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Overnight Levels of Luteinizing Hormone, Follicle-Stimulating Hormone and Growth Hormone before and during Gonadotropin-Releasing Hormone Analogue Treatment in Short Boys Born Small for Gestational Age

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Key Words

Small for gestational age · Luteinizing hormone levels · Follicle-stimulating hormone levels · Growth hormone levels · Gonadotropin-releasing hormone analogue treatment

Abstract

Aims: To evaluate if 3 months of gonadotropin-releasing hormone analogue (GnRHa) treatment results in sufficient suppression of pubertal luteinizing hormone (LH) and follicle-stimulating hormone (FSH) profile patterns in short pubertal small for gestational age (SGA) boys. To compare growth hormone (GH) profiles and fasting insulin-like growth factor (IGF)-I and IGF-binding protein-3 (IGFBP-3) levels after 3 months of GnRHa treatment with those at baseline. **Methods:** After measurement of baseline overnight profiles and IGF-I and IGFBP-3 levels, 14 short pubertal SGA boys received

leuprorelide acetate depots of 3.75 mg subcutaneously, every 4 weeks. **Results:** At baseline, mean GH levels were comparable with those of controls, whereas IGF-I and IGFBP-3 standard deviation scores (SDS) were significantly lower than zero SDS. After 3 months of GnRHa treatment, all boys showed clinical arrest of puberty. The area under the curve above zero, mean and maximum LH and FSH had significantly decreased to prepubertal levels. Peak LH during the GnRH agonist test, however, indicated insufficient pubertal suppression in 43% of boys. Overnight GH profile characteristics and IGF-I and IGFBP-3 levels did not significantly change. **Conclusions:** Puberty was sufficiently suppressed by GnRHa treatment, as shown by the prepubertal LH and FSH profiles. After 3 months of GnRHa treatment, overnight GH profile characteristics had not significantly changed, reflecting that GH levels are comparable for prepubertal and early pubertal boys.

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Introduction

Although catch-up growth occurs in most children born small for gestational age (SGA), about 10% of infants remain short throughout childhood and adulthood [1, 2]. Persistent changes in the growth hormone (GH)/insulin-like growth factor (IGF)/IGF-binding protein (IGFBP) axis might underlie this failure in catch-up growth [3–7].

In some short SGA children, puberty starts at a relatively early age for their short stature, thereby compromising adult height [8, 9]. Postponement of puberty with gonadotropin-releasing hormone analogue (GnRHa) treatment was studied in boys with central precocious puberty, and most of these boys reached an adult height in the range of their genetic height potential [10].

In healthy, older prepubertal boys, a very discrete day and night pulsatile secretion pattern of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) is detectable [11–13]. At the onset of puberty (G2), LH and FSH are secreted in a regular pattern during the daytime, with a further amplification during sleep. From early puberty onwards, increasing pulse frequency and pulse amplitude with an obvious night/day rhythm was found [11–14].

Data concerning spontaneous overnight LH and FSH profile patterns during GnRHa treatment are scarce. Therefore, our primary objective was to evaluate if GnRHa treatment results in sufficient suppression of pubertal LH and FSH profile patterns. Admitting children to a hospital in order to perform overnight LH and FSH profiles is not feasible in routine care. Therefore, all the boys in our study also underwent a GnRH agonist test during the morning after the second overnight profile. In the Netherlands, a consensus-based peak LH level below 3 IU/l (peak LH_{GnRH}; 95th percentile of prepubertal peak LH response) with testosterone levels below 1 nmol/l (upper limit of prepubertal values) during a GnRH agonist test are used as cutoff levels for sufficient pubertal suppression [15]. Our second objective was to evaluate in how many boys sufficient pubertal suppression is identified by the GnRH agonist test.

A decrease in growth velocity is a well-known phenomenon during GnRHa treatment [16–19]. There are only limited data on spontaneous GH, IGF-I and IGFBP-3 levels during GnRHa treatment [20–22]. Notably, no data are available in short pubertal boys born SGA, either before or during GnRHa treatment. Therefore, our third objective was to determine overnight GH profiles and fasting levels of IGF-I and IGFBP-3, both before and after 3 months of GnRHa treatment.

Subjects and Methods

Subjects

The study group comprised short boys born SGA who were at the beginning of puberty. They were included in a clinical trial investigating combined treatment with GnRHa and GH. All boys started GH treatment after 3 months of GnRHa treatment. Children who met the following criteria were included: (1) birth length and/or birth weight standard deviation score (SDS) below –2 for gestational age [23], (2) chronological age of 8 years or older at the start of the study, (3) current height SDS below –2.5 or a predicted adult height less than –2.5 SDS (calculated as height at the start of puberty plus 30 cm, according to Dutch references [24]) and (4) early pubertal stage defined as a testicular volume of 4 ml or more, Tanner genital stage 2 or 3 [25] and a GnRH agonist test result with a peak LH of 10 IU/l or more, indicating central puberty [26]. Children were excluded if they met one of the following criteria: (1) a complicated neonatal period with signs of severe asphyxia (defined as an Apgar score <3 after 5 min), (2) long-term complications of respiratory ventilation such as bronchopulmonary dysplasia, (3) endocrine (including idiopathic GH deficiency) or metabolic disorders, chromosomal defects, growth failures caused by other disorders (such as emotional deprivation, severe chronic illness or achondroplasia) or syndromes (except for Silver-Russell syndrome) and (4) previous or present medication that could interfere with growth or GH treatment. The study was approved by the Medical Ethics Committee of the participating centers, and written informed consent was obtained from parents or custodians and from subjects if aged 12 years or older.

Study Design

Overnight LH, FSH and GH profiles were performed in 10 boys, both before and after 3 months of GnRHa treatment (leuprorelin acetate depots of 3.75 mg subcutaneously, every 4 weeks, with an interval of 14 days between the first 2 injections). Children were admitted to the hospital and an indwelling venous catheter was inserted in an antecubital vein. For a period of 12 h (19.00–7.20 h), blood was taken every 20 min for determination of serum LH, FSH and GH levels. Children followed their normal eating and sleeping pattern. Sleep during both admissions was recorded by the same investigator (D.vdK.). All children went to bed at 22.30 h, were asleep before 23.30 h and woke up around 7.00 h the next morning. The time awake was comparable between both admissions. The next morning, a fasting blood sample was taken for measurement of IGF-I and IGFBP-3 levels.

Height was measured using a Harpenden stadiometer and expressed as SDS for calendar age [24]. The same investigator (D.vdK.) assessed pubertal stage according to Tanner during both visits, using an orchidometer [25]. Bone age was assessed by one investigator (D.vdK.), using the segmented Greulich and Pyle reference [27]. Fat mass for height at baseline was measured by dual-energy X-ray absorptiometry [28].

The inhibition of gonadotropin secretion was checked the morning after the second overnight profile by a GnRH agonist test, as follows: 0.5 mg (0.5 ml) of leuprorelin acetate was injected subcutaneously, and after 3 h, a blood sample was taken for determination of LH, FSH and testosterone levels [29, 30]. In the 4 boys in whom overnight profiles were not performed, fasting IGF-I and IGFBP-3 levels and a GnRH agonist test were performed during the second visit, after 3 months of GnRHa treat-

Table 1. Clinical characteristics before the start of GnRHa treatment

Number of boys	14
Gestational age, weeks	39.0 (37.4–40.0)
Birth weight SDS	-1.9 (-2.2 to -1.6)
Birth length SDS	-2.4 (-2.8 to -2.1)
<i>At start of GnRHa treatment</i>	
Genital stage 2, n	12
Genital stage 3, n	2
Age, years	12.7 (12.2–12.9)
Bone age, years	12.1 (11.2–12.6)
Height SDS	-2.5 (-3.4 to -2.2)
Weight SDS	-2.7 (-3.4 to -1.3)
Fat mass SDS	-0.5 (-1.4 to -0.08)

Data are expressed as medians (interquartile range in parentheses).

ment. We compared our overnight GH profile results with those found by Rose et al. [31], who performed overnight GH profiles in healthy boys with normal stature and similar pubertal stage.

Hormone Assays

Overnight LH and FSH levels were measured by chemiluminescence-based immunometric methods (Immulite 2000, Diagnostic Products Corporation, Los Angeles, Calif., USA) using WHO preparations 80/552 and 78/549 as standards. Detection limits for both assays were 0.1 IU/l, and values below 0.1 IU/l were assigned the value 0.1 IU/l. Intra- and interassay coefficients of variation (CVs) were below 4 and 7%, respectively, for LH and below 3 and 6%, respectively, for FSH. Testosterone levels were measured using a coated-tube radioimmunoassay obtained from Diagnostic Products Corporation. The detection limit for this assay is 0.1 nmol/l. Intra- and interassay CVs were below 6 and 8%, respectively.

Overnight GH levels were measured by an immunometric assay (Immulite 2000) with a lower detection limit of 0.13 mU/l. Values lower than 0.13 mU/l were assigned the value 0.13 mU/l. The results of low, medium and high standards [mean \pm intra-assay standard deviation (SD) and \pm interassay SD, respectively] were 6.8 \pm 0.23 and \pm 0.44, 14.0 \pm 0.47 and \pm 0.75, and 44 \pm 1.87 and \pm 2.94 mU/l, respectively. Intra- and interassay CVs were, respectively, 3.5 and 6.5% at a level of 6.8 mU/l, 3.4 and 5.5% at a level of 14.0 mU/l and 4.2 and 6.6% at a level of 44 mU/l. Based on standard samples, our assay was comparable with the assay used by Kamp et al. [20], who measured GH levels by polyclonal radioimmunoassay with a detection limit of 0.5 μ g/l. Their results (mean \pm SD) of low, medium and high standards were 1.1 \pm 0.1, 3.6 \pm 0.4 and 16.9 \pm 2.0 μ g/l, respectively. Intra- and interassay CVs were, respectively, 9.1 and 18.7% at a level of 1.2 μ g/l, 3.7 and 16.6% at a level of 5.1 μ g/l and 3.1 and 9.0% at a level of 18.5 μ g/l. A conversion factor of 2.6 was used to transform data from micrograms per liter to milliunits per liter [32].

Serum IGF-I and IGFBP-3 levels were measured in one laboratory using a specific radioimmunoassay [33]. Serum levels were

expressed as SDS to adjust for age and sex [34]. The intra- and interassay CVs were 4 and 6%, respectively.

Reported LH Profiles for Prepubertal Boys

Prepubertal boys with a testicular volume of 1–2 ml have a very discrete nighttime pulsatile pattern of gonadotropin secretion with mean (\pm SEM) LH levels of 0.27 \pm 0.15 IU/l and maximum LH levels of 1.06 \pm 0.24 IU/l. Mean and maximum LH levels increased to 1.34 \pm 0.42 and 3.70 \pm 1.21 IU/l, respectively, in prepubertal boys with a testicular volume of 3 ml [12].

Calculations

The area under the curve above zero (AUC₀) of LH and FSH profile patterns was calculated by the trapezoidal method. Overnight GH profiles were analyzed using the Pulsar program [33, 35].

Statistics

Because of a non-Gaussian-shaped distribution, data were expressed as medians (interquartile range in parentheses). The Mann-Whitney test was used for differences between groups. The Wilcoxon signed rank test was used to determine differences between points in time within groups. SDS were compared with zero SDS using χ^2 tests. To analyze night-to-night variation, correlation coefficients were calculated between individual time points for an individual patient. The calculated coefficients were analyzed using χ^2 tests. Spearman's correlation coefficient was used for correlations. A p value <0.05 was considered significant. Analyses were performed using the computer statistical package SPSS (version 11) for Windows.

Results

Clinical Characteristics

Table 1 shows the clinical data of all subjects. After 3 months of GnRHa treatment, none of the boys showed clinical progression of puberty and 2 boys showed clinical regression of puberty.

Overnight LH and FSH Profiles

Baseline. Characteristics of baseline overnight LH and FSH profiles are shown in table 2.

After 3 Months of GnRHa Treatment. Characteristics of overnight LH and FSH profiles after 3 months of GnRHa treatment are shown in table 2. AUC₀, mean and maximum LH and FSH levels had significantly decreased to very low levels. Mean LH levels were 0.41 IU/l and maximum LH levels were 0.72 IU/l, whereas mean FSH levels were 0.21 IU/l and maximum FSH levels were 0.38 IU/l. Thus, none of the boys had a pubertal pulsatile pattern (fig. 1, 2).

GnRH Agonist Test. After 3 months of GnRHa treatment, peak LH_{GnRH} was below 3 IU/l, with testosterone levels below 1 nmol/l in 8 out of 14 boys. Peak LH_{GnRH}

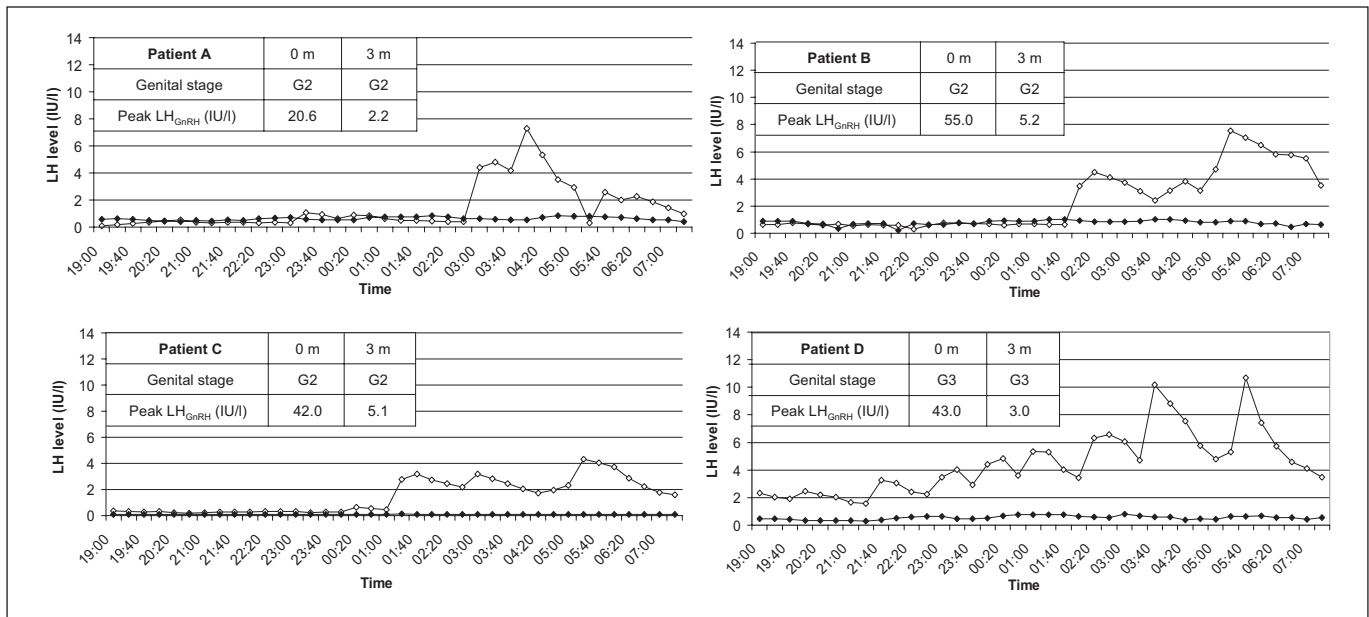


Fig. 1. Representative examples of individual overnight LH profiles in 4 boys, performed at baseline (◇) and after 3 months of GnRHa treatment (◆). Subjects A, B and C were genital stage 2,

while subject D was genital stage 3. The results of the GnRH agonist test performed after 3 months of GnRHa treatment are shown in the insets. m = Months.

varied between 3.3 and 5.2 IU/l in 6 boys (43%). Testosterone levels were below 1 nmol/l in all 6 boys, and none of these boys showed clinical progression. No significant differences in LH and FSH profiles were found between boys with a peak LH_{GnRH} above or below 3 IU/l. Testosterone levels had significantly decreased from 6.5 (1.3–9.9) nmol/l to 0.25 (0.10–0.53) nmol/l ($p = 0.001$) after 3 months of GnRHa treatment. No significant differences in testosterone levels were found between boys with a peak LH_{GnRH} above or below 3 IU/l.

Correlations between LH Profiles and the GnRH Agonist Test. At baseline, mean LH levels during the LH profile correlated positively with testosterone levels during the GnRH agonist test ($r = 0.67$, $p = 0.03$). Maximum LH levels during the LH profile correlated positively with peak LH_{GnRH} ($r = 0.69$, $p = 0.03$). After 3 months of GnRHa treatment, no correlations were found between characteristics of the LH profiles and peak LH or testosterone levels during the GnRH agonist test. Peak LH_{GnRH} and peak FSH_{GnRH} at baseline correlated respectively with peak LH_{GnRH} and peak FSH_{GnRH} after 3 months of GnRHa treatment ($r = 0.73$, $p = 0.003$ and $r = 0.70$, $p = 0.006$, respectively).

Overnight GH Profiles and IGF-I and IGFBP-3 Levels Baseline. Characteristics of the overnight GH profiles and IGF-I and IGFBP-3 levels at baseline are listed in ta-

Table 2. Characteristics of overnight LH and FSH profiles, at baseline and after 3 months of GnRHa treatment

	Baseline	3 months	p value
<i>Overnight LH profiles</i>			
AUC ₀ LH, IU/l × 12 h	36.0 (18.1–52.1)	5.2 (3.7–8.3)	0.005
Mean LH, IU/l	2.8 (1.4–4.1)	0.41 (0.29–0.66)	0.005
Maximum LH, IU/l	7.3 (4.3–9.7)	0.72 (0.43–0.92)	0.005
<i>Overnight FSH profiles</i>			
AUC ₀ FSH, IU/l × 12 h	21.0 (11.3–63.7)	2.7 (1.5–5.3)	0.005
Mean FSH, IU/l	1.7 (0.89–5.0)	0.21 (0.12–0.42)	0.005
Maximum FSH, IU/l	4.0 (1.6–6.3)	0.38 (0.22–0.93)	0.005
<i>GnRH agonist test</i>			
Peak LH, IU/l	32.4 (14.1–43.1)	2.8 (2.1–4.0)	0.001
Peak FSH, IU/l	7.2 (4.3–11.8)	0.65 (0.40–1.1)	0.001
Testosterone, nmol/l	6.5 (1.3–9.9)	0.25 (0.10–0.53)	0.001

Data are expressed as medians (interquartile range in parentheses). Reported mean prepubertal values for mean and maximum LH levels are 0.27–1.3 and 1.1–3.7 IU/l, respectively. p values are calculated compared to baseline.

ble 3. Mean GH levels in boys with genital stage 2 were comparable with mean GH levels found in boys with normal stature and similar genital stage. Since only 2 boys had genital stage 3, a comparison with boys with normal stature and similar pubertal stage was not feasible. IGF-I

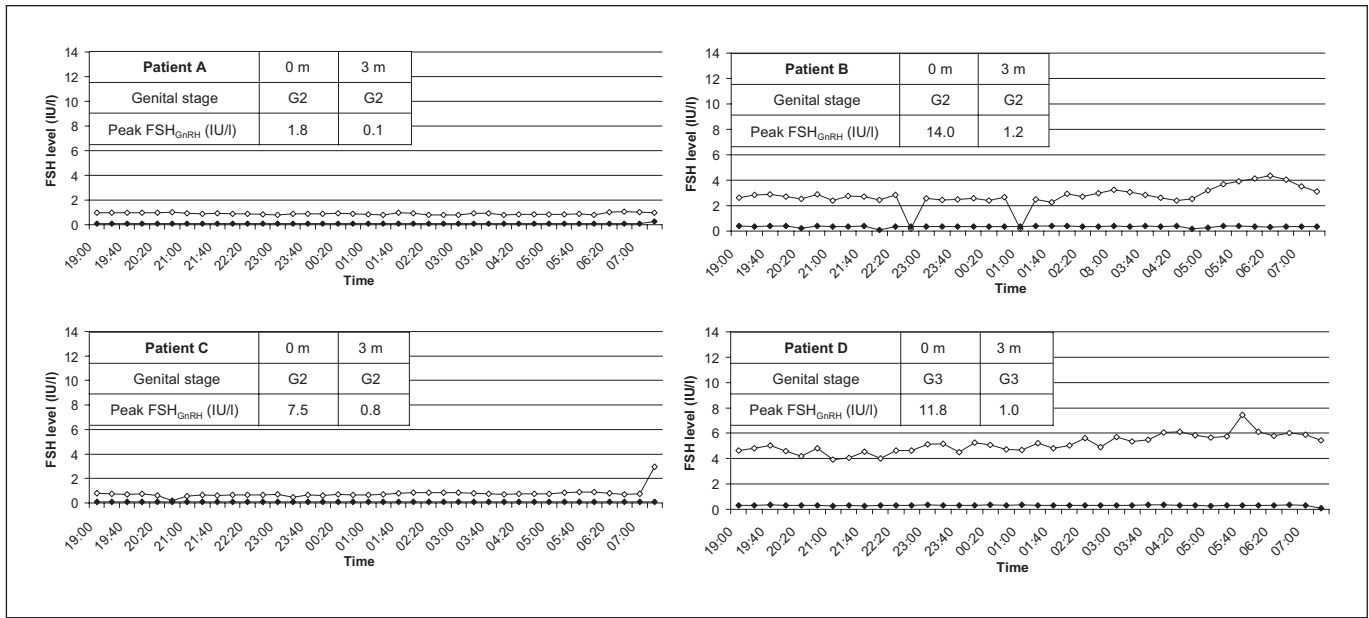


Fig. 2. Representative examples of individual overnight FSH profiles in the same 4 boys as shown in figure 1, performed at baseline (◇) and after 3 months of GnRHa treatment (◆). Subjects A, B and

C were genital stage 2, while subject D was genital stage 3. The results of the GnRH agonist test performed after 3 months of GnRHa treatment are shown in the insets. m = Months.

Table 3. Characteristics of overnight GH, IGF-I and IGFBP-3 levels, at baseline and after 3 months of GnRHa treatment

	Baseline	3 months	p value
AUC ₀ , mU/l × 12 h	135 (92.1–263)	130 (70.0–238)	0.6
Mean GH, mU/l	10.8 (7.3–20.8)	10.4 (5.6–18.8)	0.6
Maximum GH, mU/l	46.2 (32.8–137.8)	55.3 (30.9–89.7)	0.4
Pulse amplitude, mU/l	16.4 (13.7–32.5)	22.8 (11.6–32.7)	0.4
Number of GH peaks >10 mU/l	3.5 (2.8–5.0)	4.0 (3.0–4.0)	1.0
IGF-I SDS	-0.8 (-1.5 to -0.04) ^a	-0.9 (-1.4 to -0.2) ^a	0.6
IGFBP-3 SDS	-1.0 (-1.5 to -0.6) ^b	-1.2 (-1.7 to -0.7) ^b	0.1

Data are expressed as medians (interquartile range in parentheses). p values calculated comparing data at 3 months to those at baseline.

^a p ≤ 0.05 compared to zero SDS; ^b p ≤ 0.001 compared to zero SDS.

and IGFBP-3 levels were significantly lower than zero SDS (p = 0.03 and p = 0.001, respectively).

After 3 Months of GnRHa Treatment. Characteristics of overnight GH profiles, IGF-I and IGFBP-3 levels after 3 months of GnRHa treatment are listed in table 3. No significant differences were found between GH profile characteristics at baseline and after 3 months of GnRHa treatment. Mean GH levels remained comparable with those found in controls. Figure 3 shows that there was a

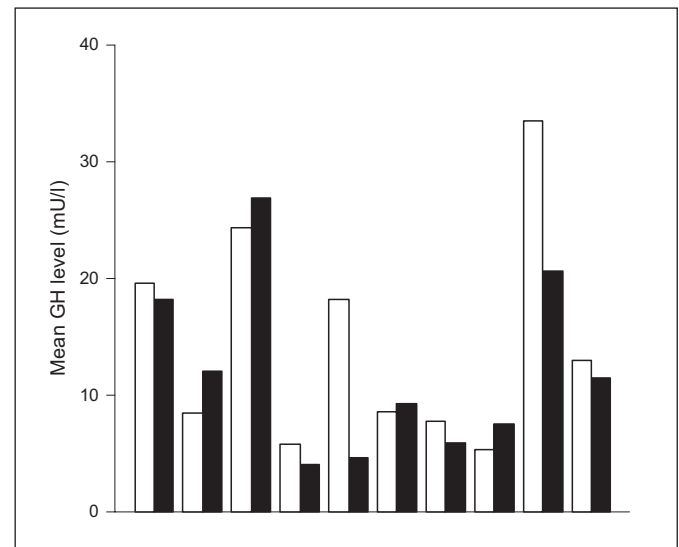


Fig. 3. Mean GH levels for each subject during overnight GH profiles, at baseline (open bars) and after 3 months of GnRHa treatment (solid bars).

wide interindividual variation in mean serum GH levels, both at baseline and after 3 months of GnRHa treatment. IGF-I and IGFBP-3 levels did not significantly change and SDS values remained significantly lower than zero

SDS ($p = 0.03$ and $p < 0.001$, respectively). In all boys, there was a significant correlation between the timing of GH peaks at baseline and after 3 months of GnRHa treatment ($r = 0.4$, $p = 0.01$).

Correlations between GH Profiles, Clinical Characteristics and the GnRH Agonist Test. Neither at baseline nor after 3 months of GnRHa treatment were correlations found between characteristics of the overnight GH profiles and age, height SDS, peak LH_{GnRH} , bone age (at baseline) or fat mass SDS (at baseline).

Correlations between GH Profiles and IGF-I and IGFBP-3 Levels. At baseline, mean GH levels correlated significantly with IGF-I SDS ($r = 0.72$, $p = 0.02$). After 3 months of GnRHa treatment, mean GH levels correlated significantly with IGF-I SDS ($r = 0.76$, $p = 0.01$) and IGFBP-3 SDS ($r = 0.64$, $p = 0.048$). Maximum GH levels correlated significantly with IGF-I SDS ($r = 0.69$, $p = 0.03$).

Discussion

In the present study, LH and FSH levels had significantly decreased to prepubertal levels in all boys, showing that treatment with leuporelide acetate depots of 3.75 mg subcutaneously every 4 weeks resulted in an adequate suppression of puberty. The GnRH agonist test performed after 3 months of GnRHa treatment falsely indicated insufficient pubertal suppression in 43% of the boys. No significant changes in overnight GH profile characteristics and IGF-I and IGFBP-3 SDS were found after 3 months of GnRHa treatment, compared to baseline.

Overnight LH and FSH Profiles

After 3 months of GnRHa treatment, all boys had clinical arrest of puberty and 2 boys had clinical regression of puberty. AUC_0 , mean and maximum LH and FSH levels had significantly decreased to very low levels. The pattern of LH and FSH profiles after 3 months of GnRHa treatment in short boys born SGA was similar to prepubertal profiles found in healthy boys [12].

Overnight LH and FSH profiles were performed for research purposes since admitting children to a hospital in order to perform overnight profiles is not suitable for routine clinical care. After 3 months of GnRHa treatment, peak LH_{GnRH} was above the cutoff level of 3 IU/l in 43% of the boys [15]. Notably, all boys had prepubertal testosterone levels (below 1 nmol/l). Furthermore, no significant differences in LH and FSH profiles were found between boys with a peak LH_{GnRH} above or below 3 IU/l. Our findings are in agreement with the only comparable

study, performed in 6 children (2 boys) with central precocious puberty [36]. In this study, children who had clinically well suppressed puberty had LH levels similar to prepubertal children.

Peak LH_{GnRH} was known before LH levels during the overnight profiles were available. The schedule of leuporelide acetate depot injections was changed to every 3 weeks instead of every 4 weeks in boys with a peak LH_{GnRH} above 3 IU/l, according to Dutch consensus guidelines. This adjustment seemed to be unnecessary once we received the results of the overnight LH profiles.

Although we found prepubertal LH profiles in all boys after 3 months of GnRHa treatment, LH and FSH profiles were determined in a rather small group. In order to determine which peak LH_{GnRH} cutoff level indicates sufficient pubertal suppression, we recommend future research in a larger study group, including boys and girls with different pubertal stages and with sufficient and insufficient pubertal suppression according to their LH profiles.

Overnight GH Profiles, IGF-I and IGFBP-3 Levels

To our knowledge, this is the first study describing baseline GH profiles in short pubertal boys born SGA. We found normal mean GH levels, compared to boys with normal stature and similar pubertal stage [20]. Overnight GH profiles have been performed in prepubertal short SGA children with conflicting results. Some authors reported significantly lower levels in short children born SGA, compared to healthy children born appropriate for gestational age [3, 4], whereas others found comparable mean GH levels [37]. The wide variability in and overlap between GH secretion seen in SGA cohorts and control populations are consistent phenomena [3, 4, 20, 37]. Within the heterogeneous SGA population, this probably reflects a continuum in GH secretion, ranging from GH deficiency to normal GH secretion. Alterations in the GH/IGF/IGFBP pathway and genetic variations found in genes involved in this pathway [38, 39] might in part explain this continuum.

Baseline IGF-I and IGFBP-3 SDS were significantly lower than zero SDS, which is in line with previous studies describing low IGF-I and IGFBP-3 levels throughout childhood in subjects born SGA [6, 40, 41]. IGF-I SDS was significantly correlated with mean GH levels, both at baseline and after 3 months of GnRHa treatment, and IGFBP-3 SDS was significantly correlated with mean GH levels after 3 months of GnRHa treatment. IGF-I and IGFBP-3 levels were found to reflect GH secretion in healthy children [42]. Our study shows that this reflection is also applicable for short boys born SGA.

After 3 months of GnRHa treatment, we found no significant changes in AUC₀, mean and maximum GH levels compared to baseline. Likewise, IGF-I and IGFBP-3 levels had not significantly changed. Rose et al. [31] showed that mean GH levels in healthy boys with testicular volumes between 5 and 10 ml remained near prepubertal levels, whereas a significant increase in spontaneous GH and IGF-I levels was found when Tanner stage 3 was reached. In our study, all boys in whom overnight GH profiles were performed had testicular volumes between 4 and 8 ml. Thus, this could well explain why we did not find significant changes in GH and IGF-I levels after 3 months of GnRHa treatment.

We found a significant trend in the timing of GH peaks within the same individual. The reproducibility of measurements of overnight GH secretion was reported to be superior to that of provocative tests [32]. The intraindividual reproducibility was found to be less profound [43]. Our results indicate the existence of an intrinsic rhythm regulating endogenous GH secretion in individual subjects.

In conclusion, treatment with leuprorelide acetate depots of 3.75 mg every 4 weeks results in an effective inhibition of central puberty, as shown by prepubertal overnight LH and FSH secretion patterns and clinical signs of pubertal arrest. The GnRH agonist test falsely indicated insufficient pubertal suppression in almost half of the boys, resulting in unnecessary adjustments in the fre-

quency of depot injections. Low IGF-I and IGFBP-3 levels were found at the start of puberty, although mean GH levels were normal for pubertal stage. Parameters of the overnight GH profile did not significantly change after 3 months of GnRHa treatment, consistent with GH levels being comparable for prepubertal and early pubertal boys.

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