CENTRAL PRECOCIOUS PUBERTY

AND

GONADOTROPIN RELEASING HORMONE AGONIST TREATMENT

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Central precocious puberty and gonadotropin releasing hormone agonist treatment

CENTRALE TE VROEGE PUBERTEIT
EN DE BEHANDELING MET EEN
GONADOTROPINEN RELEASING HORMOON AGONIST

PROEFSCHRIFT

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In gedachtenis aan mijn ouders

Dirk Hillebrand Oostdijk
en
Willy Hetty van der Hoeven

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Chapter 1

GENERAL INTRODUCTION

INTRODUCTION

In order to understand the processes occurring during precocious puberty, one needs to specify what is currently known about normal pubertal development.

1. NORMAL PUBERTY

Puberty can be defined as a maturational process of the hypothalamic-pituitary-gonadal axis, which results in the development of the gonads and, concomitantly, in the physical and physiological processes which constitute adulthood and the capacity to reproduce.
The regulation of puberty is a very complex process, involving the hormonal interaction of the cells in the hypothalamus, pituitary and gonads. A central role in this process is played by the Gonadotropin Releasing Hormone (GnRH or LHRH: Luteinizing Hormone Releasing Hormone) which is produced and secreted by the hypothalamus.

1.1 The control of the onset of puberty

The reproductive endocrine system is controlled by a hypothalamic GnRH pulse generator: a network of neurons including both the GnRH cells that discharge their products in a coordinated synchronous fashion into hypophyseal portal blood and the inputs that regulate this episodic pattern of release (Goodman *et al*, 1981). The GnRH neurons arise outside the brain in the olfactory placode. During the first trimester of gestation they migrate across the nasal septum into the septic-preoptic area and the hypothalamus (Schwanzel-Fukuda *et al*, 1992). The GnRH cells are then located in the arcuate nucleus, in the preoptic area and the medial basal hypothalamus. The axons of the GnRH cells terminate in the external layer of the anterior median eminence. GnRH, a decapeptide, first characterized by Schally *et al* (1971) and the group of Guillemin (Monahan *et al*, 1971), is secreted intermittently or in a pulsatile way from these nerve terminals into the first plexus of the hypothalamic-pituitary portal system, and carried to the anterior part of the pituitary, where the gonadotropic cells are located. GnRH stimulates these cells to release and synthesize luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Clayton *et al*, 1989). Additionally, it influences the number of GnRH receptors (Katt *et al*, 1985). The gonadotropic cells of the anterior pituitary respond not only to the signal provided by GnRH, but also to other central and anterior-pituitary-derived factors, such as neuropeptide Y (NPY), oxytocin, endothelin, γ-aminobutyric acid (GABA), galanine and pituitary adenylate cyclase-activating peptide, which potentially modulate the action of GnRH (Crowley *et al*, 1995).
In all species the functional organization of the hypothalamus is dependent on the parallel processes of neural migration and peptidergic differentiation (Wray *et al*, 1986; Foster *et al*, 1994; Ojeda & Urbanski, 1994; Plant, 1994). At the level

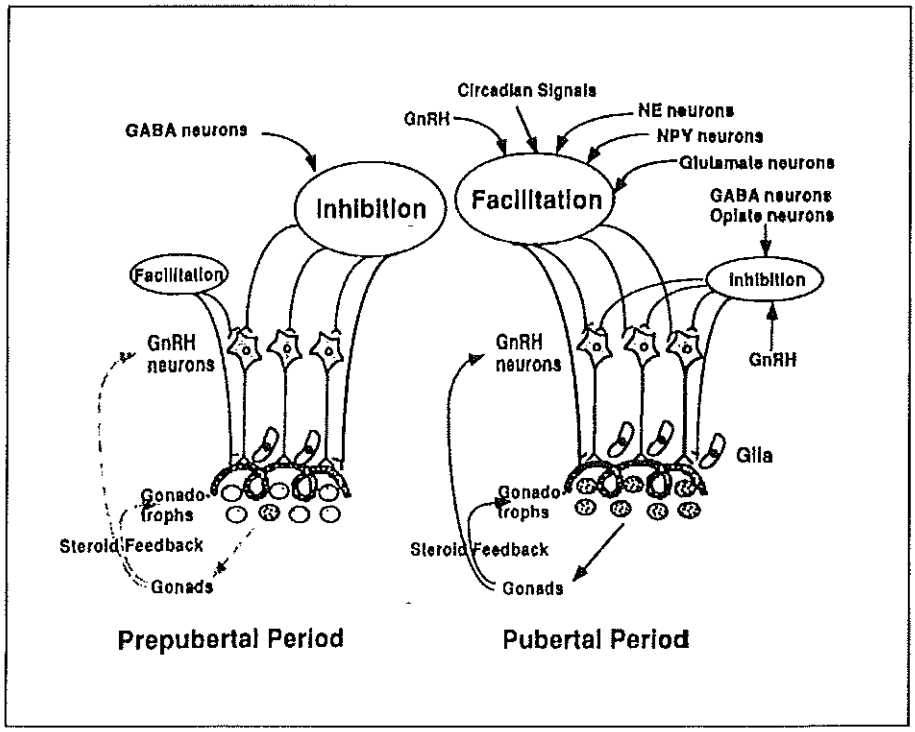

Figure 1. Schematic presentation of the mechanism controlling the onset of puberty. Although, before the onset of puberty, GnRH neurons are already mature enough to release GnRH, tonic inputs from GABA neurons inhibit GnRH release. Diminution of GABA inhibition and concurrent or subsequent increase in facilitatory inputs from glutamate, NE and NPY neurons to GnRH neurons results in the onset of puberty and a cascade of pubertal events. The importance of inhibitory and facilitatory inputs to the GnRH neuroterminal region is emphasized by heavy lines. From Terasawa (1995).

of the neuronal cytoskeleton there are processes which regulate axonal elongation, neuritic arborization and synaptogenesis (Ojeda *et al*, 1989). In a some recent reviews about synaptic connections involved in the regulation of gonadotropin release, the different populations of transmitter- and neuropeptide-containing systems and their interaction in various parts of the hypothalamus are described (Crowley *et al*, 1995; Leranth *et al*, 1995; Terasawa, 1995)(figure 1). GnRH neurons are under the stimulatory influence of at least three excitatory neurotransmitter systems. These include neuronal networks that utilize norepinephrine (NE), NPY and excitatory amino acids, especially glutamate, as neurotransmitters. The main inhibitory neurotransmitters affecting GnRH are opioid peptides and GABA. Both neuronal networks are connected synaptically to GnRH neurons (Ojeda *et al*, 1995). A network of regulatory neuritic projections surrounding the GnRH neurons permits each GnRH neuron to receive a large number of inputs and to be modulated by a

battery of aminoacids, peptides, steroids and other chemicals (Bourguignon *et al*, 1994a; Bourguignon, 1995); this results in a pattern of neuronal circuiting which can sustain synchronized pulsatile release of GnRH.
The synchronized, rhythmic, and intermittent activity of the network of GnRH neurons is inherent in the GnRH cell itself (Weiner *et al*, 1993; Bourguignon *et al*, 1994b). Structural changes or neuronal plasticity within the medial basal hypothalamus may underlie the pubertal acceleration in pulsatile GnRH release (Perera *et al*, 1995). Additionally, this functioning hypothalamic oscillator can be modulated by extrinsic cues such as photoperiod, nutritional balance, stress and lactation (Cameron *et al*, 1989; Ebling & Foster, 1989; Ferin, 1989; Foster, 1994; Reppert *et al*, 1989; Smith *et al*, 1989).
In primates this GnRH oscillator is clearly operative before birth, but is then suppressed during a prolonged juvenile phase (Knobil, 1980). Various hypotheses about the timing of puberty have been formulated in the past and were summarized by Schroor (Schroor, 1994; Schroor *et al*, 1995a): (1) changing sensitivity of the hypothalamus to sex steroids ('Gonadostat theory'); (2) continuing neuronal outgrowth which changes the stimulating input on GnRH neurons, overruling the 'intrinsic restraint'; (3) desychronization of the GnRH neurons resulting in asynchronous firing and therefore inadequate release of GnRH before puberty; (4) an increase in excitatory amino acids such as glutamate, which may play a key role in the stimulation of GnRH release; (5) nutritional factors, influencing a critical weight or fat to lean body mass ratio after which menarche occurs.
Despite continuing advances in neuroendocrine research, there are large gaps in our knowledge about both the physiological control system that dictates the timing of puberty and the neurobiological basis of the pubertal re-augmentation of pulsatile GnRH release. However, as Plant put it: 'We have a foot in the door that had hitherto closed the pathway leading to the resolution of the fascinating mystery of human puberty' (Plant, 1995).

1.2 Gonadotropins

1.2.1 Gonadotropins in the fetal and prepubertal period

The hypothalamic-pituitary-gonadal axis is already functional during fetal life: In the human fetus, LH and FSH are detectable in pituitary tissue at the fifth week of gestation (Siler-Khodr *et al*, 1974). Plasma gonadotropin levels gradually increase, until they reach maximum levels at 20 weeks of gestational age. Thereafter, the plasma levels decrease to very low levels at term, probably due to the development of the negative feedback mechanism by sex steroids and maternal steroids (Gluckman *et al*, 1983). After birth there is a transient rise in the levels of gonadotropins and sex steroids, whereafter these levels decrease to the very low prepubertal levels (Villa *et al*, 1991).
The question of whether or not gonadotropins are secreted during the prepubertal period is still under debate. Some investigators did not find

detectable serum LH levels during prepuberty even when they used a highly sensitive immunoradiometric assay (IRMA) (Wennink *et al*, 1990; Villa *et al*, 1991), whereas others who used an immunofluorometric assay (IMFA) reported gonadotropin secretion in prepubertal children with a distinct pulsatile pattern for LH (Wu *et al*, 1991; Apter *et al*, 1993; Goji, 1993). In one of these studies, in prepubertal girls an association of sleep and the appearance of LH and FSH pulses was demonstrated, suggesting that in prepubertal girls the GnRH pulse generator is functionally active and its expression is linked to the onset of sleep (Goji, 1993). Probably, the above-mentioned differences are based upon the use of a more sensitive assay.
The data on the bioactivity of LH during prepuberty are also contradictory: on the one hand bioactive LH was reported in the serum of prepubertal girls and boys (Torressani *et al*, 1983), on the other hand LH bioactivity was demonstrated in only a small number of prepubertal children, all of whom had detectable levels of LH in a radioimmunoassay (RIA) (Reiter *et al*, 1982; Reiter *et al*, 1987).

1.2.2 Gonadotropins during puberty

1.2.2.1 Basal values

During puberty, the first hormonal phenomenon is an increase of serum LH during the night, followed by a pulsatile secretion during the day, with a distinct sleep-wake pattern (Wennink *et al*, 1990; Wu *et al*, 1991; Apter *et al*, 1993). In boys, the nocturnal rise in LH levels is associated with nocturnal testosterone secretion, which occurs about 60-90 minutes after the first high-amplitude LH-pulse of the night (Boyar *et al*, 1974). In girls, the rise in serum estradiol occurs the next morning (Boyar *et al*, 1976; Goji, 1993). With the progression of puberty the LH secretion gradually increases in pulse amplitude (Wennink *et al*, 1988; Wu *et al*, 1991; Dunkel *et al*, 1992; Apter *et al*, 1993; Goji, 1993). A simultaneous increase in LH pulse frequency was reported by several investigators (Wennink *et al*, 1990; Wu *et al*, 1991; Dunkel *et al*, 1992), whereas others found that the pubertal increase in gonadotropin concentration was due exclusively to an increase in pulse amplitude (Oerter *et al*, 1990; Goji, 1993).
Bioactive LH also shows an increase during puberty. No differences have been demonstrated between the measurements of the immunoreactivity (I) and bioactivity (B); consequently the B/I ratio is normal in all stages of puberty (Schroor *et al*, 1995a). Measurement of the *in-vitro* bioactivity of LH and its use in LH B/I ratios does not give any additional information about the state of pubertal development (Schroor, 1994).

1.2.2.2 Stimulated values

In addition to the spontaneous levels of gonadotropins, the GnRH test also informs us about the maturation of the hypothalamic-pituitary-gonadal axis. During the standard GnRH test a 100-µg intravenous bolus of GnRH is administered and serial samples of serum LH and FSH are collected at 15-30 minutes intervals over a period of 1-2 hours. In the gonadotropin response,

assessed by RIA, there is a certain overlap between prepubertal and pubertal subjects (Roth *et al*, 1972; Pescovitz *et al*, 1988; Oerter *et al*, 1990). Several authors have reported the discriminatory ability of IRMA LH to distinguish prepubertal and pubertal subjects during a standard GnRH test in normal subjects (Partsch *et al*, 1990; Cavallo & Zhou, 1994). Lee (1994) reported that IFMA LH had a similar discriminatory ability. In all GnRH tests in prepubertal children, both boys and girls showed a higher FSH response compared to LH. As puberty progresses, most of the assays show a gradual increase in stimulated peak LH levels, in both boys and girls. GnRH-stimulated FSH levels do not change significantly. Some authors reported that the ratio of peak values of LH and FSH is greater during puberty than during prepuberty (Pescovitz *et al*, 1988; Oerter *et al*, 1990; Lee, 1994). Since the range of FSH at different pubertal stages is rather wide with much overlap between groups, one must be cautious about using the LH/FSH ratio as a criterion for pubertal status (Cavallo & Zhou, 1994).

1.3 Clinical presentation

Puberty presents with breast enlargement in girls and testicular enlargement in boys. In the Netherlands, in 80% of girls the first breast development occurs between the age of 9.1 and 12.3 years, and in 80% of the boys genital development starts between 9.3 and 13.4 years. In girls menarche occurs at a median age of 13.3 years (Roede & van Wieringen, 1985).
In addition to the sequence of sexual maturation in boys and girls there is a relationship between sexual characteristics and the growth spurt of puberty. Girls commence their growth spurt with the onset of breast development while boys commence their growth spurt relatively late in puberty, at a testicular volume of about 10 ml.

The pubertal growth spurt is mediated by several endocrine influences. Sex steroids exert a direct effect upon the growth cartilage and stimulate local production of insulin-like growth factor I (IGF-I) (Attie *et al*, 1990). Increased sex steroid production stimulates an increased amplitude of growth hormone (GH) secretion at puberty. The increased GH secretion in turn stimulates increased production of IGF-I. These direct and indirect effects of sex steroids cause the increased growth rate characteristic of the pubertal growth spurt.

1.4 Bone maturation

Both sex steroids, estradiol and testosterone, are present in each sex. Androgens are obligatory intermediates in estrogen biosynthesis and are produced by the adrenal glands in both sexes. Aromatase, the enzyme that catalyzes the conversion of androgen to estrogen, is present in males as well as females (Simpson *et al*, 1994). Thus the hormonal differences between females and males are relative, with females converting most and males converting least androgen to estrogen.

The two distinct sex hormones and their receptors are responsible for the sex-specific secondary sexual characteristics. Recent studies have demonstrated that estrogens are essential for normal skeletal maturation and mineralization (Conte *et al*, 1994; Smith *et al*, 1994; Morishima *et al*, 1995). Androgens, on the other hand, probably augment pubertal growth by influencing GH release and affecting local growth factor production at the growth plate (Frank, 1995).

1.5 Pelvic ultrasonography

During childhood there is a gradual increase in the volume of the ovaries: from 1 cm^3 at 2 years of age to 2 cm^3 at 12 years (Stanhope *et al*, 1985a). Others have reported that after the age of 6 years ovarian volume can increase to as much as 2.5 cm^3 (Ivarsson *et al*, 1983; Orsini *et al*, 1984). These authors also reported that the ovaries of prepubertal girls contained follicles with a diameter up to 9 mm. Cohen *et al* described greater cysts, with a diameter up to 17 mm (Cohen *et al*, 1992). Individual follicles grow and regress during childhood and there is a gradual growth of ovarian stroma. After the age of 8.5 years, the ovary develops a multicystic morphology, containing at least six follicles each with a diameter of 4 mm or greater (Orsini *et al*, 1984; Stanhope *et al*, 1985a). This pattern develops in all normal children prior to the development of dominant follicles and ovulatory menstrual cycles. In response to gonadotropin stimulation at puberty, the ovaries acquire a more ovoid shape, descend deeper into the pelvis and undergo rapid enlargement. In the post-pubertal period a volume of 1.8-5.7 cm^3 has been measured in girls in an age range of 13-20 years (Lippe & Sample, 1978; Hall, 1983; Ivarsson *et al*, 1983). In women aged 18-47 years, volumes of 2.2-13.8 cm^3 have been found (Munn *et al*, 1986). Multiple cysts representing stimulated and unstimulated follicles can be seen during each menstrual cycle. During the first 14 days of the menstrual cycle a number of immature follicles begin to mature in response to FSH. By day 5-7, the dominant follicle destined for eventual ovulation emerges, but its dominance cannot be detected by transabdominal sonography until day 8-12 of the menstrual cycle. By the time ovulation occurs, usually day 14 of a 28-day cycle, the diameter of the dominant follicle ranges between 1.7 and 2.9 cm (Siegel, 1991).

The normal neonatal uterus has a length of 2.3-4.6 cm (Nussbaum *et al*, 1986; Haber & Mayer, 1994). Immediately after the neonatal period, the uterus decreases in size because of the falling levels of maternal hormones. From infancy up till approximately 7 years of age, uterine size shows little changes; the length increases slightly to 2.5-4.0 cm and the uterus acquires a typical "teardrop" shape, with a corpus to cervix ratio of 1:2 (Ivarsson *et al*, 1983; Orsini *et al*, 1984). After 7 years of age, the uterus undergoes a gradual increase in size. A more dramatic increase in size is noted during puberty, and the corpus ultimately becomes larger than the cervix, producing the adult "pear-shaped" uterus. In the post-pubertal girl, uterine length ranges between 5 and 8 cm and maximum width varies between 1.6 and 3 cm (Lippe & Sample, 1978; Nussbaum *et al*, 1986). Griffin *et al* (1995a) reported a greater uterine length. An increase in uterus volume from 1.0 (0.3) cm^3 (mean [SD]) in the prepubertal stage to 24.6

(14.4) cm^3 at Tanner stage B4 to B5 was reported by Haber & Mayer (1994). Endometrial tissue can be observed in subjects less than 6 months of age, but otherwise it was not seen before 11.8 years of age or a Tanner stage B2 (Griffin *et al*, 1995a).

2. CENTRAL PRECOCIOUS PUBERTY

Puberty is defined as precocious when signs of sexual maturation appear at an age less than 2.5 standard deviation from the mean age, at which these signs normally occur, i.e. before 8 years of age in girls and before 9 years of age in boys.
Precocious puberty can be divided into two pathophysiological categories: 1) the normal pubertal process beginning at an earlier time, referred to as true, central, gonadotropin-dependent or gonadotropin releasing hormone (GnRH)-dependent precocious puberty, and 2) an abnormal pubertal process independent of the hypothalamic-pituitary-gonadal axis, known as pseudo-, peripheral, gonadotropin-independent, or GnRH-independent precocious puberty (Counts & Cutler, 1992).
Because this thesis is concerned chiefly with central precocious puberty (CPP), we will focus on the first category only.

2.1 History

Sexual precocity was first reported in ancient Greece and Rome. Craterus (quoted by Phlegon, 1822), who lived between 300 and 200 B.C., reported on a person who was "child, youth, old man, begot a child, and died, all within 7 years". Seneca (according to a French edition of 1942) mentioned people whose growth and development were complete long before the usual time, and Pliny (according to the edition by Rackham, 1942) described a boy from Salamis who was very tall, had a deep voice, and was sexually fully developed and died from paralysis at the age of 3 years. The first medical report is by Mandeslo (quoted by Lenz, 1913) who, in 1658, reported a girl whose menstruation had commenced at 3 years of age and who gave birth to a son when she was 6 years old. Albrecht von Haller (1766) (quoted by Ahlfeld, 1898) described the first collection of patients with sexual precocity: 13 boys and 5 girls. It was the first time that these children had been described from a more scientific point of view, and were not looked upon simply as strange monsters. Some decades later (1832), the relation between growth and sexual development was discussed by St. Hilaire (quoted by Thamdrup, 1961), who pointed out that growth ceases when sexual development is complete. In none of the reports is much written about the etiology. As quoted by Thamdrup, the first reports on etiology and classification were by Neurath (1902, 1928), Reuben & Manning (1923), Weinberger & Grant (1941) and Seckel (1946). They divided puberty into true and non-true precocious puberty, and true precocious puberty into cerebral (or

organic) and constitutional (or idiopathic), a classification used by most contemporary authors. Jolly (1955) andThamdrup (1961) were the first authors to describe large, personally examined series of patients (69 and 101, respectively) on the basis of this classification. In these reports, etiology and the natural course of the patients, with accelerated growth and reduced final height, were described extensively. In the early sixties, the treatment of true precocious puberty with medroyprogesteron acetate (Kupperman & Epstein, 1962) and cyproterone acetate (Helge *et al*, 1969) was introduced.

2.2 Incidence

The incidence of CPP in the general population is about 0.6% (Marshall & Tanner, 1969; Marshall &Tanner, 1970). In children with neurofibromatosis type 1, the incidence was reported to be 3% (Habiby *et al*, 1995) and in girls with neonatal encephalopathy the incidence was 4.3% (Robertson *et al*, 1990). Studies on hydrocephalic patients without meningomyelocele showed an incidence of 10-11% (De Luca *et al*, 1985; Kaiser *et al*, 1989). In meningomyelocele an incidence of 5-18% was reported (Meyer & Landau, 1984;Trollmann *et al*, 1996). CPP occurs most commonly in girls: a sex ratio of 23:1 (girls:boys) was described by Bridges *et al* (1994). A possible explanation as to why CPP is more common in girls is that the hypothalamic-pituitary-gonadal axis is activated by a lower dose of GnRH in girls than in boys (Stanhope *et al*, 1987). This explanation is confirmed by the existence of a much higher sex incidence of delayed puberty in boys (boys:girls = 20:1; Bridges *et al*, 1994).
The onset of CPP occurs in about 50% of the affected children before the age of 6 years (Kaplan & Grumbach, 1990).

2.3 Etiology

The hypothalamic GnRH pulse generator can be activated prematurely by central nervous system (CNS) tumors (especially hypothalamic hamartomas and astrocytomas, optic gliomas, malformations (arachnoid cyst, developmental abnormalities) and other neurogenic lesions of the CNS (infection, phakomatoses, head trauma, cranial irradiation), or nonidentifiable factors (the idiopathic form). The idiopathic form occurs in 80-90% of the girls with CPP (Kaplan & Grumbach, 1990; Counts & Cutler, 1992). In boys, the underlying cause of CPP is often an intracerebral disease, necessitating extensive investigation. A mass in the floor of the third ventricle of the posterior hypothalamus is most commonly associated with CPP. The hypothalamic hamartoma will be described in more detail in chapter 8 of this thesis.
True precocious puberty can also occur secondary to late treatment of congenital virilizing adrenal hyperplasia or other previous exposure to sex steroids (Grumbach *et al, 1978;* Pescovitz *et al*, 1984). Additionally, changes in the nutritional status can give rise to precocious puberty (Bourguignon *et al*, 1992; Proos *et al*, 1991).

2.4 Pathophysiology

As described before, the mechanism that triggers the onset of puberty is not fully understood. One can hypothesize that in idiopathic CPP the normal processes of puberty have been disturbed, in the majority of cases by unknown factors, resulting in puberty at an earlier age. Only a few data are known about possible influencing factors:

First of all, hamartomas have been described, functioning as ectopic GnRH pulse generators (Judge *et al*, 1977).

Secondly female sexual precocity has been induced experimentally in rodents and nonhuman primates by lesions of the hypothalamus (Donovan & van der Werff ten Bosch, 1956; Teresawa *et al*, 1984). This suggests that at least some of the human cases may be attributed to functional lesions that affect the neuroendocrine brain. Computed Tomography (CT) or magnetic resonance imaging (MRI) has revealed that a number of patients with idiopathic sexual precocity do in fact have discrete hypothalamic lesions (Cacciari *et al*, 1983; Robben *et al*, 1995).

In addition, again in animal studies, the glial-derived transforming growth factor alpha (TGFα), a member of the epidermal growth factor (EGF) family, and the EGF-receptor (EGFR) system have been shown to contribute to the neuroendocrine mechanism by which the lesions induce sexual precocity: Lesions of the anterior hypothalamus of juvenile rodents resulted in vaginal opening and ovulation within 7 days after injury (Junier *et al*, 1991). The initial response to injury was a change in cell structure, suggesting that the cells had reverted to a more immature functional condition (Jennes *et al*, 1985). This initial response to injury was followed by the appearance, around the lesion site, of reactive astrocytes showing increased TGFα and EGFR gene expression (Junier *et al*, 1991; Junier *et al*, 1993). Coinciding with these changes the cell structure of GnRH neurons returned to normal, and GnRH release began to increase. Since the TGFα/EGFR system plays a role in the onset of normal puberty in humans (Ma *et al*, 1992), a similar cell-cell signaling mechanism may operate in human females suffering from sexual precocity. In this case, a focal derangement in glial activity resulting in increased production of EGF-like peptides, and/or increased activation of EGFR-like receptors in the vicinity of neuroendocrine GnRH neurons, may stimulate GnRH secretion and lead to sexual precocity (Ojeda *et al*, 1995).

In the last place, information can be derived from secondary CPP, in which children who experience accelerated skeletal and somatic maturation, due to GnRH-independent precocious puberty, also experience an early onset of central puberty. Three hypothetical mechanisms can be considered (Cutler, 1995):

(a) A direct central action of sex steroids on the maturation of the neuroendocrine mechanisms that control puberty. Since the brain is a well-recognized target organ for sex steroids (Kalra, 1993; Kow *et al*, 1994) and the GnRH gene itself contains a classical estrogen response element (Radovick *et*

al, 1991), prolonged exposure to sex steroids of peripheral origin may directly accelerate the pubertal maturation of the neural system (Besecke *et al*, 1994; Mahachoklertwattana *et al*, 1994; Mitsushima *et al*, 1994; Rossmanith *et al*, 1994).
(b) Indirect action of sex steroids by means of increased GH/IGF-I secretion. Deficient GH secretion or action is associated with late onset of puberty (Merimee *et al*, 1968; Rimoin *et al*, 1968) and precocious puberty causes GH and IGF-I secretion to be elevated for chronological age (Pescovitz *et al*, 1985; Ross *et al*, 1987; Marin *et al*, 1994).
(c) An improved energy balance, specifically the greater ability of the larger body to meet glucose requirements and avoid nocturnal hypoglycemia. Several lines of evidence suggest that energy balance is monitored by the nervous system, that restricts puberty (or suppresses the reproductive axis after puberty has occurred) if the energy balance is unfavorable. For example, in ballet dancers and gymnasts, who restrict food intake to prevent weight gain at the same time as they exercise strenuously, the onset of puberty and menarche are delayed (Warren, 1990). A similar phenomenon occurs in anorexia nervosa. Conversely, nutritional rehabilitation of rats by enteral feeding after nutritional deprivation and nutritional rehabilitation of patients with disordered swallowing cause the rapid onset and progression of puberty (Feigelman *et al*, 1987; Bourguignon *et al*, 1992). Taken together, these observations suggest the existence of a central nervous system structure that monitors nutrient availability and suppresses the reproductive axis until a given threshold is reached.

2.5 Gonadotropins in CPP

2.5.1 Basal values

In contrast to basal gonadotropin values in normal puberty, we have only a few reports about basal values in CPP. In CPP, most of the data about gonadotropins are based upon stimulated values.
As in normal puberty, gonadotropins are secreted in a pulsatile manner. There is a circadian variation, with increased night-time LH pulse amplitude and increased mean LH levels in the first period of puberty (Stanhope *et al*, 1986; Stanhope et al, 1988; Schroor *et al*, 1995b). As in normal puberty, no changes have been observed in the ratio of LH bioactivity to LH immunoreactivity (Schroor, 1994).

2.5.2 Stimulated values

The GnRH stimulation test is now widely used in the evaluation of pubertal disorders. Using RIAs, in girls a diagnosis of GnRH-dependent precocious puberty can be made in almost all patients, with peak LH values above 12-15 IU/L (Partsch *et al*, 1989; Oerter *et al*, 1990; Cavallo & Zhou, 1994; Lee, 1994), and/or peak LH/FSH ratios higher than 0.66-1.0 (Pescovitz *et al*, 1988; Partsch *et al*, 1989; Oerter *et al*, 1990; Lee, 1994). Using IFMA, a peak LH higher than 6

IU/L and a peak LH/FSH ratio higher than 0.3 are reported to be consistent with CPP (Lee, 1994). Using IRMA, Cavallo *et al* (1995) found that an LH concentration of 15 IU/L and an LH/FSH ratio higher than 1.0 were appropriate cut-off points to distinguish CPP from non-CPP.
In boys, limited data are available. The criteria that have been reported, are a maximum LH peak over baseline of more than 26 IU/L, using RIA (Oerter *et al*, 1990), or more than 10 IU/L, using IFMA (Lee, 1994).
Recently, two studies have been published in which a simplified GnRH test was used with a single serum sample assayed for LH, 40 minutes after a subcutaneous GnRH bolus (Eckert *et al*, 1996), or 30-60 minutes after an intravenous bolus (Cavallo *et al*, 1995). These simplified GnRH tests were found to be just as effective as a standard intravenous GnRH stimulation test for the evaluation of CPP. In addition, in third-generation assays such as the immunochemiluminometric assay (ICMA), basal LH values seem to be strongly correlated with and highly predictive of elevated peak GnRH stimulated LH, and to be a useful screening tool for CPP (Neely *et al*, 1995a, b).
On the other hand, Pescovitz *et al* (1988) emphasized that in girls the transition to an LH-predominant response is often a relatively late development in the clinical progression of CPP and others have stated that an early phase of CPP can be characterized by demonstrable estradiol levels despite low LH secretion (Garibaldi *et al*, 1993b; Lee, 1994). Hence, a negative (prepubertal) response to GnRH stimulation cannot definitely rule out CPP with certainty, and a equivokal response should lead to reassessment after 3 to 6 months. In this context, the study of Ibanez *et al* (1994) is interesting. These authors suggest that an early phase of CPP is better distinguished by a GnRH agonist test involving a single s.c. injection (500 μg) of the GnRH agonist leuprolide acetate and LH sampling after 3 hours than by the standard GnRH test.

2.6 Clinical presentation

As in normal puberty, CPP presents with breast enlargement in girls and testicular enlargement in boys. It is accompanied by an increase in growth rate in both girls and boys, whereas in CPP in boys a growth acceleration usually occurs at a smaller testicular volume than would be expected in normal puberty. The increase in growth rate occurs in the same manner as in normally timed puberty (Ross *et al*, 1987), but it commences when insufficient childhood growth has been completed, resulting in a reduced final height. The reasons for the differences in adult heights in boys and girls with CPP are similar to those for normal men and women: women are shorter than men because the pubertal growth spurt starts earlier in the sequence of pubertal events when height is about 10 cm less than that at the take-off of the spurt in men. The spurts are of similar magnitude (25 cm and 28 cm in girls and boys, respectively [Tanner *et al*, 1966]). Therefore, this continuation of childhood growth for a further 2 years is the main cause of the final height differences.
Because puberty starts at an earlier age than in peers, psychosocial problems

like aggressive behavior and social withdrawal, occur regularly (Sonis *et al*, 1985; Schoevaart *et al*, 1990). Intellectual function and school achievement do not seem to be affected (Ehrhardt & Meyer-Bahlburg, 1994). In girls, menstruation at an early age presents practical difficulties.

2.7 Bone maturation and Height Prediction

In response to increased sex steroid secretion, an acceleration of the bone maturation occurs, which is disproportionate both in relation to chronological age and to height velocity. A possible explanation for this phenomenon is the maximal skeletal sensitivity to gonadal steroids during childhood and its decrease with advancing age (Boepple *et al*, 1988).
The rapid bone age (BA) advance results in diminishing potential for adult height: Children with CPP present with tall stature, but their height prediction is diminished at presentation and final height is reduced (Thamdrup, 1961; Sigurjonsdottir & Hayles, 1968; Bourguignon, 1988).
Only a few reports are available about height prediction in children with CPP. Zachman *et al* (1978) reported that height prediction with the Bayley-Pinneau method (Bayley & Pinneau, 1952), using BA assessed by the method of Greulich and Pyle (1959) was the best method for children with CPP and that other methods grossly overestimate final height. A recent report has confirmed these conclusions by demonstrating a good relationship between between final height and the initial height prediction (Bar *et al*, 1995). On the other hand, the last author and Kirkland *et al* (1981) demonstrated that this method was inaccurate either, especially when bone age was extremely advanced.
The reason why methods other than the Bayley-Pinneau method grossly overestimate final height is that in normally growing children BA has relatively little impact on the estimation of final height until puberty is reached. Thus, chronological age (CA)-based models (e.g.Tanner-WhitehouseTW-II equations [Tanner *et al*, 1983]) assign relatively little weight to BA in young children. Instead, they rely largely upon the actual measured height, and upon the assumption that tall children will be tall adults in the future. In children with an advanced BA and consequently far less residual growth potential, this assumption does not hold (Boepple *et al*, 1993). The failure of CA-based methods in CPP has resulted in reliance upon the height prediction tables of Bayley-Pinneau, which are based more on BA than on CA. Most of the authors reporting on height prediction in CPP have used this method. Other investigators have adopted an alternative approach: the calculation of a standard deviation score, or z-score, for height based upon the BA of the child, since this calculation is possible at any age , also at BAs < 7 years (Werder *et al*, 1974; Sabbe-Claus *et al*, 1983).

2.8 Pelvic ultrasonography

The sequence of sexual maturation in CPP is similar to that in normal puberty. The ovaries are enlarged with the typical multifollicular pattern, that is seen after the onset of puberty. It has been suggested that the number of follicles with diameters over 4 mm, exceeds that of normal puberty (Stanhope *et al*, 1985b).
Gonadal production of sex steroids causes uterine enlargement and endometrial proliferation, and the uterus adopts the "pearshaped" configuration. Uterine length and ovarian volumes are increased compared to values in prepubertal girls, although there is overlap (Schoenfeld *et al*, 1990; Haber *et al*, 1995; Griffin *et al*, 1995b). The last-mentioned authors reported that a uterine length above the 97th centile for age, i.e. at least more than 4.0 cm, and the presence of a midline endometrial echo are the best ultrasound parameters for discriminating precocious puberty from the prepubertal situation and other forms of sexual precocity such as premature thelarche and "thelarche variant" (both described in section 2.10), and adrenarche (Griffin *et al*, 1995b).
Gonadotropin suppression by GnRH agonists causes reduction in uterine length to prepubertal size and, after prolonged treatment, reversion to the prepubertal configuration. In addition, it causes reduction in ovarian volume and number and size of ovarian follicles (Schoenfeld *et al*, 1990; Adams *et al*, 1993; Ambrosino *et al*, 1994).

2.9 Diagnosis

The diagnosis CPP is based first of all on the early development of secondary sexual characteristics, increased height velocity and accelerated bone maturation (BA one year above chronological age or above 2 SD). A GnRH test will reveal responses, as has been described in section 2.5.2: 'Stimulated gonadotropins in CPP'.
Estimation of sex steroids, especially in early puberty, will often provide inadequate information about the presence of puberty. For estradiol, the following reasons have been reported: (1) the use of an assay which is inadequate to measure low concentrations of estrogens; (2) basal estradiol levels may be the same in girls with CPP as in normal girls (Bidlingmaier *et al*, 1977); (3) intra-individual variations in plasma estradiol levels are common in girls with CPP, just as in normal puberty (Brauner *et al*, 1991). The highest estrogen concentrations can be measured at the end of the morning. In boys, elevated testosterone levels can be missed, if sampling is not performed in the early morning or at night.
Transabdominal ultrasound examination of the pelvis, as described in the 'Ultrasound section' (section 2.8), is performed in girls with sexual precocity to establish the size of the uterus and the aspect of the ovaries and to exclude adrenarche, large solitary ovarian cysts (as can be observed in girls with McCune Albright syndrome [Foster *et al*, 1986]), ovarian tumors and, in combination

with other parameters, premature thelarche (section 2.10). In young girls (< 6 years of age) and all boys, MRI of the brain always has to be performed, to rule out the possibility of a cerebral organic cause.

2.10 Differentiation between CPP and premature thelarche

Premature thelarche is characterized by breast development without any other signs of puberty. Growth velocity is normal, bone age is not advanced and final height is not affected. The mechanism underlying premature thelarche is unknown but has been hypothesized to result from a number of factors: increased breast sensitivity to estrogen, transient estrogen secretion by follicular cysts of the ovary, increased estrogen production from adrenal precursors, increased dietary estrogen, or transient partial activation of the hypothalamic-pituitary-ovarian axis with excessive FSH secretion (Pescovitz *et al*, 1988). In these girls, Stanhope *et al* (1986) described a characteristic pattern of FSH secretion unlike that seen in normal puberty where LH predominates. A similar stimulated pattern of predominant FSH-responses to GnRH has been reported by Pescovitz *et al* (1988) in girls with isolated breast development, without bone age advancement and/or growth acceleration. However, when bone age advancement and/or growth acceleration were present, the authors observed various patterns of LH and FSH responses to LHRH, varying from FSH-predominant to LH-predominant responses. Stanhope & Brook (1990) also described a less predominant FSH-pattern in girls with premature thelarche and bone age advancement and/or growth acceleration. This form of premature thelarche has been called the "Thelarche variant" (Stanhope & Brook, 1990) or the "Slowly progressive variant of precocious puberty in girls" (Fontoura *et al*, 1989) or "Exaggerated thelarche" (Garibaldi *et al*, 1993a).

In premature thelarche, the ovaries when examined with ultrasound do not show the typical multifollicular pattern associated with puberty. Typically, fewer than five follicles are seen, but their size frequently exceeds 10 mm. There is a relationship between breast size and number and size of follicles. Breast changes are frequently associated with increased follicular development, and a reduction in breast size follows reduced ovarian activity (Stanhope *et al*, 1986).

Pelvic ultrasound data about how to differentiate between premature thelarche and CPP are contradictory. Some authors state that differentiation between these abnormalities is possible on the basis of greater uterine lengths and ovarian volumes in CPP compared to girls with premature thelarche (King *et al*, 1993; Haber *et al*, 1995). Others point to an overlap in ovarian volumes, but to a good differentiation between these abnormalities on the basis of uterine length (Adams *et al*, 1993; Griffin *et al*, 1995b). In contrast, Ibanez *et al* (1994) report no increased uterine length in about 50% of the girls with a pubertal LH response after GnRH testing and clinically progressive puberty. Therefore, ultrasound findings can be helpful in differentiating CPP and premature thelarche or the "thelarche variant", but they have to be compared with other parameters, such as serum gonadotropin concentrations.

The above-mentioned pattern contributes to the hypothesis that premature thelarche and central precocious puberty may represent different positions along a continuum of hypothalamic GnRH neuron activation (Pescovitz *et al*, 1988).

2.11 Treatment

2.11.1 The goals of treatment

It is often difficult to decide whether to treat or not to treat a child with sexual precocity. Often the decision hinges upon the somewhat arbitrary distinction between the timing of early (but still normal) and precocious sexual maturation. However, once the decision to treat has been made, the goals of therapy of CPP can be summarized as follows: The main goal is to minimize the differences between the patient and age-matched peers. More specifically this means:

(1) halting or regressing secondary sexual development;
(2) preventing menses in girls;
(3) restoring age-appropriate behavior;
(4) restoring the pattern of growth so that it is more in keeping with a child's genetic potential.

2.11.2 Progestational agents

In the past, children with CPP have been treated with progestational or anti-androgenic drugs such as medroxyprogesterone acetate or cyproterone acetate, which suppress gonadotropins and sex steroids. Both drugs were rather effective in halting the advancement of secondary sexual characteristics and in preventing menstruation. However, they did not effectively halt the advancement of skeletal maturation, so final height was not improved (Werder *et al*, 1974; Kauli *et al*, 1984; Styne & Grumbach, 1986; Sorgo *et al*, 1987). Besides, long-term administration of these drugs led to some undesirable side effects like weight gain and fatigue, caused by adrenal suppression, probably due to the fact that the drugs are structurally rather similar to glucocorticoids. Severe side effects secondary to adrenal suppression have been reported too (Savage & Swift, 1981).

2.11.3 GnRH agonists

2.11.3.1 Pituitary desensitization

Under normal physiological conditions GnRH is secreted in a pulsatile fashion by the hypothalamus. GnRH binds to GnRH receptors of the gonadotropic cells of the anterior pituitary gland, giving rise to a cascade of stimulatory events which results in a release of the gonadotropins LH and FSH. Continuous stimulation of the pituitary by GnRH leads to a drop in the gonadotropin levels, after an initial rise (Belchetz *et al*, 1978; Schuiling *et al*, 1976). The pituitary becomes refractory if it is persistently stimulated by GnRH. This process is called desensitization, and this property is used to suppress gonadotropin levels

in the treatment of CPP with GnRH analogs, but also in metastatic prostate and breast cancer, and gynaecological disorders (Santen and Bourguignon, 1987; Conn & Crowley, 1991). The process of pituitary desensitization can be explained partly by a reduction in the concentration of GnRH receptors in the pituitary by a process of down-regulation, because the reduction in the number of receptors is transient (Conn, 1986; Jinnah & Conn, 1986). Postreceptor effects seem to be more important mechanisms in suppressing gonadotropin release during constant infusion of GnRH or during GnRH agonist therapy. The continuous infusion of GnRH terminates some specific aspects of the activation pathway by negative feedback mechanisms (Stojilkovic *et al*, 1994).

2.11.3.2 Structure-related characteristics

Because native GnRH has a half-life only a few minutes in plasma, the use of this decapeptide is not very practical. For this reason long acting and potent analogs have been synthesized. Several structure-activity relationships were identified during attempts to synthesize these analogs. The major site of metabolic degradation of native GnRH is at the sixth amino acid position. The insertion of dextro- or levoamino acids at this position enhances biological activity. The substitution of hydrophobic amino acid molecules at the sixth position enhances biopotency still further. In the agonists that are generally used in the treatment of CPP, these substitutions are: D-His(ImBzl) (histrelin); D-Leu (leuprorelin); D-Nal(2) (nafarelin); D-Ser(tBu) (buserelin and goserelin); D-Trp (deslorelin and triptorelin). Substitution of the 10th amino acid of native GnRH by proethylamide increases the affinity of binding for the receptor and biopotency of the GnRH analog. This substitution has been used in all the above mentioned agonists, with the exception of triptorelin. This modification also retards the rate at which the GnRH analog dissociates from its receptor. Structural modifications at the 10th position also result in delayed plasma clearance of the GnRH analogs when compared to native GnRH (Karten & Rivier; 1986).

Another important factor determining the efficacy of GnRH agonists is their bioavailability. The rate and the degree of absorption of the GnRH agonists are critical, since sustained GnRH receptor occupancy is required for the process of receptor and postreceptor desensitization. The subcutaneous route of administration allows > 94% absorption and sustained plasma levels. In contrast, only 2-5% of the administered dose is absorbed via the intranasal route. Sustained plasma levels may be achieved over a 1-2 month period by the use of by very long-acting biodegradable preparations such as triptorelin (Happ *et al*, 1987; Broekmans *et al*, 1993), leuprorelin depot (Parker *et al*, 1991) and goserelin (Ahmed *et al*, 1986). In this thesis treatment with triptorelin is described. Triptorelin is slowly released from microcapsules into the blood to give a plasma concentration of 200-500 pg/ml. This therapeutic concentration is maintained for 4 weeks and following a repeat injection, the steady state concentration remains approximately 400 pg/ml (Happ *et al*, 1987). Injection of triptorelin raises the plasma concentrations to maximum within 4 hours. Desensitization

of the pituitary starts within 24 hours and pituitary responsiveness is completely absent in the second week (Broekmans *et al*, 1993).
All the above-mentioned GnRH agonists are highly potent, although potencies may vary, depending on the route of administration and delivery systems. When choosing a GnRH agonist dosage one should bear in mind that no complications or adverse effects have ever been reported in connection with an overdose of these drugs. However, inadequate pituitary/gonadal suppression may prevent full therapeutic efficacy of these compounds.

2.11.3.3 Treatment in CPP
Treatment with GnRH agonists can influence the outcome of the goals, mentioned in section 2.11.1, positively: CPP represents one of the earliest and most successful therapeutic applications of GnRH agonists. It was introduced in 1981 (Crowley *et al*, 1981). Well documented therapeutic effects are: regression or arrest of physical signs of puberty, behavioral improvements in affected children, and restraint of the rapid rate of growth and skeletal maturation (Crowley *et al*, 1981; Mansfield *et al*, 1983; Luder *et al*, 1984; Styne *et al*, 1985; Brauner *et al*, 1985; Pescovitz *et al*, 1986; Roger *et al*, 1986; Bourguignon *et al*, 1987; Drop *et al*, 1987; Oostdijk *et al*, 1990; Neely *et al*, 1992). Auxological data have suggested that improved height potential can be attained after long-term therapy with daily subcutaneously administered GnRH-agonists (Manasco *et al*, 1989; Kreiter *et al*, 1990; Oerter *et al*, 1991; Paul *et al*, 1995) and depot preparations (Kauli *et al*, 1990; Brauner *et al*, 1994; Oostdijk *et al*, 1995).
The introduction of GnRH agonists is generally considered to be a major improvement in the treatment of children with CPP.

3. STRUCTURE OF THE THESIS

This thesis describes the short-term and long-term results of treating central precocious puberty with a slow-release GnRH agonist, triptorelin (D-Trp^6-LHRH, Decapeptyl-CR 3.75). In addition, we present provisional data concerning an attempt to improve final height in very short children with CPP. Finally, we describe the effect of early onset of puberty on the growth of foreign-born adopted children.
In *chapter 2* the short-term results during the first 18 months of treatment with triptorelin are evaluated. The suppressive action on clinical features, gonadotropins and sex steroids, and auxological parameters such as height velocity and bone maturation are shown. In *chapter 3* the influence of the slow-release long-acting GnRH agonist triptorelin on the above-mentioned parameters is compared with the short-acting GnRH agonist buserelin. *Chapters 4 and 5* present the long-term (4 and 5 years) results of triptorelin treatment on growth velocity, bone maturation and height prediction and some preliminary results about the addition of GH therapy to GnRH agonist treatment (*chapter 4*). In addition, *chapter 5* also describes the effects on growth velocity, bone

maturation and height prediction during the first 18 months after the discontinuation of treatment. The hormonal changes during triptorelin treatment and after discontinuation are described in *chapter 6*. In *chapter 7*, the auxological effects are described in more detail until the point of final height was reached. In addition, this chapter shows the effects on the menstrual cycle and the pelvic ultrasound data.
Chapter 8 describes the examination of the brain by magnetic resonance imaging and the abnormalities which were demonstrated in 30 children with CPP.
In *chapter 9* the results are described of the combined treatment with triptorelin and GH in very short CPP children. *Chapter 10* concerns a study in adopted children, in which puberty starts rather early and final height seems to be influenced negatively by this event, analogous to the situation in CPP.
Chapter 11 discusses the significance of the data presented. Conclusions are drawn, recommendations are made, and suggestions are given for future research.
Chapter 12 presents a summary of the thesis in English and Dutch.

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Chapter 2

TREATMENT OF CHILDREN WITH CENTRAL PRECOCIOUS PUBERTY BY A SLOW-RELEASE GNRH AGONIST

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ABSTRACT

A total of 82 patients, (74 girls, 8 boys) are presently participating in an international multicentre trial for treatment of central precocious puberty (CPP) with a slow release Gonadotropin-releasing hormone (GnRH) agonist depot preparation: Decapeptyl-Depot (DD). Of these patients, 53 (3 boys) were previously untreated (group 1) and 29 (5 boys) have been treated before with either a short-acting GnRH analogue (Buserelin) or cyproterone acetate (group 2). Fifty-one patients (44 girls, 7 boys) were treated with DD for 12 months or more. Basal plasma luteinizing hormone (LH) levels (mIU/ml) decreased in both groups after 1 year of therapy.The LH response to intravenous GnRH was reduced in both groups. Basal plasma follicle stimulating hormone (FSH) levels (mIU/ml) decreased in both groups. Stimulated FSH levels were reduced in both groups after one year of DD treatment. Plasma oestradiol levels in the girls decreased to prepubertal levels in both groups. In all patients the clinical signs of precocious gonadarche such as breast development and menstruations (girls) and an increased testis volume (boys), did not further progress and sometimes regressed in several patients. Growth velocity decreased in the girls of group 1 from 9.0 ± 0.72 cm/yr (mean ± SEM) in the last half-year before treatment to 6.3 ± 0.50 in the first half-year ($p < 0.01$) and to 4.5 ± 0.23 cm/yr in the second half-year of treatment ($p < 0.01$). After 12 months a stabilization of growth velocity was observed. The ΔBA/ΔCA ratio decreased during treatment in this group of girls, resulting in an improvement of adult height prediction from 161.9 ± 3.3 cm (mean ± SEM) at the start to 164.1 ± 3.5 cm after 18 months of therapy ($p < 0.05$). No change in height prediction was observed in group 2. At present we consider one i.m. injection of DD every 4 weeks as the treatment of choice in children with CPP.

INTRODUCTION

Until the early 1980s central precocious puberty (CPP) was treated exclusively with synthetic progestational agents such as medroxyprogesterone acetate and cyproterone acetate [7, 17, 20, 23]. This treatment was neither fully effective in suppressing gonadotropin secretion [8, 11] nor in stopping the rapid bone maturation and thus in improving final height [19]. An alternative therapeutic approach became possible with the synthesis of the potent agonist analogues of Gonadotropin-releasing hormone (GnRH). These agonists lead to pituitary desensitization and to total refractoriness to further stimulation by natural GnRH. Several studies using various GnRH agonists have been published recently, indicating that gonadal activity in children with CPP remains fully suppressed for prolonged periods of time with different results in advancement of bone age and adult height prediction [2, 4, 5, 9, 12, 14, 16, 21, 22]. In most of these studies children were treated by daily subcutaneous injections of the GnRH analogue. We here report the results of a large international multicenter trial, performed over a 12-18 month period, with a slow-release preparation of GnRH agonist Decapeptyl Depot (DD). In Decapeptyl the glycine in position 6 is substituted by D-tryptophane. Otherwise the molecule is identical to the genuine GnRH. It is placed in biologically degradable microspheres of lactide-glycolide copolymer similar to synthetic catgut material whereby efficient agonist plasma levels are maintained for more than 4 weeks after intramuscular injection [15]. As is well known, the clinical signs of puberty are partly due to gonadal steroids. Circulating androgens result from gonadal and adrenal secretion and from extraglandular conversion of steroid precursors. These androgens might influence auxological parameters such as growth and bone maturation. For this reason we correlated androgen serum levels (androstenedione and dehydroepiandrosterone-sulfate) with the auxological data.

PATIENTS AND METHODS

Patients

This multicentre trial involved 82 Patients with CPP, 74 girls and 8 boys. The patients were divided in two groups: untreated or naive patients (group 1: 50 girls and 3 boys) and pretreated patients (group 2: 24 girls and 5 boys), i.e. pretreated with oral cyproterone acetate (CPA) ($n = 6$), with daily intranasal or subcutaneous administration of a short-acting GnRH agonist (Buserelin, Hoechst) for a period varying from 3 to 24 months ($n = 23$) (Table 1). We considered these 29 patients as one group as basal and stimulated luteinizing hormone (LH) concentrations were in the same range at the start of therapy and no difference was seen in the response to treatment.
Of this total of 82 patients the growth data of the patients which have been treated for 12 months or longer (44 girls, 7 boys), have been included in this

interim evaluation. In addition, only the results of those patients were used of which the data of a particular parameter were known at each moment of control during the treatment period. Because of the small number of boys only the results of the girls are described in detail.
Inclusion criteria for the study were the following: The diagnosis of CPP based upon: (1) the appearance of secondary sex characteristics in girls before the age of 8 years and in boys before the age of 9 years; (2) a pubertal response of plasma LH in a standard GnRH test (100 ug i.v.) (LH > 12.3 mIU/ml) [13]; (3) an accelerated growth velocity above the 97th percentile for chronological age at least 6 months prior to treatment (group 1: DD; group 2: CPA or Buserelin); and (4) an advancement of bone maturation at least 1 year of height age and/or $\Delta BA/\Delta HA \geq 1$. The chronological age at start of therapy was less than 9 years in girls and less than 10 years in boys. Exclusion criteria were the following: (1) the diagnosis of pseudoprecocious puberty; (2) a bone age > 12 in girls or > 13 years in boys; (3) a progressive brain tumour; (4) bleeding disorders (not compatible with i.m. injections); (5) diseases or other treatment influencing growth and/or activity of pituitary-gonadal axis. Written parental informed consent after thorough explanation of the study protocol was obtained before the start of treatment.

Table 1. *Study group (girls: mean ± SEM, boys: mean and range)*

		n	Chronological age (yrs)	Bone age (yrs)
Girls	Group 1	50	6.5 ± 0.3	9.2 ± 0.3
	Group 2	24	6.9 ± 0.3	10.0 ± 0.3
Boys	Group 1	3	6.9 (4.8 - 9.4)	12.0 (6.0 - 13.5)
	Group 2	5	5.1 (4.8 - 7.5)	11.9 (6.25 - 12.5)

Methods

Decapeptyl-Depot was provided and distributed to all clinical investigators by Ferring (Federal Republic of Germany/The Netherlands). The single injection dose was 75 (50-100) ug/kg body weight. The first three injections were given intramuscularly at 2 weekly intervals and thereafter every 4 weeks.
Height and pubertal stages (according to Tanner) were obtained every 12 weeks; an X-ray of the hand every six months. Bone age was determined by one observer according to the method of Greulich and Pyle [6] and adult height prediction according to the tables of Bayley and Pinneau [6].
Plasma concentrations of LH and follicle stimulating hormones (FSH) were determined at both laboratory centers (Amsterdam, Kiel) by commercially

available polyclonal antibody radioimmunoassays (RIA) (Amerlex, Amersham, U.K.; Serono Diagnostika, FRG). Quality characteristics were similar. The intra-assay coefficients of variation for LH and FSH ranged from 3.8%-7.3% and 3.2%-5.8%, respectively. LH and FSH interassay variability was 4.5%-12.5% and 4.2%-14%, respectively. Plasma oestradiol was measured with the use of two commercially available RIA kits (Institut für Reaktorforschung, Würenlingen, Switzerland and Diagnostic Products Corporation, Los Angeles, California, respectively). The intra-assay and interassay coefficients of variation were 4%-16.7% and 11%-11.4%, respectively.

Plasma testosterone was determined by individual specific RIA after extraction with methylene chloride and subsequent isolation by Sephadex LH-20 chromatography [18]. Androstenedione and dehydroepiandrosterone-sulfate (DHEAS) were measured at the start of therapy and after 12, 24, and 48 weeks. In the serum of Dutch girls androstenedione was assayed after extraction with pentane using kits from Diagnostic Products Corporation (lower limit of assay sensitivity 0.7 nmol/l, intra-assay coefficient of variation 5.4%, interassay variability 8.9%). DHEAS was measured using a no extraction, solid phase ^{125}I RIA (Coat-a-Count, Diagnostic Products Inc.) (lower limit of assay sensitivity 0.2 umol/l, intra-assay coefficient of variation 4.5%, interassay variability 5.6%). Decapeptyl levels in serum were determined using a RIA after acetone/petroleum ether extraction. The assay was sensitive for a range between 1.0 and 173.5 nmol/l serum. Mean intra-assay coefficients of variation were below 6%, interassay coefficients of variation were 14.4%.

Liver and renal function was monitored using standard laboratory methods. Thyroxine and cortisol were determined by RIA methods (Travenol, Cambridge; USA, Serono).

Statistical analysis for hormonal results was performed with non parametric methods (Wilcoxon test). Growth data were statistically analysed by the Friedman test (a non parametic two way analysis of variance) to investigate whether a significant tendency for a parameter was present during the whole treatment period. If significancy existed according to Friedman, the one sample Student *t*-test for paired observations was used for analysing further differences between the interval periods of treatment. All data are expressed as mean ± 1 standard error of mean (SEM), unless mentioned otherwise.

RESULTS

Clinical signs

In girls vaginal bleeding ceased permanently within 1 month after start of DD in each of the 12 girls having menarche before start of DD-treatment. In the girls a variable reduction of breast tissue occurred and the consistency became weaker. Pubic hair did not change significantly during the first 12 months of

treatment in both groups. During DD therapy in boys, pubic hair decreased and testicular volume regressed by 0-4 ml. Greasy hair, acne and sweaty odor also diminished.

Pituitary-gonadal axis

The mean basal plasma LH levels did not change during the first 12 months of treatment in group 1, but decreased significantly in group 2 (Table 2). Before treatment the GnRH stimulated levels of LH were markedly elevated in group 1 (44.6 ± 3.6 mIU/ml) and in group 2 (25.0 ± 4.9 mIU/ml). Continuous sampling showed suppression of pulsatile gonadotropin secretion after 3 months (example of 1 patient - Fig. 1a, b). After 3 months the LH response to GnRH i.v. was prepubertally low (< 12.3 mU/ml) [13] in all children tested and remained suppressed after 1 year of therapy. In contrast to LH, basal plasma FSH values in girls were in the pubertal range at the time of diagnosis. During DD therapy both basal FSH and stimulated LH and FSH levels decreased significantly in group 1 and 2 (Table 2).

Table 2. *Plasma levels of basal and stimulated peak LH and FSH (mIU/ml) and oestradiol (E2, pmol/l) in group 1 and 2 (mean ± SEM) before and after 6 and 12 months of DD treatment.*

Group 1	LH		FSH		E2
	Basal	Peak	Basal	Peak	
Before	3.2 ± 0.3	44.6 ± 3.6	4.7 ± 0.3	17.2 ± 1.5	101 ± 12.3
(*n*)	(50)	(44)	(50)	(44)	(46)
6 months	3.1 ± 0.4	5.3 ± 0.8	2.0 ± 0.2	3.1 ± 0.7	44 ± 6.1
(*n*)	(41)	(15)	(41)	(15)	(39)
12 months	2.6 ± 0.4 NS	3.5 ± 0.6***	1.8 ± 0.2***	1.8 ± 0.3***	43 ± 4.6***
(*n*)	(30)	(9)	(30)	(9)	(27)
Group 2	**Basal**	**Peak**	**Basal**	**Peak**	
Before	2.9 ± 0.4	25.0 ± 4.9	3.4 ± 0.3	9.1 ± 1.6	89 ± 22
(*n*)	(24)	(15)	(23)	(14)	(21)
6 months	2.4 ± 0.5	3.5 ± 0.6	1.7 ± 0.2	1.4 ± 0.3	37 ± 4
(*n*)	(20)	(7)	(18)	(7)	(17)
12 months	1.7 ± 0.2*	3.1 ± 0.8**	1.8 ± 0.3**	2.3 ± 0.5*	35 ± 4(NS)
(*n*)	(14)	(7)	(14) (6)	(10)	

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$ compared to pretreatment values
NS = not significant; *n* = number of patients

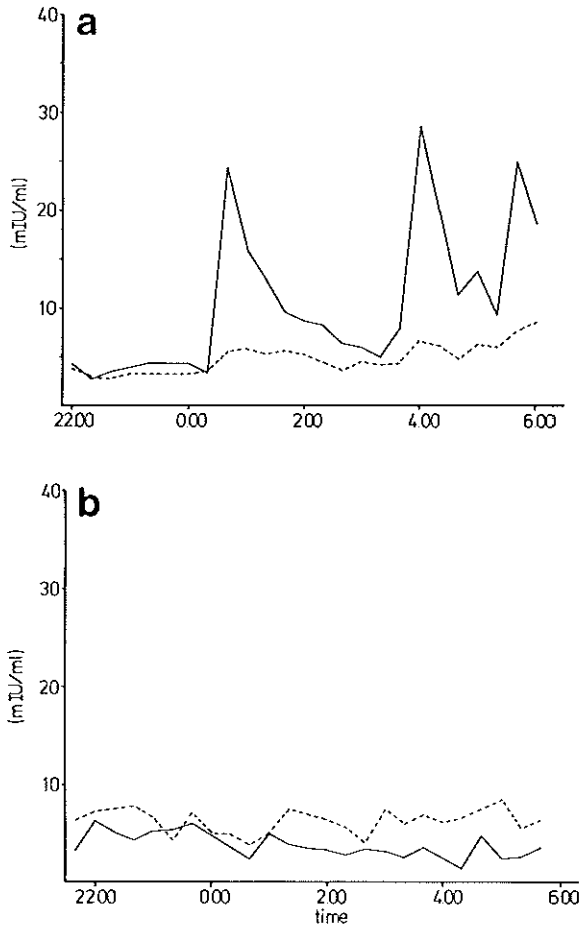

Figure 1. LH (–) and FSH (- - -) spontaneous night profile **(a)** before and **(b)** after 6 months of DD therapy.

Mean plasma oestradiol was in the mid-pubertal range before treatment and decreased rapidly and significantly to prepubertal levels [1] during treatment in both groups (Table 2). With the exception of ten patients, prepubertal oestradiol levels (< 50 pmol/l) were reached after the first 4 weeks of treatment (i.e. after 2 DD injections). Testosterone levels showed the same pattern. Serum levels of Decapeptyl showed wide variations, when determined 2 weeks and 4 weeks after a DD injection (Fig. 2). There were no significant differences between the 2 and the 4 week levels. At each time point there were Decapeptyl levels below the sensitivity of the assay in some patients, but without any clinical or laboratory evidence for incomplete suppression of the pituitary-gonadal axis.

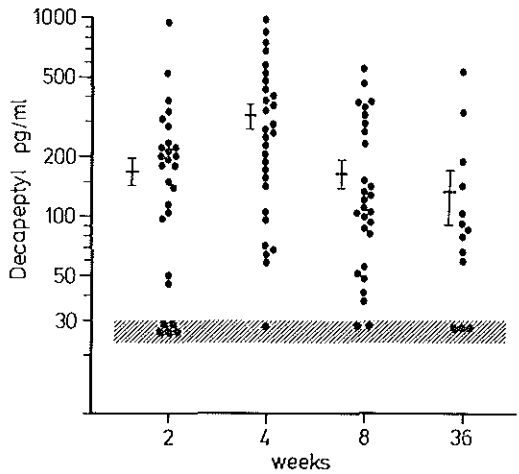

Figure 2. Serum levels of Decapeptyl (pg/ml) at 2, 4, 8 and 36 weeks of DD treatment after intramuscular injection. The *hatched area* indicates the sensitivity of the assay.

Standard laboratory parameters

DD treatment showed no effect on blood count, liver or renal function.

Androgens

In most young children the DHEAS values at start of treatment were low and showed little changes. In some older patients levels were higher at the start of therapy and remained elevated. In others there was a marked increase in DHEAS levels, indicating start of adrenarche (DHEAS > 2.0 umol/l). No correlation could be observed between the rate of bone maturation and rate of DHEAS increase.

Androstenedione levels showed a significant decrease from 1.97 ± 1.58 to 1.04 ± 0.56 nmol/l (mean ± SD) in the girls of group 1 after 3 months of therapy (Friedman, $n = 19, p < 0.01$). This pattern could not be observed in group 2. No correlation was found between the rate of bone maturation and the individual androstenedione levels.

Growth response

The growth velocity of the individual patients in cm/yr calculated over 6 months intervals are given in Fig. 3a, b. There was an obvious decrease in growth velocity during the first 6 months of therapy in most of the girls in group 1, with a stabilization after 1 year. As expected, in group 2 there was only a slight decrease

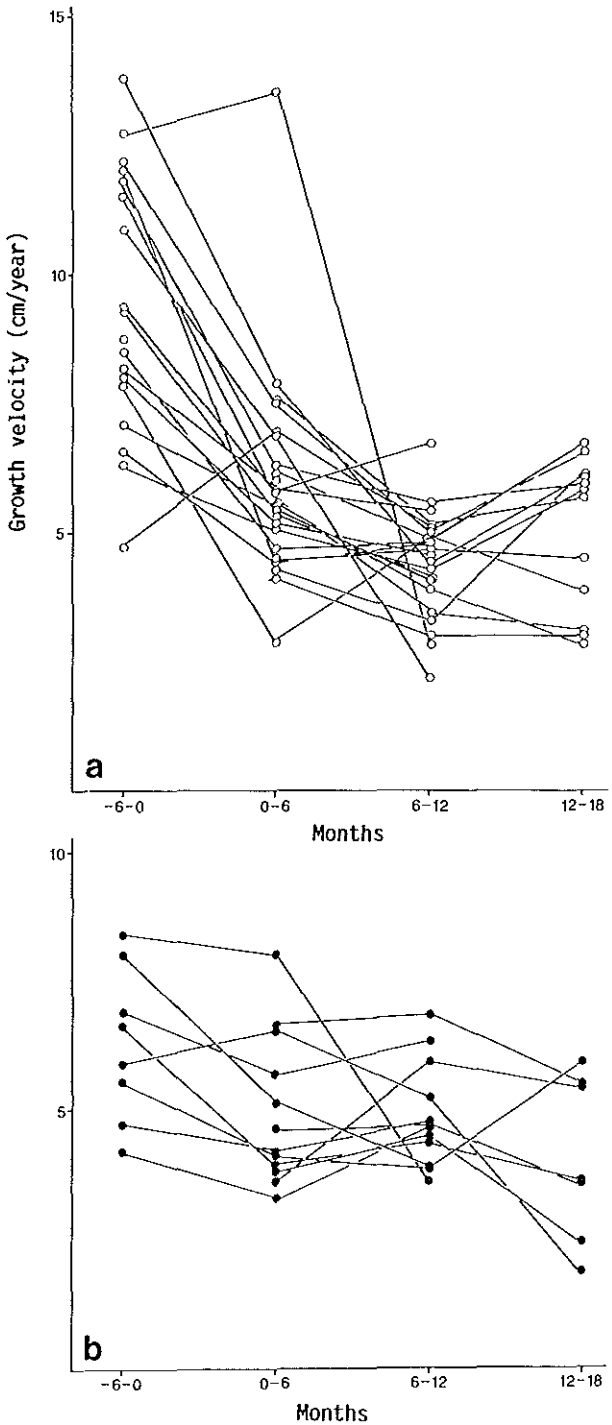

Figure 3. **(a)** Growth velocity in girls of group 1; **(b)** Growth velocity in girls of group 2.

in growth velocity. Mean growth rates were calculated: in 27 girls all the growth data were available for the period 6 months before start of therapy throughout 12 months of treatment. In all girls together and in group 1 ($n = 19$) and 2 ($n = 8$) separately, growth velocity decreased significantly during this treatment period (Friedman, $p < 0.01$). The decrease was most obvious in the girls of group 1 in which a significant decrease in growth velocity was observed from 9.0 ± 0.72 cm/yr before to 6.3 ± 0.5 cm/yr after 6 months of treatment (Student t, $p < 0.01$), and to 4.5 ± 0.23 cm/yr after 1 year of treatment ($p < 0.001$). In 11 girls, of which all the growth data from pretreatment period throughout 18 months were available, the growth velocity decreased significantly (Friedman, $p < 0.01$) until 12 months with a stabilization between 12 and 18 months (4.8 ± 0.52 cm/yr). The same pattern was observed in boys. As a measure of bone maturation we used the ratio bone age versus chronological age ($\Delta BA/\Delta CA$). We consider a value of 1.0 as normal. In the treatment period 0-18 months there was a significant decrease in $\Delta BA/\Delta CA$ (Friedman, $n = 20$, $p < 0.001$). The most obvious decrease was noticed in the period 12-18 months in the group 1 girls (Student t, $p < 0.01$). There was no significant change in the girls of group 2 (Fig. 4).

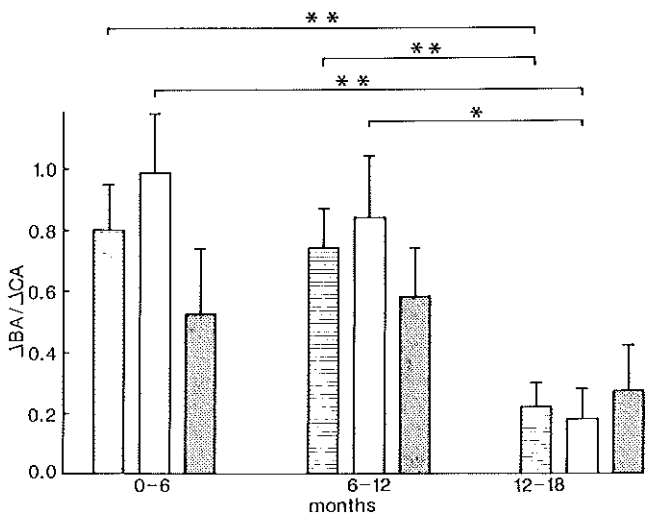

Figure 4. $\Delta BA/\Delta CA$ ratio in girls (mean, SEM). ⊟ All girls ($n = 20$); □, group 1 ($n = 12$); ■, group 2 ($n = 8$). * $p < 0.05$; ** $p < 0.01$.

One of the goals of treatment is to improve final height. For this reason adult height prediction was calculated every 6 month of treatment according to the method of Bayley and Pinneau [6]. In the girls with results known up to 12 months, a significant tendency to improvement in adult height prediction was

seen in all the girls together (Friedman, $n = 39, p < 0.01$) and in girls of group 2 ($n = 14, p < 0.05$). The same pattern was observed in the girls with results known up to 18 months ($n = 20$), with also a significant tendency to improvement in group 1 (Friedman, $n = 12$, $p < 0.05$). In all girls with results known up to 18 months, the adult height prediction increased from 160.7 ± 2.6 cm to 163.2 ± 2.5 and in group 1 from 161.9 ± 3.3 cm to 164.1 ± 3.5 cm (Fig. 5). The same pattern of increase was observed in boys.

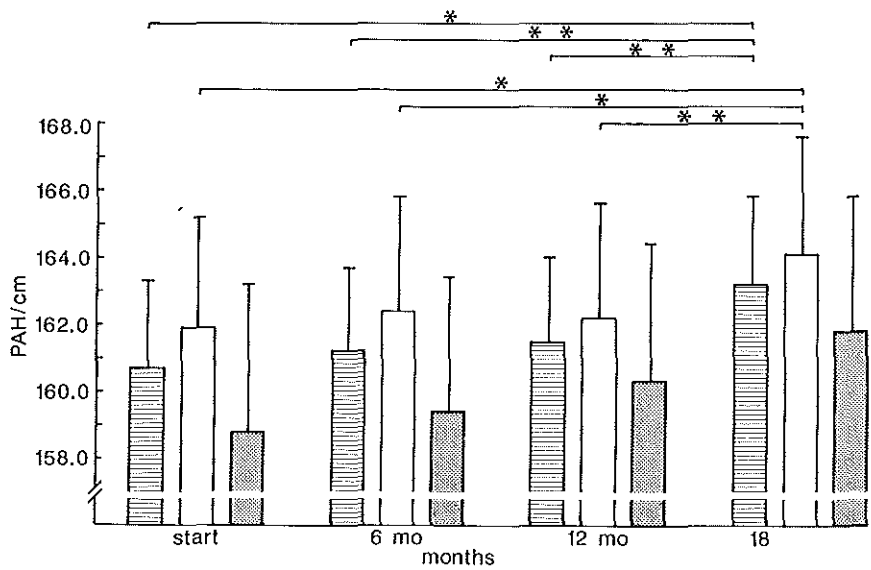

Figure 5. Predicted Adult Height (PAH) in girls (mean, SEM). Symbols as for Fig. 4.
* $p < 0.01$; ** $p < 0.05$.

DISCUSSION

The two major objectives of treatment of children with CPP is first to halt further progression or preferably to induce regression of pubertal development, and second to improve final height.

Our 1st year results of treatment with the slow-release GnRH agonist Decapeptyl indicate an effective and sustained suppression of gonadotropins and serum oestradiol/testosterone levels. This resulted in an arrest or even regression of pubertal signs. The treatment has been well tolerated, despite the short-lasting pain of the injection site. So far the treatment has been used without systemic or local side-effects.

The other main goal of treatment is to improve final height. Several studies using various GnRH agonists have been recently published, with different results

concerning final height prognosis, ranging from no improvement [3, 5, 21] to a slight increase [2, 4, 12, 14, 16, 22].
In our study, improvement of final height prognosis from 161.9 to 164.1 cm was reached in the naive girls after 18 months of therapy. This improvement is due to a decrease in the ΔBA/ΔCA ratio and a stabilization of growth velocity towards a normal prepubertal growth rate (mean 4.8 cm/yr) after 12 months of therapy.
In the patients previously treated with CPA or short acting GnRH analogue, no significant decrease of ΔBA/ΔCA was seen. This factor and a more advanced bone age of these girls at start of therapy (as compared to the mean bone age of group 1) might explain the observation of a slight improvement of predicted adult height.
One has to keep in mind that the final height prediction is purely a calculated value based on height measurements and bone age estimations, especially difficult in children with precocious puberty. So far only one study [10] has been reported on the achieved final height after treatment of 8 girls with a GnRH agonist, in 7 of whom the final height was greater than height prediction at start of therapy.
Effective GnRH analogue treatment of children with precocious puberty has confirmed the clinical observation that adrenarche and the maturation of gonadal function at puberty are independent but associated processes controlled by different mechanisms. In our study we observed an age-appropriate onset and progression of adrenarche during GnRH analogue suppression of gonadarche using serum DHEAS levels as a marker. In addition, DHEAS levels were appropriate for chronological age but not for bone age (data not shown). In contrast, we found a decrease of androstenedione levels suggesting a partly ovarian origin of this weak androgenic steroid.
Boepple et al [2] found no correlation between growth velocity and DHEAS levels but did establish a correlation between the rate of bone age advancement and DHEAS levels. Thus, the patients with the highest DHEAS levels exhibited the smallest increases in predicted height, whereas the patients who were preadrenarchal and remained so throughout GnRH treatment, experienced the greatest advances in height prognosis. Our preliminary results allow no such conclusion to be drawn. Concomitant CPA treatment has been advocated in girls with precocious puberty and rapid progression of adrenarche [9], although no firm correlation between androgen levels and growth rate and bone maturation in children with CPP has been established.
At this point in the study we may conclude that treatment with DD has the important practical advantage that it is administered once per 4 weeks intramuscularly instead of a short acting GnRH agonist that has to be given every day once or twice subcutaneously or even three to six times intranasally.
In addition, DD may improve final height prognosis in naive girls with CPP. Only long-term follow up, until final height has been reached, will reveal whether the growth course will differ from that described in untreated patients or patients treated with CPA [19, 23].

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Chapter 3

COMPARISON OF COMPLETE AND INCOMPLETE SUPPRESSION OF PITUITARY-GONADAL ACTIVITY IN GIRLS WITH CENTRAL PRECOCIOUS PUBERTY: INFLUENCE ON GROWTH AND PREDICTED FINAL HEIGHT

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ABSTRACT

The question as to whether treatment with short-acting or with slow-release gonadotropin-releasing hormone (GnRH) agonists has different effects on growth and bone maturation when treating girls with central precocious puberty has not yet been studied. In a meta-analysis, we compared 21 naive girls with central precocious puberty who were treated with buserelin with 22 naive girls with central precocious puberty who received Decapeptyl in depot form. Treatment lasted for at least 18 months. At the start of therapy, chronological age, bone age, growth velocity and pubertal stage in the two groups were very similar. During the first 6 months of treatment, significantly more phases of incomplete suppression of pituitary-gonadal activity occurred in the buserelin group. As a result, growth velocity and bone maturation (Δ bone age/Δ chronological age) remained significantly higher than in the Decapeptyl Depot group ($p < 0.0001$ and $p < 0.01$, respectively). In contrast to the Decapeptyl Depot group, the height standard deviation score (SDS) for bone age in the buserelin group dit not change significantly in the first 6 months of treatment, and the predicted adult height decreased. Between the 6th and 18th months of therapy, the development of growth rate, Δ bone age/Δ chronological age, height SDS for bone age and predicted adult height in both groups became almost identical. However, the rate of growth and bone maturation in the buserelin group remained faster than in the Decapeptyl group, though not significantly so. The mean predicted adult height had risen significantly after 18 months in the Decapeptyl Depot group but not in the group treated with buserelin. Treatment with the slow-release GnRH agonist Decapeptyl Depot is more effective for children with central precocious puberty than therapy with the short-acting agonist buserelin, not only with regard to long-term compliance but also for auxological reasons, since Decapeptyl Depot suppresses gonadotropins and oestradiol faster and more completely at least during the first 6 months of treatment.

INTRODUCTION

For some years gonadotropin releasing hormone (GnRH) agonists have been considered the therapy of choice for children with central precocious puberty. Throughout the world a large number of different substances are being used and tested in clinical trials. Through long-term occupation of the receptors, these agonists result in a desensitization of the pituitary and a lack of response to further stimulation by endogenous GnRH [1, 2]. Their effectiveness in suppressing gonadotropins and sexual steroids has been demonstrated by various authors [3-12]. The GnRH agonists which have been most commonly used in Europe so far are buserelin (Suprefact, Hoechst AG, Frankfurt) and Decapeptyl Depot (Ferrin Arzneimittel GmbH, Kiel). Buserelin, a shorter-acting agonist [13] (elimination half-life about 75 min, biological effectiveness about 3 hours) is administered once or twice daily as a subcutaneous injection or three to six times a day as a nasal spray [3-5, 7, 10, 12, 14-17]. Decapeptyl Depot consists of biodegradable microspheres to which the Decapeptyl agonist is absorbed [18], and is injected intramuscularly once a month.
It is known that the suppression of pituitary-gonadal activity with buserelin therapy is very variable [12-16, 19], whereas Decapeptl Depot results in long-term complete suppression [6, 8, 9, 11, 20]. Conspicuously low growth rates have been reported with complete suppression through Decapeptyl Depot therapy [8, 9, 11, 20] which could negatively affect the final adult height of the children. On the other hand, treatment with the depot drug resulted in slower bone maturation, so that the length of growing time available was considerably increased [8, 9, 11, 20]. This means that it is possible to increase final height. Which of these two opposing trends will take priority during the period of therapy, and which type of therapy - complete (Decapeptyl Depot) or incomplete (buserelin) suppression - will have the more favourable effect on the auxological parameters, is still completely unclear since comparative studies of the two agonists have not yet been carried out in children with central precocious puberty.
For this reason we examined growth and bone maturation in two comparable groups of girls with central precocious puberty in a meta-analysis. The girls were treated in multicentre studies [8, 9, 12, 15, 20] for at least 18 months with either buserelin of Decapeptyl Depot.

MATERIAL AND METHODS

Patients

The original data of 43 girls, who had not been treated before the onset of therapy with GnRH agonists, were evaluated. The diagnosis of central precocious puberty was based upon the following criteria:

(1) the appearance of secondary sexual characteristics before the chronological age of 8 years;
(2) increased growth velocity;
(3) accelerated bone age; i.e. at least 1 year ahead of height age or Δ bone age/Δ height age > 1.0, and
(4) pubertal increase of LH of above 12.3 mIU/ml in a GnRH bolus test (60 μg/m² i.v.) [21].

The chronological age of the patients at the start of therapy was not more than 8.5 years and bone age was not more than 12 years. In most cases an observation period of at least 6 months preceded the start of therapy in order to exclude transitory forms of premature pubertal development. Children with meningomyelocele, progressive brain tumour or with gonadotropin-independent pseudoprecocious puberty (e.g. McCune-Albright syndrome or congenital adrenal hyperplasia) were excluded.

21 girls were treated in two multicentre studies in Germany and Holland [12, 15] for at least 18 months with buserelin. The data of 22 girls from the ongoing German-Dutch multicentre study [8, 9, 20] who had completed 18 months of treatment with Decapeptyl Depot were used as a comparison.

Table 1 gives a survey of the most important clinical data of both patient groups. Both groups were comparable with regard to clinical and auxological parameters, such as age, bone age, stage of puberty and growth rate, with no significant differences in each parameter.

Table 1. *Clinical data of patients in the buserelin and the Decapeptyl Depot groups at the start of therapy.*

Chronological age years	Bone age years	Height SDS_{CA}	Pubertal stage B[a], P	Menses *n*[b]	Growth rate cm/year	E_2 pmol/l
Buserelin						
6.5 ± 1.9	9.5 ± 1.9	1.8 ± 2.0	B2-B4 P1-P4	5	10.2 ± 2.9	155 ± 159
Decapeptyl Depot						
6.6 ± 1.2	9.5 ± 1.9	2.0 ± 1.5	B2-B5	5	10.3 ± 4.1	93 ± 65
NS	NS	NS		NS	NS	NS

Mean values ± SD are given. NS = not significant.
a Breast development stage B and development stage P of pubic hair according to Tanner [21].
b Number of patients with menses before start of therapy.

Methods

Buserelin was injected subcutaneously in a dosage of 1 × 10 µg/kg to 2 × 20 µg/kg per day [12, 15]. Some patients received 2-4 doses (each 150 µg) intranasally in the morning or at midday in addition to the subcutaneous dose at night. At the start of therapy Decapeptyl Depot was injected three times at intervals of 2 weeks and subsequently once a month intramuscularly in dosage of 75 µg/kg (with a maximum dosage of 3.2 mg, the contents of one ampoule) [20]. If one calculates the monthly requirement of GnRH agonist, the girls in the buserelin group needed up to 12 times as much medication as those in the Decapeptyl Depot group. Bone age was determined by experienced paediatric radiologists or endocrinologists using the Greulich and Pyle method [23]. The predicted adult height was calculated according to Bayley and Pinneau [24]. Height and X-rays of the left hand were evaluated at 6-months intervals. The calculation of standard deviation scores (SDS = deviation from the mean age, expressed in standard deviations) was based on German and Dutch longitudinal growth standards [25, 26]. Hormone plasma levels were determined by specific radioimmunoassays in centralised laboratories as previously described in detail [12, 15, 17, 20].

Statistical comparisons were carried out with non-parametric methods: the Mann-and-Whitney U test, Wilcoxon test, Friedman test, with multiple comparisons conducted according to Newman-Keul. Comparison of the frequency of periods of incomplete suppression was made with the Fisher exact test.

RESULTS

Hormonal Data

Phases of incomplete clinical suppression (increase of vaginal discharge, enlargement of breasts) were characterised by increased LH in the GnRH test (< 12.3 mU/ml) and by oestradiol (E_2) plasma levels above the prepubertal reference range (< 50 pmol/l; fig. 1). These periods of increased oestrogen influence were seen more frequently during the first 6 months of treatment with buserelin than subsequently with Decapeptyl Depot (fig. 1). Significantly more frequent clinical and biochemical "escape phenomena" with mean E_2 levels of 100 and 67 pmol/l were observed in the buserelin group than in the Decapeptyl Depot group at 3 and 6 months of treatment, respectively (fig. 1). Even after 18 months of therapy, periods of insufficient suppression still occurred in the buserelin group but not in the Decapeptyl Depot group (fig. 1). It therefore appears justified to speak of incomplete suppression in the buserelin group.

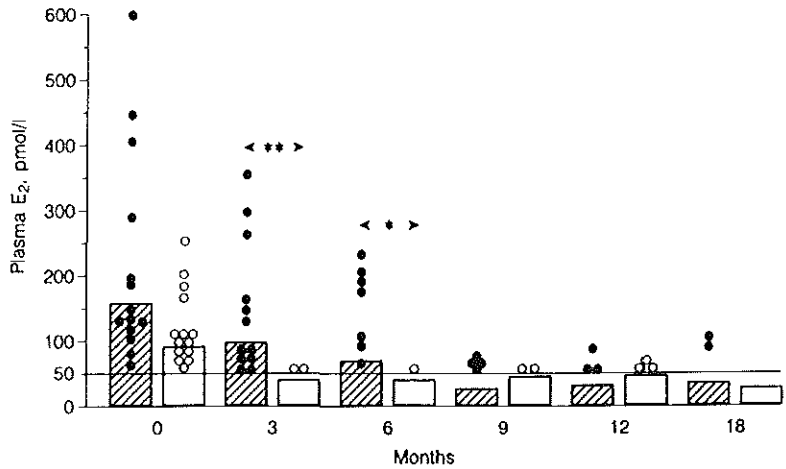

Figure 1. Mean plasma E_2 (pmol/l) before and during therapy with buserelin (▨) or Decapeptyl Depot (□). Individual E_2 levels above the pre-pubertal reference range (i.e., < 50 pmol/l) are depicted: ● = buserelin patients; ○ = Decapeptyl Depot patients. * $p < 0.05$; ** $p < 0.01$.

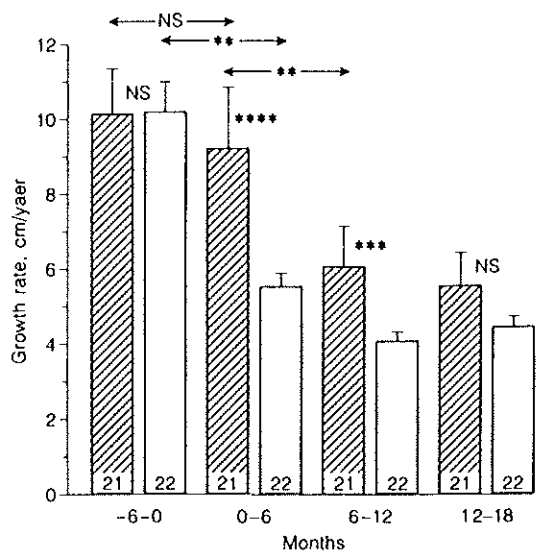

Figure 2. Half-yearly growth rates (mean ± SD) for the buserelin group (▨) and the Decapeptyl Depot group (□).
The number at the base of each bar shows the number of patients.
NS = not significant. ** $p < 0.01$, *** $p < 0.01$, **** $p < 0.0001$.

Auxological Data

Growth velocity (fig. 2) of the buserelin patients remained pathologically raised during the first 6 months of therapy (9.3 ± 3.7 cm/year), whereas if fell significantly in the Decapeptyl Depot group to the normal value for age (5.6 ± 1.9 cm/year; $p < 0.01$). Throughout the first year of therapy, growth rates in the buserelin group remained significantly higher than in the Decapeptyl Depot group (fig. 2). The buserelin patients were still growing slightly faster after 18 months.

The advance of bone maturation, expressed as the ratio of Δ bone age/Δ chronological age (fig. 3), remained pathologically elevated in the buserelin group after 6 months of treatment (1.64 ± 0.9), whereas it fell significantly ($p < 0.01$) in the Decapeptyl Depot group into the normal range (about 1.0). During the course of therapy, bone maturation normalized also in the buserelin group, but throughout the 18 months the ratio remained higher than in the Decapeptyl Depot group.

The courses of height SDS of chronological age and bone age reflected the findings on growth velocity and bone maturation (fig. 4). The height SDS for chronological age in the buserelin group remained elevated (about + 2) throughout the entire observation period, whereas a continuous significant fall into the normal range ($p < 0.01$) was seen in the Decapeptyl Depot group. In contrast, the initial decreased height SDS for bone age rose in both groups. However, this rise was more continuous and marked in the Decapeptyl Depot group than in the buserelin group ($p < 0.001$ vs. $p < 0.05$), which showed no clear increase during the first 6 months of therapy (fig. 4).

Comparing the groups at start of therapy, the predicted adult height means were very similar (buserelin: 157.6 ± 11.4 cm; Decapeptyl Depot: 158.2 ± 9.4 cm). Whereas the predicted adult height in the buserelin group decreased after

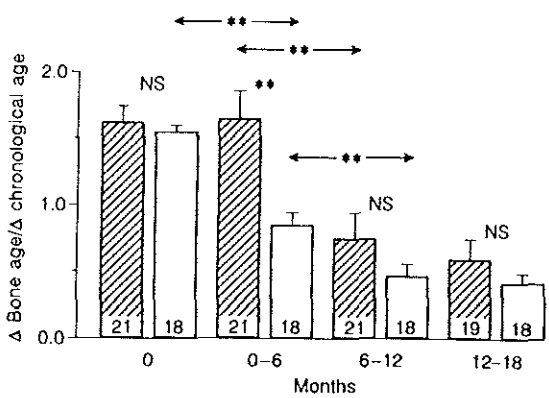

Figure 3. Half-yearly ratios of Δ bone age/Δ chronological age (mean ± SEM) for the buserelin (▨) and the Decapeptyl Depot group (□). NS = not significant. ** $p < 0.01$.

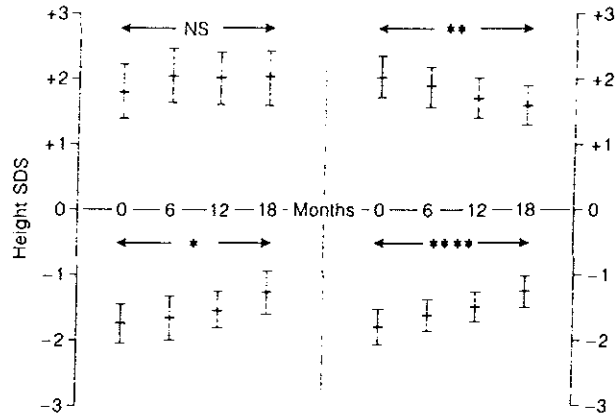

Figure 4. Height SDS (mean ± SEM) for chronological age (upper half) and for bone age (lower half) before and during therapy in the buserelin group (left side) and the Decapeptyl Depot group (right side).
NS = not significant, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

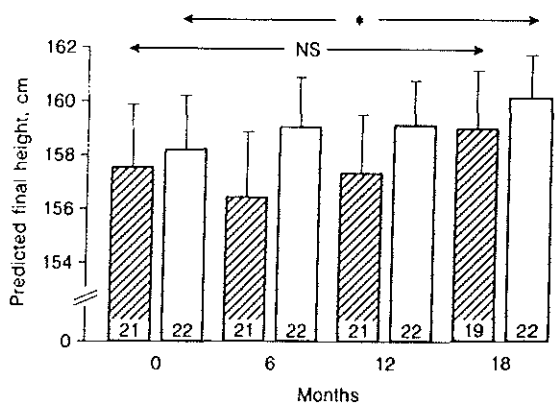

Figure 5. Predicted final heights (mean ± SD) according to Bayley and Pinneau [24] before and during therapy with buserelin (▨) or Decapeptyl Depot (□).
NS = not significant, * $p < 0.05$.

6 months, it increased continuously in the Decapeptyl Depot group throughout the 18 months (fig. 5). Although there were no statistically significant differences between the two groups at any point, the increase in the mean predicted adult height of the buserelin patients was still insignificant after 18 months but was significant for the Decapeptyl Depot patients. The difference in the mean predicted adult height of the two groups increased slightly from 0.6 cm before therapy to 1.0 cm at the end of 18 months' treatment.

DISCUSSION

Based upon the clinical observation that the efficacy of gonadotropin suppression varies with the different types of galenic preparation of the GnRH agonists, the auxological parameters of two comparable groups of girls with central precocious puberty were compared in a meta-analysis. The girls were treated either with the short-acting buserelin (daily subcutaneous injections and/ of several daily intranasal applications) or with the slow-release Decapeptyl Depot (monthly intramuscular injections) [8, 9, 12, 15, 20].

Incomplete suppression of pituitary-gonadal activity in the girls treated with buserelin was shown by the many "escape periods" with raised levels of E2 and clinical signs of increased oestrogen activity [14, 16]. The occurrence of phases of incomplete suppression, due either to insufficient nasal absorption, resistance to the drug or lack of compliance, was also demonstrated by measuring the plasma levels and the excretion of buserelin in the urine [13, 19].

Continuous or intermittent exposure of the epiphyseal growth plates to pubertal E_2 levels, and thus to increased growth hormone levels, resulted not only in a significantly higher growth rate but also in much more rapid bone maturation in the patients treated with buserelin when compared with those treated with Decapeptyl Depot. This development of growth rate, with a slow drop to the normal range for age, has been described by other authors [4, 10, 17]. A fast and conspicuous drop in growth rate to the prepubertal range, as occurred with our Decapeptyl Depot group, has also been described by the French working group [11] and was also observed in a large number of children in the Dutch/German Decapeptyl Depot study [20].

During the first year of therapy with buserelin there was no improvement in the mean height SDS for bone age, even with a good growth rate, since bone maturation was not slowed down. In the Decapeptyl Depot group, on the other hand, the height SDS increased significantly after only 6 months of treatment, in spite of a clearly lower growth rate, due to the conspicuous slowing down of bone maturation. These findings agree with those of Rappaport et al. [10], and Rime et al. [16] who observed no real improvement in the height SDS for bone age in courses of therapy running for different lengths of time up to 3 years (–1.5 before therapy, –1.2 after 3 years [10]). Other studies have also reported on the effect on bone age development during buserelin treatment [3, 7, 14].

An improvement of predicted final height of patients treated with buserelin has been described by many authors [3-5, 7, 10, 17, 27]. The same applies to therapy with Decapeptyl Depot [6, 8, 9, 11, 20, 28, 29], as was confirmed by our patients. The fact that treatment with GnRH agonists results in an improvement of predicted adult height has, thus, often been described in the literature.

If one considers the development of predicted adult height over a longer period of therapy, a significant improvement should also be seen in buserelin patients as long as continuous good compliance is guaranteed. The differences in mean predicted adult height between the two groups were at no time significant and always less than 2 cm. The development of the height SDS for bone age between

the two groups should also equal out over a longer period of therapy, as was implied by the trend seen during the 18 months of treatment. In a recent preliminary report, however, Nizzoli et al. [29] showed that the differences between short-acting and long-acting GnRH agonists concerning their effect on bone maturation and growth may even persist until final height. In their patients adult height was significantly higher in the Decepeptyl Depot group than in the buserelin group.

In conclusion it can be said that when comparing buserelin treatment with Decapeptyl Depot therapy there are clear differences, particularly during the first 6 months, in the development of growth rate, bone maturation and predicted final height. The therapeutically desired increase in the height SDS for bone age occurs faster with Decapeptyl Depot therapy. In the course of further treatment, up to a period of 18 months, the results level out so that there are no significant differences between the above-named parameters in the two groups. If clinical and/or auxological reasons necessitate rapid and complete suppression, or treatment is only intended to last for 1-2 years (e.g. with 7- to 9-years-old girls), than Decapeptyl Depot is also the drug of preference compared with buserelin with regard to improvement of predicted adult height and the prevention of further acceleration of bone maturation.

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Chapter 4

LONG-TERM RESULTS WITH A SLOW-RELEASE GONADOTROPHIN RELEASING HORMONE AGONIST IN CENTRAL PRECOCIOUS PUBERTY

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ABSTRACT

As part of an ongoing international multicentre study, 19 children (14 girls, 5 boys) with central precocious puberty (CPP) were treated with a slow-release gonadotrophin-releasing hormone (GnRH) agonist, triptorelin, for 4 years. After 3 years of treatment, height velocity stabilized at 4.0 cm/year. Predicted adult height (mean ± SD) increased from 158.9 ± 6.8 to 164.9 ± 6.6 cm in girls ($n = 14$, $p < 0.01$), and from 174.4 ± 18.5 to 184.3 ± 17.1 cm in boys ($n = 4$, $p < 0.05$). In 12 additional girls who started the multicentre study but discontinued triptorelin treatment after 2.2 ± 0.5 years, menses started 9.8 ± 3.7 months after cessation of treatment in all but one patient. Height velocity increased over the first 6 months after discontinuation of treatment, from 3.6 ± 0.1 to 5.4 ± 2.5 cm/year, and remained higher than pretreatment values in the second 6 months, but decreased subsequently. Bone maturation increased, and no significant improvement in predicted adult height was observed. Therefore, for auxological reasons it may be advisable to continue triptorelin treatment for as long as possible. Concomitant growth hormone (GH) therapy was initiated in three girls with CPP with height velocities of 3.2-3.6 cm/year after 3 years of treatment with triptorelin and with predicted adult heights of less than the third centile of Dutch girls. Prior to the administration of GH, all patients had subnormaal 24-hour GH profiles and GH responses to arginine provocation. GH treatment increased height velocity markedly in all girls, and improved predicted adult height.

It is concluded that triptorelin therapy improves predicted adult height. In children with CPP and genetic short stature, with a markedly decreased height velocity during triptorelin therapy, concomitant administration of a GnRH agonist and GH may have advantages. Further extensive studies are required.

INTRODUCTION

Since the early 1980s, agonist analogues of gonadotrophin-releasing hormone (GnRH) have been considered to be the treatment of choice for children with central precocious puberty (CPP). Administration of these agonists leads to pituitary desensitization and to total refractoriness to further stimulation by natural GnRH. Several studies, mostly investigating the short-term effects of a number of GnRH analogues administered by a variety of different routes, have been published [1-7]. In most of these studies, children were treated with daily s.c. injections of a GnRH analogue [1-5]; in other studies, a slow-release preparation was used [6, 7].

It has been clearly demonstrated that the use of the slow-release GnRH agonist, triptorelin (D-Trp6-LHRH, Decapeptyl-CR 3.75, Cytotech SA, Martigny, Switzerland) results in sustained suppression of the secretion of the gonadal steroids, and an arrest of pubertal development [7, 8]. However, several important questions regarding the auxological effectiveness of prolonged treatment remain unanswered. It has become apparent that such treatment results in a marked decrease in height velocity. However, an improvement in predicted adult height, which is dependent on a concurrent, relative decrease in the rate of bone maturation, has been observed in some but not all patients [3, 6, 8]. Due to the advancement in bone maturation and a diminished growth rate during GnRH agonist treatment in some children with CPP, especially those with genetic/familial short stature, the adult height prognosis remains poor. In addition, a decrease in the spontaneous secretion of growth hormone (GH) has been reported during GnRH agonist treatment [9-11].

In order to investigate the long-term effects of a slow-release GnRH agonist, a study of the treatment of CPP children with triptorelin was initiated, and the auxological data after 4 years of treatment are presented here. Although definitive data on final heights are not yet available, growth after the discontinuation of such treatment has been reviewed. A pilot study was set up to examine the effects of combined GnRH agonist and GH therapy in short, slowly growing children with CPP. The individual auxological data of three of these children after 18 months of combined treatment, are presented.

PATIENTS AND METHODS

Patients

As part of an ongoing international multicentre study, auxological data were obtained in 19 children (14 girls, 5 boys) with CPP treated for 4 years with triptorelin, 60-120 μg/kg i.m., every 4 weeks. The diagnosis of CPP was based upon criteria detailed previously [8]. The hormonal data, including the sustained suppression of the LH response following intravenous GnRH administration, have also been reported for this group of CPP patients [8]. The mean

chronological ages (CA) at the start of therapy were 6.7 ± 1.4 years (± SD) and 5.9 ± 3.7 years in girls and boys, respectively; mean bone ages (BA) were 10.0 ± 1.0 years and 10.9 ± 4.4 years, respectively. Eleven children (9 girls, 2 boys) had received no previous treatment for CPP prior to the study (group 1). Eight children (5 girls, 3 boys) had received treatment previously, either with cyproterone acetate (2 boys), or with a short-acting GnRH agonist (Buserelin, Hoechst) administered as a daily s.c. injection for 7-36 months (group 2).
Auxological data were also obtained for 12 additional girls who started the multicentre study and discontinued treatment after a mean period of 2.2 ± 0.5 years. Treatment was stopped when psychosocial indications for the suppression of pubertal development were no longer present and the predicted adult height was greater than the third centil for Dutch children [12].
Three girls (aged 6.4-7.8 years) with predicted adult heights less than the 3rd centile [12], and height velocities less than the 25th centile for their age [13] after 3 years' treatment with triptorelin were transferred to a regimen of triptorelin plus concomitant human GH (Genotropin Kabi Pharmacia AB, Sweden) at a daily dosage of 4 IU/m² body surface area, for 18 months.

Measurements

Height and pubertal stage (Tanner criteria) were assessed every 3 months in all patients; X-rays of the hands were taken every 6 months. BAs were determined by one observer, according to the method of Greulich and Pyle [14]; adult height was predicted according to Bayley and Pinneau [15].
Before the start of concomitant treatment with GH, the spontaneous secretion of GH in plasma was measured over a 24-hour period, starting at 09.00 h, after a light breakfast. Blood was sampled according to the Cormed-Kowarski method [16], via a non-thrombogenic catheter (Cormed) inserted into the antecubital vein and connected to a constant withdrawal pump. Samples of blood were taken during 72 periods of 20 minutes each; all samples were analyzed in duplicate in the same assay. Details of the methods used for measurement of GH in plasma have been published previously [17]. The 24-hour GH profiles were analysed using the Pulsar program developed by Merriam and Wachter [18]. Peak selection criteria appropriate for the assay conditions employed were established and used as described previously [17].
Following completion of blood sampling, a standardized arginine GH provocation test was performed: arginine, 0.5 g/kg, was infused over a period of 30 minutes. Blood samples were taken at 15-minute intervals, from 15 minutes before, to 180 minutes after the start of the infusion, and GH levels were determined as before.

Statistical analysis

All results are expressed as mean ± SD, unless otherwise indicated. Non-parametric statistical methods were used tot assess significance (Friedman test).

The Wilcoxon matched-pairs signed-rank test was used for within-group changes.

RESULTS

Auxological data after 4 years of GnRH agonist treatment

The auxological effects of triptorelin during the initial treatment period have been reported previously [8]. As expected, the height velocities of all the girls studied decreased significantly over the first year of treatment with triptorelin (Fig. 1); the same pattern was observed in boys. In group 1 height velocity decreased gradually form 5.6 ± 1.4 cm/year to a significantly lower value of 4.0 ± 0.8 cm/year in the fourth year of treatment.

Bone maturation of boys and girls, measured as the ratio of the change in BA to the change in CA (ΔBA/ΔCA), decreased significantly from 0.7 ± 0.3 in the first year of treatment to 0.5 ± 0.3 in the second year. Thereafter it remained in the same range: at 0.4 ± 0.3 in the third year and 0.5 ± 0.3 in the fourth year.

The relatively greater slowing of bone maturation as compared with height velocity resulted in a significant increase in predicted adult height, from 158.9 ± 6.8 cm at the start of treatment with triptorelin to 164.9 ± 6.6 cm after 4 years in girls ($n = 14$, $p < 0.01$) (Fig. 2), and from 174.4 ± 18.5 to 184.3 ± 17.1 cm in boys ($n = 4$, $p < 0.05$).

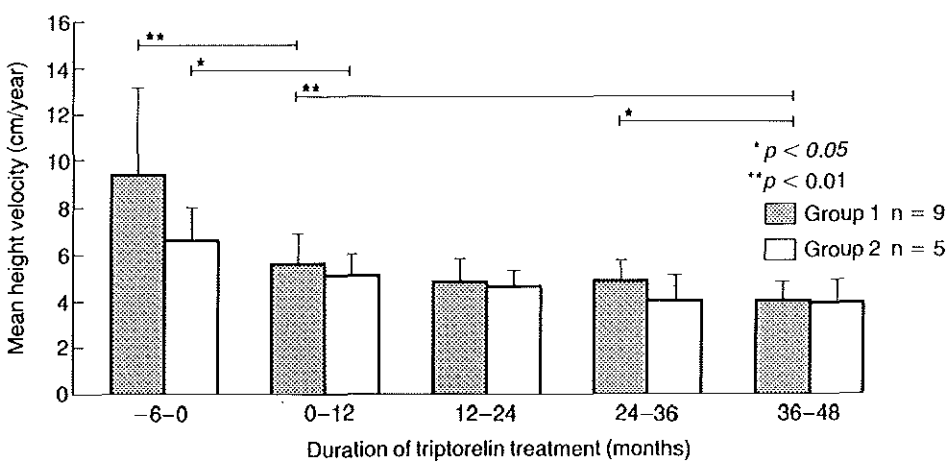

Figure 1. Mean height velocity in girls ($n = 14$) with CPP during GnRH agonist treatment. The first pair of colomns indicates mean height velocity for the 6 months prior to the start of treatment.

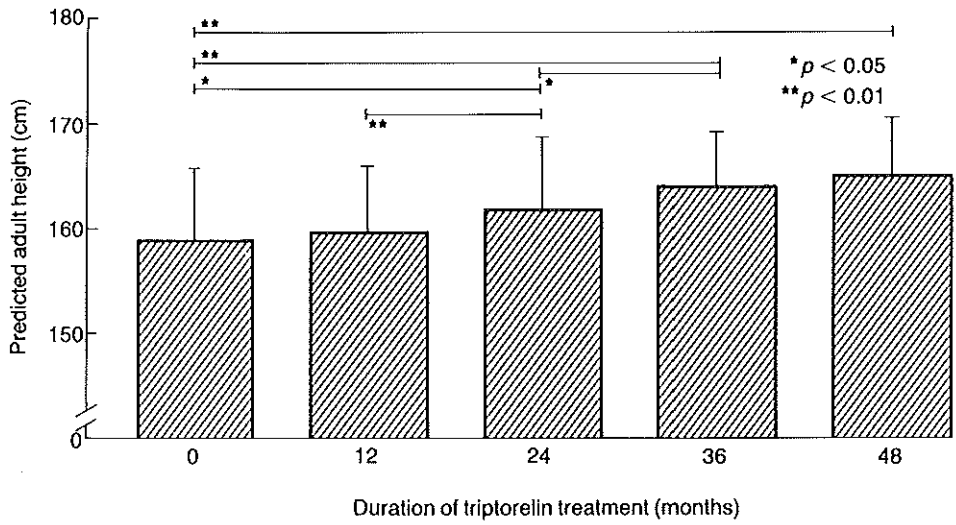

Figure 2. Mean predicted adult height in girls ($n = 14$) with CPP during GnRH agonist treatment.

Auxological data after discontinuation of GnRH agonist treatment

The mean CA of 12 girls who discontinued treatment with triptorelin after a mean period of 2.2 ± 0.5 years was 10.5 ± 0.5 years; BA was 12.3 ± 0.6 years. During treatment, an improvement in predicted adult height was observed, but this increase was not significant (Table 1). The patients were followed up for12 months ($n = 12$), 18 months ($n = 8$) and 24 months ($n = 4$) after discontinuation of treatment. Progression of breast development and pubic hair were observed in all cases. In 11 girls, menses started 9.8 ± 3.7 months (range 4-15 months) after discontinuation of treatment. The remaining patient had not reached menarche 15 months after cessation of treatment. In 5 girls, luteinizing hormone-releasing hormone (LHRH) tests were performed after cessation of triptorelin treatment: all were within the pubertal range [19].

There were slight, but not significant, changes in bone maturation and predicted adult height within the first 12 months after discontinuation of triptorelin treatment, compared with the last year of treatment (Table 1). Height velocity increased significantly in the first 6 months following cessation of treatment, from 3.6 ± 1.0 to 5.4 ± 2.5 cm/year ($p < 0.05$). During the next 6 months height velocity decreased slightly, but still remained higher than that recorded in the second year of triptorelin treatment.

In the girls followed up at 18 months after discontinuation of treatment, height velocity showed an initial increase in the first and second 6-month periods, and a significant decrease to 3.3 ± 1.7 cm/year over the subsequent 6 months. In the 4 girls followed up for 24 months after discontinuation of therapy, height velocity

decreased over the final 6-month period to 2.8 ± 1.9 cm/year; predicted adult height was unchanged.

Table 1. *Auxological data for 12 girls with CPP before and after discontinuation of treatment with triptorelin.*

		During triptorelin treatment		After cessation of triptorelin	
	Start of treatment	Year 1	Year 2	0-6 months	6-12 months
HV (cm/year)	8.0 ± 3.3	5.1 ± 1.3	3.6 ± 1.0	5.4 ± 2.5	4.7 ± 1.8
ΔBA/ΔCA	–	0.6 ± 0.5	0.8 ± 0.5	1.0 ± 0.7	1.0 ± 0.4
PAH (cm)	161.6 ± 8.6	163.9 ± 8.6	163.7 ± 7.7	163.7 ± 8.2	162.2 ± 7.8

PAH, predicted adult height.

Auxological data from patients receiving concomitant GnRH and GH treatment

Concomitant therapy with GH was started in 3 girls who showed a decrease in height velocity after 3 years of treatment with triptorelin, to 3.2-3.6 cm/year, and a predicted adult height less than the 3rd centile for Dutch girls. CA at the start of triptorelin therapy was 6.4-7.8 years; CA and BA at the start of GH administration were 9.6-10.9 years and 11.75-12.5 years, respectively. The GH response to arginine infusion was less than 20 mU/l. Twenty-four-hour GH profiles before the start of GH therapy showed regular but subnormal GH peaks; no peak greater than 20 mU/l was seen during the night and none of the profiles showed a day-night rhythm. Further characteristics of the profiles are given in Table 2.

Height velocity increased markedly in all three girls after the start of GH therapy. Predicted adult height also continued to improve (Table 3). During triptorelin treatment alone for 3.0 years, the predicted adult height increased at a mean rate of 0.7, 3.5 and 2.5 cm/year in patients 1, 2 and 3, respectively. During subsequent concomitant therapy with triptorelin and GH for 18 months, predicted adult height increased at a rate of 1.8, 4.3 and 2.7 cm/year, respectively.

DISCUSSION

The data show that 4 years' treatment with a slow-release preparation of a GnRH agonist (triptorelin) in children with CPP results in a stabilization of height velocity at 4.0 cm/year, and an ongoing improvement in predicted adult height from 158.9 ± 6.8 to 164.9 ± 6.6 cm in girls, who approached their target height of 166.3 ± 5.6 cm. These results are comparable with those of a number

Table 2. *Pertinent data obtained from measurement of 24-hour GH profiles in three girls with CPP and predicted adult height below the third centile after 3 years' treatment with triptorelin.*

Characteristics of the 24-hour GH profiles	Patient 1	Patient 2	Patient 3	Normal values[1]
Mean GH (mU/l)	2.9	3.1	2.0	> 6.0
Maximal GH (mU/l)	11.0	14.0	12.3	> 20.0
AUC_b[2] (mU/l·24 hours)	88.4	97.2	34.2	180-600

1 Normal values were derived from ref. 24.
2 AUC_b area under the curve above baseline.

Table 3. *Auxological data for three girls receiving combined GnRH agonist and GH treatment.*

	Patient 1			Patient 2			Patient 3		
	Height velocity	BA (years)	PAH (cm)	Height velocity	BA (years)	PAH (cm)	Height velocity	BA (years)	PAH (m)
At start of triptorelin treatment	6.7	10.25	149.8	6.9	11.0	133.0	6.7	11.5	146.5
After 3 years of triptorelin treatment	3.6	11.75	152.0	3.2	12.0	143.5	3.5	12.5	153.9
After 12 months of triptorelin + GH treatment	5.3	12.0	156.6	6.3	12.25	148.4	6.8	12.75	157.6
After 18 months of triptorelin + GH treatment	5.9	12.25	157.4[1]	6.7	12.5	150.0[1]	4.0	13.25	157.9[1]

PAH, predicted adult height
1 Target height of patients 1, 2 and 3: 154.5, 166.0 and 163.0, respectively.

of studies which have investigated the effects of long-acting GnRH agonists [1, 2, 4]. The predicted adult height for the girls is higher than the final heights reported for girls with untreated CPP [20] or for girls with CPP treated with cyproterone acetate [21]. Whether the final heights of these girls will reach predicted values will depend on the accuracy of the calculations of predicted adult height in children with advanced BAs, and on the height velocity and rate of bone maturation after discontinuation of treatment with triptorelin.
Twelve girls who discontinued treatment after 2.2 ± 0.5 years showed progressive bone maturation ($\Delta BA/\Delta CA > 1.0$) and a slight, though not significant, decrease in predicted adult height 1 year later. It would therefore appear that the gain in

predicted adult height with GnRH agonist therapy is obtained during treatment and not thereafter. For auxological reasons it may thus be advisable to continue GnRH agonist therapy for as long as possible.

It is of interest to note that this group of girls showed no significant improvement in predicted adult height during triptorelin therapy. One explanation for this phenomenon could be that the girls were older and had higher BAs at the start of treatment compared with those who continued treatment for 4 years.

In some patients, height velocity decreased to less than 4.0 cm/year during treatment with triptorelin and predicted adult height showed either no change or only a slight improvement, which is very unfavourable in children with genetic short stature. In children with delayed puberty, GH secretion is reduced during the years immediately preceding spontaneous puberty. In addition, in some recent studies have been described that the secretion of GH over 24 hours, which is markedly elevated in untreated girls with CPP [22], is reduced to prepubertal levels after 2-6 months of GnRH agonist therapy in most patients [9, 10, 11, 23]. In our patients, 24-hour GH profiles after 3 years of treatment with triptorelin were subnormal, and the responses of GH secretion to arginine stimulation were low. It was therefore considered that addition of GH therapy to triptorelin treatment might improve final height prognosis. This was confirmed in three girls with CPP and predicted adult heights below the third centile, who showed a significant increase in height velocity in the first year of concomitant GH and triptorelin therapy. In two of them, height velocity remained high after 18 months of treatment. Bone maturation was not progressive, and for this reason the combination of triptorelin and GH therapy resulted in a greater improvement in predicted adult height than treatment with triptorelin alone. Is is therefore our intention to continue concomitant therapy with triptorelin and GH in these children.

The subnormaal 24-hour GH profiles and the auxological results in these patients strengthen the argument that GH secretion is subnormal during GnRH analogue therapy. Subnormal GH secretion could explain the subnormal height velocity observed during treatment, but it offers no explanation for the fact that serum IGF-I values remain elevated during GnRH agonist treatment [24]. Our results give support to the idea that concomitant GH and GnRH agonist therapy can be beneficial for children with CPP and familial short stature with a low height velocity and predicted adult height. However, more extensive studies are required. Only when final height has been attained in a large number of patients definite conclusions can be drawn, concerning the auxological effectiveness of GnRH analogue treatment of children with CPP.

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Chapter 5

GROWTH AND PUBERTAL DEVELOPMENT DURING AND AFTER TREATMENT WITH A SLOW-RELEASE GNRH-AGONIST IN CENTRAL PRECOCIOUS PUBERTY

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ABSTRACT

The auxological data of 25 patients (21 girls, 4 boys) with central precocious puberty (CPP), treated for 4 years with a slow-release GnRH-agonist [Decapeptyl-controlled release (D-CR) 3.75] every 4 weeks intramuscularly, and of 6 patients (3 girls, 3 boys), treated for 5 years, are presented.

After 3 years of D-CR a stabilization of height velocity (HV) at about ± 4 cm/year was observed. Bone maturation (ratio of change in bone age to change in chronological age; ΔBA/ΔCA) slowed down to a mean ΔBA/ΔCA ratio of 0.5 ± 0.2 (mean ± SD) measured over 48 months. As a result, predicted adult height (PAH) improved from 156.3 ± 7.4 to 162.2 ± 6.8 cm in girls ($p < 0.001$) and from 174.4 ± 18.6 to 184.3 ± 17.1 cm in boys after 4 years. In the 5th year an ongoing improvement of PAH was observed.

Twenty additional girls discontinued D-CR for at least 12 months after treatment with D-CR for 2 years or more. In 11 girls menses started after 10.6 ± 3.1 months; nine girls had no menarche after 12-16 months. HV increased in the first and second 6 months to a level of about 6.0 cm/year, decreased in the third 6 months after cessation to the level before discontinuing D-CR and decreased further afterwards. Bone maturation (ΔBA/ΔCA) increased progressively in the first 18 months after discontinuation, with a stabilization at about 1.3. PAH did not change in the first 12 months after discontinuation of D-CR, but showed a decrease afterwards.

We conclude that D-CR treatment is very effective in the long-term suppression of gonadal activity of children with central precocious puberty, resulting in a stabilization of height velocity and bone maturation. After treatment the resumption of gonadal activity results in progression of puberty. Preliminary auxological data suggest that the remaining growth potential may be reduced.

INTRODUCTION

Agonists of gonadotropin-releasing hormone (GnRH) have been used since about one decade in the treatment of central precocious puberty (CPP). Different routes of administration have been used, resulting in a suppression of the gonadarche. Previously, we and others have reported that, as a result of the suppressed secretion of the gonadal steroids, the height velocity (HV) is decreased as compared to pubertal pretreatment values and is maintained at a low prepubertal rate of approximately 4 cm/year [1-4]. Concomitantly the rate of bone maturation is decreased, resulting in an improvement of predicted adult height (PAH) in some but not all patients [1-7]. In this report we present the individual data of 25 children with CPP treated for up to 5 years with the slow-release GnRH agonist *D*-Trp^6-luteinizing-hormone-releasing-hormone (Decapeptyl-controlled release [D-CR]). As the calculation of PAH is based on the presumption that the remaining growth potential is unaffected by gonadal-suppressive therapy, it is highly relevant to study growth after discontinuation of the GnRH agonist treatment. Therefore, we report the auxological data of 20 girls who have been treated for 2 years or more with this slow-release GnRH agonist and present follow-up data up to 36 months after discontinuation of treatment.

PATIENTS AND METHODS

Patients

As part of an ongoing international Dutch/German multicenter study in children with CPP treated with D-CR 3.75 (triptorelin; Cytotech, Martigny, Switzerland) every 4 weeks intramuscularly (60-120 ug/kg), auxological data were obtained in 25 patients (21 girls, 4 boys) treated for 4 years and in six patients (3 girls, 3 boys) treated for 5 years. The diagnosis CPP was based upon criteria as detailed previously [8]. The hormonal data, including the sustained suppression of the luteinizing hormone response after GnRH administration, have been reported for this group of patients [8]. In the group with 4-year results mean chronological age (CA) at the start of D-CR was 6.7 ± 1.3 years in girls (n = 21) and 6.2 ± 1.5 years in boys (n = 4); mean bone age (BA) at the start of therapy was 10.2 ± 0.9 and 10.9 ± 2.6 years, respectively. Fifteen patients (14 girls, 1 boy) had received no previous treatment for CPP prior to the study; 10 patients (7 girls, 3 boys) had received treatment previously, either with cyproterone acetate (CPA; 2 boys) or with a short-acting GnRH agonist (buserelin, Hoechst, Frankfurt, FRG) for a period of 7-36 months. In the group with 5-year results, the mean CA at the start of therapy was 5.5 ± 0.8 years in girls and 5.7 ± 1.0 years in boys with a BA of 9.5 ± 1.0 and 10.5 ± 2.5 years, respectively.

Auxological data were also obtained from 20 additional girls who started the

multicenter study but discontinued treatment after at least 2 years. The periods of follow-up ranged from 12 to 36 months. Treatment was stopped when psychosocial indications for the suppression of pubertal development were no longer present.

Methods

Height and pubertal stage, according to Tanner, were assessed every 3 months. X-rays of the left hand were taken every 6 months and determined by one observer, according to the method of Greulich and Pyle [9]; adult height was predicted according to Bayley and Pinneau [10]. As a measure of bone maturation we used the ratio of the change in bone age to the change in chronological age (ΔBA/ΔCA) between the start and after 4 years of treatment. We consider a value of 1.0 as normal. All X-rays of the hand obtained every 6 months were determined serially. Therefore, the ratio ΔBA/ΔCA is a reflection of bone age development during this 4-year period and is not a reflection of only two X-rays of the hand obtained at 0 and 48 months.
Target height (TH) was calculated with the formula ([height father] + [height mother] ± 12)/2 ± 3 cm according to Tanner [11], adjusted for the Dutch/German population.
All results are expressed as mean ± standard deviation. Non-parametric statistical methods of analysis were used to assess significance (Friedman-test). The Mann-Whitney U test and Wilcoxon matched-pairs signed rank test were used for between-group and within-group changes, respectively. The correlations between various parameters were calculated by Spearman's correlation test.

RESULTS

Auxological data after 4 and 5 years of GnRH-agonist treatment

In the girls treated for 4 years ($n = 21$), HV decreased significantly from 9.9 ± 4.1 cm/year in the 6 months before D-CR to 5.2 ± 1.0 cm/year in the 1st year ($p < 0.001$), with a gradual decrease to 3.7 ± 0.7 cm/year in the 4th year ($p < 0.001$). In the boys ($n = 4$), HV decreased from 9.6 ± 4.5 cm/year before treatment to 5.1 ± 2.6 cm/year in the first year and 4.4 ± 0.3 cm/year in the 4th year (n.s.).
The mean ΔBA/ΔCA ratio calculated over 4 years of D-CR treatment was 0.5 ± 0.2 (range 0.2-0.8) in girls and 0.5 ± 0.3 (range 0.2-1.0) in boys, i.e. significantly lower than normal. In the girls HV decreased, but ΔBA/ΔCA decreased even more, resulting in a significant increase in PAH from 156.3 ± 7.3 cm at the start of therapy to respectively 158.1 ± 6.7 ($p < 0.01$), 160.0 ± 7.7 ($p < 0.001$), 161.8 ± 6.8 ($p < 0.001$) and 162.2 ± 7.5 cm ($p < 0.001$) after the 1st, 2nd, 3rd and 4th year, respectively. This constitutes an increase of almost 6 cm over the 4 years treatment period. The PAH of 162.2 cm after 4 years of treatment

is well within the range of the TH of 164.8 ± 6.4 cm. The actual height rose from 131.8 ± 7.5 at the start to 149.4 ± 6.9 cm after 4 years. In boys a nonsignificant increase of the PAH from 174.4 ± 18.6 to, respectively, 176.9 ± 20.9, 180.6 ± 20.2, 183.5 ± 18.5 and 184.3 ± 17.1 cm after 4 years was observed. These values are well comparable with the range of the TH of 176.3 ± 4.9 cm. This constitutes an increase of almost 10 cm over the 4 years treatment period.
As illustrated in table 1, HV was significantly higher in the girls with BA < 10 years at the start of therapy than in the girls with BA 10-11 and 11-12 years ($p < 0.01$). The ΔBA/ΔCA ratio was highest in the girls with the youngest BA as compared with the girls with oldest BA ($p < 0.01$). As a result of this antagonizing phenomenon the ΔPAH did not show significant differences.
In the group of 6 patients treated for 5 years, the mean HV in the 5th year was maintained at the same level as in the 3rd and 4th year: 4.0 ± 1.1 cm/year ($n = 3$) in girls and 4.0 ± 1.5 cm/year ($n = 3$) in boys. In addition, an ongoing improvement of PAH was observed (n.s.).

Clinical and auxological data after cessation of GnRH-agonist treatment

After discontinuation of GnRH-agonist therapy, 20 girls were followed up to 36 months (table 2). In all girls progression of breast and pubic hair development

Table 1. *Auxological data during 48 months of treatment with D-CR for 21 girls divided in 3 different groups, according to the BA at the start of treatment.*

BA at start years	n	HV cm/year	ΔBA/ΔCA	ΔPAH cm	TH cm
< 10	6	5.2 ± 0.3	0.64 ± 0.06	4.4 ± 2.7	163.4 ± 3.0
10-11	11	4.3 ± 0.4	0.50 ± 0.14	5.7 ± 4.2	164.5 ± 7.7
11-12	4	3.8 ± 0.6	0.33 ± 0.11	8.4 ± 3.7	168.3 ± 6.5

Table 2. *Characteristics for girls treated with D-CR and with a follow-up period of 12-36 months after discontinuation of D-CR.*

Period after cessation of D-CR	Patients	Duration of D-CR	At start of D-CR		At cessation of D-CR	
months		years	CA (years)	BA (years)	CA (years)	BA (years)
12	20	3.1 ± 0.8	8.1 ± 1.0	10.9 ± 0.8	10.8 ± 0.8	12.4 ± 0.5
18	8	2.7 ± 0.6	8.0 ± 0.6	10.6 ± 0.7	10.6 ± 0.5	12.2 ± 0.3
24	6	2.4 ± 0.5	8.1 ± 0.5	10.4 ± 0.5	10.5 ± 0.6	12.1 ± 0.2
30	5	2.3 ± 0.5	8.1 ± 0.5	10.3 ± 0.5	10.4 ± 9.7	12.1 ± 0.2
36	2	1.9 ± 0.2	8.2 ± 0.5	9.9 ± 0.5	11.0 ± 0.0	12.3 ± 0.4

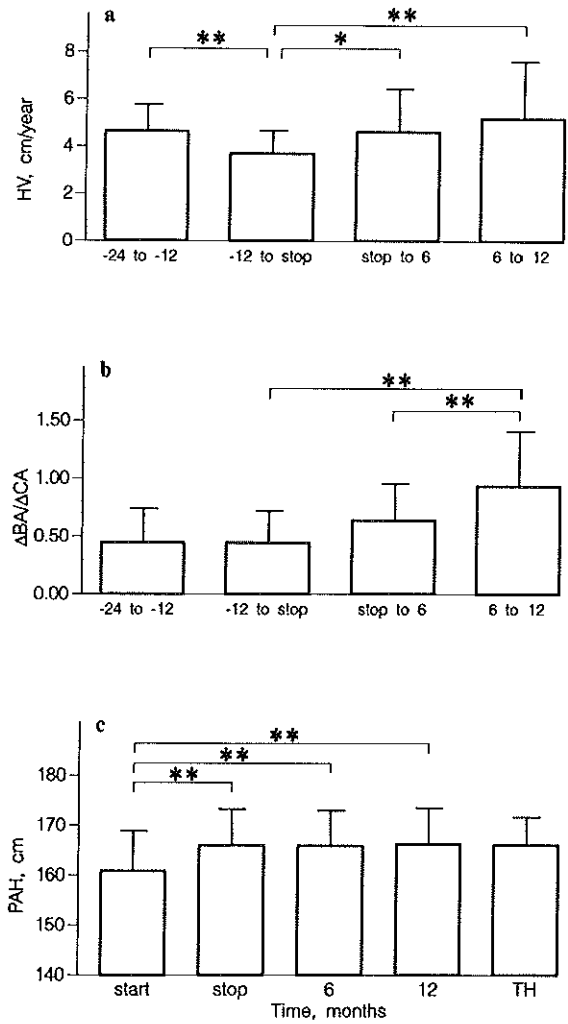

Figure 1. Mean HV (**a**), bone maturation (**b**) and PAH (**c**) for 20 girls before and 12 months after discontinuation of D-CR treatment.
* $p < 0.05$; ** $p < 0.01$.

was observed. Menses started 10.6 ± 3.1 months after discontinuation (range 5.0-15.0 months) in 11 girls. The other 9 girls did not reach menarche at the last visit of 12-16 months after discontinuation.

In the 20 girls with a follow-up period of 12 months, a significant increase in HV was observed from 3.8 ± 1.0 to 4.8 ± 1.8 cm/year in the first 6 months after cessation; in the second 6 months HV did not change significantly (figure 1a). ΔBA/ΔCA increased immediately from 0.46 ± 0.26 to 0.93 ± 0.48 ($p < 0.01$) within the first 12 months after discontinuation (figure 1b). No change in PAH

was observed (figure 1c). In the group of 8 girls with a follow-up of 18 months and in the 6 girls with 24-months results, the same pattern of increase of mean HV and ΔBA/ΔCA and a nonsignificant decrease of PAH in the 1st year after cessation was observed. After 1 year of discontinuation, HV decreased and ΔBA/ΔCA increased in the same way as was observed in the 5 girls who discontinued D-CR for 30 months (figure 2a, b). In these 5 girls PAH decreased from 165.3 ± 8.9 to 162.5 ± 7.6 cm (Friedman $p < 0.05$). After 30 months of

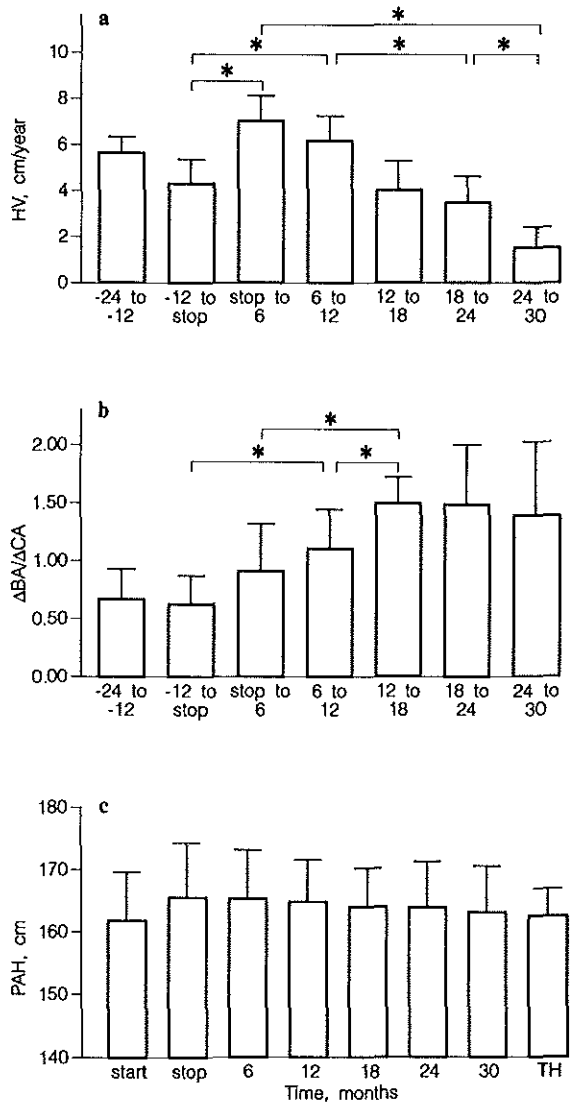

Figure 2. Mean HV (a), bone maturation (b) and PAH (c) for 5 girls before and 30 months after discontinuation of D-CR treatment. * $p < 0.05$

discontinuation the PAH of 162.5 cm was in the same range as at the start of D-CR treatment and as theTH (figure 2c).
No significant correlation was found between CA or BA at the time of discontinuation of treatment or the duration of D-CR treatment and the ΔPAH after treatment.

DISCUSSION

Treatment of girls with central precocious puberty with a slow-release preparation of a GnRH agonist (D-CR 3.75) results in a stabilization of the HV at about 4 cm/year and an improvement in PAH from 156.3 ± 7.4 to 162.2 ± 6.7 cm after 4 years, i.e. within the range of the TH of 164.8 ± 6.4 cm. These data are comparable with the 4-year results of a smaller number of children treated with D-CR [1] and with the long-term results of studies with the daily subcutaneously administered GnRH agonists [3, 4, 12]. The PAH is also higher than the observed final height described for girls treated with CPA [13] or untreated girls [14, 15], but in the same range as the untreated and CPA-treated girls described in a recent study [16]. It should be noted that the PAH is a theoretically calculated parameter. So, differences between PAH and observed final height could be due to such methodologic problems. PAH is influenced by HV and bone maturation. In our study, bone maturation slowed markedly during treatment, as did HV. The mean values of HV and bone maturation were different when girls were divided in different BA groups, based on the BA at the start of therapy: in the girls with a younger BA at the start of treatment (BA < 10 years), HV was significantly higher but bone maturation was more progressive during treatment compared with the girls with an older BA (BA 11-12 years), as was observed by Boepple et al. [2, 12] too. This resulted in a similar gain of PAH, although in our study the oldest BA group seems to have a better improvement of PAH (table 1).
The calculation of PAH is also based on the presumption that the remaining growth potential is unaffected during and after discontinuation of gonadal-suppressive therapy. In our study, after discontinuation of D-CR, HV increased in the 1st year with a decrease afterwards, but bone maturation progressed immediately. This resulted in an ongoing decrease of PAH after 18 months of discontinuation. Thirty months after cessation PAH was in the same range as PAH before the start of D-CR, suggesting that the remaining growth potential may be reduced. At this moment no correlation could be demonstrated between BA or CA at the moment of discontinuation of treatment and ΔPAH. For this reason the question at which BA or CA GnRH-agonist treatment should be discontinued, still remains a clinical dilemma.
Our results are not comparable with the data from other studies, in which no decrease in PAH was observed in girls off treatment [6, 17, 18]. At this moment there is only one report on final height in a limited number of girls after treatment with a slow-release GnRH agonist indicating a positive influence on

final height [6]. Only when final height will be attained in a larger number of patients a definitive answer about the influence of GnRH agonist treatment on height can be given.

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Chapter 6

HORMONAL EVALUATION DURING AND AFTER LONG-TERM TREATMENT WITH A SLOW-RELEASE GNRH-AGONIST OF CHILDREN WITH CENTRAL PRECOCIOUS PUBERTY; EFFECT ON FINAL HEIGHT

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INTRODUCTION

For more than a decade children with central precocious puberty (CPP) have been treated with gonadotropin releasing hormone (GnRH) agonists. While GnRH agonists with variable potencies and with different routes of administration have been used [2, 5, 8, 9, 10, 12, 18, 19], most published studies report results of agonists given by daily injections or nasal insufflations. Only limited data are available about slow-release depot preparations [8, 12, 19]. In this paper we present the data from a Dutch-German multicenter study in children with CPP who were treated with the slow-release GnRH agonist triptorelin. We report hormonal data during treatment, recovery of the hypothalamic-pituitary-gonadal axis and gonadal function after discontinuation of treatment. In addition, auxological data up to final height are presented.

PATIENTS AND METHODS

One hundred and four patients (93 girls, 11 boys) with central precocious puberty are participating in a Dutch-German multicenter study, using the slow-release GnRH agonist triptorelin (D-Trp^6-LHRH) given intramuscularly every four weeks (mean dosage ± SD has been 96 ± 25 ug/kg/4 weeks). Inclusion criteria for this protocol have been described previously [13]. Hormonal data are presented for 76 patients (71 girls, 10 pretreated with buserelin before starting triptorelin and 5 boys, 1 pretreated). Because of the small number of boys we will focus on the results of the girls. For both sexes, chronological age (CA) at start was 6.5 ± 2.0 years. The patients were treated for a period of 3.2 ± 1.6 years (range 12-72 months) and had a follow-up period of 2.8 ± 1.8 years after discontinuation of triptorelin. Hormonal evaluation including a GnRH-test took place before the start of triptorelin, every six months during treatment and at least one year after discontinuation of treatment. The GnRH test was performed as a capacity test using 60 ug Luliberin per square meter of body surface i.v. (Lutrelef, Ferring, Kiel, Germany). Plasma samples were collected before (basal) and 30 min (stimulated) after injection of Luliberin. All hormonal samples were centralized and determined by the same assay. All hormonal data are presented cross-sectional and expressed as mean ± SEM.
Plasma gonadotropin levels were determined using monoclonal immunoradiometric assays (MAIA Clone, Serono Diagnostika, Freiburg, Germany) [14]. Stimulated LH levels of less than 5.0 IU/L and stimulated follicle-stimulation hormone (FSH) values less than 15.0 IU/L were considered prepubertal [14]. Plasma estradiol was measured by a sensitive double-antibody RIA (Diagnostic Products Corporation, Los Angeles, CA, USA). In girls, estradiol values less than 15 pg/ml were considered prepubertal. Plasma testosterone was determined by RIA after extraction with methylene chloride and subsequent isolation by Sephadex LH-20 chromatography [16]. In boys, testosterone values less than 35 ng/dl were considered prepubertal.

Dehydroepiandrosterone-sulphate (DHEAS) was measured using a nonextractive, solid-phase RIA (Diagnostic Products Corporation).
Auxological data were obtained in 31 patients (26 girls and 5 boys), who achieved final height (FH). All auxological data are expressed as mean ± SD. Height was expressed as SD score for chronological age (SDS_{CA}), using the most recent Dutch national growth references [15]. Because of the small number of boys only the results for the girls are described in detail. CA for the girls at the start of treatment was 7.8 ± 0.8 years, bone age was 10.8 ± 0.7 years and height SDS 2.2 ± 0.9. Girls were treated for at least two years, with a treatment period of 3.3 ± 1.0 years. After discontinuation of triptorelin they were seen every year till FH was achieved. FH was considered to be achieved when bone age was ≥ 15.0 years in girls and ≥ 16.0 years in boys and/or when height velocity was less then 1.0 cm/year over a period of one year. Bone age was determined by one observer according to the method of Greulich and Pyle and adult height prediction according to the tables of Bayley and Pinneau [6]. When FH had been achieved, pelvic ultrasonography was performed with Acuson equipment (MountainView, California) using 3.5 or 5 MHz transducers.
For the statistical analysis, Student's *t*-test was used for within-group changes of the growth data.
Written informed parental consent was obtained before the start of treatment. The study was approved by the local Ethical Committees.

RESULTS

Hormonal evaluation

Before the start of treatment, stimulated LH levels among both sexes were 36.4 ± 3.2 IU/L, which is considerably higher than stimulated LH values during normal puberty (range B_2-B_4: 3.3-33.2 IU/L [14]). After six months of therapy mean stimulated LH levels were suppressed (< 5.0 IU/L), and they remained suppressed during treatment (Fig. 1). The percentage of children with a stimulated LH value less than 5.0 IU/L varied from 76 till 100% (Table 1). In the children with biochemical escapes (LH > 5.0 IU/L) it was retrospectively found that the preceding injection intervals had exceeded the scheduled 28 days by at least 72 hours (poor-compliance families). Twelve months after discontinuation of treatment, stimulated LH levels were 21.1 ± 3.2 IU/L, i.e. in the normal pubertal range.
Basal and stimulated FSH levels showed a similar pattern, although stimulated FSH before therapy (14.8 ± 1.1 IU/L) were in the normal pubertal range, as they were a year after discontinuation (9.5 ± 0.6 IU/L).
Before the start of treatment, plasma estradiol levels were elevated (19 ± 3 pg/ml, $n = 58$). They declined to prepubertal values (6 ± 1 pg/ml after six months, $n = 64$) and remained suppressed during treatment. The percentage of girls with prepubertal estradiol levels during therapy varied from 97 to 100% during the

treatment period from 6 to 42 months. After 48 months 82% of the girls had estradiol levels less than 15 pg/ml. After discontinuation of triptorelin, estradiol levels increased to pubertal values. None of the girls with LH levels > 5.0 IU/L had clinical signs of puberty or elevated estradiol levels > 15 pg/ml.
In boys, plasma testosterone levels decreased from 132 ± 44 ng/dl before therapy to 6 ± 3 ng/dl after one year of therapy and remained in the prepubertal range throughout treatment.
Plasma DHEAS levels increased gradually from 411 ± 47 ng/ml (n = 55) before therapy to 1200 ± 183 ng/ml (n = 11) after four years of treatment.

Table 1. *Basal and peak LH and FSH levels (IU/L) and the percentage of suppression during triptorelin therapy among female and male patients.*

		LH			FSH	
		basal	peak	% peak values < 5 IU/l	basal	peak
pretreatment	(n = 61)	3.2 ± 0.4	36.4 ± 3.2		4.6 ± 0.3	14.8 ± 1.1
month 6	(n = 71)	2.3 ± 0.2	3.7 ± 0.4	76%	1.5 ± 0.2	2.6 ± 0.4
month 12	(n = 71)	1.5 ± 0.2	3.0 ± 0.4	78%	1.4 ± 0.1	3.1 ± 0.8
month 18	(n = 65)	1.0 ± 0.1	1.8 ± 0.3	96%	1.5 ± 0.1	2.1 ± 0.3
month 24	(n = 59)	1.8 ± 0.7	2.5 ± 0.5	84%	2.2 ± 0.4	3.4 ± 0.6
month 30	(n = 33)	0.7 ± 0.1	1.0 ± 0.3	100%	1.3 ± 0.1	2.7 ± 0.4
month 36	(n = 32)	0.9 ± 0.3	1.4 ± 0.5	93%	1.3 ± 0.1	2.1 ± 0.3
month 42	(n = 24)	0.9 ± 0.4	1.3 ± 0.5	100%	1.0 ± 0.1	1.9 ± 0.3
month 48	(n = 21)	0.7 ± 0.2	0.9 ± 0.2	100%	1.2 ± 0.2	1.5 ± 0.3

Auxological Evaluation

Bone age (BA) at the start of therapy was 10.0 ± 0.7 years and, after a treatment period of 3.3 ± 1.0 years, the BA was 12.4 ± 0.6 years. The girls had a follow-up period of 3.9 ± 1.0 years after discontinuation of therapy. At the final analysis CA was 15.0 ± 1.1 years and BA 15.9 ± 0.6 years. As shown in Table 2, the predicted adult height (PAH) increased significantly during therapy ($p < 0.001$), but after discontinuation PAH decreased, although FH was significantly higher than PAH at the start of treatment ($p < 0.001$). A mean height gain of 4.5 cm was achieved.
FH was also significantly lower than target height (TH) (168.2 ± 6.7 cm). A negative correlation was observed between BA at the end of treatment and FH ($r = -0.52$, $p = 0.003$). There was also a negative correlation between CA and BA at the end of treatment, and the height increment after treatment ($r = -0.49$, $p = 0.006$, and $r = -0.85$, $p < 0.001$ respectively). No correlation was observed between FH on the one hand, and on the other, CA or BA at the start of therapy, or the duration of therapy. Stepwise multiple regression analysis revealed that height at start of treatment was the most important factor influencing FH.

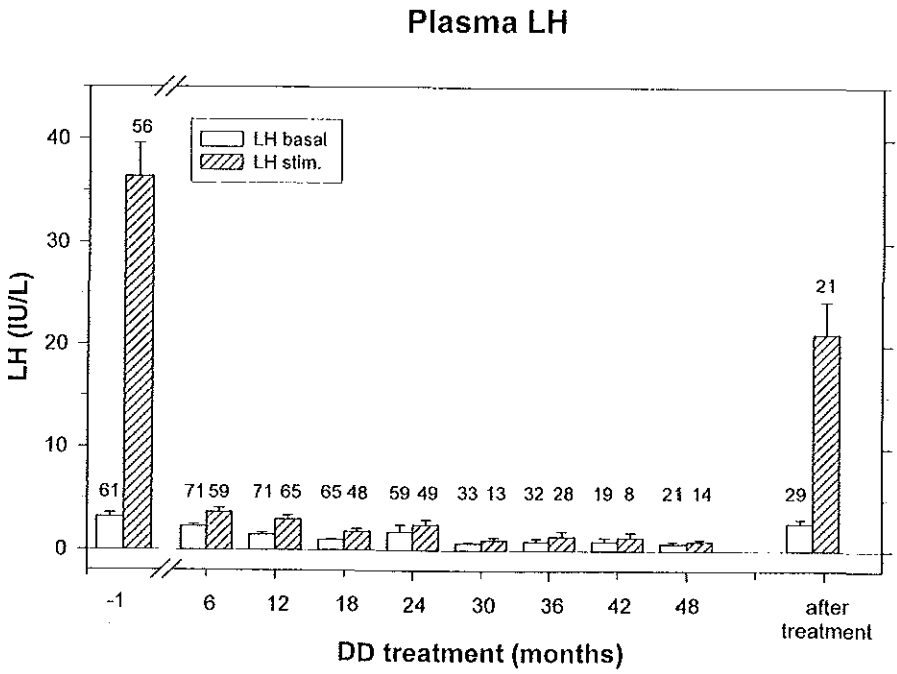

Figure 1. Basal and stimulated plasma LH levels (mean ± SEM) (Patient numbers on top of the bars).

Table 2. *Predicted adult height (PAH) and final height (FH) in girls (n = 26)*

	cm	sds_{CA}
PAH at start of therapy	157.1 ± 7.1	-1.8 ± 1.14
PAH at end of therapy	163.0 ± 7.7	-0.85 ± 1.24
FH	161.6 ± 6.8	-1.2 ± 1.16

Menses and pelvic ultrasound evaluation

Menarche occurred at a mean period of 1.1 ± 0.9 yrs after discontinuation of therapy at a CA of 12.3 ± 1.3 years. At the final visit menses were regular in 23 out of the 26 girls.

Sonography of the uterus revealed a volume of 40.5 ± 18.3 cm^3 (n = 13), which is at the upper limit of the normal adult volume [7]. Ovarian sonography showed that the volumes of the left and right ovaries were 7.8 ± 4.5 cm^3. These volumes are in the normal range (reference value: 7.4 ± 4.8 cm^3 [7]).

In three girls one of the ovaries was larger than normal. In two of these three girls, two to three cysts with a diameter ≤ 0.6 cm were visualised, which is a normal pubertal pattern [1]. In the other girl, 10 cysts ≤ 0.6 cm were observed. Menses were regular in this girl. In the ovaries of the remaining 14 girls a normal pubertal pattern with a maximum of two to six cysts with a diameter ≤ 0.9 cm were observed. Cysts with a diameter of 2.0 cm were seen in only in 2 of these girls, but these were observed 12-14 days after the first day of the menstruation, i.e. at the time of ovulation.

DISCUSSION

During the last decade a variety of GnRH agonists have been used in the treatment of CPP. Triptorelin, as a slow-release depot preparation, has been available in Europe since 1985 [8, 12, 19]. We have previously demonstrated good suppression of the hypothalamic-pituitary-gonadal axis in the first year of therapy [12]. In this paper, the data, as presented in Fig. 1 and Table 1, demonstrate suppression of the hypothalamic-pituitary-gonadal axis throughout treatment in almost all patients except in the non-compliant ones. Plasma estradiol levels were also suppressed into the prepuberal range. Therefore, we can conclude that triptorelin in a dosage of 96 ± 25 μg/kg/4 weeks i.m. is a potent GnRH agonist in children with CPP.

GnRH testing at the start of therapy showed an exaggerated pubertal LH-peak after 30 minutes. The same phenomenon has been reported by the group of Delemarre-Van de Waal [17]. We conclude that girls with CPP do have an abnormal LH response compared with normal pubertal girls at the same stage of puberty. We also conclude that a normal recovery of the hypothalamic-pituitary-gonadal axis occurs after treatment with triptorelin because a normal LH response to GnRH was demonstrated after discontinuation of therapy. The occurrence of regular menses in most of the girls supports this observation.

To date, only limited information on FH is available [3, 4, 8, 10, 11], predominantly after therapy with GnRH agonists used daily. In this study, we have demontrated a mean height gain of 4.5 cm, measured as a difference between PAH at the start of therapy and FH (Table 2). The height gain seemed to be higher during therapy than afterwards, as PAH at the end of therapy was higher than FH. This phenomenon is most probably caused by the decreasing rate of bone maturation during therapy and its re-acceleration after discontinuation of therapy. It should be emphasized that the decrease of bone maturation during therapy occurred despite increasing adrenal androgen (DHEAS) production; thus DHEAS does not seem to have a significant influence on bone maturation. After therapy, plasma concentrations of gonadotropins returned to normal basal and stimulated pubertal levels (Fig. 1), as did estradiol levels. In CPP the rise of gonadotropins and sex steroid levels after discontinuation of therapy is probably less gradual than in normal puberty so that higher levels are reached faster, resulting in a faster bone maturation

than in normal puberty, a shorter period of growth and a FH lower than PAH at the time of discontinuation of therapy.

The height gain we demonstrated is comparable with the reported height gain of 2.4 to 6.4 cm in other studies [3, 4, 8, 10, 11]. The greatest height gain was reported by Oerter *et al* [10]. In that study, the mean age of the girls at the start of therapy was younger (7.2 years), the mean duration of treatment longer (4.0 years), and notably pretreatment height SDS was greater (2.6 SDS). Since, in our study height at the start of therapy was the most important factor influencing FH, this difference probably explains the difference in height gain between our study and that of Oerter *et al* [10].

In our study, menarche occurred 1.1 ± 0.9 years after discontinuation. This was similar to data reported by others [3, 8, 10]. In contrast to Adams *et al* [1], we did not observe polycystic ovaries (PCO).

Thus, in conclusion, slow-release triptorelin suppresses CPP reliably on a long-term basis and is able to increase FH. After the discontinuation of therapy, a normal recovery of the hypothalamic-pituitary-gonadal-axis occur with no sign of abnormalities such as PCO.

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Chapter 7

FINAL HEIGHT IN CENTRAL PRECOCIOUS PUBERTY AFTER LONG-TERM TREATMENT WITH A SLOW-RELEASE GNRH AGONIST

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ABSTRACT

Objective - To study the resumption of puberty and the final height achieved in children with central precocious puberty (CPP) treated with the GnRH agonist triptorelin.

Patients - 31 girls and five boys with CPP who were treated with triptorelin 3.75 mg intramuscularly every four weeks. Girls were treated for a mean (SD) of 3.4 (1.0) years and were followed up for 4.0 (1.2) years after the treatment was stopped.

Results - The rate of bone maturation decreased during treatment and the predicted adult height increased from 158.2 (7.4) cm to 163.9 (7.5) cm at the end of treatment ($p < 0.001$). When treatment was stopped the bone maturation accelerated, resulting in a final height of 161.6 (7.0) cm, which was higher than the predicted adult height at the start of treatment ($p < 0.001$). Height at the start of treatment was the most important factor positively influencing final height ($r = + 0.75$, $p < 0.001$). Bone age at cessation of treatment, negatively influenced final height ($r = -0.52$, $p = 0.03$). A negative correlation between bone age and height increment following Rx was demonstrated ($r = -0.85$, $p < 0.001$). Residual growth capacity was optimal when bone age at cessation of treatment was 12 to 12.5 years or younger. Body mass index increased during treatment ($p < 0.001$) and remained high on cessation. At final height the ratio of sitting height to subischial leg length was normal. Menarche occurred at 12.3 (1.1) years, and at a medium (range) of 1.1 (0.4-2.6) years. The ovaries were normal on pelvic ultrasonography.

Conclusions - Treatment of CPP with triptorelin increases final height, with normal body proportions, and seems to increase body mass index. The best results were achieved in girls who were taller at the start of treatment. Puberty was resumed after treatment, without the occurrence of polycystic ovaries.

INTRODUCTION

For more than a decade children with central precocious puberty (CPP) have been treated with gonadotrophin releasing hormone (GnRH) agonists with variable potencies and different routes of administration. GnRH agonist treatment causes hormonal suppression and thereby influences secondary sexual characteristics, growth, and bone maturation [1- 8].
Some investigators suggest that delaying puberty does not improve final height prognosis, as the decreased rate of epiphysial maturation is offset by a decrease in growth hormone secretion and growth rate [9].
Recently, various studies reporting final height and the reversibility of the gonadal axis on discontinuation of treatment have been published [10-17]. Most of these results are based upon agonist treatment given by daily subcutaneous injections or intranasal application. It was suggested that auxological improvement depends on a continuous and full suppression of gonadal steroid production [18]. Furthermore, improved results have been documented in children with clearly young (bone) ages [19].
We have shown that treatment of children with CPP with the slow-release GnRH agonist triptorelin is effective in continuous suppression of gonadal activity [7, 20].
In this paper we present the data on the resumption of puberty and the adult height attained in children with CPP who were treated with the slow release GnRH agonist triptorelin.

METHODS

Patients

Thirty six patients (31 girls and five boys) with CPP achieved a final height following treatment with triptorelin (D-Trp6-LHRH) at a mean dose of 96 (SD 25) µg/kg intramuscularly repeated every four weeks. The diagnostic criteria for CPP have been described in the past and are summarized as follows: the appearance of secondary sexual characteristics before the age of 8 and 9 years in girls and boys respectively, a bone age/height age ratio ≥ 1, including a pubertal luteinizing hormone (LH) response in a GnRH test [7]. Twenty eight patients (25 girls and three boys) received only triptorelin, whereas eight patients (six girls and two boys) were treated with buserelin subcutaneously (27-30 months) or cyproterone acetate orally (6-40 months) before starting triptorelin.
A computerised tomographic (CT) scan or magnetic resonance imaging (MRI) of the brain, or both, was performed in all patients: in 24 girls CPP was considered idiopathic, in four girls there was infantile encephalopathy, two girls had been operated on for an astrocytoma, and in one girl a harmatoma was found. In none of the girls with organic CPP growth hormone secretion was evaluated. Three of the five boys were suffering from neurofibromatosis, one

boy was operated on for an arachnoid cyst and in one boy the CPP was idiopathic. None of the boys with neurofibromatosis underwent radiotherapy or surgery. Because no growth impairment was observed in these three boys, growth hormone secretion was not evaluated. The boy operated on for an arachnoid cyst was evaluated before GnRH agonist treatment was started, and a normal growth hormone response to arginine stimulation (GH > 20mU/l) was shown.
The patients were treated for at least 2 years. After discontinuation of triptorelin they were evaluated each subsequent year until the final height was achieved. Final height was considered to be attained when the bone age was ≥ 15.0 years in girls and ≥ 16.0 years in boys or when height velocity was < 1.0 cm/year for a minimum period of one year, or both. Once the final height was attained, pelvic ultrasonography was performed using Acuson equipment (Mountain View, California) with 3.5 or 5 MHz transducers.

Growth evaluation

On initiating the study and subsequently every three months during the treatment and every six to 12 months after the treatment was discontinued, the patients underwent a physical examination including measurement of height, sitting height, weight, and recording of secondary sexual characteristics. Weight was expressed as a quotient using the body mass index (BMI) (weight/height^2) and BMI SD score for chronological age (BMI SDS_{CA}) and bone age (BMI SDS_{BA}), using French reference values [21].
Height was expressed as SD score (SDS) for chronological age and bone age, using national growth references [22]. When measured parental heights were available, target height (TH) was calculated using the following formula: (sum of the height of the parents ± 12)/2 + 3 cm. Target height range was defined as target height ± 1 SD. For adult females 1 SD amounts to 6.2 cm [22].
The ratio of sitting height to subischial leg length was expressed as SD score for chronological age (SH/LL SDS_{CA}) using reference values obtained from the Zürich longitudinal growth study [23].
Bone age (BA) was determined by one observer (WO) according to the method of Greulich and Pyle and predicted adult height was determined using the tables of Bayley and Pinneau [24].
Strictly speaking, adult height prediction is only applicable under physiological circumstances, and has an inherent tendency to overestimation in CPP. However, changes in predicted adult height can reasonably be used as a measure of efficacy using treatment such as GnRH agonist therapy. In this study we have defined change in predicted adult height as the difference between final height and the predicted adult height at the start of treatment.

Statistical analyses

All results are expressed as mean (SD), unless indicated otherwise. In the auxological evaluation the Wilcoxon signed rank test was used to determine differences within each group and the Mann-Whitney U test was used for determining differences between groups. Pearson correlation analysis was carried out to assess linear associations. To determine the effects of a number of auxological variables upon final height, a stepwise multiple regression analysis was performed using final height as the dependent variable. The variables were chronological age, bone age, height and BMI (all at the start of treatment); bone age at the time of discontinuation of treatment; target height, duration of treatment, and chronological age at attainment of final height.
Written informed parental consent was obtained before the start of treatment. The study was approved by the ethics committees of the participating university hospitals.

RESULTS

During treatment, regression in secondary sexual characteristics occurred because of prepubertal oestradiol and testosterone levels in girls and boys, respectively, as has been reported in the past [7, 20]. No local or systemic side effects were observed. One girl developed type 1 diabetes mellitus several months after discontinuation of triptorelin.

Auxological evaluation

No significant differences in chronological age, bone age, height, predicted adult height at the start of treatment, or in final height and target height were found between the group of previously untreated patients compared to the previously treated group (Mann-Whitney U test). Therefore, all data were compiled together.

Baseline data

In girls, chronological age at the start of triptorelin treatment was 7.7 (0.8) years (table 1). The onset of puberty as reported by the parents was 6.0 (2.0) years. In boys, chronological age at the start of treatment was 7.9 (1.9) years.
The actual height at the start of treatment in girls is reported in table 1. It was 140.1 (8.4) cm (+ 2.10 [2.21] SDS) in boys.
Patients were treated for a period of 3.4 (1.1) (F) and 4.2 (0.8) (M) years, respectively, and were followed up for 4.0 (1.2) (F) and 3.9 (0.9) (M) years after the discontinuation of treatment.

Table 1. *Effect of triptorelin treatment in girls on mean (range or SD) chronological age, bone age, actual height, predicted adult height, height SD score for chronological age and bone age, sitting height/leg length SD score for chronological age and body mass index SD score for chronological and bone age, evaluated at the start and at the end of treatment and at final height.*

	n	Start of treatment	End of treatment	Last visit
Chronological age (years)	31	7.7(5.1-9.2)	11.1(9.4-13.1)	15.1(12.7-17.5)
Bone age (G&P) (years)	31	10.8(9.4-12.5)	12.5(11.5-13.5)	16.0(15.0-17.0)
Actual height (cm)	31	137.3(117.9-152)	152.4(140.6-161.8)	161.6(146-174.9)[a,b]
Predicted adult height (cm)	31	158.2(145.5-176)	163.9(148.7-181.6)[a]	-
Height SDS_{CA}	31	1.60(1.21)	0.58(1.10)[c]	-1.08(1.12)[c,d]
Height SDS_{BA}	31	-1.31(0.95)	-0.71(0.98)[c]	-1.08(1.12)[d,e]
Sitting height/Leg lenght SDS_{CA}	15	-0.36(1.53)	0.06(1.21)	-0.08(0.97)
Body mass index as: - SDS_{CA}	30	1.70(1.31)	1.80(1.41)	1.44(1.46)
- SDS_{BA}	30	0.66(1.05)	1.29(1.29)[c]	1.44(1.46)[c]

a = $p < 0.001$ compared with predicted adult height at the start of treatment; b = $p < 0.001$ compared with predicted adult height at the end of treatment; c = $p < 0.001$ compared with the start of treatment; d = $p < 0.001$ compared with the end of treatment; e = $p < 0.05$ compared with the start of treatment.

Table 2. *Height velocity and bone maturation (Δ bone age/Δ chronological age) during and after discontinuation of triptorelin treatment in girls*

	n	Heightvelocity (cm/year)	*n*	Δ Bone age/ Δ Chronological age
Whole treatment period	31	4.5 (0.9)	31	0.52 (0.21)
Last year of treatment	25	3.7 (1.0)	19	0.47 (0.34)
1rst year after stopping treatment	25	4.8 (1.8)*	19	0.81 (0.36)*
2nd year after stopping treatment	25	2.9 (1.5)$#	19	1.34 (0.36)$#

* = $p < 0.001$ compared with the last year of treatment; $ = $p < 0.01$ compared with the 1rst year after stop of treatment: # = $p < 0.01$ compared with the last year of treatment.

Height velocity

Before initiating treatment the height velocity was 8.0 (2.3) cm/year in girls and 10.4 (4.8) cm/year in boys.

In girls, during treatment height velocity decreased to low values in the last year of treatment (table 2). An increase in height velocity was only observed in the first year after discontinuation of treatment. In boys the same pattern was observed (data not shown).

Change in bone maturation and predicted adult height

Bone age at the start of treatment was 10.8 (0.7) years in girls (table 1) and 11.0 (2.6) years in boys. In girls, bone maturation expressed as Δ bone age/Δ chronological age ratio decreased during treatment (table 2) resulting in an improvement of predicted adult height at the start of treatment compared to predicted adult height at the point of discontinuation of treatment (table 1) (fig 1). After discontinuation of treatment bone maturation accelerated inmediately in the first year, with higher Δ bone age/Δ chronological age ratios in the second year (table 2).
In boys, the Δ bone age/Δ chronological age ratio during treatment was 0.56 (0.34), resulting in an improvement of predicted adult height from 177.4 (12.6) cm at the start of treatment to 182.5 (15.7) cm at the point of cessation of treatment (fig 1). After discontinuation of treatment the same pattern was observed as in girls (data not shown).

Final height

In girls, the changes in height velocity and bone maturation resulted in a final height of 161.6 (7.0) cm, which was less than that predicted at cessation of treatment (163.9 [7.5]) and less than target height (168.7 [6.4] cm)(+ 0.06 [1.03] SDS)(n = 26), but higher than predicted adult height at the start of treatment (158.2 [7.4] cm) (fig 1)(table 1). A positive change in predicted adult

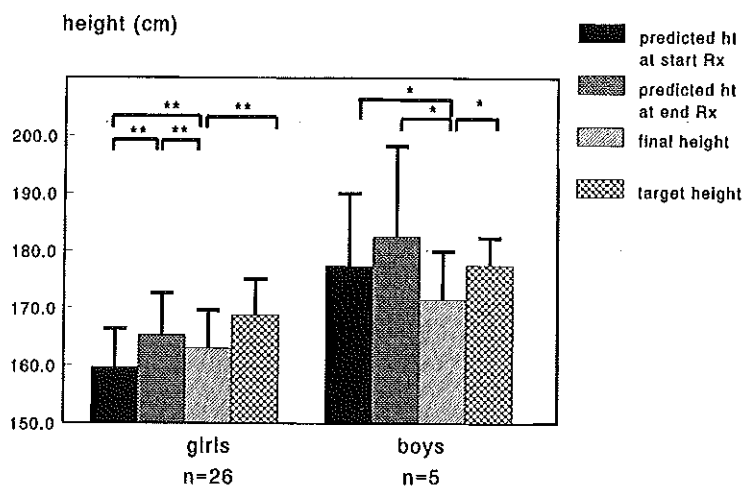

Figure 1. Predicted, final and target height in girls (n = 26) and boys (n = 5) during and after treatment with triptorelin
*: $p < 0.05$, ** $p < 0.01$

height of 3.5 (4.2) cm (range -4.8 to + 10.5 cm) was achieved. The change in predicted adult height was higher when treatment was stopped at a BA ≤ 12.5 years than at a BA > 12.5 years (4.6 [3.0] cm, $n = 21$ *vs* 0.8 [3.8] cm, $n = 10$ respectively; $p < 0.05$).
In the five boys, final height was 171.5 (8.5) cm (–1.57 [1.27] SDS) which, simarly, was less than predicted at cessation of treatment, but also less than predicted adult height at start of treatment.

Correlation between final height and other variables

In girls a positive correlation was shown between final height and height at the beginning and at the end of treatment ($r = + 0.75$, $p < 0.001$, and $r = + 0.84$, $p < 0.001$ respectively). A negative correlation was observed between final height and bone age at the end of treatment ($r = -0.52$, $p = 0.03$). Furthermore, a negative correlation between chronological age and bone age at the end of treatment and the height increment after treatment was found ($r = -0.45$, $p = 0.005$, and $r = -0.85$, $p < 0.001$ [fig 2] respectively). No linear correlation was observed between final height and chronological age or bone age at start of treatment or the duration of treatment. A weak correlation was demonstrated between final height and target height ($r = + 0.32$, $p = 0.05$).
Stepwise multiple regression analysis revealed that final height (y) was influenced most significantly by height at the start of treatment (x_1). Other significant factors were the bone age at the point of starting of treatment (x_2) and at discontinuation of treatment (x_3) and the duration of treatment (x_4) ($y = + 0.91 \times x_1 - 3.6 \times x_2 - 5.0 \times x_3 + 1.5 \times x_4 + 132.5$; $R^2 = 0.89$; residual SD = 2.46; $p < 0.001$).
Twenty four of the 31 girls showed a positive change in predicted adult height (median: 5.2 cm, range: 0.5 to 10.5 cm) (group A); however, seven of the 31 girls (group B) reached a final height below predicted adult height at start of treatment (median: –2.9 cm, range –0.9 to –4.8 cm). The most important differences between these groups were a younger age at start of treatment in group A (7.5 [0.8] *vs* 8.4 [0.6] years, $p = 0.01$), a higher bone age/chronological age ratio at start of treatment in group A (1.45 [0.15] *vs* 1.29 [0.08], $p = 0.01$) and a longer period of treatment in group A (3.7 [1.1] years *vs* 2.5 [0.7] years, $p = 0.02$).
In 26 girls target height was available. Sixteen of these 26 (62%) girls achieved final height within the target height range whereas 10 did not.

Change in body proportions and composition

The SDS of the ratio of sitting height to subischial leg length at final height was normal in girls at 0.07 (0.98) ($n = 26$). In 15 girls the ratio SHLL SDS_{CA} was known during treatment and at final height (table 1). No significant changes were observed.

BMI SDS_{CA} in girls at start of treatment was higher than the reference population and it did not change significantly during and after treatment (table 1). BMI SDS_{BA} at start of treatment was normal and increased during treatment (table 1). It did not change after discontinuation of treatment. The same pattern was observed in boys.

We compared the BMI SDS data of our patients with those from a group of 23 girls treated daily with subcutaneously buserelin, another GnRH agonist, for two years [3]. In this buserelin group (chronological age: 6.0 [1.7] years; bone age: 9.9 [2.2] years at the start of treatment) BMI SDS_{CA} and BMI SDS_{BA} were calculated at the start and at the end of buserelin therapy (BMI at final height was not known in this group of girls). BMI SDS_{CA} at the start and at the end of the buserelin treatment was 1.07 (1.00) and 1.30 (1.05) respectively, not significantly different from the equivalent data in our triptorelin group. BMI SDS_{BA} at the start and at the end of buserelin was 0.28 (0.64) and 0.06 (0.75) respectively. BMI SDS_{BA} at the start of buserelin treatment was not significantly different from BMI SDS_{BA} at the start of triptorelin. On the other hand BMI SDS_{BA} at the end of therapy was significantly higher in the triptorelin group than in the buserelin group ($p < 0.001$) (There was no difference in bone age at the start of therapy between the two groups).

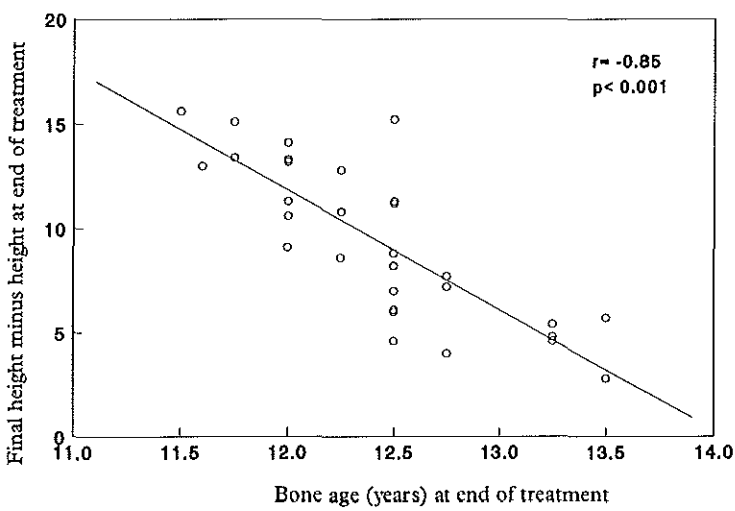

Figure 2. Final height minus height at the end of triptorelin treatment as a function of bone age (years) at the end of triptorelin treatment in girls ($n = 31$).

Menses and pelvic ultrasound evaluation

Menarche occurred at a period of 1.1 yrs (median) (range: 0.4 to 2.6 yrs) after discontinuation of treatment and at a chronological age of 12.3 (1.1) years. At the final consultation, five of the 31 girls were using oral contraceptives. The menstrual cycles were regular in 26 out of the 31 girls.
Pelvic ultrasonography was performed in 22 girls. Four of them were using contraceptives and in all four girls normal ovaries were observed. In the remaining 18 girls, a uterus volume of 33.9 (15.6) cm^3 was demonstrated, which represents the upper limit of the normal adult volume. The volumes of the ovaries were within the normal range at 7.9 (3.9) cm^3 compared to reference value of 7.4 (4.8) cm^3 [25].
In four girls, one of the ovaries was larger than normal. In two of these four girls, 2–3 cysts with a diameter ≤ 0.6 cm were visualized, coinciding with the norm for this stage of puberty [17]. In the remaining two girls, 8–10 cysts ≤ 0.6 cm were observed. The menstrual cycles were regular in these four girls. In the ovaries of the remaining 14 girls, a maximum of two to six cysts with a diameter ≤ 0.9 cm were observed. In two of the 14 girls, cysts were documented with a diameter of 2.0 cm. However, this was observed 12 to 14 days after the first day of the menstruation, that is at the point of ovulation.

DISCUSSION

This study shows that adult height in girls can be increased by treatment with the slow-release GnRH agonist triptorelin, although less than would be expected at the point of discontinuation of treatment.
Final height in our girls was 161 (7.0) cm, greater than the final height of untreated girls in the studies of Thamdrup [26] (151.3 [8.8] cm, $n = 26$), Sigurjonsdottir and Hayles [27] (152.7 [8.0] cm, $n = 34$) and Paul *et al* [19] (152.7 [8.6] cm, $n = 93$). The positive change in predicted adult height of 3.5 cm lies between the values of 2.4 to 6.2 cm reported in two other studies where daily subcutaneous preparations were used [11, 12]. Using triptorelin a greater improvement in predicted adult height of 6.5 (1.4) cm was reported by Brauner *et al*, despite the fact that the inclusion criteria used were similar to ours [13]. The most remarkable positive change in predicted adult height – 10 cm – was described by Paul *et al* in a group of girls treated before 5 years of age [19]. This tendency for young patients to obtain a greater positive change in predicted adult height was also observed in our population: in the girls who had a positive change in predicted adult height of 5.2 cm (range: 0.5 to 10.5 cm) (group A), chronological age was significantly lower than in those girls who did show a negative change in predicted adult height. Therefore age at the onset of treatment seems to play an important role. Only four girls in our group were younger than 7 years of age at the start of treatment; hence, we could not show

a significant relationship between age at start of treatment, and the final height obtained.

Height at start of treatment proved to be the most important positive factor influencing final height, as demonstrated by multiple regression analysis and by a positive correlation coefficient ($r = +0.75$), a characteristic that is also described in normal puberty [28]. Final height showed a weak association with target height, as also shown by Paul *et al* [19]. Only 62% of the girls with a known target height reached a final height within the target height range. However, this weak association between target height and final height is similarly identified in the normal population (0.45) [29].

In contrast to other studies, no improvement of adult height was demonstrated in boys [11, 19]. The most plausible explanation for this difference is that three out of the five boys were suffering from neurofibromatosis, a condition which itself is associated with growth impairment, and in which the development of complications such as optic nerve glioma may lead to growth hormone insufficiency. On the other hand, in some cases growth impairment has been described independent of growth hormone levels [30]. In our patients growth hormone secretion was not evaluated.

The difference in final height as predicted at the point of discontinuation of treatment and the actual final height attained is caused by a moderate growth acceleration after cessation of treatment and a definite accelerated bone maturation within the same period. The same tendency has been described by Oerter *et al* [11]. As suggested before, the post-therapy level of gonadotrophins and sex steroids rises more rapidly than in normal puberty and higher levels are reached in a shorter period of time [20]. This results in a shorter period of growth and eventually a final height that is less than the predicted adult height at the point of discontinuation of treatment.

In addition to height at start of treatment, bone age at the point of discontinuation of treatment is another important negative factor contributing to final height. As shown in fig 2, residual growth capacity is greater before to a bone age of 12.0 to 12.5 years. A better change in predicted adult height was observed in children in which treatment was stopped at a BA $\leq$ 12.5 years. For auxological reasons discontinuation of GnRH treatment might be considered at a bone age of 12 to 12.5 years.

Triptorelin treatment did not influence body proportions, as normal ratios of sitting height to subischial leg length were observed at the point when final height was reached.

In girls, BMI SDS_{CA} was increased at start of treatment and did not change during or after discontinuation of treatment. On comparison of our data with those of Marti-Henneberg *et al*, who described the normal course of BMI during early, normal and late puberty, the increased BMI SDS_{CA} at start of treatment appears to be a natural event [31]. Since children with CPP who had been exposed to sex steroids underwent a pubertal growth spurt and subsequently developed an accelerated bone age, it would be better to use the index BMI SDS_{BA}. Using this index, a comparison can be made between the girls with CPP

and those girls with normal age of onset of puberty at similar bone ages. This index was normal at start of treatment and increased during treatment, suggesting that triptorelin increases BMI, as no change in BMI SDS_{BA} was observed during buserelin treatment.
In our patients a rapid resumption of puberty was observed, menarche occurring 1.1 years (range 0.4 to 2.6 years) after withdrawal of treatment, at a mean age of 12.3 (1.1) years [20]. This is comparable with the results of other investigators [8, 32]. Ultrasound evaluation demonstrated normal ovaries 4.0 (1.2) years after discontinuation of treatment. In contrast to the report by Adams *et al*, no evidence of polycystic ovaries was found [17].
We conclude, therefore, that treatment with the depot GnRH agonist triptorelin increases final height in girls without giving rise to abnormal body proportions. The best results are obtained in girls with a greater height at the point of starting treatment. After withdrawal of therapy, resumption of puberty occurs rather rapidly, without evidence for development of polycystic ovaries. Triptorelin seems to increase BMI. From an auxological point of view discontinuation of triptorelin in girls might be considered at a bone age of 12.0 to 12.5 years.

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Chapter 8

IDIOPATHIC ISOSEXUAL CENTRAL PRECOCIOUS PUBERTY: MAGNETIC RESONANCE FINDINGS IN 30 PATIENTS

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ABSTRACT

The purpose of this prospective study was to define the incidence of magnetic resonance imaging (MRI) abnormalities in the brain in patients with idiopathic central precocious puberty without any additional neurologic signs and symptoms, and to evaluate the routine use of gadolinium contrast in these patients. Thirty patients (29 girls, 1 boy; age range 1.9–11.9 years) with idiopathic central precocious puberty were studied. MRI of the brain in axial, coronal and sagittal planes was performed before and after administration of gadopentetate dimeglumine, with special attention to the region of the third ventricle.

There are three major findings: (1) the height of the pituitary gland is increased up to adult size compared to normal individuals; (2) in four patients (13%) major structural abnormalities were found: three hamartomas of the tuber cinereum and one gliomatous process extending from the chiasm to the optic tract; and (3) the routine use of gadopentetate dimeglumine did not reveal new abnormalities although the lack of enhancement made a positive contribution to diagnostic certainty.

We conclude that contrast enhanced MR examination is a safe and reliable method for the exclusion of abnormalites in children with precocious puberty and for the follow-up of those patients in whom abnormalities are present.

INTRODUCTION

Idiopathic central precocious puberty (CPP) in girls is caused by an unidentified dysfunction in the cerebro-hypothalamo-pituitary axis, resulting in premature telarche, menarche, advanced skeleton maturation and growth acceleration. This occurs in the absence of neurological signs and symptoms and causative diseases as gonadal, adrenal or gonadotrophin producing tumors. Moreover, neurofibromatosis Type I and McCune Albright syndrome must be excluded.
Nowadays the pituitary area can be imaged in great detail by magnetic resonance imaging (MRI) and lesions that were previously undetected by computed tomography (CT) can be visualized because of the superior tissue contrast and multiplanar imaging capability of MRI. In addition to its greater sensitivity, the lack of ionizing radiation promotes MRI as the imaging standard in paediatric patients.
Several studies have reported on MR abnormalities in the hypothalamo-pituitary area; most being case reports [1-10].
The purpose of this study was to define the incidence of MR abnormalities in the brain in patients with idiopathic CPP without any additional neurologic signs and symptoms, and to evaluate the routine use of gadopentetate dimeglumine (Gd-DTPA) in these patients.

MATERIALS AND METHODS

Patients

In 1990 the records of all 54 patients with idiopathic CPP who were regularly visiting the outpatient clinic of Sophia's Children's Hospital and the University Hospital of Leiden were reviewed.
The diagnosis of idiopathic CPP was based upon: (1) the appearance of secondary sex characteristics in girls before the age of 8 years and in boys before the age of 9 years; (2) an accelerated height velocity above the 90th percentile for chronological age; (3) bone age exceeding calendar age by more than one year; (4) a pubertal response of plasma LH in a standard LHRH test and (5) normal findings on CT examination.
These patients were asked to participate in the study. Thirty patients agreed and MR was subsequently performed; 23 refused or did not respond, and 1 patient was untraceable.
Most patients were girls ($n = 29$) except for 1 boy; mean chronological age was 9.3 years (1.9–11.9 years); mean bone age was 11.2 years (3.75–13.75 years). Mean age at onset of puberty was 5.4 years (0.8–8.3 years). All patients were treated with Decapeptyl-CR® at the time of the MR examination and were in good health without any neurological signs or symptoms.

Methods

Patients were examined on either a 0.5T Philips Gyroscan (Philips Medical Systems, Best, The Netherlands) (26 patients) or a 1.5T Philips Gyroscan (four patients). Scan sequences included T_1 weighted (SE 450–600/TE 21–30) sagittal and coronal 3 mm slices of the hypothalamo-pituitary area before and after intravenous injection of 0.1 mmol/kg Gd-DTPA (Schering AG, Berlin, Germany) and T_2 weighted (SE 2000–2500/TE 100) axial 0.5 cm slices of the total brain. Interslice gap was 10% of slice thickness. The matrix was 205 × 256, the field of view was 18 cm (pixel height 0.7 mm, pixel width 0.9 mm).
The height of the pituitary gland was determined by measuring the greatest distance between the base and the top of the gland on a midsagittal [12] and coronal image. The height of the gland as a function of age in patients with CPP was compared with normal individuals by using covariance analysis. Normal values of pituitary height were obtained from a study of Argyropoulou *et al* [13]. In their study the same measurement technique was used, but small differences in matrix and field of view resulted in a pixel height of 1 mm compared with a pixel height of 0.7 mm in our study. This did not affect the results of the covariance analysis.
The formula for the volume of an ellipsoid was used for calculation of the volume of the hamartomas: Volume = Length × Width × Height × /6.
To assess interobserver and intraobserver variability the pituitary gland height was measured independently by two experienced radiologists (interobserver variability) and one radiologist made two seperate measurements (intraobserver variability).
Skeletal maturation was determined according to the method of Greulich and Pyle [14].

RESULTS

The MR findings are summarized in Table 1. In three patients a tumor of the tuber cinereum, protruding in the prepontine cistern, was present. In two patients the mass was pedunculated and in one patient it appeared to be sessile. In all cases the tumours were isointense with grey matter on T_1 weighted images and hyperintense on T_2 weighted images and did not enhance after administration of Gd-DTPA (Fig. 1). These characteristics are compatible with hamartoma [6–8, 10]. The largest hamartoma measured 14 × 16 × 14 mm (volume 1.7 ml) and the smallest measured 7 × 6 × 7 mm (volume 0.15 ml).
One patient showed a pathologically increased signal intensity in the optic chiasm, lamina terminalis and right uncus on T_2 weighted images (Fig. 2). There was no change in size or appearance during 3 years of follow-up.
The results of the measurements of the pituitary gland are given in Table 2 and Figure 3.

Table 1. *MR findings in the hypothalamo-pituitary area of the study population*

MR Findings	Number of Patients
Hamartoma tuber cinereum	3 (10%)
Low grade glioma	1 (3%)
Normal	26 (87%)
Total	30

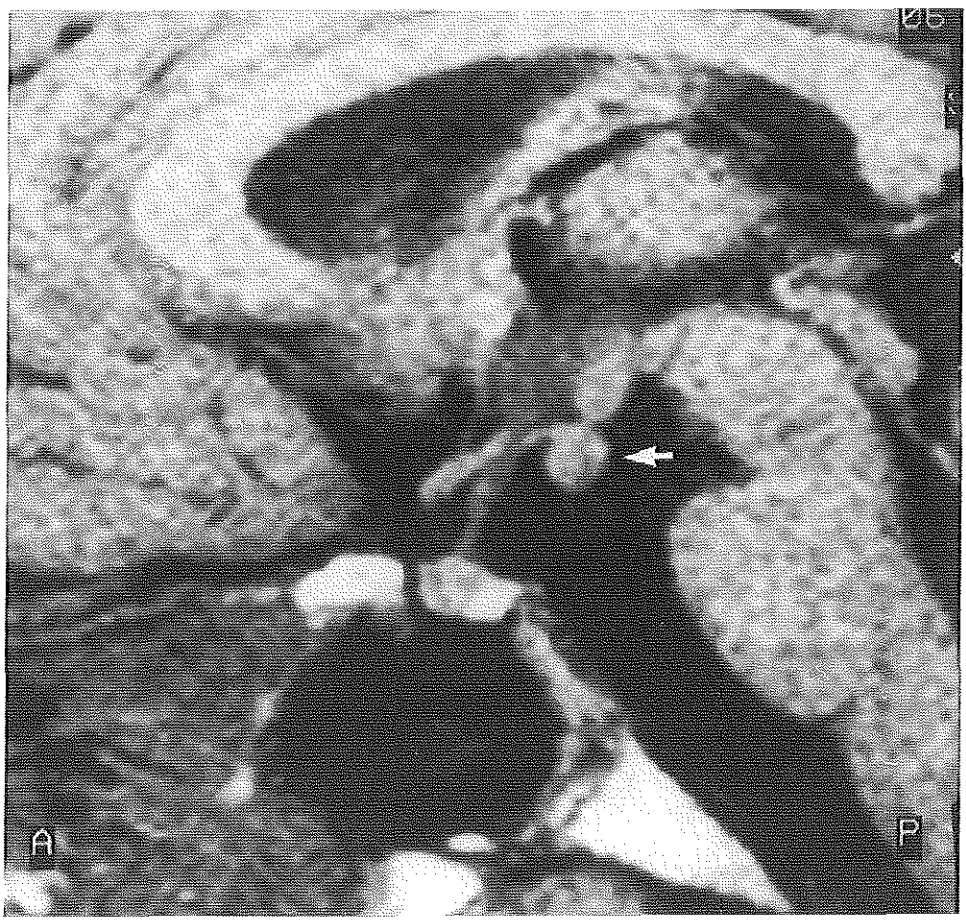

Figure 1. T_1 weighted (SE 450/30) midsagittal MR image of the hypothalamic region shows a small pedunculated hamartoma of the tuber cinereum (arrow). Enhanced image (not shown) showed no change in signal intensity.

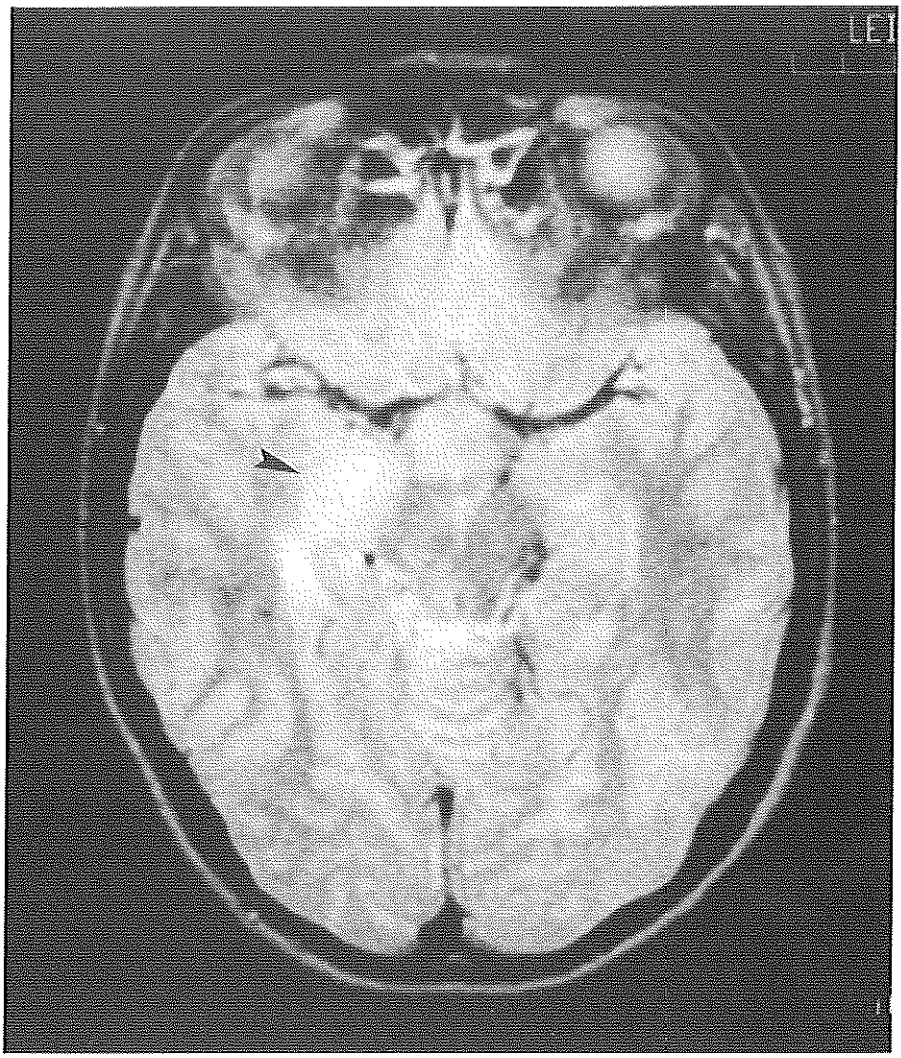

Figure 2. T_2 weighted (SE 2500/100) axial MR image shows increased signal intensity in the right uncus, with mild mass effect (arrowhead).

The mean difference between coronal and sagittal measurements was 0.4 mm, standard deviation 0.4 mm, range 0.0–1.2 mm. All patients had homogeneous signal intensities in the pituitary gland before and after intravenous injection of Gd-DTPA without any evidence of tumour. Moreover, in all patients the stalk and posterior pituitary gland were present and considered normal. No adverse or allergic reactions occurred after intravenous administration of Gd-DTPA.

The mean difference between two observers (intraobserver variability) was 0.14 mm (standard deviation 0.18, range 0.00–0.60 mm). The mean difference between two separate measures by the same observer (intraobserver variability) was 0.19 mm (standard deviation 0.12 mm, range 0.00–0.40 mm).

Table 2. *Pituitary measurements in the study population*

	n	Mean	SD	Range
Height of the Pituitary Gland				
Midsagittal	30	6.2	1.1	4.4–8.9
Coronal image	30	6.1	1.0	4.3–8.3

All values are given in mm. *n*, number of patients; SD, standard deviation

DISCUSSION

Pituitary gland and stalk

In the first year of life there is no significant change in gland height [15,16]. From 1 year of age to puberty a positive correlation between age and gland height is found until a plateau is reached at the age of 15–20 years (mean gland height 6.1 mm)[13]. Maximal gland height in normal males does not exceed 8.0 mm and in females 9.0 mm [12]. After the age of 20 years the height gradually decreases [12, 17]. In patients with CPP a positive correlation was also found between chronological age and pituitary gland height. Moreover, the average gland height was 1.2 mm (± 0.2 mm) higher compared with normal girls; this difference is highly significant ($p < 0.001$). Even if the age is corrected for advanced maturation (bone age) the difference remaines highly significant (Δ 0.9 mm, $p < 0.001$) (Fig.3).

Gland enlargement during puberty is a well-known fact [12, 13, 18] and may be related to hypersecretion of gonadotropins during this period. Gland enlargement in CPP patients supports the concept that this mechanism is also present in CPP caused by a premature activation of the hypothalamo-pituitary-gonadal axis [19]. Kao *et al* compared the pituitary gland height in 13 CPP patients with 19 age matched controls [20]. Although he did not correct the age for advanced maturation, he reported gland enlargement in CPP patients (mean height 5.84 mm). These findings are compatible with our study. All patients in Kao *et al's* study were examined before hormonal treatment, whereas our patients were being treated with LH-releasing hormone (LHRH) agonist. Apparently hormonal treatment does not reduce the gland size to prepubertal values.

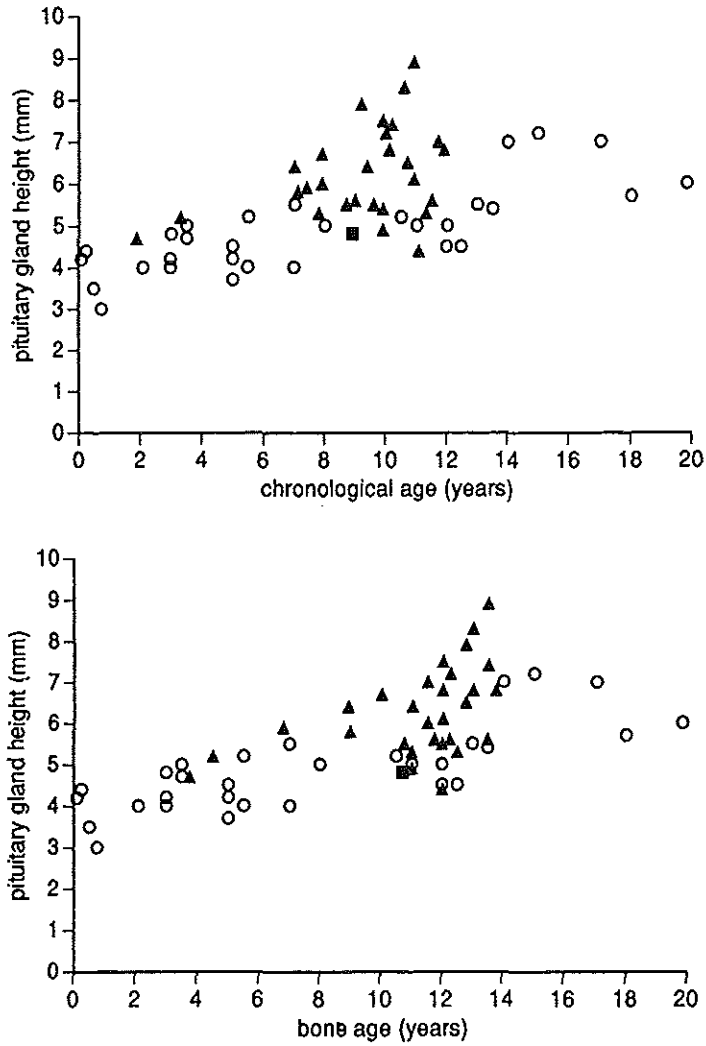

Figure 3. Height of pituitary gland as a function of age.
○= Normal girls (n = 30)[13],▲= Girls with CPP (n = 29),■= Boy with CPP (n = 1).
(a) Gland height compared with chronological age. The gland is higher in CPP patients; mean difference, 1.2 mm (± 0.2), $p < 0.001$.
(b) Gland height compared with age, corrected for maturation (bone age). The mean difference remains significant: 0.9 mm (± 0.2), $p < 0.001$.

Hypothalamic hamartoma

Hamartomas of the hypothalamus are congenital malformations characterized by heterotopic and hyperplastic tissue located in the proximity of the tuber cinereum and mammillary bodies. The MR appearance (isointense on T_1 weighted and hyperintense on T_2 weighted images) is well known [6-8, 10]. It is a common detectable lesion associated with precocious puberty, although the mechanism causing puberty is unknown. Precocious puberty is present in 74% of patients with histologically proven hypothalamic hamartoma [21]. Several large CT and pneumoencephalotomographic studies have reported on the incidence of hamartomas in patients with isosexual precocious puberty, varying from 19–33% [21-23]. Cacciari *et al* report an incidence of 14% in an MR study of 21 patients, but it is not clear if these patients had idiopathic CPP [1].
Our patient group consists of idiopathic CPP patients without any neurological signs or symptoms and without other syndromes or disease states that are known to cause CPP. The incidence of hypothalamic hamartomas is 10%. All patients with hypothalamic hamartomas had early onset of symptoms before the age of 3 years, similar to other reports [1, 23, 24].
Kao *et al* reported that CPP patients with a hypothalamic tumour (including hamartoma) have normal or reduced anterior pituitary gland size and show absence of the posterior pituitary lobe [20]. We were not able to confirm this finding; all three patients with hypothalamic hamartoma had enlargement of the anterior pituitary gland and a normal posterior lobe.
With current endocrinological testing and MRI a diagnosis of hamartoma can be made with a high degree of probability, thus obviating the need for surgical confirmation. Surgical intervention is necessary only if the lesion enlarges on subsequent MR studies [6, 22].

Miscellaneous findings

In one patient hyperintense areas in the region of chiasma, lamina terminalis, uncus and optic tract were found. These abnormalities remained stable during a follow-up period of 3 years and resembled low-grade optic glioma, as frequently seen in paediatic patients with neurofibromatosis Type I. Indeed, precocious sexual development is the most common endocrinopathy associated with neurofibromatosis in childhood [25]. This patient, however, had no other signs of neurofibromatosis. The mechanism by which these gliomas cause CPP is probably interruption and dysfunction of hypothalamic-pituitary or pineal-hypothalamic pathways [25, 26].

CONCLUSION

In several patients with CPP, considered idiopathic based on clinical and biochemical findings, definite abnormalities were present. These abnormalities

include hypothalamic hamartoma and low-grade glioma and are known to cause precocious puberty. Therefore, precocious puberty in these patients was not in fact idiopathic.

Gd-DTPA did not improve the sensitivity of the MR examination although the lack of enhancement of the hamartomas added to the diagnostic certainty with which these lesions were considered. Moreover, in the patients without abnormalities on the non-enhanced scans, the lack of enhancement added to the diagnostic confidence that small lesions had not been overlooked.

MR provides optimal imaging for initial evaluation of patients with CPP and for follow-up of patients with abnormalities.

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Chapter 9

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

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ABSTRACT

GnRH agonist (GnRHa) treatment slows bone maturation in patients with central precocious puberty (CPP). However, height velocity is also decreased and may fall to subnormal levels in a subset of CPP patients. This combination of subnormal growth rate and slow bone maturation may prevent an improvement of adult height prediction and ultimately of final height. We performed a prospective pilot study in girls with CPP to investigate the auxological effects of the addition of growth hormone (GH) to depot GnRHa treatment in patients characterized by a low growth rate (< 25th centile) and/or a low height standard deviation score (SDS: ≤ -1.0), and a compromised height prediction (< 3rd centile of normal, i.e. below 156 cm). These patients represent the most negative selection in terms of height potential. After a mean duration of 2.9 ± 0.2 years (± SEM) of GnRHa therapy alone (Decapeptyl Depot®, 75 µg/kg i.m every 4 weeks) eight patients with a chronological age (CA) of 10.0 ± 0.5 years and a bone age (BA) of 11.8 ± 0.4 years were enrolled in the study. They received recombinant human GH (Genotropin®) in a dose of 4 IU/m^2 per day (0.07 mg/kg per day, 7 days a wk s.c). Duration of combination treatment was 3.2 ± 0.3 years. Height velocity SDS increased from −1.86 ± 0.25 during the year prior to GH therapy to + 0.43 ± 0.45 ($p < 0.05$) during the 1st year of GH. Height SDS for BA was improved from −2.51 ± 0.39 to −1.15 ± 0.47 ($p < 0.05$). There was no acceleration of bone maturation by GH treatment ($\Delta BA/\Delta CA$ during GnRHa 0.60 ± 0.08 vs 0.45 ± 0.08 during combination treatment; NS). The ratio BA/CA was completely normalized at the end of GnRHa + GH therapy (1.00 ± 0.03) with four patients now showing a BA below their CA. Consequently, predicted adult height increased by a mean of 7.8 ± 1.5 cm from 150.5 ± 1.9 to 158.2 ± 2.3 cm during combination treatment ($p < 0.01$). It is noteworthy that an increase in adult height prediction was seen in all eight patients. Target height was statistically not different from last height prediction. We conclude that the addition of GH to GnRHa treatment of subnormally growing girls with CPP has beneficial effects on several auxological parameters. In combination with GnRHa, GH has no negative effect on bone maturation. By this combination therapy, predicted adult height within the genetic target height range is made possible in girls with severely compromised auxological data at start of treatment. Whether final height is similarly improved by this treatment regimen will be determined after long-term follow-up.

INTRODUCTION

Agonistic Gonadotropin-releasing hormone (GnRH) analogues are widely used in the treatment of children with the central precocious puberty (CPP) [1, 2]). While this treatment modality is highly efficient in suppressing the pituitary-gonadal axis, leading to a deceleration of bone maturation, the effects on growth are sometimes less positive. During treatment with GnRH agonists (GnRHa), height velocity decreases markedly to a mean in the low normal range [3-8]. However, in a subset of patients height velocity drops to subnormal levels [1, 7, 9]. This may jeopardize one of the goals of suppressive treatment in CPP, namely the increase of final height into the genetically determined range. In recent years there have been controversial discussions how to achieve a better final height in these slowly growing children who even may present with additional factors which compromise adult height. Low-dose estradiol would be a theoretical possibility to increase height velocity, would there not be the acceleration of bone maturation even with very low doses. A second treatment option could be the addition of growth hormone (GH) to the GnRHa therapy. At a supraphysiological dose GH would have the potential to increase growth rates. The effect on bone maturation, however, cannot be predicted. We performed a prospective study in girls with CPP who were on treatment with depot GnRHa for at least 12 months. The hypothesis was that GH would increase height velocity in patients with a low growth rate and/or a low height SDS and a compromised height prediction during GnRHa treatment. If bone maturation was not to be accelerated by GH, height prediction would increase in these patients with unfavourable auxological characteristics to start with.

MATERIAL AND METHODS

Patients

This was a prospective pilot study on eight girls with CPP. They were selected from our large multicentre trial of 104 girls with CPP [6-8]. The purpose of this study was to investigate the auxological effects of the addition of recombinant human GH to the treatment with a depot GnRHa. Included were patients with CPP who presented after at least 1 year of depot GnRHa treatment with either a low height velocity or a subnormal body height, both in combination with a compromised height prediction. The protocol was approved by the ethics committees of the Universities of Kiel, Germany and Rotterdam, The Netherlands. Parents or guardians of all patients signed informed consent forms before starting the protocol of combination treatment. The patients were considered to have CPP if pubertal development started before their eighth birthday. Additional diagnostic criteria were an acceleration of BA more than 1 year above CA and a pubertal gonadotropin response to a standard gonadotropin releasing hormone (GnRH) test. Clinical and laboratory

characteristics of patients at start of GnRHa treatment are given in Table 1. All patients showed a peak LH/FSH ratio > 1 which is evidence for the active and progressive form of CPP [10, 11].

For the inclusion into the combination treatment study, two tracks were available. The inclusion criteria for Track A were the following: (1) CA at start of GnRHa treatment ≥ 9 years; (2) BA at start of combination treatment < 14 years; (3) Treatment duration with GnRHa ≥ two years; (4) Ratio BA/Height age (HA) at start of combination treatment ≥ 1.2; (5) Predicted adult height (PAH) by the method of Bayley and Pinneau [12] < 3rd centile of normal adult women; (6) Height velocity during the preceding 12 months < 25th centile.

Inclusion criteria for Track B were: (1), (2), and (5) as for Track A; (3) Treatment duration with GnRHa ≥ 1 year; (4) Ratio BA/Height age (HA) at start of GnRHa treatment ≥ 1.2; (6) height SDS for CA ≤ −1.0; (7) Growth data available for the last year of GnRHa treatment. Three patients fulfilled the criteria of either Track A or B, respectively, and two patients fulfilled the criteria of both tracks. From the clinical and auxological characteristics of our patients it is evident that these patients belong to those with the worst starting point prior to treatment in terms of height potential and growth dynamics. Furthermore, our patients clearly fulfilled the rigorous conditions for GnRHa treatment in CPP patients as recently proposed by Rosenfield [13].

Table 1. *Clinical, hormonal, and auxological data of girls with central precocious puberty at start of GnRH agonist treatment*

Patient	Chronol. age (years)	Bone age (years)	Breast stage	Pubic hair stage	Target height (cm)	Height (SD)	Height velocity (cm/yr)	Predicted adult height (cm)	LH basal/ stimulated (IU/L)	FSH basal/ stimulated (IU/L)	Estradiol (pmol/l)
EH	8.7	9.9	4	1	n.a.	−0.61	10.2	158.7	3.1/23.6	7.6/11.4	36
NC	4.5	7.5	4	2	n.a.	1.0	8.6	149.9	2.6/76.5	4.7/31.4	< 18
JM	6.0	10.3	2	3–4	169	2.09	7.7	151.6	2.4/19.0	3.0/8.2	90
BS	9.0	10.5	3–4	2	161	−1.02	8.6	152.7	1.8/17.7	4.6/14.8	114
MM	8.1	11.3	4	3	158.5	−0.75	7.2	143.9	9.0/33.1	7.0/9.7	103
RR	8.1	10.3	3	2	159	−1.09	12.0	149.8	3.3/16.9	1.6/7.8	116
WR	6.4	10.3	2	1	163	2.15	6.6	155.2	4.0/75.0	5.0/14.0	190
AA	6.5	10.5	3	3	169	−0.85	7.4	137.2	1.9/34.0	0.8/15.8	60

n.a. not available

Patients with growth failure due to disorders of cardiopulmonary, gastrointestinal, skeletal, and central nervous systems were excluded. Further exclusion criteria comprised incomplete, gonadotropin-independent precocious pseudopuberty, progressive brain tumors or other malignant diseases, and bleeding disorders incompatible with frequent injections.

Prior to GH treatment spontaneous and stimulated GH secretion were tested during GnRHa therapy. Mean nocturnal plasma GH levels showed a range from

1.0 to 3.9 ng/ml (normal prepubertal range: > 3 ng/ml [42]; normal in one patient). Maximal plasma GH after stimulation with arginine ranged from 4.0 to 42.2 ng/ml (normal range: > 10 ng/ml; normal in three patients). IGF-I ranged from 231 to 348 ng/ml (normal prepubertal range: 94-330 ng/ml).

Methods

Suppression of the pituitary-gonadal axis was achieved by treatment with the depot GnRHa Decapeptyl Depot® (Ferring, Germany and The Netherlands). It was injected intramuscularly every 4 weeks in an average dose of 75 µg/kg body weight. GH (Genotropin® ,Pharmacia, Germany and The Netherlands) was administered subcutaneously in a daily dose of 4 IU/m² body surface (0.07 mg/kg), 7 days per week.
BA was estimated in a blinded fashion by an experienced pediatric radiologist employing the method of Greulich and Pyle [14]. PAH was calculated according to Bayley and Pinneau [12]. As standards for height and height velocity German longitudinal normative data were used [15, 16]. The normative data of Prokopec and Belisle [17] were used for body-mass index (BMI) . Target height was calculated according to the formula (sum of heights of the parents ± 12)/2 + 3 cm. Gonadotropins, estradiol, GH, and IGF-I were determined by RIA or immunoradiometric assay at the respective centers.

Statistical analysis

Statistical comparison between data at start of GnRHa, at start of additional GH therapy, and at end of combination treatment was done by Friedman's ranked analysis of variance. For the evaluation of statistical differences between each of two time points Duncan's multiple range test was used. Correlation analysis between two parameters was performed using Spearman's rank correlation test. Data are presented as mean and SEM. $P < 0.05$ was considered significant.

RESULTS

Clinical and hormonal suppression

Mean CA and BA at the start of GnRHa treatment, at start of additional GH treatment, and at the end of treatment was 7.2 ± 0.55 and 10.1 ± 0.39, 10.0 ± 0.48 and 11.8 ± 0.39, and 13.1 ± 0.44 and 13.1 ± 0.47 years, respectively.
Breast development at start of GnRHa therapy ranged between Tanner stage B 2 and 4 (median 3.5). After treatment with GnRHa alone, breast stage had decreased in four of eight patients to a median of 3 (range 2 to 4). Until the end of combination therapy, a further decrease was noted in one patient (median 3, range 1 to 4).

BMI increased from 18.1 ± 0.99 at start of GnRHa to 19.5 ± 0.9 at start of GH, and to 22.6 ± 1.0 at the end of treatment. However, there was no change in BMI SDS (1.15 ± 0.6, 0.9 ± 0.4, and 1.1 ± 0.5, respectively).
Patients were treated during 2.9 ± 0.2 years with GnRHa alone (range 1.5 to 3.5 years). Combined GnRHa plus GH treatment lasted 3.2 ± 0.3 years (range for seven patients 3 to 4 years, one patient was treated for 1 year only). During treatment 17ß-estradiol (E2) decreased from a pretreatment mean plasma level of 129 ± 45 pmol/l to 23 ± 5 pmol/l at start of GH treatment. E2 was in the prepubertal range throughout treatment in all but one patient. This patient showed repeated increases of E2 to borderline pubertal levels between 59 and 77 pmol/l without evidence for clinical escapes. All GnRH tests were fully suppressed in terms of plasma LH and FSH responses in this patient. Furthermore, this girl did not differ from the others in terms of her auxological response to treatment. In five patients GnRH tests were performed regularly for the control of suppression. Stimulated LH was suppressed sufficiently in 26 of 29 GnRH tests. In the patients not monitored by GnRH tests E2 was suppressed to prepubertal levels in all but one instance. Clinical escapes from suppression were not seen. IGF-I levels increased from 297 ± 20 ng/ml before GH treatment (n = 6) to 616 ± 42 ng/ml after the first year of combination treatment (n = 6). After three years of combined therapy IGF-I levels were 695 ± 45 ng/ml.

Auxological effects

Individual growth curves of 4 patients are shown in Fig. 1. Height velocity decreased from 8.5 ± 0.6 cm/year before treatment to 3.5 ± 0.3 cm/year during the last year of GnRHa therapy ($p < 0.01$) and increased to 6.2 ± 0.4 cm/year during the first year of combination treatment ($p < 0.01$ vs pretreatment and vs last year of GnRHa). During combination treatment height velocity showed a continuous decrease until it reached 3.3 ± 0.5 cm. The corresponding height velocity standard deviation scores were + 3.19 ± 0.94, −1.86 ± 0.25 and + 0.43 ± 0.45, respectively (Fig. 2). The height velocity SDS during combination therapy decreased to −1.0 ± 0.30. Height velocity SDS during combination treatment did not correlate with TH. The increase in height velocity in the first year of combination treatment showed no correlation with the GH response to arginine stimulation or to the mean nocturnal GH level.
Height SDS for CA decreased during GnRHa treatment (NS) and showed a further small decrease during combination therapy (NS; Fig. 2). Height SDS for BA was low at start of treatment (−3.04 ± 0.34). A small increase was seen during GnRHa therapy (NS). The combination treatment resulted in a significant increase of the height SDS for BA by almost 2 SD to −1.15 ± 0.47 ($p < 0.05$; Fig. 2).
The rate of bone maturation expressed as ΔBA/ΔCA was accelerated to 1.32 ± 0.25 before start of treatment (two bone age determinations, 4 to 6 months apart, were available before start of GnRHa treatment in six patients). GnRHa treatment (whole period) led to a significant reduction in ΔBA/ΔCA to

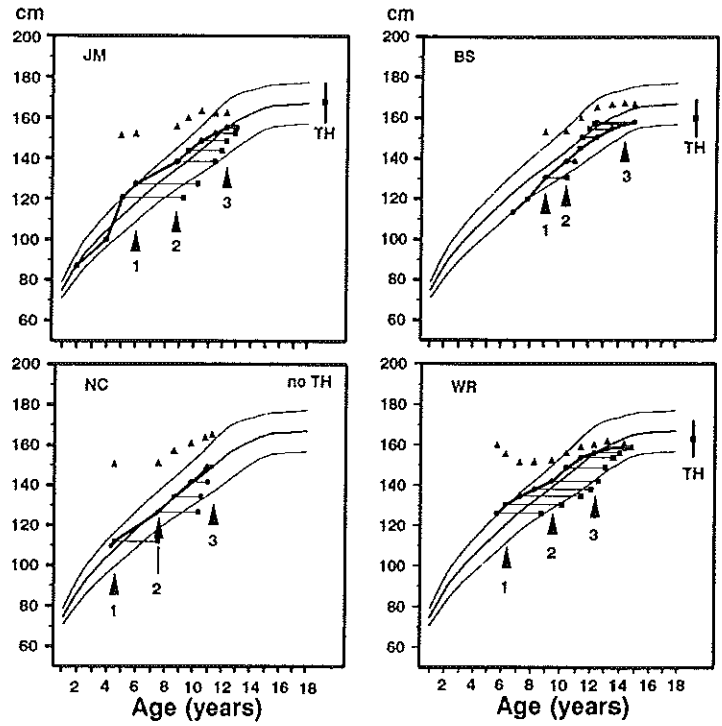

Figure 1. Four individual growth curves of girls with central precocious puberty receiving GnRH agonist treatment and combined GnRH agonist plus GH treatment. Circles represent height for age, squares height for bone age, and triangles height prediction. TH = target height. 1: start of GnRH agonist therapy, 2: start of additional GH therapy, 3: end of treatment. Note that patients BS and NC showed bone ages 2.0 and 0.3 years below chronological ages at last examination, respectively. Patients BS and WR have reached their target height range (2.6 and 4 cm short of TH).

0.60 ± 0.08 ($p < 0.01$). The addition of GH to the treatment regimen did not result in a reacceleration of skeletal maturation (ΔBA/ΔCA 0.45 ± 0.08 for the period of combination therapy; NS vs GnRHa treatment; $p < 0.01$ vs pretreatment period). In addition, there was no significant difference between the ratio ΔBA/ΔCA during the last year of GnRHa treatment and the first year of combined GnRHa plus GH treatment (0.56 ± 0.11 vs 0.50 ± 0.09; NS). This marked reduction of bone maturation yielded a completely normal ratio of BA/CA at the end of treatment (1.0 ± 0.03) (Fig. 3). In parallel, the ratio BA/HA decreased significantly from 1.45 ± 0.07 at start to 1.13 ± 0.05 at end of treatment ($p < 0.05$). It is remarkable that at the end of treatment four of the eight patients showed a BA below their corresponding CA (range of difference −0.3 to −1.7 years). The remaining four patients showed bone ages 0.1 to 2.0 years ahead of their CA.

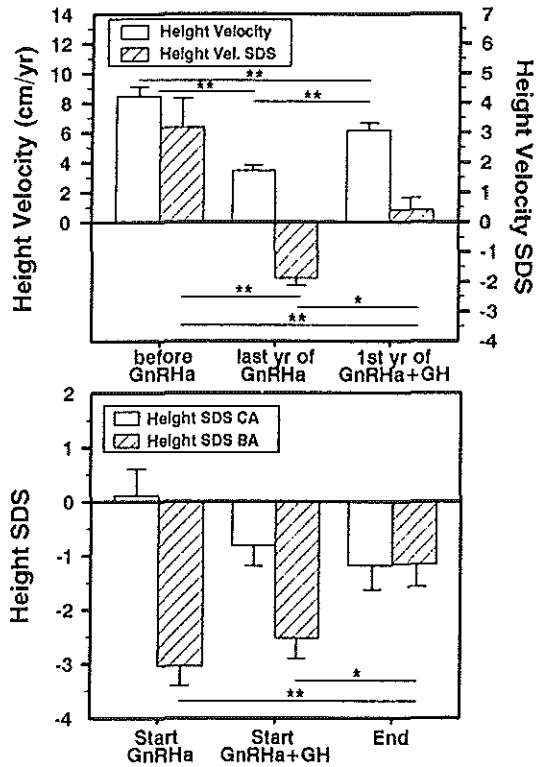

Figure 2. Height velocity (open bars) and height velocity standard deviation score (SDS; hatched bars) are shown in girls with central precocious puberty before GnRH agonist treatment, during the last year of GnRH agonist therapy, and during the first year of additional GH treatment (upper panel). Note the normalization of height velocity by GH treatment.
Height SDS for chronological age (CA; open bars) and bone age (BA; hatched bars) at start of GnRHa treatment, at start of additional GH treatment, and at end of treatment (lower panel). Height SDS for BA is improved by almost two SD.
* $p < 0.05$, ** $p < 0.01$

Height prediction did not change during GnRHa treatment (Fig. 3). However, it increased significantly during combination therapy by a mean of 7.8 ± 1.5 cm (range 1.1 to 14.2 cm; $p < 0.05$). Linear regression analysis showed a significant correlation between the gain in PAH during combination therapy with the duration of this treatment period ($p < 0.02$). It is important to note that an increase in height prediction during combination therapy was seen in all eight patients. Overall gain in PAH was 8.4 ± 2.1 cm (range –2.0 to + 14.8 cm). Target height and height prediction at the end of treatment were not statistically different, whereas TH was significantly higher than PAH at the start of GnRHa

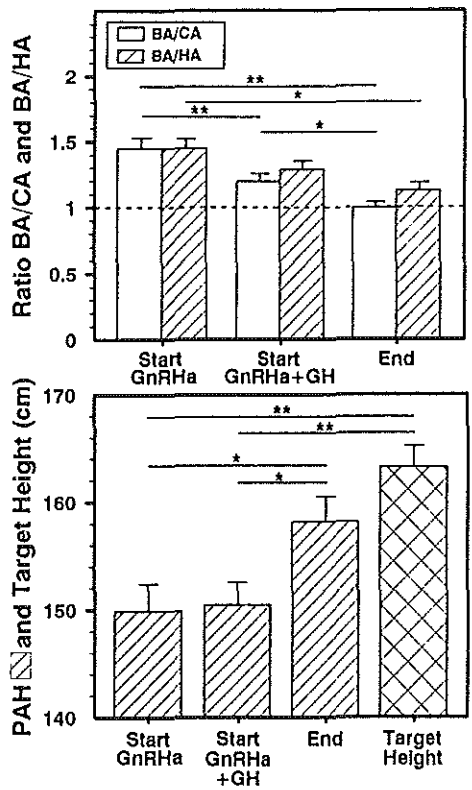

Figure 3. Ratio BA/CA (open bars) and ratio BA/Height age (HA; hatched bars) at start of GnRH agonist treatment, at start of additional GH treatment, and at end of treatment (upper panel). Note that the ratio BA/CA is normal (1.0) at the end of treatment.
Predicted adult height (PAH; hatched bars) at start of GnRH agonist treatment, at start of additional GH treatment, and at end of treatment (lower panel). Target height is shown by the right-hand column for comparison. PAH is improved by GnRH agonist plus GH treatment by a mean of 8.4 cm.
* $p < 0.05$, ** $p < 0.01$

treatment ($p < 0.01$) and PAH at the start of combination therapy ($p < 0.01$; Fig. 3).

DISCUSSION

The purpose of this study was to determine the effects of additional GH treatment on bone maturation, height velocity, and adult height prediction in GnRHa-treated girls with CPP. The inclusion criteria were chosen to include

patients with unfavourable auxological characteristics, particularly adult height prediction below the 3rd centile (i.e. < 156 cm), and a low height velocity during GnRHa treatment. Thus, this study comprises a subset of patients from our large CPP trial of 104 patients [6-8] with the worst prognosis before and during GnRHa treatment and an unfavourable auxological response to GnRHa treatment. The data whether GnRHa treatment leads to an increase in final height in girls with CPP are contradictory. In a large survey of GnRHa-treated (n = 131) and untreated (or medroxyprogesterone or cyproterone acetate-treated) CPP patients (n = 64) from Europe and the US it was recently shown that adult height was not improved significantly in the group of all treated girls in comparison to the control group [18]. However, a beneficial though not significant effect on final height was noted in children with an early onset of disease and early start of treatment (< 6 years). In contrast to this survey a number of studies from single centers have shown an increase in final height compared to the pretreatment height prediction [chapter 7][19-22] or to the historical untreated groups [23] from Thamdrup [24], Sigurjonsdottir and Hayles [25], Werder *et al* [26], Bovier-Lapierre *et al* [27], and Lee [28]. The mean increases ranged from + 2.4 cm (19) to + 6.9 cm [20]. Most studies included heterogeneous groups of children in respect to the auxological starting point. Only Brauner *et al* [20] used an impaired adult height prediction (< 156 cm) as an inclusion criterion. In their group the benefit of GnRHa treatment was the highest reported in the literature (mean + 6.9 cm). Our results (mean change in PAH + 7.8 cm) are comparable with this study of Brauner *et al* [20]. It has to be taken into account, however, that our patients were a selected group with even worse auxological characteristics than those of Brauner *et al* [20]. To our knowledge there is no study in the literature dealing with combination treatment in a negative selection of CPP patients. Therefore, the present study is of importance in demonstrating for the first time that combined treatment with depot GnRHa and GH has the potential to exert significant positive effects in auxological terms in CPP patients who otherwise would have a very unsatisfactory outcome. These results are in good agreement with preliminary reports on combination therapy in presumably comparable groups of CPP children [29, 30]. Lee [29] demonstrated an increase in height prediction (+ 4.3 ± 1.2 cm) and a normal height velocity (6.1 ± 1.0 cm/year) in three patients with CPP and initially impaired height potential after 6 to 26 months of combination treatment. Tuvemo *et al* [30] showed that height velocity was significantly higher in the group receiving combination therapy than in the group with GnRHa therapy alone while bone maturation was comparable in both groups of girls with CPP. Additional evidence for a positive effect of combination therapy comes from a study of 10 girls with acute lymphoblastic leukemia who developed CPP several years after cranial irradiation [31]. Eight of these received combination treatment with GnRHa and GH at replacement dose for 4 years. Height SDS for bone age increased significantly. Height prediction increased by a mean of 2.8 cm. However, all of these patients had GH insufficiency in response to insulin-induced hypoglycemia. Recently,

Saggese *et al* [31] reported on short-term (12 months) results of combined GnRHa and GH treatment in CPP girls after a mean of 2.1 year of GnRHa treatment alone. The results of this study were well comparable to ours in respect to the positive effects of additional GH to height velocity (an increase from 3.0 to 6.0 cm/year), to PAH (an increase from 152.0 to 155.9 cm), and to height SDS for BA (an increase from −1.6 to −1.04). However, the selection of patients was different since PAH at start of GnRHa treatment was 2.7 cm below TH in his study compared to 13.4 cm in our study. Despite these encouraging results it seems important to make efforts to optimize the combination treatment regimen. Even better results may be achieved with different or stepwise increasing doses of GH, or with a longer treatment duration.

GnRHa treatment, in particular when depot preparations have been used suppresses the pituitary-gonadal axis effectively and leads to low prepubertal sex steroid secretion [33]. In parallel, height velocity is decreased to the low normal range with some patients growing at subnormal rates. While spontaneous GH secretion is markedly elevated in children with CPP before treatment [34] it is reduced to prepubertal levels after a few months of GnRHa treatment [35-38]. Other authors did not find a decrease in GH secretion after initiation of GnRHa therapy and interpreted the fall in height velocity as a consequence of sex steroid withdrawal [39, 40]. Thus, it is not completely clear whether declining GH levels are the reason for the low height velocity. Kamp *et al* [41] reported that during GnRHa treatment 38% of CPP patients had subnormal responses to standard GH stimulation tests and 34% had a mean nighttime GH level below the 95% confidence limit for normal prepubertal children. However, no correlation between the GH levels and the growth characteristics were found. Therefore, differences in growth rates of GnRHa-treated children cannot be explained by different spontaneous or stimulated GH levels. The possibility that statural growth may be independent of the GH-IGF axis is further supported by constantly elevated IGF-I levels during GnRHa treatment [39, 40, 42]. Both spontaneous and stimulated GH secretion were inversely correlated with BMI [41]. At time of GH testing mean BMI SDS was 0.9 in our patients and thus elevated to a similar extent as in the group of Kamp *et al* [41]. Therefore, the subnormal GH levels in some of our patients could be due rather to their increased BMI than to true GH deficiency. In addition, IGF-I levels were normal or increased for age before start of additional GH treatment in our patients. Before start of treatment there was no clinical evidence for GH deficiency in our patients. Therefore we used a supraphysiological dose of GH rather than a substitution dose. Cara et al. [43] studied two groups of CPP patients who were tested for GH deficiency before starting therapy. The GH deficient group showed a positive response to three years of combination therapy (height prediction + 10 cm) which is comparable to our results.

We conclude that girls with CPP who showed severely compromised height prediction and growth impairment during GnRHa therapy alone, are good candidates for additional GH treatment. Combination treatment offers the potential to normalize height velocity and increase height prediction and

possibly final height into the genetic target height range. The question whether underlying GH insufficiency is the main reason for the favourable response to combination therapy cannot be answered at this point.

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Chapter 10

THE IMPACT OF EARLY PUBERTY ON FINAL HEIGHT IN FOREIGN BORN, ADOPTED CHILDREN IN THE NETHERLANDS

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ABSTRACT

In a group of 859 children (465 girls, 394 boys), adopted from Colombia, India, Indonesia and South Korea to the Netherlands, growth and puberty were analysed retrospectively. In 167 of these children final height data were available. In the last mentioned group, mean (SD) age was 2.7 (1.9) years, height was –1.8 (1.5) SDS and weight for height was –0.4 (1.3) SDS, at the moment of arrival in the Netherlands. In the first two years after arrival a catch-up growth occurred, resulting in a mean height of –0.7 (1.0) SDS after two years (n = 75). After these two years, till the age of 6 to 8 years, i.e. before the onset of puberty, no change in height occurred (–0.6 [0.8] SDS [n = 34]). Despite this catch-up growth, final height was –1.2 (1.0) SDS. The loss of height must have occurred between the period just before puberty and the attainment of final height. Final height was similar to the country of origin. Mean menarcheal age occurred at 12.0 (1.5) years, i.e. at a lower age than in the countries of origin. Regression analysis revealed that in girls menarcheal age was positively related to final height ($p < 0.001$). These data suggest that, at least in adopted girls, the reduced final height is caused by an early puberty.

INTRODUCTION

Since the early seventies adoption of foreign born children to the Netherlands occurs with increasing numbers [1]. In the first months after arrival the most important problems are malnutrition, infectious diseases and dermatological problems. In the following years short stature and early puberty have been noticed regularly. Little is known about timing of puberty and final height of these adopted children.
Generally, worldwide secular changes have resulted in an increasing adult height, due to an overall improvement of the nutritional and health situation in all socio-economic groups of the population, and also to a decrease in the number of families living in less favourable conditions [2, 3]. From studies regarding growth during the first two years after arrival, we know that catch-up growth is observed in most of the adopted children [1, 4, 5]. Therefore, in view of higher standards of living, an improvement of final height in adopted children as compared to the peers in their country of origin should be expected. However, in Indian girls adopted to Sweden mean final height was similar to the country of origin [6]. In the same study a lower mean menarcheal age than in the country of origin was observed, suggesting a relationship between early puberty and lack of improvement of final height.
In the present study we evaluated height and weight evolution during the prepubertal period, the onset of puberty, and final height in children, adopted from different developing countries to the Netherlands and we studied whether there were differences between the countries of origin. In addition, determinants for the onset of puberty and final height were evaluated.

SUBJECTS AND METHODS

Subjects

We had the opportunity to participate in a follow-up study evaluating the prevalence of behavioural and emotional problems in adopted children [7]. The original population of this study consisted of 1363 children, born in Colombia, India, Indonesia or South Korea between January 1st, 1972 and December 31st, 1975, and adopted to the Netherlands. These children and their parents were approached by means of a questionnaire containing questions about growth (height and weight) and development of puberty. At that moment, the children had an age of 15 to 18 years. Nine hundred and thirty four of the 1363 (68.5%) responded to this questionnaire. The reasons for non-responding have been described in detail [7]. Seventy five of these children were excluded in a first selection because they just started puberty, and were not expected to have reached final height. Eventually 859 (465 females, 394 males) adoptees were used for analysis of puberty. We have called this group: 'the questionnaire group'.
These 859 children received a second writing in which parents were asked to

collect growth data of their children from infant welfare centres and school medical health services. The children were invited for a visit to measure final height. Two hundred and seven adolescents were willing to be measured by means of a Harpenden stadiometer. One hundred and sixty seven (128 females, 39 males) of these adolescents had reached final height. We called this group: 'The final height group'. In 34 children of this 'final height group' height data were available in the age range of 6 to 8 years. We called this group: 'The age group 6–8'. The main reason for not participating were the active role the adopted adolescents had to play her/himself to visit a hospital to be measured and the aversion to be involved in an additional research project. We evaluated whether 'the final height' group was representative for 'the questionnaire group'. This evaluation is discussed as 'Validation of representativeness of the final height group' at the results section.
Written informed parental consent was obtained before collecting the data. The study was approved by the Ethical Committees from the University Hospitals of Leiden and Rotterdam.

Methods

In 'the final height group', height and weight data at the time of arrival, and one and two years after arrival were collected retrospectively. Measurements within the first four months after arrival were considered as height and weight data at the time of arrival. Height and weight were expressed as standard deviation score (SDS) for chronological age (H-SDS and WFA-SDS respectively), using National Center for Health Statistic (NCHS) references [8, 9]. Weight was also expressed as weight-for-height-SDS (WFH-SDS) [8, 9].
Final height (FH) was considered to be reached when one of the following criteria was met: a) no height increment between our height measurement and an earlier available height measurement at least one year before (n = 126) and/or b) chronological age above 16 years in girls and 17 years in boys (n = 6) and/or c) (in girls) three or more years post-menarche at the last measurement if other growth data were not available (n = 35).
Catch-up growth was defined as the difference in H-SDS at one or two years after arrival ($H\text{-}SDS_{1yr}$ and $H\text{-}SDS_{2yrs}$ respectively) and the H-SDS at the moment of arrival ($H\text{-}SDS_{arrival}$). Influences of pubertal growth spurt on catch-up growth was eliminated: in girls growth data at one and two years after arrival were excluded when menarche occurred within four years after the two years measurement. For the same reason all boys with a chronological age above 7.0 years at the moment of arrival and/or a history with early puberty, were excluded.

Statistics

Results are expressed as mean (SD), unless mentioned otherwise.
Baseline differences between the countries and sexes were tested by ANOVA,

followed by Student t-test. The repeated measures of height and weight evolution were analysed by mixed model ANOVA [10]. All effects, including differences between countries or sexes, were analysed with F tests. In case of significance, differences were tested with paired and unpaired Student t-tests.
Univariate regression analysis was performed to distinguish the independent determinants at arrival and 1 year after arrival, which might be related to the dependent determinants: final height SDS ($H\text{-}SDS_{final}$), delta $H\text{-}SDS_{final}$ versus $H\text{-}SDS_{arrival}$ ($\Delta\ H\text{-}SDS_{final\text{-}arrival}$) and menarcheal age. The evaluated independent determinants were: age, $H\text{-}SDS_{arrival}$, $WFH\text{-}SDS_{arrival}$, $WFA\text{-}SDS_{arrival}$, $\Delta\ H\text{-}SDS_{1yr\text{-}arrival}$, $\Delta\ WFH\text{-}SDS_{1yr\text{-}arrival}$, $\Delta\ WFA\text{-}SDS_{1yr\text{-}arrival}$, and additionally in girls, menarcheal age. In a multivariate regression analysis we studied the influence of menarcheal age on final height.
Significance level α was 0.05.

RESULTS

The questionnaire group

Age at arrival

In the group of 859 adolescents (465 females, 394 males) median age at the time of arrival was 2.7 years with a range of 0.1–10.6 years (mean: 2.9 [2.1]).

Puberty

Mean age of menarche in girls was 12.0 (1.5) years (n = 446). The distribution of menarcheal ages was normal (figure 1). In 6.7% of the girls menarcheal age was below the age of 10 years. Ages at menarche differed significantly between the four countries ($p < 0.01$), with the exclusion of India compared to Colombia. The lowest age at menarche was observed in girls originating from India and the highest in girls born in S-Korea (table 1). Age at menarche was significantly lower in all adopted girls compared with Dutch girls (median: 13.3 years) ($p < 0.01$) [11]. In girls born in India and Indonesia it was also lower compared with the median age at menarche in the country of origin ($p < 0.05$)(table 1).
Insufficient data concerning the pubertal development of puberty in boys were available.

The final height group

Growth parameters at arrival

In the group of 167 children (128 girls, 39 boys) age at arrival was 2.7 (1.9) yrs (range: 0.1–7.9 yrs). This was similar to the larger 'questionnaire group'.
In 111 children (88 girls, 23 boys) of 'the final height group' growth data were

available at the moment of arrival (table 2). We did not find any differerences between the growth data of girls and boys at baseline. Therefore, growth data of girls and boys were combined.
No difference in the age at arrival between the four countries could be demonstrated.
In the total group of children H-SDS$_{arrival}$ was –1.8 (1.5). The highest H-SDS$_{arrival}$ was seen in children originating from Indonesia compared to Colombia, India and S-Korea ($p < 0.001$, $p = 0.02$ and $p = 0.003$, respectively) (figure 2).
In the total group of children WFH-SDS$_{arrival}$ was –0.4 (1.3). No differences in WFH-SDS$_{arrival}$ were seen between the four countries. WFA-SDS$_{arrival}$ was –1.4 (1.3). Children originating from Indonesia and S-Korea had the highest WFA-SDS$_{arrival}$.

Table 1. *Age at menarche in adopted girls (in years)*

Country of origin	Mean (SD)	n	95% confidence interval of the mean	ref.-value (median)
Colombia	11.6 (1.5)	77	11.3 –11.9	
India	11.2 (1.5)	82	10.9 –11.5	13.7[12]
Indonesia	12.0 (1.4)	38	11.5 –12.5	12.9[26]
South Korea	12.4 (1.4)	259	12.2 –12.6	
Total	12.0 (1.5)	446		

Table 2. *Baseline characteristics (mean (SD)) of 111 adopted children*

	Colombia	India	Indonesia	South-Korea	ANOVA
number	17	20	15	59	
girls/boys	11/6	18/2	10/5	49/10	
age at adoption (yrs)	3.4 (2.1)	2.9 (2.0)	2.6 (2.4)	2.6 (1.5)	$p > 0.05$
height (SDS)	–2.5 (1.2)	–2.1 (2.1)	–0.7 (1.1)	–1.8 (1.2)	$p < 0.01$
weight for height (SDS)	–0.6 (1.7)	–0.8 (1.4)	–0.6 (0.9)	–0.1 (1.1)	$p > 0.05$
weight for age (SDS)	–1.9 (1.0)	–1.9 (1.6)	–1.0 (1.0)	–1.2 (1.3)	$p < 0.05$

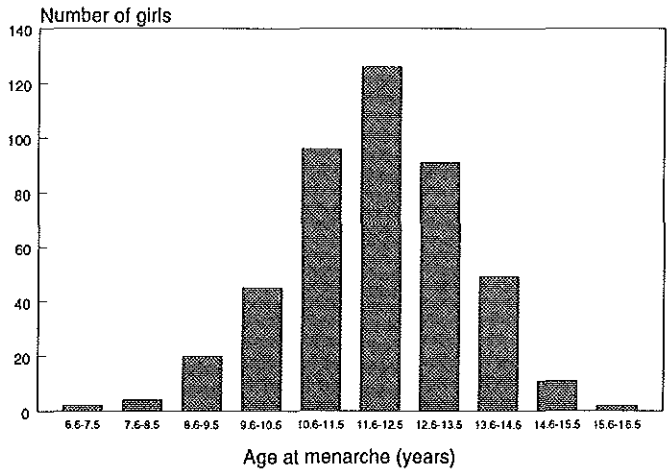

Figure 1. Distribution of the age at menarche in adopted girls (n = 446)

Growth parameters during the first two years after arrival

In 94 (73 girls, 21 boys) of the 111 children with growth data at arrival, growth data were available one year after arrival. In 75 (61 girls, 14 boys) of them growth data were also available two years after arrival (table 3). In 7 girls the growth data at one and two years after arrival could not be used, because of interference of catch-up growth by puberty. We did not find any differences between the growth data of girls and boys.

During the first two years after arrival H-SDS increased to −1.1 (1.1) SDS_{1yr} and −0.7 (1.0) SDS_{2yrs} ($p < 0.001$). The changes in H-SDS in the first two years after arrival were different between the four countries ($p = 0.001$) (figure 2), with the lowest increment of 0.3 (0.8) SDS in the group originating from Indonesia compared to Colombia, India and S-Korea ($p = 0.002$, $p = 0.23$ and $p = 0.003$ respectively) (table 3). In 86 of the 94 children (91.5%) a positive catch-up growth in the first one or two years after arrival was observed.

WFH-SDS increased to + 0.1 (1.1) SDS and + 0.0 (1.0) SDS one and two years after arrival respectively ($p < 0.001$). DeltaWFH-SDS during the first two years after arrival were similar in the four countries.

During the first two years after arrival WFA-SDS increased to −0.6 (1.1) SDS_{1yr} and −0.5 (1.0) SDS_{2yrs}, respectively ($p < 0.001$). WFH-SDS_{2yrs} differed between the countries ($p < 0.001$)(table 3). Delta WFA-SDS in the first two years after arrival were different between the countries ($p = 0.04$), with the highest increment in the children originating from Colombia compared to India, Indonesia and S-Korea ($p = 0.03$, $p = 0.002$ and $p = 0.08$ respectively) (table 3).

Table 3. *Characteristics (mean [SD] [n]) of adopted children 2 years after arrival*

	Colombia	India	Indonesia	South-Korea	ANOVA
height (SDS)	− 0.6 (1.1) (10)	− 0.9 (1.1) (13)	− 0.4 (0.8) (10)	− 0.7 (1.0) (42)	$p > 0.05$
weight for height (SDS)	− 0.1 (0.4)(9)	− 0.8 (1.1) (12)	− 0.6 (0.7) (9)	+ 0.5 (0.8) (34)	$p < 0.001$
weight for age (SDS)	− 0.5 (0.7) (9)	− 1.3 (0.9) (13)	− 0.8 (0.7) (10)	− 0.2 (0.9) (36)	$p < 0.05$
Δ height (SDS)	+ 1.6 (0.8) (10)	+ 0.8 (1.3) (13)	+ 0.3 (0.8) (10)	+ 1.1 (0.8) (42)	$p < 0.05$
Δ weight for height	+ 1.2 (1.4) (8)	+ 0.3 (1.0) (12)	+ 0.4 (0.9)(9)	+ 0.5 (0.9) (33)	$p > 0.05$
Δ weight for age	+ 1.6 (0.8) (8)	+ 0.6 (1.0) (13)	+ 0.4 (0.6) (10)	+ 1.1 (1.1) (35)	$p < 0.05$

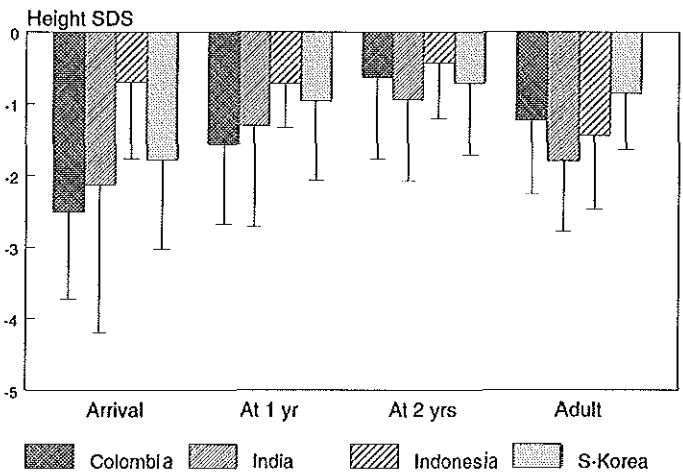

Figure 2. Height-SDS at arrival, one and two years after arrival and at final height, in adopted children (n = 111) in relation to country of origin.

Height at the age of 6 to 8 years

To analyse if there was any change in H-SDS in the period after 2 years following arrival but still before puberty, the H-SDS data were studied in those children in whom height data were also available at the age of 6 to 8 years ('the age group 6–8')($H\text{-}SDS_{6-8}$). The data of the girls with a menarche within 4 years after the age of 6 to 8 years were not used, to exclude pubertal growth. Thirty-four (25 girls, 9 boys) of the 75 children with height data available at 1 and 2 years after arrival, were included in this part of the analysis.

The height data of 'the age group 6–8' are presented in figure 3. H-SDS increased gradually after arrival till the age of 6–8 yrs. No significant change was observed between $H\text{-}SDS_{2yrs}$ and $H\text{-}SDS_{6-8}$.

H-SDS$_{arrival}$ and the course of H-SDS from arrival till FH in this group, did not differ from the course of H-SDS in the remaining part of 'the FH group'. Also WFH-SDS$_{arrival}$, WFA-SDS$_{arrival}$, sex and country distribution did not vary between these groups. Age at arrival was lower in 'the age group 6–8' compared with the remaining part of 'the FH group' (2.0 [1.3] vs 3.3 [2.1] yrs respectively, $p < 0.001$). In addition, age at menarche was higher in 'the age group 6–8' (12.4 [1.0] vs 11.3 [1.4] yrs respectively, $p < 0.001$) as well as H-SDS$_{final}$ (–0.9 [0.9] vs –1.3 [1.0] SDS respectively, $p = 0.040$), but this can be explained by the fact that girls with a menarche within 4 years after the age of 6 to 8 years, were omitted from 'the age group 6–8'.

Growth parameters at adulthood

From the moment of arrival till FH, H-SDS changed significantly ($p < 0.001$): H-SDS$_{final}$ (–1.2 [1.0]) was higher than H-SDS$_{arrival}$ ($p < 0.001$), but lower than H-SDS$_{2yrs}$ ($p = 0.002$). H-SDS$_{final}$ was not significantly different between girls and boys. The highest H-SDS$_{final}$ was observed in the children from S-Korea (figure 2). This was significantly higher compared to Colombia, India and Indonesia ($p = 0.02$, $p < 0.001$ and $p = 0.002$ respectively).
Mean FH in girls was 156.2 (6.1) cm (H-SDS$_{final}$: –1.1 [1.0]) ($n = 128$) and in boys 168.2 (6.3) cm (H-SDS$_{final}$: –1.4 [0.9]) ($n = 39$), 8.5 and 7.5 cm respectively below the adult height according to NCHS references. Mean FH of the studied adoptees from Colombia and India was not significantly different from the mean

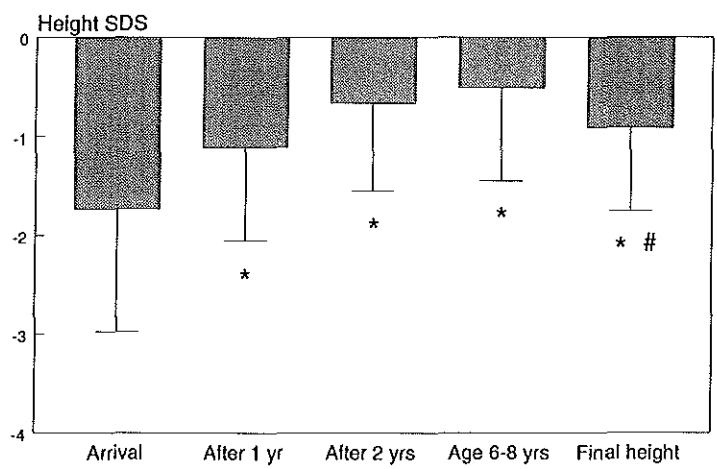

Figure 3. Height-SDS at arrival, one and two years after arrival, at the age of 6 to 8 years and at final height in the 'age group 6–8' ($n = 34$).
* $p < 0.001$ compared to H-SDS at arrival
\# $p = 0.002$ compared to H-SDS$_{2yrs}$ and to H-SDS$_{6-8}$.

adult height in their country of origin [12, 13] (figure 4a and 4b). Children from S-Korea were slightly but not significantly taller than in their country of origin [14]. Adult height data from Indonesia were not available.
Mean adult height was also lower compared to Dutch standards (14 and 12 cm below mean adult height in girls and boys, respectively) [11]. In 49% of the children FH was below the third centile and in 98.8% below the 50th centile according to Dutch standards. The questionnaire revealed that 44% of these children considered themselves short.
As shown in figure 3, in 'the age group 6-8' H-SDS$_{final}$ was lower than H-SDS$_{2yrs}$. Moreover, H-SDS$_{final}$ was also lower than H-SDS$_{6-8}$, indicating loss of height occurring between the age just before onset of puberty and FH, for H-SDS$_{2yrs}$ and H-SDS$_{6-8}$ were in the same range.
For adults NCHS references forWFA are available, but not forWFH. Compared to these references WFA$_{final}$ was −1.0 (1.0) SDS, i.e. higher than WFA$_{arrival}$ (−1.4 [1.3] SDS) (p = 0.005), but lower than WFA$_{2yrs}$ (0.5 [1.0] SDS) (p = 0.013).

Determinants influencing final height and age at menarche

In Table 4 the univariate analysis of independent determinants influencing H-SDS$_{final}$, Δ H-SDS$_{final-arrival}$, and age at menarche, have been summarized. H-SDS$_{final}$ was significantly positively related to H-SDS$_{arrival}$, WFH-SDS$_{arrival}$ and WFA-SDS$_{arrival}$. Another significant influencing determinant was the age at menarche: each increase of menarcheal age with 1.0 year, resulted in a increase of H-SDS$_{final}$ with 0.27 SDS. Δ H-SDS$_{final-arrival}$ was significantly negatively related to H-SDS$_{arrival}$ and WFA-SDS$_{arrival}$. Additionally, it was significantly positively related to Δ H-SDS$_{1\ yr-arrival}$, Δ WFA-SDS$_{1\ yr-arrival}$, and age at menarche. No

Table 4. Univariate analysis of determinants for H-SDS$_{final}$, delta H-SDS$_{final-arrival}$ and age at menarche. The estimated mean change (SE of the mean) in delta H-SDS$_{final-arrival}$, H-SDS$_{final}$ and age at menarche are presented, if the determinant increases with 1 unit.

Determinants	H-SDS$_{final}$	Δ H-SDS$_{final-arrival}$	Age$_{menarche}$
Age arrival (yrs)	−0.05 (0.04)	0.02 (0.07)	−0.06 (0.08)
Height SDS arrival	0.22 (0.06) ***	−0.78 (0.06) ***	−0.10 (0.10)
Weight for height SDS arrival	0.20 (0.07) **	0.13 (0.12)	0.10 (0.12)
Weight for age SDS arrival	0.24 (0.07) ***	−0.41 (0.10) ***	−0.03 (0.11)
Δ Height SDS 1 yr-arrival	−0.08 (0.12)	1.01 (0.15) ***	−0.07 (0.18)
Δ Weight for height SDS 1 yr-arrival	0.06 (0.10)	0.06 (0.16)	0.05 (0.15)
Δ Weight for age SDS 1 yr-arrival	0.07 (0.12)	0.53 (0.18) **	−0.02 (0.17)
Age menarche (yrs)	0.27 (0.06) ***	0.42 (0.09) ***	–

* $p <= 0.05$
** $p <= 0.01$
*** $p <= 0.001$

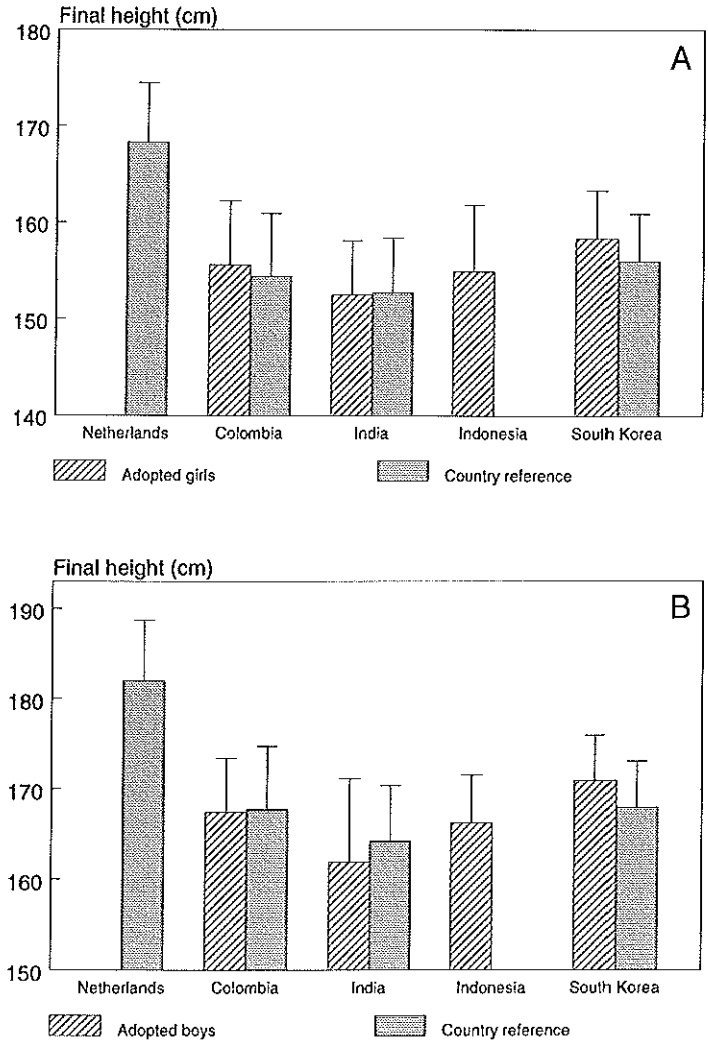

Figure 4. Upper pannel: **A.** Final height in cm (mean ± sd) in adopted girls.
Lower pannel: **B.** Final height in cm (mean ± sd) in adopted boys.

determinant influencing age at menarche could be demonstrated, even after correction for country of origin.

The influence of age at menarche on H-SDS$_{final}$ in girls, was studied in a multivariate linear regression analysis, with correction for country of origin and H-SDS$_{arrival}$ ($R^2 = 0.45$; $n = 88$; $p < 0.001$). The model revealed that age at

menarche and H-SDS$_{arrival}$, were strongly related to H-SDS$_{final}$ (both $p < 0.001$). The contribution of the various countries was not significant.
The same analysis was performed with Δ H-SDS$_{final\text{-}arrival}$ as the dependent determinant. Similar results were obtained ($R^2 = 0.76$; $n = 88$; $p < 0.001$).

Validation of representativeness of 'the final height group'

To find out whether selection has influenced the results we compared the height data of 'the FH group', measured by ourselves, with height data of the questionnaire group. These data were obtained by asking the parents to measure their children. In the population of 859 children FH documented by the parents was 157.4 (6.0) cm ($n = 310$) in girls with a chronological age of 16 to 18 years and 170.4 (6.3) cm ($n = 134$) in boys with a chronological age of 17 to 18 years. No significant difference between FH documented by the parents and FH measured by ourselves (156.2 (6.1) cm [$n = 128$] in girls and 168.2 (6.3) cm [$n = 39$] in boys) was observed. Besides, no differences could be observed in mean age at arrival and mean age at menarche in girls between these groups. Since no significant differences were found in these mentioned items, it seems reasonable to assume that the 167 children are representative for the original group of 859 children.

DISCUSSION

The purpose of this study was to obtain a better understanding of growth and pubertal development of foreign-born adopted children in the Netherlands. In the literature most authors have described growth in the first years after arrival [1, 4, 5]. Only two reports about menarche and final height have been published [5, 6].
As recently reviewed by Hauspie [3] a secular increase in height is still occurring in most countries. However, several developing countries or areas do not exhibit evidence of a secular trend in height and a negative secular trend is reported in groups with a declining socio-economic and health status. With this knowledge, we were very interested to find out what would happen in children, adopted from developing countries and transported from low to high standards of living. In our study, in all adopted children, H-SDS$_{arrival}$ was below the mean of NCHS references. WFH$_{arrival}$ was just below the mean. Usually, WFH was considered as a rather good parameter for acute malnutrition and height as a rather good parameter for chronic malnutrition [15,16]. However, from a recent study we know that none of the parameters as WFH, WFA or height are optimal parameters for evaluating the nutritional status, and that midupper-arm circumference and clinical examination should be a nesessary part of any comprehensive nutritional status [17]. Our data suggest that the children of our population had a chronic but not a acute malnutrition, but the exact determination of the nutritional status was not possible.

In the first two years after arrival a catch-up in height and weight for age, but not in weight for height, was observed in all adopted children. After these two years height did not appear to change much, as height SDS at the age of 6–8 years was similar to height SDS 2 years after arrival.

Mean FH in adopted girls and boys was 8.5 and 7.5 cm respectively lower than the FH according to NCHS references and 14 and 12 cm respectively lower compared to Dutch standards. In children adopted from Colombia and S-Korea FH was in the same range as the mean adult height in the country of origin. The FH in Indian adopted girls (152.5 cm) was equal to the mean FH in less privileged Indian girls (152.7 cm), but 6.7 cm lower than FH in privileged Indian girls (159.2 cm) [12]. This all despite the fact that catch-up growth in the first two years after arrival was observed and, in the 34 children in whom data were available, height remained at the same level in the prepuberal period. In view of this catch-up growth and the existence of a secular trend in Western countries, an increase in adult height, compared with the country of origin, would be expected. The knowledge that children of immigrants have shown a gradual catch-up growth and have become taller than their parents and their peers in their country of origin as a result of better environmental conditions [18–20], also contributes to this expectation. In contrast, loss of height was demonstrated, occurring between the period just before onset of puberty and FH. Insufficient data were available to find out, whether this was true for the whole population. The presence of an early age at menarche suggests that this height loss probably occurs as a consequence of a shortened period of growth before puberty [21], rather than by a reduced pubertal growth spurt.

Summarized, various auxological differences in children originating from the four countries were found: (1) a difference in $H\text{-}SDS_{arrival}$, with a different course in H-SDS till final height and significant differences in $H\text{-}SDS_{final}$; (2) differences in WFA at arrival, but not at final height; (3) no differences in WFH.

There were also differences in ages at menarche between the four countries, with lowest menarcheal ages in girls originating from India. In the adopted girls menarche occurred significantly earlier than in Dutch girls. In adopted girls from India and Indonesia mean age at menarche was also significantly lower than in the girls living in their country of origin. We are not able to comment on the age at menarche in the girls originating from Colombia or S-Korea because of lack of reference data from these countries.

We established a positive correlation between age at menarche and FH. Multiple regression analysis confirmed the strong influence of age at menarche on FH. As data about puberty were obtained by a questionnaire, no judgement could be given about the onset of puberty in boys and its influence on final height.

In animal studies Bourguignon showed an association between an increase in growth rate resulting from unrestricted feeding after nutritional deprivation and an accelerated hypothalamic and testicular maturation in rats [22]. However, such an effect was only observed when refeeding occurred before weaning, indicating that hypothalamic maturation was sensitive to changes in nutritional conditions and growth rate during a critical period preceding the onset of

puberty. In a study in Indian girls adopted to Sweden a faster weight and height catch-up and a higher age at the moment of arrival were associated with earlier menarche [5], indicating that a fast catch-up growth after the age of 3 years could give rise to a change in the maturation of the hypothalamic-pituitary-gonadal axis. For this reason we have looked at potential parameters influencing menarcheal age, as age, height and weight at arrival and height and weight changes after arrival. Although height catch-up occurred and a slightly positive change in WFA was observed in the first two years after arrival, multiple regression analysis with respect to age at menarche did not reveal such influencing variables. An explanation for this finding might be an earlier age at arrival in the Netherlands compared with the Indian girls adopted to Sweden (2.9 versus 3.7 years) and a better nutritional status at arrival in the Dutch adopted children (WFA-SDS: –1.4 versus –2.2) [5, 6]. With respect to these data catch-up in weight probably might be more important than height catch-up in influencing central nervous system maturation. On the other hand, we have also observed a relatively early puberty in our population (age at menarche 12.0 years, versus 11.5 years in the Swedish population [6]), suggesting that also other unknown factors, for instance intra-uterine nutritional circumstances, are influencing central nervous system maturation.
We conclude that under higher standards of living catch-up growth after adoption does not result in an increment of FH. Early puberty appears to be the most important factor compromising FH, a phenomenon that is known from precocious puberty as well [23]. Treatment with GnRH-agonists, probably combined with growth hormone therapy [24, 25] might be considered to delay puberty and to increase FH.

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Chapter 11

GENERAL DISCUSSION AND CONCLUSIONS

General discussion and conclusions

In the last twenty years there has been tremendous progress in research into GnRH. GnRH was isolated and characterized by Schally and Guillemin in 1971. The time between discovery and clinical use was remarkably short. GnRH analogs have been developed and numerous clinical therapies designed to stimulate or suppress reproductive processes have been introduced. Initially, treatment focused on metastatic prostate and breast cancer, but later on GnRH agonists were also introduced to treat gynaecological disorders, for instance endometriosis, uterine fibroids, polycystic ovary syndrome, and ovulation induction. Since 1981, GnRH agonists have been used in the treatment of CPP (Crowley *et al*, 1981). In the Netherlands the GnRH agonist buserelin was introduced for this indication in 1983 (Drop *et al*, 1987). Like most of the GnRH agonists used up till then, the drug had to be administered subcutaneously (s.c.) once a day, sometimes even twice a day. Therefore, in 1986, there was great interest in the action of the new slow-release GnRH agonist, triptorelin, since it could be administered intramuscularly (i.m.) every 4 weeks. Up till then, preliminary data were available about the use of this GnRH agonist to treat children with CPP (Roger *et al*, 1986).
The short-term and long-term results of treatment with this GnRH agonist triptorelin are described in this thesis. Most of the results are based upon girls, as the number of boys studied was rather small.

Short-term results with triptorelin

Treatment with triptorelin resulted in the suppression of serum concentrations of gonadotropins and sex steroids. LH responses to GnRH testing are the most definitive way to determine whether adequate gonadotropin suppression has occurred. For the estimation of serum gonadotropins, validated assays have to be used, such as IRMA (Cavallo *et al*, 1995), IFMA (Lee, 1994; Wu *et al*, 1996) or ICMA (Neely *et al*, 1995). The value of these tests has already been discussed in the introduction to this thesis. Generally, a suppressed response after GnRH testing is considered as adequate suppression. This is at variance with a dose-finding study of leuprolide acetate depot in which nocturnal LH sampling was found to be more sensitive than GnRH testing alone for assessing suppression of the hypothalamic-pituitary-gonadal axis (Cook *et al*, 1992). However, it is important to know that in their study Cook *et al* used a less sensitive radio-immunoassay for estimation of LH.
As described in chapters 2 and 6, basal and stimulated LH and FSH levels were shown to be suppressed within six months after the start of treatment and sex steroids within four weeks. Secondary sexual chacacteristics came to a halt or regressed, vaginal bleeding stopped and behavior became less emotional and less aggressive. Additionally, growth velocity normalized, the rate of bone maturation decreased and adult height prediction improved. In summary, all the goals of treatment for CPP were reached.

Our data seem to be comparable with the short-term results of other GnRH agonists, administered daily s.c. (Mansfield *et al*, 1983; Brauner *et al*, 1985; Styne *et al*, 1985; Boepple *et al* , 1985; Comite *et al*, 1986; Lee & Page, 1989), but seem to be better than the variable suppressive results with daily s.c. preparations described by some other authors (Holland *et al*, 1986; Drop *et al*, 1987; Sizonenko *et al*, 1990). Short-term results with intranasally (i.n.) administered GnRH agonists seem to be less favourable (Stanhope *et al*, 1985; Holland *et al*, 1986; Bourguignon *et al*, 1987). Similar short-term results with i.m. triptorelin were obtained by other investigators (Roger *et al*, 1986; Brauner *et al*, 1992; Marcondes *et al*, 1993). The data reported about another depot preparation, leuprolide depot, that is administered s.c, also seem similar, although higher dosages had to be used more frequently (Parker *et al*, 1991; Clemons *et al*, 1993; Carel *et al*, 1995). All the above-mentioned reports are based upon various GnRH agonists with different biopotencies plasma concentrations and possible failure of compliance (Holland *et al*, 1986; Sizonenko *et al*, 1990; Filicori *et al*, 1993). Furthermore, investigators used different hormonal assays and scored bone ages at a wide variation. Therefore, it is impossible to make a fair and accurate comparison between different GnRH agonists.
In conclusion, short-term results indicate that all the goals of treatment for CPP are reached and that a slow-release GnRH agonist seems to be superior to GnRH agonists administered intranasally and sometimes seem to be superior to GnRH agonists administered daily subcutaneously.

Long-term auxological results

Treatment with triptorelin for a period of four to five years resulted in sustained suppression of gonadotropins and sex steroids. During treatment height velocity decreased to rather low prepubertal values. Additionally, the rate of bone maturation decreased. In general, the decrease in bone maturation exceeded the decrease in height velocity and resulted in an improvement of predicted adult height at the moment when therapy was discontinued: In girls a positive change in predicted adult height of 5.9 and 4.5 cm was observed after treatment for a period of 4 to 3.3 years, respectively. Height SDS for bone age, another parameter indicating height prognosis as has been discussed in the introduction, also showed an improvement.
An increase in height velocity was observed only in the first year after therapy had been discontinued. Bone maturation accelerated immediately after cessation, with a further advancement in the second year. Finally, in girls, a 'height gain' (= difference in adult height minus predicted adult height at the start of treatment) of 3.5 cm was achieved. Adult height was below target height. In boys, no improvement in adult height was observed. In contrast to our study, others have described an improvement in adult height in boys (Boepple *et al*, 1991; Paul *et al*, 1995). The most plausible explanation for this difference is that most of the boys in our study were suffering from neurofibromatosis, a condition which in itself is associated with growth impairment.

The height gain of 3.5 cm in girls in our study lies between the values of 2.4 – 6.2 cm reported in two other studies where daily s.c. preparations were used (Boepple *et al*, 1991; Oerter *et al*, 1991). Using triptorelin a greater improvement in predicted adult height, namely 6.5 cm has been reported by Brauner, despite the fact that the inclusion criteria used were similar to ours (Brauner *et al*, 1994). The most remarkable positive change in predicted adult height with an s.c. preparation, namely 10 cm, was described in a group of girls treated before they were 5 years old (Paul *et al*, 1995). Also Kletter & Kelch (1994) described better results in younger children. This tendency of young patients to obtain a greater positive change in predicted adult height was observed in our population too. Therefore, age at the onset of therapy seems to play an important role.
Height SDS at the start of therapy proved to be the most important positive factor, influencing adult height. In addition, bone age at the point of discontinuation of therapy is another important, negative factor contributing to final height. Height gain seems to be greater when bone age at the point of discontinuation of triptorelin was lower than 12.5 years.
It is concluded, that in girls treatment with triptorelin improves adult height, but height is not restored to its genetic potential. Height and age at the onset of therapy and bone age at the point of discontinuation of treatment seems to play an important role. Discontinuation of triptorelin treatment might be considered at a bone age of 12 to 12.5 years.

Complete or partial suppression

Several years ago, when no final height data were available about children who had been treated with GnRH agonists, there was a discussion about whether suppression of the pituitary-gonadal axis by a GnRH agonist needed to be complete for a child to obtain optimum adult height (Boepple *et al*, 1993). Complete suppression is defined as a prepubertal LH response after GnRH testing and when estradiol levels are below 50 pmol/l or testosterone levels are below 0.5 nmol/l. Those advocating incomplete suppression argued that low, but detectable levels of estrogens are essential for adequate GH secretion (Bourguignon, 1988). These differences of opinion led us to perform our comparative retrospective study of triptorelin and buserelin administered s.c. or i.n. We concluded that the rate of growth and bone maturation during the first 18 months of treatment was faster in the buserelin group and that height prediction did not improve significantly, in contrast to the triptorelin group. This suggested the possibility of a greater adult height in the triptorelin group. Because the medication for most of our buserelin treated children was changed and buserelin was replaced by triptorelin, no final height data are or will be available. Consequently it is not possible to draw a final conclusion about the effect of complete or partial suppression.
In contrast to our results, Heinrichs *et al* (1994) did not find any significant

increase in predicted adult height when they compared i.m. triptorelin treatment with i.n. buserelin in a short-term study, although they found that stimulated gonadotropins were more successfully suppressed during triptorelin treatment. In a comparative study of Antioniazzi *et al* (1994) obtained a better result in adult height with i.m. triptorelin than with i.n. buserelin. In that study suppression by triptorelin was considered more complete than with i.n. buserelin. In the studies of Boepple *et al* (1991) and Oerter *et al* (1991) with s.c. preparations, the results of predicted adult height at the moment of discontination and the adult height results be comparable with those of our study of final height. The degree of suppression appeared to be complete in these studies. In contrast, therapies for CPP using progesterone, which did not achieve complete suppression, failed to improve predicted or actual final height (Werder *et al*, 1974; Lee, 1981; Sorgo *et al*, 1987). Final heights in GH- plus GnRH-deficient patients (with no spontaneous puberty) exceeded final heights in isolated GH-deficient patients (Burns *et al*, 1981), which is an additional argument in favour of striving for complete suppression. In contrast, some other phenomena such as a subnormal height velocity and a decreased GH secretion during complete suppression as a result of GnRH agonist therapy, are arguments that support the idea that partial suppression during GnRH agonist therapy might be preferable.
We conclude, based upon our short-term and final height results and the above-mentioned data from the literature, that complete suppression seems to be preferable to partial suppression.

Resumption of puberty after discontination of GnRH agonist therapy

After treatment with triptorelin has been discontinued the hypothalamic-pituitary-gonadal axis recovered rather rapidly, and gonadotropins and sex steroids returned to normal pubertal values and, in girls, menarche occurred after about one year. These results are comparable with those of others (Manasco *et al*, 1988; Kreiter *et al*, 1990; Jay *et al*,1992). Menstrual cycles became regular in the majority of the girls. Pelvic ultrasonography of the internal genitalia showed no abnormalities, and no signs of polycystic ovaries, such as has been reported by some authors (Adams *et al*, 1993; Bridges *et al*, 1995). Because the patients in our studies were too young for reproduction, no conclusions can be made about fertility. Data about fertility in children with CPP, untreated and following treatment with cyproterone acetate and GnRH agonists, have been reported by some authors, but all these reports are based upon only a few cases (Murram *et al*, 1984; Cisternino *et al*, 1992; Jay *et al*, 1992). *We conclude*, that gonadotropin and sex steroid secretion and menses resume normally after long-term treatment with triptorelin is discontinued. Follow-up studies have to be done in order to characterize fully the reproductive function in CPP patients during adolescence and adulthood.

Combined treatment with triptorelin and GH

During treatment with GnRH agonists height velocity decreases to low normal ranges and may fall to subnormal values in a subset of CPP patients. Whereas spontaneous GH secretion is markedly elevated in children with CPP before treatment, some authors have reported that after a few months of GnRH agonist treatment it is reduced to prepubertal levels (Mansfield *et al*, 1988; Stanhope *et al*, 1988; DiMartino-Nardi *et al*, 1991). Other authors did not find a decrease in GH secretion and interpreted the drop in height velocity to be a direct consequence of the withdrawal of sex steroids (Attie *et al*, 1990; Sklar *et al*, 1991). In our population, GH secretion was suppressed in most of the girls during GnRH agonist treatment. Thus, it is not completely clear whether declining GH levels are the reason for low height velocity. Kamp *et al* (1991) reported subnormal GH responses in one third of CPP patients treated with a GnRH agonist. They did not find a correlation between the GH levels and growth characteristics but they demonstrated that both spontaneous and stimulated GH secretion were inversely correlated with BMI. Although the exact cause of the decrease in height velocity was not known, the purpose of our provisional study on the combination of triptorelin and GH (chapter 3 and 9) was to determine the effects of additional GH treatment on bone maturation, height velocity and adult height prediction in GnRH agonist-treated girls with CPP. We included only patients with unfavourable auxological characteristics, such as an adult height prediction below the 3rd centile (i.e. < 156 cm)(Roede & van Wieringen, 1985) and a low height velocity during GnRH agonist therapy. In these patients height prediction did not change during triptorelin treatment. However, prediction increased significantly during combination therapy (8.4 [2.1] cm, chapter 9). Our preliminary results are in line with preliminary reports in comparable groups of children with CPP (Lee, 1992; Tuvemo, 1992; Saggese *et al*, 1995).

We conclude that girls with CPP showing severely compromised height predictions and growth impairment during treatment with triptorelin, may be candidates for additional GH treatment. Combination therapy may be successful in normalizing height velocity and increasing height prediction and possibly in increasing adult height. At this point we cannot say whether underlying GH insufficiency is the main reason for the favourable response to combination therapy.

Side effects

During all of our studies triptorelin had no effect on blood count, liver or renal function. No local side effects, suggestive of drug sensitivity, were observed and no sterile abcesses were seen at the injection site, although other authors have reported these in 3–13 % of children who recieved subcutaneous depot injections of leuprolide acetate (Manasco et al, 1993; Carel et al, 1995). Triptorelin did not influence body proportions. Body mass index seems to

increase with triptorelin treatment. In our population after discontinuation of triptorelin there was normal resumption of puberty and there were no signs of polycystic ovaries such as reported by Adams (Adams et al, 1993).
Recently, attention has focused on bone mass in children with CPP. Adolescence is a crucial time for bone development, and estrogens are known to play an important role in the increase in bone mass observed during puberty. Peak bone mass achieved during this period is an important determinant of postmenopausal osteoporosis (Riggs & Melton, 1988). Hypo-estrogenic conditions such as natural menopause and GnRH agonist administration in premenopausal women are characterized by bone mass reduction, although recovery has been reported after the discontinuation of GnRH agonists. GH plays an important role too, as GH-deficiency is associated with decreased bone density in both growing and adult individuals (Kaufman *et al*, 1992). The results of studies about bone density in treated and untreated children with CPP are contradictory. In all studies bone density was increased before the start of treatment. In two studies a reduction in bone density was reported one year after a 12 month period of GnRH agonist treatment (Saggese *et al*, 1993; Antoniazzi *et al*, 1995). In contrast, Neely *et al* (1995b) did not observe any reduction in bone density during a 24 month period of GnHR agonist treatment. No reduction in bone density was observed 18 months after the discontinuation of GnRH agonist treatment (Boepple *et al*, 1995; Lee *et al*, 1995). However, these latter studies did not include longitudinal evaluations. Therefore, no conclusion about bone mass during and after GnRH agonist therapy can be made. Longitudinal studies are needed in order to determine whether bone loss occurs during GnRH agonist therapy and whether it is relevant for the attainment of peak bone mass at the time that growth is completed.
We conclude, that the only side effect observed during triptorelin treatment is the increase in BMI. We did not study bone mass development. Literature data are contradictory and longitudinal studies need to be performed.

Recommendations on whether to treat or not to treat

First of all it is very important to make a correct diagnosis and to differentiate between CPP, premature thelarche and GnRH-independent forms of precocious puberty. A pubertal LH response to GnRH confirms the diagnosis of CPP. A low LH response to GnRH does not rule out an early phase of CPP. The condition should be reassessed after 3 to 6 months when clinical signs of puberty progress. The use of a GnRH agonist test, as advocated by Ibanez *et al* (1994), may be used as an additional test in the early diagnosis of precocious puberty. Uterine length and endometrial echo measurements by pelvic ultrasonography can provide additional parameters for differentiating between CPP and premature thelarche.
Once CPP has been diagnosed, the possible existence of central nervous system abnormalities has to be checked using MRI, since MRI provides optimal imaging for the initial evaluation of patients with CPP. In children without any

additional neurologic signs and symptoms, no gadopentetate dimeglumine has to be used.
In young children, i.e. under the age of 7 years, treatment with a GnRH agonist should be started without delay. In older children, i.e. between 7 and 9 years, GnRH agonist treatment should be considered seriously when puberty advances rapidly or when psychological problems are present.
GnRH testing is the best way to monitor adequate suppression by GnRH treatment. A simplified GnRH test can be considered, which considers the use of a single serum sample assayed for LH, 30 to 60 minutes after an intravenous bolus (Cavallo *et al*, 1995) or 40 minutes after a subcutaneous GnRH bolus (Eckert *et al*, 1996).
We conclude that triptorelin should be considered seriously in the treatment for CPP, since this slow-release GnRH agonist leads to an optimal compliance and a complete suppression, without major side effects.

Height and puberty in adopted children

In most children with short stature in the Dutch population puberty is delayed. Foreign-born children adopted and brought to the Netherlands, also have a short stature, but as described in chapter 10, the girls develop puberty at an earlier age than the girls in their country of origin. In contrast to our expectations, adult height was not greater than that of peers in their country of origin, although catch-up growth was observed in the first two years after arrival and positive secular changes are known to occur in countries with advancing standards of living. In a subpopulation of the adopted children we demonstrated that height loss occurred in the period between the start of puberty and the attainment of final height. Besides, menarcheal age was positively related to final height .
Therefore, *we conclude* that the reduced final height in adopted girls is caused by early puberty, analogous to the situation in CPP.

Prospective study

Since adult height in adopted children is reduced and puberty occurs early, at least in girls, suppression of puberty in these children has to be considered similarly to suppression of puberty in CPP, since both short stature and early puberty can cause psychological problems. Combination therapy with GH should be considered too, in view of the results of our preliminary combination therapy study on CPP, as described in chapter 9. Only a controlled prospective study, that considers GnRH agonist therapy alone on the one hand and the combination of GnRH agonist and GH on the other hand can reveal whether suppression of puberty by GnRH agonists in children with early puberty (< 10 years in girls and < 11 years in boys) will result in an improvement in adult height and psychosocial stability. The results of that study might be also relevant for other groups of children with short stature, treated with GH, such as children

with GH deficiency, idiopathic short stature and chronic renal failure, in whom puberty also may occur (relatively) early and compromise their genetically determined adult height.

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Chapter 12

SUMMARY/SAMENVATTING

SUMMARY

This thesis descibes the results of treating central precocious puberty (CPP) with the slow-release Gonadotropin Releasing Hormone (GnRH) agonist, triptorelin. In addition, the diagnostic value of examining the brain of children with CPP by means of magnetic resonance imaging is emphasized. Finally, a description is given of the effect that the early onset of puberty can have on the growth of foreign-born adopted children.

Chapter 1 begins with an overview of various aspects of normal puberty reported in the literature, in order to understand the processes occurring during precocious puberty.The complex maturational changes in both the organization and the activation of GnRH neurons and the hypothalamic-pituitary-gonadal axis, which result in the onset of puberty, are described. Despite ongoing advances in neuroendocrine research, the complex changes that together trigger the onset of puberty still puzzle the experts. In addition, the various factors influencing precocious puberty are described, but no precise answer can yet be given about the pathophysiology of precocious puberty. Basal and stimulated gonadotropin values in normal and precocious puberty are found to be a useful tool for diagnosing (precocious) puberty. Clinical presentation and pelvic ultrasound findings under normal and pathological circumstances are discussed. The influences of sex steroids on bone maturation are described to understand why adult height potential is found to be compromised in the presence of supranormal sex steroid concentrations. In addition, the problems of height prediction in CPP are discussed. Finally, the desensitization of the pituitary by various GnRH agonists is described to understand the therapeutic application of these drugs in CPP.

Chapters 2 and 6 describe the successful suppressive action of triptorelin on gonadotropins, sex steroids, secondary sex characteristics, height velocity and bone maturation in children with CPP. After just 18 months of treatment with triptorelin an impovement in adult height prediction is obtained.

Chapter 3 compares the results of the slow-release agonist triptorelin and the short-acting GnRH agonist buserelin. During the first 6 months of treatment, significantly more phases of incomplete suppression of pituitary-gonadal activity occurred during treatment with buserelin than during treatment with triptorelin. As a result, height velocity and bone maturation remained significantly higher in the buserelin group than in the triptorelin group. No improvement in height prediction occurred in the buserelin group, in contrast to the situation in the triptorelin group. Therefore, treatment of children with CPP with triptorelin seems to be more effective than treatment with buserelin.

In **chapters 4 and 5** the long-term (over 4 and 5 years) results of treatment with triptorelin are presented; these show an ongoing suppression of puberty, resulting in a stabilization of height velocity at about 4 cm/year and a decreasing bone maturation. As a result height prediction improved during treatment. Concomitant growth hormone (GH) therapy was initiated in three girls with subnormal growth rates and with very low adult height predictions three years

after triptorelin therapy was initiated (**chapter 4**). Prior to the administration of GH the GH values were measured and found to be subnormal. GH treatment increased height velocity and improved adult height prediction.
In addition, in **chapter 5** the effects during the first 18 months after discontinuing of triptorelin therapy are described. Height velocity increased in the first year after discontinuation, but decreased in the next six months. Bone maturation increased progressively, indicating that the remaining growth potential was reduced in children with CPP, treated with a GnRH agonist.
The hormonal changes after discontinuation are described in **chapter 6**. After withdrawal of triptorelin the hypothalamic-pituitary-gonadal axis recovered rather rapidly, resulting in an increase in gonadotropins and sex steroids to normal pubertal values and, in girls, leading to menarche after about one year. Menstrual cycles became regular in the majority of the girls. Pelvic ultrasonography of the internal genitalia did not reveal any abnormalities.
Chapter 7 describes in more detail what happened when triptorelin was withdrawn, until final height was reached. At the moment of withdrawal a height gain of 5.7 cm was expected. However, bone maturation accelerated, resulting in a mean height gain of 3.5 cm compared to the height prediction at the start of treatment. Height at start of therapy proved to be the most important factor contributing to final height. Bone age at the point of discontinuation was another important negative factor influencing final height. Height gain seemed to be greater when bone age at the point of discontinuation of triptorelin was lower than 12.5 years.
In **chapter 8** magnetic resonance (MR) findings for 30 patients with CPP are presented. Three major findings were demonstrated: (1) an increase in the height of the pituitary gland compared to normal individuals, (2) major structural abnormalities in 13% of the children (three hamartomas and one gliomatous process) and (3) no improvement in the sensitivity of the MR examination by the routine use of gadolinium contrast. We concluded that MR examination is a safe and reliable method for the exclusion of abnormalities in children with CPP.
Chapter 9 reports preliminary data of the combined treatment with GH and triptorelin in girls with unfavourable auxological characteristics, such as an adult height prediction below the third centile and a low height velocity during GnRH agonist treatment. In these eight patients height prediction did not change during triptorelin treatment. However, during combination therapy a mean increase of 8.4 (SD: 2.1) cm in height prediction was observed. The question of whether underlying GH insufficiency is the main reason for the favourable response to combination therapy cannot be answered at this point.
Chapter 10 gives the final height data and ages at menarche for foreign-born children adopted in the Netherlands. At the moment of arrival, height standard deviation score (H-SDS) was below the mean according to NCHS references. In the first two years after arrival catch-up growth occurred, with a higher H-SDS after two years compared to that at arrival. In a subgroup of the children there was no change in H-SDS after these two years, up to the age of 6 to 8

years, i.e. before the onset of puberty. Despite this catch-up growth, final height SDS was lower than H-SDS two years after arrival and at the age of 6 to 8 years. The loss of height must have occurred during the period just before puberty and the attainment of adult height. Final height was in the same range as in the country of origin. Mean menarcheal age was 12.0 (SD: 1.5) years, thus earlier than in the country of origin. Menarcheal age was positively related to final height. These data suggest that at least in adopted girls, the reduced final height is caused by early puberty, analogous to the situation in CPP.

Finally, **chapter 11** reports the results of the studies and discusses their implications for the management of children with central precocious puberty. In addition, recommendations are made and suggestions for future research are given.

SAMENVATTING

Dit proefschrift beschrijft de resultaten van de behandeling van kinderen met een centrale pubertas precox (CPP) met behulp van triptorelin, een slow-release gonadotropinen releasing hormoon (GnRH) agonist. Daarnaast wordt aandacht besteed aan de diagnostische waarde dat het onderzoek van de hersenen met behulp van magnetisch resonantie (MR) onderzoek heeft bij kinderen met CPP. Tot slot wordt een beschrijving gegeven van het effect welke vroege puberteit kan hebben op de groei van in het buitenland geboren adoptiekinderen.

Hoofdstuk 1 begint met een overzicht van verschillende aspecten die in de literatuur over normale puberteit worden beschreven, om ook de processen te kunnen begrijpen die tijdens te vroege puberteit optreden. De complexe veranderingen worden beschreven, die optreden in de maturatie van zowel de organisatie als de activatie van de GnRH neuronen en de hypothalamus-hypofyse-gonaden-as, welke uiteindelijk resulteren in het begin van de puberteit. Ondanks het feit dat er nog steeds vorderingen worden gemaakt op het gebied van het neuro-endocriene onderzoek, is het complex van veranderingen, die tesamen de aanzet geven tot het begin van de puberteit, nog niet ontrafeld.

Daarnaast worden de verschillende factoren beschreven, welke een te vroege puberteit kunnen veroorzaken. Ook ten aanzien van de pathofysiologie van pubertas precox moet echter geconstateerd worden, dat het exacte mechanisme niet bekend is. Besproken wordt wat de waarde is bij het diagnostiseren van (te vroege) puberteit, van basale en gestimuleerde serum gonadotropinen concentraties, gemeten onder verschillende condities en met behulp van verschillende assay-methoden. In het zelfde licht worden de klinische presentatie en de bevindingen bij echografisch onderzoek van de inwendige geslachtsorganen onder normale en pathologische omstandigheden beschreven.

De invloed van geslachtshormonen op de botmaturatie wordt besproken, om te begrijpen om welke reden de volwassen eindlengte beperkt blijft in aanwezigheid van supranormale concentraties van geslachtshormonen. Tevens wordt het probleem van de lengte voorspelling bij CPP besproken. Tot slot wordt de desensitisatie van de hypofyse onder invloed van verschillende GnRH agonisten toegelicht, om de klinische toepassing van deze middelen te verduidelijken.

De **hoofdstukken 2 en 6** beschrijven de succesvolle suppressieve werking van triptorelin op de gonadotropinen, geslachtshormonen, secundaire geslachtskenmerken, groeisnelheid en botmaturatie bij kinderen met CPP. Na een behandelingsduur van 18 maanden met triptorelin wordt een verbetering van de voorspelde volwassen eindlengte gezien.

Hoofdstuk 3 vergelijkt de resultaten van de slow-release agonist triptorelin met de kort-werkende GnRH agonist buserelin. Tijdens de eerste 6 maanden van behandeling, treden significant meer fasen van incomplete onderdrukking van de hypofyse-gonaden activiteit op tijdens behandeling met buserelin, dan tijdens behandeling met triptorelin. Ten gevolge hiervan blijven de groeisnelheid en mate van botmaturatie significant hoger in de buserelin groep, in vergelijking met de triptorelingroep. In tegenstelling tot de triptorelingroep, treedt er geen

verbetering van de lengtevoorspelling in de buserelingroep op. Om die reden leek behandeling van kinderen met CPP met behulp van triptorelin effectiever te zijn, dan behandeling met behulp van buserelin.
In de **hoofdstukken 4 en 5** worden de lange termijn resultaten (d.w.z. na 4 en 5 jaar) van de behandeling met triptorelin gepresenteerd. Deze laten een voortdurende onderdrukking van de puberteit zien, resulterend in een stabilisatie van de groeisnelheid van ongeveer 4 cm/jaar en een afname van de botmaturatie. Ten gevolge hiervan verbeterde de eindlengte voorspelling. Drie jaar na het starten van de triptorelin behandeling werd bij drie kinderen, die een subnormale groeisnelheid èn zeer slechte eindlengte voorspelling hadden, tevens groeihormoon (GH) therapie gestart (**hoofdstuk** 4). De GH secretie die voorafgaand aan de GH behandeling werd bepaald, bleek subnormaal te zijn. GH behandeling verhoogde de groeisnelheid en de verbeterde de voorspelling van de eindlengte.
Daarnaast worden in **hoofdstuk** 5 de effecten van de eerste 18 maanden na het staken van de behandeling met triptorelin beschreven. De groeisnelheid nam in het eerste jaar na staken toe, maar nam reeds af in de daarop volgende zes maanden. De botmaturatie nam progressief toe, hetgeen een aanwijzing was dat het resterende groeipotentieel gereduceerd is bij kinderen met CPP, die met een GnRH agonist behandeld worden.
De hormonale veranderingen na het staken van de triptorelin behandeling, worden beschreven in **hoofdstuk** 6. Na het onttrekken van triptorelin herstelt de hypothalamus-hypofyse-gonaden-as vrij snel, hetgeen een stijging van de gonadotrofinen en geslachtshormonen tot normale waarden veroorzaakt en bij meisjes na ongeveer één jaar aanleiding geeft tot het optreden van de menarche. In het merendeel van de meisjes trad vervolgens een regelmatige menstruele cyclus op.
Hoofdstuk 7 beschrijft meer gedetailleerd en tot en met het bereiken van de eindlengte, wat er plaats vindt nadat de triptorelin behandeling gestaakt is. Op het moment van staken werd een lengte winst van gemiddeld 5,7 cm verwacht. De botmaturatie bleek echter dusdanig te versnellen dat de gemiddelde lengtewinst, in vergelijking met de lengtevoorspelling aan het begin van de behandeling, 3,5 cm bedroeg. De lengte op het moment dat de behandeling gestart werd, bleek de belangrijkste factor te zijn die bijdraagt aan de eindlengte. De botleeftijd op het moment van het staken van de behandeling was een andere belangrijke, negatieve, factor die de eindlengte beinvloedt. De lengtewinst die bereikt wordt, lijkt groter te zijn als de skeletleeftijd bij het staken van de behandeling kleiner of gelijk is aan 12,5 jaar.
In **hoofdstuk** 8 worden de bevindingen gepresenteerd, welke met behulp van magnetisch resonantie (MR) onderzoek bij 30 kinderen met CPP gevonden werden. Er waren drie belangrijke bevindingen: (1) de lengte van de hypofyse was groter in vergelijking met normale kinderen, (2) in 13% van de kinderen werden grote structurele afwijkingen gezien (drie harmatomen en één gliomateus proces) en (3) de sensitiviteit van het MR onderzoek nam niet toe door toevoeging van gadolinium contrast. Geconcludeerd wordt dat MR

onderzoek van de hersenen een veilige en betrouwbare methode is om afwijkingen bij kinderen met CPP uit te sluiten.
Hoofdstuk 9 beschrijft de voorlopige data over de gecombineerde behandeling van GH en triptorelin bij meisjes met slechte auxologische kenmerken, zoals een voorspelling van de volwassen eindlengte onder de derde lengtepercentiel en een lage groeisnelheid tijdens GnRH agonist behandeling. Tijdens de gecombineerde behandeling werd een toename van gemiddeld 8,4 (SD: 2,1) cm in de lengtevoorspelling waargenomen. De vraag of een onderliggende GH-insufficientie als de belangrijkste reden voor de gunstige respons op de combinatietherapie moet worden gezien, kan op dit moment niet beantwoord worden.
Hoofdstuk 10 geeft een beschrijving van de volwassen eindlengten en de leeftijd, waarop de menarche optreedt bij in het buitenland geboren geadopteerde kinderen. Op het moment van aankomst in Nederland, lag de lengte standaard deviatie score (lengte-SDS) onder het gemiddelde volgens de NCHS referenties. In de eerste twee jaar na aankomst trad een inhaalgroei op, waardoor de lengte-SDS twee jaar na aankomst hoger lag dan bij aankomst. In een sub-groep van deze kinderen bleef de lengte-SDS na deze twee jaar tot een leeftijd van 6 à 8 jaar, d.w.z. tot vóór het begin van de puberteit, onveranderd. Ondanks de inhaalgroei lag de eindlengte-SDS lager dan de lengte-SDS twee jaar na aankomst en lager dan de lengte-SDS op de leeftijd van 6 à 8 jaar. Het verlies in lengte-SDS moet zijn opgetreden in de periode die gelgen is tussen het moment vlak voor het optreden van de puberteit en het bereiken van de volwassen lengte. De eindlengte van de kinderen bleek hetzelfde te zijn als de eindlengte van kinderen in het land van herkomst. De gemiddelde menarche leeftijd trad op de leeftijd van 12,0 (SD: 1,5) jaar op, d.w.z. op een jongere leeftijd dan in het land van herkomst. De menarche leeftijd was positief gerelateerd aan de volwassen eindlengte. Deze data geven aan, dat de gereduceerde volwassenen lengte, in ieder geval bij adoptiemeisjes, veroorzaakt lijkt te worden door vroege puberteit, analoog aan de situatie bij CPP.
Tot slot beschrijft **hoofdstuk 11** de resultaten van de studies en hun implicaties voor het beleid bij kinderen met centrale pubertas precox. Vervolgens worden aanbevelingen gedaan en suggesties gegeven voor toekomstig onderzoek.

LIST OF ABBREVATIONS

BA	bone age
BMI	body mass index
BP	Bayley & Pinneau
CA	chronological age
CPA	cyproterone acetate
CPP	central precocious puberty
CT	computed tomography
D-CR	Decapeptyl-controlled release
DD	Decapeptyl depot
DHEAS	dehydroepiandrosterone sulphate
FH	final height
FSH	follicle stimulating hormone
Gd-DTPA	gadopentetate dimeglumine
GH	growth hormone
GnRH	gonadotropin releasing hormone
GnRHa	gonadotropin releasing hormone agonist
GP	Greulich & Pyle
HA	height age
H-SDS	height standard deviation score
HV	height velocity
ICMA	immunochemiluminometric assay
IFMA	immunofluorometric assay
IGF-I	insulin–like growth factor I
i.m.	intramuscularly
i.n.	intranasally
IRMA	immunoradiometric assay
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
MRI	magnetic resonance imaging
NCHS	national center for health statistics
PAH	predicted adult height
PCO	polycystic ovaries
RIA	radioimmunoassay
s.c.	subcutaneously
SD	standard deviation
SDS	standard deviation score
SEM	standard error of mean
SH/LL ratio	sitting height/leg length ratio
TGFα	transforming growth factor alpha
TH	target height
WFA	weight for age
WFH	weight for height

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CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 7 november 1951 te 's-Gravenhage. Zij behaalde in 1970 het Gymnasium ß diploma aan het Dalton Lyceum te Voorburg. In hetzelfde jaar begon zij aan haar studie geneeskunde aan de Rijksuniversiteit te Leiden. Zij behaalde het doctoraal examen in juni 1975. Het artsexamen werd in januari 1977 afgelegd. In de daaropvolgende maand begon zij met de opleiding kindergeneeskunde in het Sint Joseph Ziekenhuis te Eindhoven (Opleider destijds: Dr. C. de Monchy). De opleiding werd vanaf oktober 1979 vervolgd in het Wilhelmina Kinderziekenhuis te Utrecht (Opleider destijds: Prof.Dr. J.W. Stoop). In juni 1981 vond haar registratie als kinderarts plaats. Daarna werd aangevangen met het fellowship kinderendocrinologie (Opleider: Prof.Dr. J.V.L. Van den Brande). In februari 1983, nog tijdens haar fellowship, begon zij haar werkzaamheden binnen de kinderendocrinologie op de afdeling kindergeneeskunde van het Academisch Ziekenhuis te Leiden (Hoofd destijds: Prof.Dr. L.J. Dooren; vanaf 1994: Prof.Dr. J.M. Wit). Sinds maart 1984 is zij als stafarts aan deze afdeling verbonden en als kinderarts-endocrinoloog werkzaam. Aldaar verrichtte zij in de loop der jaren, in multicenter verband en in nauwe samenwerking met Prof.Dr. S.L.S. Drop, hoogleraar kinderendocrinologie, verbonden aan het Sophia Kinderziekenhuis te Rotterdam, het in dit proefschrift beschreven onderzoek.
De auteur is gehuwd met Jan Deetman. Zij hebben twee kinderen: Joost en Suzanne.