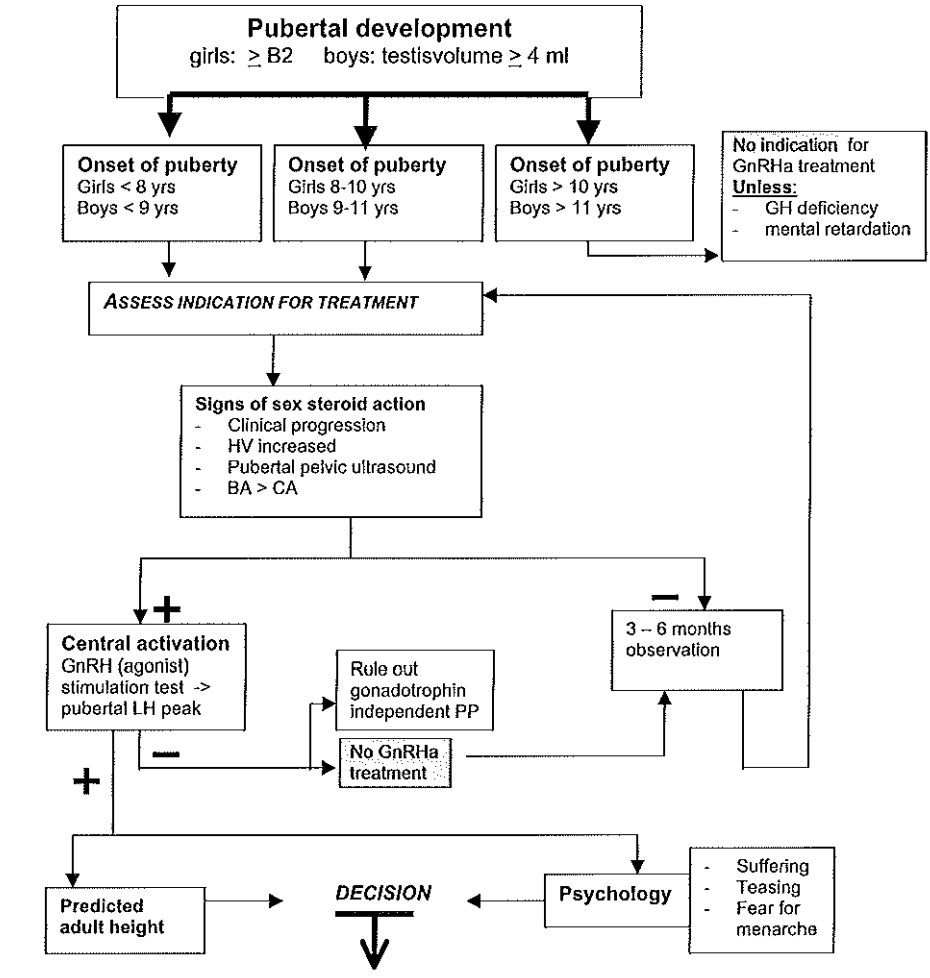
Prospective studies should elucidate the question whether the factors retrospectively found to improve FH outcome represent reliable predictive factors. The obvious ethical problems connected to these kind of studies need careful attention.

The issue of the timing of discontinuation of treatment for auxological reasons has not been resolved yet and would require a prospective randomised trial design.

Further research could also involve other patient groups in which gonadal suppression could be considered, e.g. IUGR children treated with growth hormone or children with renal insufficiency on GH treatment.

The use of the GnRHa stimulation test during treatment as a means to evaluate efficacy of pubertal suppression needs further validation.

Figure: Flow diagram of considerations regarding eventual initiation of treatment in children with pubertal development



Age group :	girls < 8 yrs boys < 9 yrs				girls 8-10 yrs boys 9-11 yrs			
PAH compromised [1]	+	+	-	-	+	+	-	-
Psychological reason	+	-	+	-	+	-	+	-
GnRHa treatment	+	+	+	- / #	+/▲	+/▲	+	-

[1] < - 2 SDS #: follow clinical course and PAH; ▲: treatment in study, consider combination GnRHa and GH when PAH << - 2 SDS, HV decreases or PAH deteriorates during GnRHa treatment

To alleviate the treatment for children, new modes of GnRHa administration that have been introduced in adult oncology, for example a 3 months depot of leuprolide acetate, need to be studied in order to establish its efficacy and safety.

Whether aromatase-inhibitors deserve a place in the treatment of precocious puberty remains to be investigated, as no central inhibition will occur during this treatment. On the other hand, aromatase inhibitors are shown to inhibit oestrogen action effectively.

The impact of either short stature or early puberty on quality of life during adolescence and adulthood and the effect of any endocrine intervention deserve further scientific attention.

References

1. Herman-Giddens ME, Slora EJ, Wasserman RC, et al. Secondary sexual characteristics and menses in young girls seen in office practice: A study from the pediatric research in office settings network. Pediatrics. 1997;99:505-512.
2. Mokdad AH, Serdula MK, Dietz WH, Bowman BA, Marks JS, Koplan JP. The spread of the obesity epidemic in the United States, 1991-1998. JAMA 1999;282(16):1519-22.
3. Fredriks AM, van Buuren S, Wit JM, Verloove-Vanhorick SP. Body index measurements in 1996-7 compared with 1980. Arch Dis Child 2000;82(2):107-12.
4. Carel JC, Roger M, Ispas S, et al. Final height after long-term treatment with Triptorelin slow release for central precocious puberty: Importance of statural growth after interruption of treatment. J Clin Endocrinol Metab 1999;84:1973-1978.
5. Heger S, Partsch CJ, Sippell WG. Long-term outcome after depot gonadotropin-releasing hormone agonist treatment of central precocious puberty: final height, body proportions, body composition, bone mineral density, and reproductive function. J Clin Endocrinol Metab 1999;84(12):4583-90.
6. Oostdijk W, Rikken B, Schreuder S, et al. Final height in central precocious puberty after long-term treatment with a slow-release GnRH agonist. Arch Dis Childh 1996;75:292-297.
7. Arrigo T, Cisternino M, Galluzzi F, et al. Analysis of the factors affecting auxological response to GnRH agonist treatment and final height outcome in girls with idiopathic central precocious puberty. Eur J Endocrinol 1999;141(2):140-4.
8. Thamdrup E. Precocious sexual development. Copenhagen: Munksgaard; 1961.
9. Mul D, Oostdijk W, Otten BJ, et al. Final height after Gonadotrophin Releasing Hormone agonist treatment for Central Precocious Puberty: The Dutch experience. J Ped Endocrinol Metab 2000;13(Suppl 1):765-772.
10. Galluzzi F, Salti R, Pasquini E, La Cauza C. Adult height comparison between boys and girls with precocious puberty after long-term gonadotrophin-releasing hormone analogue therapy. Acta Paediatr 1998;87:521-527.
11. Lebrethon MC, Bourguignon JP. Management of central isosexual precocity: diagnosis, treatment, outcome. Curr Opin Pediatr 2000;12(4):394-9.
12. Palmert MR, Malin HV, Boepple PA. Unsustained or slowly progressive puberty in young girls: initial presentation and long-term follow-up of 20 untreated patients. J Clin Endocrinol Metab 1999;84:415-423.
13. Fontoura M, Brauner R, Prevot C, Rappaport R. Precocious puberty in girls: early diagnosis of a slowly progressing variant. Arch Dis Child 1989;64(8):1170-6.
14. Bourguignon JP, Belgian study group for paediatric endocrinology. Variations in duration of pubertal growth: a mechanism compensating for differences in timing of puberty and minimizing their effects on final height. Acta Paediatr Scand 1988;347:16-24.
15. Municchi G, Rose SR, Pescovitz OH, Barnes KM, Cassorla FG, Cutler Jr GB. Effects of Deslorelin-induced pubertal delay on the growth of adolescents with short stature and normally timed puberty: preliminary results. J Clin Endocrinol Metab 1993;77:1334-1339.
16. Carel JC, Hay F, Coutant R, Rodrigue D, Chaussain JL. Gonadotropin-releasing hormone agonist treatment of girls with constitutional short stature and normal pubertal development. J Clin Endocrinol Metab 1996;81(9):3318-22.

17. Mericq MV, Eggers M, Avila A, Cutler GB, Jr., Cassorla F. Near final height in pubertal growth hormone (GH)-deficient patients treated with GH alone or in combination with luteinizing hormone-releasing hormone analog: results of a prospective, randomized trial. J Clin Endocrinol Metab 2000;85(2):569-73.
18. Ibanez L, Potau N, Zampolli M, et al. Use of Leuprolide acetate response patterns in the early diagnosis of pubertal disorders: comparison with the Gonadotropin Releasing Hormone test. J Clin Endocrinol Metab 1994;78:30-35.
19. Feuillan PP, Jones JV, Barnes K, Oerter Klein K, Cutler Jr GB. Reproductive axis after discontinuation of Gonadotropin- Releasing Hormone analog treatment of girls with precocious puberty: long term follow-up comparing girls with hypothalamic hamartoma to those with idiopathic precocious puberty. J Clin Endocrinol Metab 1999;84:44-49.
20. Saggese G, Pasquino AM, Bertelloni S, et al. Effect of combined treatment with gonadotropin releasing hormone analogue and growth hormone in patients with central precocious puberty who had subnormal growth velocity and impaired height prognosis. Acta Paediatr 1995;84:299-304.
21. Tatò L, Saggese G, Cavallo L, et al. Use of combined Gn-RH agonist and hGH therapy for better attaining the goals in precocious puberty treatment. Horm Res 1995;44 (suppl.3):49-54.
22. Partsch CJ, Oostdijk W, Sippell WG, et al. Combined treatment with a depot GnRH agonist and GH in girls with central precocious puberty and low height velocity: effects on growth and bone maturation. In: Oostdijk W, editor. Central precocious puberty and gonadotropin releasing hormone agonist treatment. Rotterdam: Thesis Erasmus University; 1996. p. 121-134.
23. Pasquino AM, Pucarelli I, Segni M, Matrunola M, Cerrone F. Adult height in girls with central precocious puberty treated with Gonadotropin-Releasing Hormone analogues and growth hormone. J Clin Endocrinol Metab 1999;84:449-452.
24. Job JC, Toublanc JE, Landier F. Growth of short normal children in puberty treated for 3 years with growth hormone alone or in association with Gonadotropin-releasing Hormone agonist. Horm Res 1994;41:177-184.
25. Saggese G, Cesaretti G, Barsanti S, Rossi A. Combination treatment with growth hormone and gonadotropin-releasing hormone analogs in short normal girls. J Pediatr 1995;126(3):468-73.
26. Tuvemo T, Gustafsson J, Proos LA. Growth hormone treatment during suppression of early puberty in adopted girls. Swedish Growth Hormone Advisory Group. Acta Paediatr 1999;88(9):928-32.
27. Pasquino AM, Pucarelli I, Roggini M, Segni M. Adult height in short normal girls treated with gonadotropin-releasing hormone analogs and growth hormone. J Clin Endocrinol Metab 2000;85(2):619-22.
28. Lanes R, Gunczler P. Final height after combined growth hormone and gonadotrophin-releasing hormone analogue therapy in short healthy children entering into normally timed puberty. Clin Endocrinol 1998;49:197-202.
29. Balducci R, Toscano V, Mangiantini A, et al. Adult height in short normal adolescent girls treated with Gonadotropin-Releasing Hormone analog and growth hormone. J Clin Endocrinol Metab 1995;80:3596-3600.
30. Sheehy A, Gasser T, Molinari L, Largo RH. Contribution of growth phases to adult size. Ann Hum Biol 2000;27(3):281-98.
31. Palmert MR, Mansfield MJ, Crowley WF, Jr., Crigler JF, Jr., Crawford JD, Boepple PA. Is obesity an outcome of gonadotropin-releasing hormone agonist administration? Analysis of growth and body composition in 110 patients with central precocious puberty. J Clin Endocrinol Metab 1999;84(12):4480-8.
32. Kaplowitz PB, Oberfield SE. Reexamination of the age limit for defining when puberty is precocious in girls in the United States: implications for evaluation and treatment. Drug and Therapeutics and Executive Committees of the Lawson Wilkins Pediatric Endocrine Society. Pediatrics 1999;104(4 Pt 1):936-41.
33. Kelnar CHJ. Cost-benefit analysis is the key. Arch Dis Childh 2000;83:176-178.
34. Considerations related to the use of recombinant human growth hormone in children. American Academy of Pediatrics Committee on Drugs and Committee on Bioethics. Pediatrics 1997;99(1):122-9.
35. Haverkamp F, Ranke MB. The ethical dilemma of growth hormone treatment of short stature: a scientific theoretical approach. Horm Res 1999;51(6):301-4.

Summary

This thesis contains the results of several studies on growth promoting treatment around puberty in adopted and non-adopted children. Indications for treatment as well as short-term and final results were subject of the studies and presented in this thesis.

After a general introduction (**chapter 1**), the thesis starts with 2 articles on the timing of puberty in normal children.

In the Netherlands the fourth nation-wide growth study from 1997 provided new data and new reference curves on the occurrence of puberty in healthy children (**chapter 2.1**). The P_{50} of the age of menarche has decreased slightly from 13.28 years in 1980 to 13.15 years in 1997. The age of onset of puberty in girls defined by the presence of Tanner breast stage 2 (B2) changed from 10.5 yrs in 1980 to 10.7 yrs in 1997. In boys an increase in the age of Tanner G-stage 2 was observed from 11.3 yrs in 1980 to 11.5 yrs in 1997. With regard to testicular volume, the 4 ml volume is reached in 50% of the boys at 11.5 years, compared to about 12 years in 1965. Based upon these data, there is no need to adapt the current definitions for precocious puberty in The Netherlands.

The influence of body mass index (BMI) on the age of menarche was analysed; we found that, besides chronological age, height and weight contributed to the timing of menarche. Further, we found a high agreement between the pubic hair stages and stages of pubertal development.

We *conclude* that the decrease in age of puberty has been stabilised between 1980 and 1997.

In **chapter 2.2** the literature on changes in the age of onset of puberty and of menarcheal age is reviewed, concluding that a positive secular trend is observed in most European countries towards earlier puberty. The role of environmental factors such as improvement of socio-economic circumstances and public health is discussed. The influence of estrogen-like containing food elements needs further research.

In **chapter 3** a new stimulation test with the GnRH-agonist leuprolide-acetate was evaluated for use in the diagnosis of central precocious puberty. This GnRH-agonist stimulation test was compared with the test used to date, a GnRH stimulation test.

We showed that the GnRH-agonist stimulation test was able to detect central activation of the hypothalamo-pituitary-gonadal (HPG) axis in more cases than the GnRH stimulation test. This offers the opportunity to eventually start treatment in children with central activation at an earlier age or Tanner stage. Besides this, the test is simpler and less invasive for the child as no intravenous access is needed for administration of GnRHa.

Once treated with a GnRH agonist it is important to monitor the gonadal suppression carefully. We studied pubertal suppression in children treated for 18 months with the GnRH agonist leuprolide acetate (**chapter 4**). The puberty suppression score (PSS) was developed as an instrument to monitor the efficacy of treatment. The parameters used in this score are: Tanner stage progression, changes in H-SDS, sex steroid levels and bone age progression. The PSS appeared to correlate with changes in height prediction during 18 months of treatment. Leuprolide acetate, in an usual dose of 3.75 mg per month, was shown to suppress puberty effectively.

Final height (FH) results of GnRHa treatment were studied in a large population of Dutch children. They were previously treated with GnRHa for Central Precocious Puberty (CPP) and had reached final height.

In **chapter 5.1** the results were presented with emphasis on *girls*. We showed that GnRH treatment results in a mean FH that is 7.4 (5.7) cm above the mean initial height prediction. Expressed in SD scores, a FH-SDS of –0.93 (1.17) was found, significantly lower than TH-SDS, but still in the target range. In this study the mean age at start of treatment was 7.7 (1.3) years and the mean treatment period was 3.4 (1.3) years.

Several factors were found that influenced the effect of treatment. Chronological age at start of treatment correlated positively with height gain, whereas bone age at start of treatment and BA advance at discontinuation of treatment were negatively associated with height gain.

In **chapter 5.2**, we combined FH data of *boys* from The Netherlands, Italy and France previously treated with GnRHa for CPP. We showed that mean FH was at mean 5.8 (8.4) cm above the initial prediction. Expressed in SD scores, FH was not significantly different from target height. In multiple regression analysis BA at start (negatively) and BA advance at start of treatment (positively) were the most significant contributors to height gain.

From our studies we conclude that in children with precocious puberty a FH in the normal range can be obtained with GnRHa treatment.

In adopted children early puberty is frequently seen. We performed a randomised clinical study in 30 adopted children with early puberty to evaluate the effect of treatment with GnRHa alone or in combination with growth hormone. The children were randomised for Gonadotrophin Releasing Hormone agonist (GnRHa) treatment alone or in combination with growth hormone (GH) for 3 years. This study is described in **chapter 6.**

The results concerning growth (**chapter 6.1**) showed that after 3 years of treatment girls treated with GnRHa alone gained 5.7 (SD 3.8) cm in predicted adult height. In the girls on combination treatment mean height gain was 10.1 (3.8) cm, which was a significant difference between the groups. In both groups a gradual decrease in height velocity was observed, despite the absence of a decrease in insulin-like growth factor I (IGF-I). We suggest that the decrease in HV is not mediated by changes in the GH axis.

The evaluation of psychosocial functioning of parents and children in this study (**chapter 6.2**) showed no increased levels of emotional or behavioural problems and normal self-perception in adopted children, and no increased levels of family stress as reported by the parents.

The motivation for treatment appeared to be adequate in the fast majority of cases. Only in one of 30 cases a family was not allowed to participate in the trial (see **chapter 6.3**). There seems to be no reason to a particular approach to adoptive parents in clinical research.

A randomised trial with an untreated control group described in **chapter 7** demonstrates the efficacy of combined treatment with GnRHa and GH in 36 children with idiopathic short stature and IUGR. The mean increase in FH was 10.4 (SEM 1.2) cm in girls and 6.5 (0.9) cm in boys. In the untreated controls height gain was 2.4 (1.7) cm and –3.9 (2.1) cm respectively ($p < 0.001$). Growth hormone treatment preserved a normal height velocity during GnRHa treatment. IGF-I and II levels did not differ significantly between the untreated controls and the children treated with GnRHa and GH.

When children with GH deficiency (GHD) enter into puberty at an early (bone) age or short stature, GnRHa treatment can be used to extend the prepubertal growth period. We studied

final height data in these patients in a retrospective analysis (**chapter 8**). We concluded that in GHD patients with the onset of puberty at small stature or early (bone) age GnRHa treatment results in a FH in the normal range (mean –1.31 (0.39) SDS).

Finally, questions regarding the ethical background of growth promoting treatment with GH and/or GnRHa in non-GHD children were addressed in **chapter 9**. The availability and use of GH in several patients without a classical GH deficiency renders questions with regard to medical treatment of healthy children, the effectiveness of GH as treatment to improve the quality of life or psycho-social well being of children and the costs of GH treatment for the society.

We introduce an approach in which the suffering of a particular child could be the reason to treat him or her with GH although no GHD is present. The need for valid instruments to estimate suffering before treatment and the effect of the intervention with GH or GnRHa on suffering is pointed out.

In **chapter 10** the findings of our studies are discussed and recommendations for clinical practice as well as for future research are provided.

Samenvatting voor de niet-medicus[1]

ONDERWERP VAN HET PROEFSCHRIFT

Dit proefschrift heeft als onderwerp de behandeling van (te) vroege puberteit bij kinderen. Zowel te *vroege* puberteit als *vroege* puberteit worden in dit proefschrift genoemd. *Te vroege* puberteit, ook wel *pubertas praecox* genoemd, kenmerkt zich bij meisjes door het beginnen van borstontwikkeling vóór het 8e jaar en bij jongens door een vergroting van de zaadballetjes vóór het 9e jaar. Van *vroege* puberteit wordt gesproken als deze lichamelijke puberteitskenmerken bij het meisje tussen de 8 en 10 jaar en bij de jongen tussen de 9 en 11 jaar beginnen op te treden.

NORMALE PUBERTEIT

Met het oog op de juiste hantering van de termen *vroege* en *te vroege* puberteit moet duidelijk zijn op welke leeftijd de puberteit gewoonlijk begint. Dit kan onderzocht worden met grote landelijke of regionale onderzoeken bij gezonde kinderen. Uit de gegevens die deze onderzoeken opleveren, kan afgeleid worden welke leeftijd normaal is voor bijvoorbeeld het begin van borstontwikkeling of de eerste ongesteldheid.

In Nederland is in 1997 een landelijk onderzoek uitgevoerd, dat vergeleken kan worden met soortgelijke onderzoeken in 1965 en 1980. Hierdoor is het mogelijk om in Nederland het verloop van de puberteitsontwikkeling over een periode van ruim dertig jaar te bestuderen. De resultaten van deze studie staan beschreven in **hoofdstuk 2.1**. De normale leeftijd waarop meisjes beginnen met de puberteit is ongeveer 10 jaar en 8 maanden, bij jongens is dit 11 jaar en 6 maanden. De eerste menstruatie bij meisjes treedt op de leeftijd van 13 jaar en 2 maanden op, dit is iets eerder dan in 1980 werd vastgesteld: toen lag de leeftijd op 13 jaar en 3 maanden.

Het totaalbeeld van 1965 tot 1997 laat een lichte vervroeging zien van de leeftijd waarop de puberteit begint tussen 1965 en 1980, en geen verdere vervroeging meer van 1980 tot 1997.

In dit onderzoek keken we ook naar de verhouding tussen het optreden van pubisbeharing ("schaamhaar") en de echte kenmerken van beginnende puberteit, namelijk borstontwikkeling bij meisjes en toename van de testisgrootte bij jongens. Het bleek dat voor het vaststellen van puberteit pubisbeharing geen goede maat is.

Tenslotte is met de cijfers uit de studie uit 1997 onderzocht wat de invloed van lengte en gewicht is op het moment van optreden van de eerste ongesteldheid. Samengevat vonden wij dat langere en zwaardere meisjes vroeger in de puberteit komen.

De gevonden resultaten uit deze studie maken duidelijk dat de bovengenoemde leeftijden in de definitie van te vroege puberteit niet aangepast hoeven te worden.

In Europa zijn de afgelopen tientallen jaren in diverse landen dergelijke onderzoeken uitgevoerd; een overzicht daarvan wordt gegeven in **hoofdstuk 2.2**. De trend in de meeste westerse landen is dat de puberteit steeds vroeger begint. De rol van de gezondheidszorg en welvaartssituatie spelen hierbij waarschijnlijk de belangrijkste rol. Ook allerlei stoffen in het milieu of in de voeding met een werking die lijkt op vrouwelijk hormoon zouden kunnen bijdragen aan de steeds vroeger optredende puberteitsontwikkeling. Dit moet echter verder worden onderzocht.

[1] Dit is geen letterlijke vertaling van de 'summary'

DIAGNOSE VAN (TE) VROEGE PUBERTEIT

Voorafgaand aan een eventuele behandeling moet de diagnose van (te) vroege puberteit wel duidelijk zijn. We willen er zeker van zijn dat er bij het kind sprake is van een normale, door de hersenen gestuurde puberteit (*centrale puberteit*). Alleen het moment van aanvang van de puberteit is om wat voor reden dan ook vervroegd.

Of er inderdaad sprake is van vervroegde puberteit werd tot op heden met een stimulatietest, de GnRH-test onderzocht, waarbij het kind gedurende een uur een infuus inkreeg en waarbij de GnRH-testvloeistof werd ingespoten.

Inmiddels is er een wat eenvoudiger test, de z.g. GnRHa-test waarbij in het onderhuidse vet een kleine hoeveelheid GnRHa-testvloeistof wordt ingespoten. Na drie uur kan dan door één bloedafname worden beoordeeld of er sprake is van centrale puberteit.

In **hoofdstuk 3** onderzochten wij of de GnRHa-test in vergelijking met de GnRH-test de centrale puberteit even goed of zelfs beter kan aantonen. Meer kinderen met een lichamelijke puberteitsontwikkeling hadden een positief testresultaat bij de GnRHa-test dan bij de GnRH-test. Dit maakt het mogelijk om met een eenvoudiger test sneller de diagnose te stellen en eerder behandeling in te stellen. Je hoeft dan minder frequent de GnRH-test te herhalen om zekerheid te krijgen, wat met deze test nogal eens nodig was.

BEHANDELING VAN (TE) VROEGE PUBERTEIT

Er kunnen twee redenen zijn om bij kinderen met een te vroege lichamelijke puberteitsontwikkeling de puberteit met medicijnen te remmen. In de eerste plaats leidt een erg vroege puberteit ertoe dat het kind vervroegd is uitgegroeid en daardoor niet de lengte haalt die het bij een normaal optredende puberteit had kunnen bereiken. Dit komt omdat de geslachtshormonen in het bloed ervoor zorgen dat de groeischijven in de botten gaan sluiten. In de tweede plaats kan (te) vroege puberteit aanleiding geven tot psychologische problemen. Te denken valt daarbij aan het gevoel van 'anderszijn', schaamte en angst voor ongesteldheid. Dit is met name een probleem als het kind qua ontwikkeling nog niet toe is aan de puberteit.

Sinds een aantal jaren kan de puberteit met behulp van medicijnen worden geremd. Dit gebeurt met de z.g. GnRH-agonisten ('puberteitsremmers') die via een injectie eenmaal in de maand worden toegediend. Dit zijn zogenaamde depot preparaten waarbij het actieve medicijn over een periode van 4 weken langzaam aan het bloed wordt afgegeven. Een kortwerkende vorm van dit medicijn wordt – zoals hiervoor beschreven – gebruikt in de GnRHa-stimulatie test.

Een van de GnRH-agonisten die voor de behandeling wordt gebruikt is het leuprolide acetaat. In het kader van dit proefschrift werd bij een groep kinderen onderzocht hoe effectief dit middel de puberteit kan onderdrukken (**hoofdstuk 4**). Daarbij werd een bepaalde score gehanteerd, de Puberty Suppression Score (PSS). Deze score kan poliklinisch gebruikt worden om iets zeggen over hoe effectief de puberteit wordt geremd. Daarnaast zegt de score ook iets over de winst die de behandeling op korte termijn kan geven wat betreft lengte.

In dit proefschrift staan in de **hoofdstukken 5.1** en **5.2** de *lange*-termijn resultaten genoemd van de behandeling met GnRH-agonisten bij te vroege puberteit. Gekeken is naar wat de uiteindelijke lengte van een kind geworden is vergeleken met de lengte die voorspeld was bij het begin van de behandeling. Wij zagen dat bij meisjes de gemiddelde eindlengte ruim 7 cm groter was dan de voorspelde lengte bij start. Dus, om een voorbeeld te geven, een meisje waarvan bij het begin van de behandeling voorspeld werd dat zij 146 cm lang zou worden, haalde uiteindelijk een lengte van 153 cm. Uit de gegevens van dit onderzoek

concludeerden wij verder dat voor een goed resultaat tijdig begonnen moet worden met de behandeling. Juist bij kinderen met een snel verlopende vorm van te vroege puberteit en snelle botijping kan GnRHa-behandeling winst in lengte opleveren.

Bij jongens komt te vroege puberteit veel minder vaak voor en daarom hebben we gegevens van een groep Nederlandse jongens uitgebreid met gegevens van Italiaanse en Franse jongens. Ook bij jongens met te vroege puberteit bleek de GnRHa-behandeling winst op te leveren: de eindlengte was 5.8 cm hoger dan de voorspelde eindlengte bij start van de behandeling.

BEHANDELING VAN VROEGE PUBERTEIT BIJ ADOPTIEKINDEREN

Tijdens de behandeling van te vroege puberteit met GnRH-agonisten wordt bij een aantal kinderen een te sterke afname van de groeisnelheid gezien. Omdat je behandelt om juist lengte te winnen is dat een ongewenst effect. De gedachte kwam daardoor op of de toevoeging van groeihormoon deze groei, en daarmee ook de prognoses voor de eindlengte, zou kunnen verbeteren. Deze vraagstelling werd onderzocht bij een groep geadopteerde kinderen. Bij kinderen die uit het buitenland geadopteerd zijn wordt relatief vaak een vroeg optredende puberteit gezien. De precieze oorzaak daarvan is niet bekend. De onderzoeksgroep bestond uit 30 kinderen die geadopteerd waren uit India, Sri Lanka, Zuid Korea of Colombia. Bij hen was de puberteit vroeg begonnen (meisjes voor de leeftijd van 10 en jongens voor de leeftijd van 11 jaar) en ze hadden een prognose voor een kleine eindlengte.

Deze studie bestaat uit drie onderdelen: een groeistudie (**hoofdstuk 6.1**), een psychologisch onderzoek (**hoofdstuk 6.2**) en een onderzoek naar de motivatie van ouders en kinderen voor deelname aan de studie en behandeling (**hoofdstuk 6.3**).

In de ***groeistudie*** zijn de kinderen in twee groepen verdeeld en met elkaar vergeleken: groep A werd alleen met puberteitremmer (GnRH-agonist, in dit geval triptoreline) behandeld, terwijl in groep B de behandeling bestond uit zowel GnRH-agonist als groeihormoon (GH). De studieperiode was 3 jaar en de kinderen werden om de drie maanden op de polikliniek gezien. Wij zagen na de 3 jaar behandeling een toename van de voorspelde eindlengte in groep A van *5,7* cm (van 149.8 naar 155.6 cm) en in groep B van *10,1* cm (namelijk van 146.8 naar 157.0 cm). Dit was een statistisch significant verschil, wat betekent dat het niet door het toeval bepaald is maar een echt behandeleffect is. We onderzochten ook groeifactoren in het bloed om te zien welke rol die spelen bij het verschil in effect en het bleek dat de GH toediening zorgde voor hogere waarden van de groeifactoren in het bloed. Dit geeft een verklaring voor de hogere groeisnelheid die in groep B werd gevonden.

In het ***psychologisch*** onderzoek ging het erom te bestuderen welke invloed het vroeg in de puberteit zijn en de behandeling hebben op het psychologisch functioneren van de kinderen. Tegelijk werd ook gekeken naar de invloed die het op de ouders heeft en ook naar de verwachtingen die ouders en kinderen van de behandeling hebben. De uitkomst liet zien dat de behandelde geadopteerde kinderen niet méér gedrags- of emotionele problemen hebben dan hun niet-behandelde geadopteerde leeftijdsgenoten, en dat er tijdens de behandeling geen toename van deze problemen wordt gezien. De kinderen in de studie hebben een normaal zelfbeeld en voelen zich geaccepteerd door leeftijdsgenoten, zowel voor als aan het eind van de behandelperiode. Aan het eind van deze periode werd vastgesteld dat het gevoel van acceptatie zelfs hoger was dan het gemiddelde van Nederlandse kinderen. Ouders blijken reëlere verwachtingen van behandeling te hebben dan kinderen. Het hebben van een kind met vroege puberteit veroorzaakt geen grote stress in de gezinssituatie.

Het ***motivatie***onderzoek werd verricht om te kijken hoe de motivatie van ouders en kinderen was om deel te nemen aan de behandeling en het onderzoek. De achterliggende reden voor dit onderzoek was dat door sommigen gevreesd werd dat adoptieouders té gemotiveerd zouden kunnen zijn en dat die motivatie soms tegen de wensen van het kind in zou gaan. Van overmotivatie is in onze evaluatie niets gebleken. Van de onderzochte ouder-/kind paren is er slechts één afgewezen. Wij concluderen dan ook dat adoptieouders niet anders benaderd dienen te worden in onderzoek of behandeling dan ouders van niet-geadopteerde kinderen.

ANDERE STUDIES MET COMBINATIE GNRHA EN GROEIHORMOON

De studies die beschreven staan in de hoofdstukken 7 en 8 onderzochten de vraagstelling naar het effect van de gecombineerde behandeling met GnRHa en GH op verschillende manieren. In **hoofdstuk 8** wordt een evaluatie beschreven van kinderen met een groeihormoontekort die behandeld worden met GH en ook met puberteitsremmer omdat ze vroeg of klein in de puberteit zijn gekomen. De resultaten van de uitgegroeide kinderen tonen dat het remmen van de puberteit gedurende een bepaalde periode de uiteindelijke lengte doet toenemen in vergelijking met kinderen bij wie de puberteit niet is geremd.

De studie bij kinderen met kleine gestalte (al dan niet na groeivertraging tijdens de zwangerschap) die in **hoofdstuk 7** staat is een bijzondere studie omdat behalve een groep behandelde kinderen ook een zogenaamde controlegroep werd bestudeerd die geen behandeling kreeg. Het vergelijken van deze twee groepen maakt het mogelijk het behandeleffect nauwkeurig in kaart te brengen.

De studie liet zien dat kinderen met de gecombineerde behandeling van GnRH-agonist en groeihormoon, in vergelijking met een onbehandelde groep een winst in voorspelde eindlengte liet zien van 10,4 cm bij jongens en 8,0 cm bij meisjes. Dit is een veelbelovend resultaat maar er moet afgewacht worden wat het resultaat is als de kinderen zijn uitgegroeid. Dat geldt ook voor de studie bij uit het buitenland geadopteerde kinderen die hierboven werd beschreven.

Tenslotte wordt in dit proefschrift (in **hoofdstuk 9**) vanuit de invalshoek van de ethiek gekeken naar het behandelen van kinderen met groeihormoon (GH), met name bij die kinderen die geen echt tekort hebben aan GH maar door een andere oorzaak (bijvoorbeeld gebruik van puberteitsremmer) slecht groeien.

Er kan op verschillende manieren naar gekeken worden. Allereerst vanuit het uitgangspunt dat als er geen echt tekort is aan groeihormoon, het kind niet ziek is en dus geen medicijn – groeihormoon- zou moeten krijgen. Een tweede manier om het te bekijken is dat als de ouders zo'n behandeling graag willen, waarom zou een dokter het dan niet toestaan? Een derde mogelijkheid, waarvoor wij in het artikel kiezen, is dat uitgegaan moet worden van het lijden van het kind, en dat bekeken moet worden of het geven van groeihormoon daar echt iets aan kan doen. De moeilijkheid daarbij is dat er weinig onderzoek is gedaan naar de mate van lijden van kleine kinderen en evenmin naar het 'helend' effect van groeihormoon. Wij benadrukken het belang van het ontwikkelen van goede instrumenten om 'lijden' in kaart te kunnen brengen en daarmee te evalueren wat het effect van groeihormoon behandeling is.

In **hoofdstuk 10** worden de resultaten van de verschillende studies besproken en worden er aanbevelingen gedaan voor de praktijk en voor verder onderzoek.

Abbreviations

α-MSH	Alpha-Melanocyte Stimulating Hormone
ACTH	Adrenocorticotropic Hormone
AF	Alkaline Phosphatase
BA	Bone Age
BMD	Bone Mineral Density
BMI	Body Mass Index
BP	Bayley & Pinneau
CA	Chronological Age
CART	Cocaine- and Amphetamine Regulated Transcript
CBCL	Child Behaviour Check List
CPP	Central Precocious Puberty
DEXA	Dual Energy X-ray Absorptiometry
DHEA-S	Dehydroepiandrostenedione Sulphate
E2	Estradiol
FH	Final Height
FSH	Follicle Stimulating Hormone
FT4	Free Thyroxine
GAD 67	Glutamic Acid Decarboxylase 67
GABA	Gamma Aminobutyric Acid
GH	Growth Hormone
GHD	Growth Hormone Deficiency
GnRH	Gonadotrophin Releasing Hormone
GnRHa	Gonadotrophin Releasing Hormone agonist
GP	Greulich & Pyle
HbA1c	Glycosyated Hemoglobin
HPG	Hypothalamo-Pituitary-Gonadal
HV	Height Velocity
IFMA	Immuno Fluorometric Assay
IGF	Insulin-like Growth Factor
IGF-BP	IGF-Binding Protein
IRMA	Immuno Radiometric Assay
ISS	Idiopathic Short Stature
IU	International Units
IUGR	Intrauterine Growth Retardation
LH	Luteinizing Hormone
LHRH	Luteinizing Hormone Releasing Hormone
LMS	statistical method for smoothing [smooth (L), mean (M), coeff. of variation (S)]
MMC	Meningo-myelocele
MPH	Midparental Height
MRI	Magnetic Resonance Imaging
NF	Neurofibromatosis
NMDA	N-methyl-D-aspartate
nmol	nanomol
NVOS	Nijmeegse Vragenlijst voor de Opvoedingssituatie
17-OHP	17 – Hydroxyprogesterone
PAH	Predicted Adult Height
PCO	Polycystic Ovary
PHV	Peak Height Velocity

PICP	Carboxyterminal Propeptide of type I Procollagen
PIIINP	Aminoterminal Propeptide of type III Procollagen
pmol	picomol
POMC	Pro-opiomelanocortin
PSS	Puberty Suppression Score
RIA	Radio Immuno Assay
SD	Standard Deviation
SDS	Standard Deviation Score
SE	Standard Error (SEM: standard error of the mean)
SH	Sitting Height
SPPC	Self-perception Profile for Children
T	Testosterone
TGF-α	Transforming growth factor–alpha
TH	Target Height
TSH	Thyroid Stimulating Hormone

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Curriculum Vitae

The author of this thesis was born on October 3rd 1968 in Barneveld. He attended secondary school in Amersfoort (gymnasium, gereformeerde scholengemeenschap) were he passed his exam in 1987.
From 1987 to 1994 he studied Medicine at the Free University Amsterdam. While studying in Amsterdam he was member and president of a christian students society. In 1994 he obtained his medical degree (cum laude).
He performed research in the Free University Hospital at the departments of paediatric haematology and oncology (head: Prof. Dr. A.J.P. Veerman) and paediatric endocrinology (head: Prof. Dr. H.A. Delemarre-van de Waal) as well as in child and adolescent psychiatry at the Academic Medical Centre Amsterdam (head: Prof. Dr. J.A.R. Sanders-Woudstra). Further, he was involved in research on anxiety disorders in the Valeriuskliniek (supervision: Dr. A.J.L.M. van Balkom).
For a short period he teached nurses-in-training. In 1995 he served in the army as a teacher in medical skills and knowledge. Thereafter, he wrote his first book for the education of nurses in general paediatrics.
From March 1st 1996 the research presented in this thesis was performed at the department of Paediatrics / Subdivision Endocrinology (head: Prof. Dr. S.L.S. Drop), of the Sophia Children's Hospital Rotterdam in close collaboration with Dr. W. Oostdijk, paediatric endocrinologist from the Department of Paediatrics, Leiden University Medical Centre. Besides the research concerning early puberty he coördinates a clinical trial on the effects of growth hormone treatment on growth and bone mineral density in children with rheumatic disease.
At Jan. 1st 2001 he will start his training in Paediatrics first as AGNIO at the Sophia Children's Hospital Rotterdam (Head: Prof. Dr. H.A. Büller).
Dick lives with his wife Annemiek in the northern part of the city of Rotterdam.

List of publications

▶ BOOKS / BOOK SECTIONS

D Mul, SMPF de Muinck Keizer-Schrama: LHRH behandeling van (te) vroege puberteit. *In*: Therapeutisch handelen in kindergeneeskunde en kinderverpleegkunde II, redactie: LWA van Suijlekom- Smit, Rotterdam 1996, ISBN 90-801804-6-7

D Mul: Kindergeneeskunde. Serie "Bouwstenen voor gezondheidszorgonderwijs". Bohn Stafleu Van Loghum 1998, ISBN 90-313-2661-x

MF Schutte, OP Bleker, G Huygebaert, D Mul, EMV Hertogs (red): Verloskunde, gynaecologie en kindergeneeskunde. Serie "Bouwstenen voor gezondheidszorgonderwijs". Bohn Stafleu Van Loghum 1998, ISBN 90-313-2580-5

D Mul: Aspecten van groei bij adoptiekinderen. *In*: syllabus PAOK symposium "Mondiale kindergeneeskunde" Red. LWA van Suijlekom-Smit en AJCM van der Velden, Rotterdam 1998. ISBN 90-76220-03-4

D Mul, SMPF de Muinck Keizer-Schrama: Te vroege en te late puberteit. *In*: syllabus PAOK symposium "Gynaecologie en andrologie, ook in de praktijk van de kinderarts" Red. LWA van Suijlekom-Smit en WA van der Zijden, Rotterdam 1999. ISBN 90-76220-05-0

D. Mul, IM van der Sluis, SMPF de Muinck Keizer-Schrama: Endocrinologische aspecten van corticosteroid geinduceerde osteoporose en groeiretardatie. *In*: Nascholingsweek Kindergeneeskunde Leiden 1999 (Red. JM Wit et al.); Boerhaave Commissie voor Postacademisch Onderwijs Geneeskunde Universiteit Leiden, 1999 ISBN 90-6767-417-6

▶ ARTICLES

Mul D, Schouten-van Meeteren AYN, Kamp GJ van, Tan KEWP: Tumormarkers in retinoblastoma. Med Pediatr Oncol 1999, 32(2): 146-147

Mul D, Pieters R, Veerman AJP: Te weinig en te grote bloedplaatjes op de kinderleeftijd: de "giant platelet'syndromen. NTvG 1999, 143 (38) 1918-1922

Mul D, de Muinck Keizer-Schrama SMPF, Oostdijk W, Drop SLS: Auxological and biochemical evaluation of pubertal suppression with GnRH-agonist leuprolide acetate in early and precocious puberty. Horm Res 1999, 51 (6): 270-276

Mul D, Oostdijk W, Otten BJ, Rouwé C, Jansen M, Delemarre-van de Waal HA, Waelkens JJJ, and Drop SLS: Final height after Gonadotrophin Releasing Hormone agonist treatment for central precocious puberty: the Dutch experience.
J Ped Endocrinol Metab 2000;13(suppl1): 765-772

Bolt LLE, Mul D,: Growth hormone in short children: beyond medicine?
Acta Paediatr (*accepted for publication*)

GA Kamp, D Mul, JJJ Waelkens, M Jansen, HA Delemarre-van de Waal, L Verhoeven-Wind, M Frölich, W Oostdijk and JM Wit: A randomized controlled trial of three years Growth Hormone (GH) and GnRH agonist treatment in children with Idiopathic Short Stature and Intrauterine Growth Retardation. J Clin Endocrinol Metab (*accepted for publication*)