ISSN 0025-7680

ORIGINAL ARTICLE

MEDICINA (Buenos Aires) 1996; 56: 241-246

GROWTH ACCELERATION IN CHILDREN WITH CHRONIC RENAL FAILURE TREATED WITH GROWTH-HORMONE-RELEASING HORMONE (GHRH)

TITANIA PASQUALINI[1], JORGE FERRARIS[2], PATRICIA FAINSTEIN-DAY[3], ALFREDO A. EYMANN[2], SILVIA MOYANO CATURELLY[1], SUSANA RUIZ[2], JOSE RAMIREZ[2], RAUL GUTMAN[3]

[1] Sección de Endocrinología, Crecimiento y Desarrollo; [2] Sección de Nefrología y Hemodiálisis, Departamento de Pediatría; [3] Servicio de Endocrinología y Medicina Nuclear, Hospital Italiano de Buenos Aires.

Summary Growth retardation is a prominent clinical manifestation in children with chronic renal failure (CRF). Nine children with CRF (3 on conservative treatment; 3 on dialysis and 3 after renal transplantation) aged 1.6 to 14.0 (x ± SE: 8.1 ± 1.4) years, were treated with twice daily subcutaneous injections of 26 ± 2.4 µg/kg/day growth-hormone-releasing-hormone [GHRH (1-29) NH_2 Serono (Geref)] during 3 to 6 months. Mean serum urea and creatinine remained stable, although 2 patients on conservative treatment showed a moderate increase in serum creatinine. At the start of the study, heigth SDS was - 2.2 ± 0.2 (x ± SE), growth velocity was 4.5 ± 1.0 cm/year (- 2.3 ± 0.6 DS for chronological age) and growth hormone (GH) response to acute GHRH test (1 µg/kg IV) was 62 ± 17.5 ng/ml. Five patients increased height velocity from 3.8 ± 0.7 to 8.0 ± 1.2 cm/year (paired t test, $p < 0.05$). The peak GH response to GHRH was significantly higher in the group of growth non-responders than in the responders ($p < 0.05$). In conclusion, 5 out of 9 short children with CRF, 3 on conservative treatment, 1 on dialysis and 1 post renal transplantation, showed improved growth in response to GHRH therapy. No consistent effect on renal function was detected. GHRH may be an alternative therapy to increase growth velocity in patients with CRF.

Key words: GHRH, growth, renal disease.

Growth retardation is a prominent clinical manifestation in children with chronic renal failure (CRF) and after renal transplantation (rTx)[1,3]. Van Diemen-Steenvoorde et al[1] demonstrated that height at transplantation was more than 2 standard deviation score (SDS) below the mean in 34.2% of prepubertal children. After rTx, 37% of the patients who attained adult height had SDS less than -2[1]. The challenge for those in care of children with CRF is to provide a therapeutic milieu that will not only sustain life but also facilitate attainment of normal adult height.

Previous reports have shown a significant increase in growth velocity during growth hormone (GH) treatment in children with CRF[4,9]. The identification and synthesis of a growth-hormone-releasing analogue (GHRH) that stimulates GH secretion made possible and alternative form of therapy[10,13]. This is the first report on GHRH therapy in children with CRF before and after rTx.

Materials and Methods

Patients

Nine patients (8 boys and 1 girl) were treated at the Pediatric Department of the Hospital Italiano in Buenos

Received: 27-XII-1995 Accepted: 24-IV-1996

Postal address: Dr. Titania Pasqualini, Departamento de Pediatría, Hospital Italiano, Gascón 450, 1181 Buenos Aires, Argentina.

ABBREVIATIONS:
CRF: chronic renal failure
rTx: renal transplantation
SDS: standard deviation score
GH: growth hormone
GHRH: growth-hormone-releasing hormone
IGF-1: insulin-like growth factor I

Aires. Inclusion criteria were as follows: height and/or linear growth velocity SDS less than -2.0 for chronological age, Tanner pubertal stage 1 and stable renal function during the previous year. At the start of the study mean age was 8.1 years (range 1.6 to 14.0), mean bone age 5.2 years (range 1 to 10). CRF was secondary to hemolytic uremic syndrome (4 patients), obstructive uropathy (2 patients) and renal dysplasia, diffuse mesangial glomerulosclerosis or focal and segmental glomerulosclerosis (1 patient each). Three patients were on conservative treatment, 3 on dialysis and 3 had undergone rTx, 6.2, 4.0 and 3.0 years before the study (Table 1). Immunosuppressive treatment included azathioprine, cyclosporin and minimal doses of corticosteroids.

Treatment regimens

Patients were given twice daily subcutaneous injections of GHRH (1-29) NH_2 Serono (Geref) during 3 to 6 months. GHRH mean dose was 26 ± 2.4 µg/kg/day. The GHRH treatment was supplied by Ares Serono and the protocol was approved by the Ethical Committee of the Hospital Italiano. Assent was obtained from the children studied, and informed consent was signed by parents.

Anthropometric measurements

Height was measured by the same trained person at 1-month intervals. Anthropometric measurements were matched against the Argentine National Growth Charts[14], with children at the 50th percentile and of the same chronological age and gender used for calculation of height SDS and growth velocity SDS.

Bone age was determined by the method of Greulich and Pyle[15].

Laboratory studies

Laboratory studies were performed before GHRH therapy. Children were admitted to the hospital from 6 PM to 12 AM for the following hormonal assays and functional tests: nocturnal spontaneous GH secretion and GH response to intravenously administered GHRH. Blood samples for measurement of spontaneous GH secretion were obtained every 30 minutes for 12 hours (7 PM to 7 AM). Care was taken not to disturb the children's sleep during blood sampling. At 7 AM, patients received, intravenously, 1 µg per kilogram of body weight of GHRH and blood for GH assay was collected at 5, 10, 15, 30, 60, 90 minutes afterwards.

Serum creatinine, urea, glycemia, hematocrit, calcium, phosphorus, alkaline phosphatase, cholesterol and urinary analysis were performed monthly during the follow-up period.

Plasma GH levels were measured in duplicate by radioimmunoassay[16]. All GH samples belonging to the same patient were analyzed in the same assay. The mean level of GH was estimated as the average of all

TABLE 1.— *Auxological data, types of treatment, mean 12-hour nocturnal GH serum levels and GH responses to the acute GHRH test in patients treated with GHRH*

Patient No/Sex	Type of treatment	Chron Bone Age (years)		Height (SDS)	GH $\bar{x}$ (ng/ml)	GH response to GHRH peak (ng/ml)	GH response to GHRH Area (ng/ml/h)
1/M	conservative	3.6	2.0	- 1.2	7.2	42.8	2442
2/M	conservative	8.6	5.0	- 2.6	2.9	22.8	1558
3/M	conservative	12.4	10.0	- 2.3	4.1	24.0	1200
4/M	hemodialysis	11.0	8.0	- 3.0	2.8	31.5	2285
5/F	renal Tx	7.4	4.3	- 2.6	1.7	36.7	1589
6/M	renal Tx	14.0	8.0	- 2.9	2.5	55.0	2925
7/M	renal Tx	10.5	6.5	- 1.7	2.2	191.4	12454
8/M	CAPD	1.6	1.0	- 2.0	2.9	78.6	5507
9/M	CAPD	3.6	2.3	- 1.6	3.7	72.7	5298

Chron: chronological age.
Height: SDS for chronological age.
Type of treatment: hemodialysis 3 times/week;
CAPD: continuous ambulatory peritoneal dialysis.
Patients 1 to 5 improved growth with GHRH treatment

25 values obtained. Undetectable levels were considered equal to the lower detection limit value of 0.75 ng/ml.

Statistical analysis

Results were analyzed by Student t tests and linear regression. Values are reported as mean and ranges, or as mean ± SE, $p < 0.05$ being considered statistically significant.

Results

Auxological data, type of treatment, mean 12-hour nocturnal GH levels and GH response to the acute GHRH test are shown in Table 1. Children with renal transplant had the lowest mean spontaneous GH levels.

All patiens received GHRH treatment during three months or longer. Growth velocity increased from 4.5 ± 1.0 cm/year to 6.1 ± 1.0 cm/year. A significant improvement in height velocity (cm/year or SDS) was seen in 5 patients (patients 1 to 5) (Fig. 1), with an increase in mean height velocity from 3.8 ± 0.7 to 8.0 ± 1.2 cm/year (paired t test, $p < 0.05$) and from - 2.0 ± 1.0 to 2.4 ± 0.6 SDS (paired t test, $p < 0.005$).

Acute intravenous administration of GHRH elicited a GH response in all subjects (Table 1). Peak values were reached between 15 and 90 minutes after injection with a mean peak level of 62 ± 17.5 ng/ml and a mean secretory area of 3917 ± 1187 ng/ml/hour. Peak GH value and GH area under the curve in response to the GHRH intravenous test

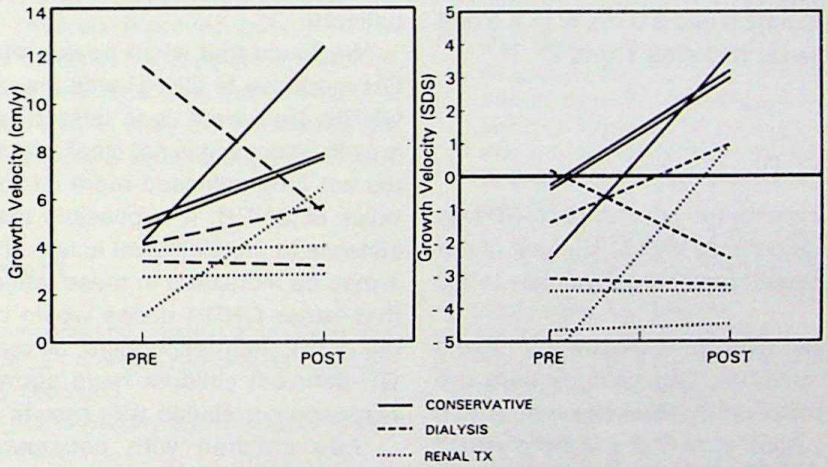

Fig. 1.— Growth velocity pre and post GHRH treatment. Five out of nine patients, 3 on conservative treatment, 1 on dialysis and 1 post rTx, increased growth velocity significantly.

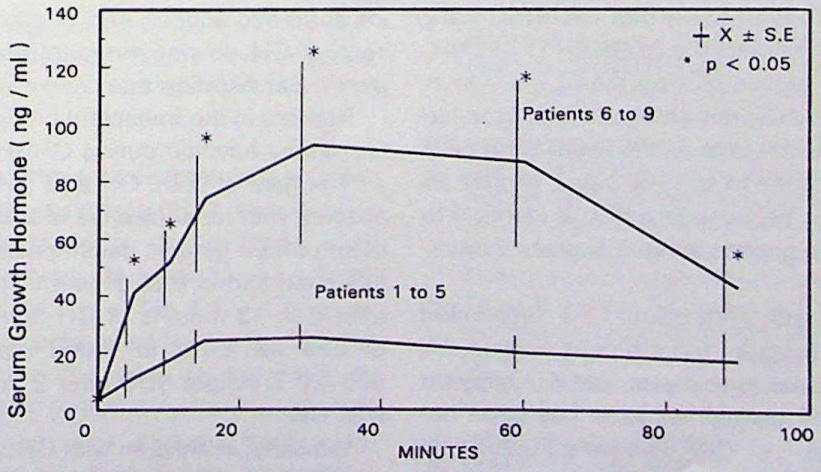

Fig. 2.— Growth hormone response to acute IV GHRH test. Patients 1 to 5, who increased growth velocity significantly, showed a lower growth hormone response as compared to patients 6 to 9, who did not grow. Peak GH response: 31.5 ± 3.8 vs 99.4 ± 31.0 ng/ml; GH area under the curve: 1815 ± 236 vs 6546 ± 2054 ng/ml/h).

was significantly higher in the group of patients who did not improve growth velocity as compared to patients who did ($p < 0.05$) (Table 1 and Fig. 2). Thus, an inverse correlation could be established between the GH area under the curve after intravenous GHRH and the growth velocity during GHRH treatment ($r = - 0.6$, $p < 0.05$).

No significant changes were observed during treatment in serum urea, glycemia, hematocrit, calcium, phosphorus, cholesterol and urinary analysis. Serum alkaline phosphatase showed a significant increase from 375 ± 54 UI/L (x ± SE) before treatment to 523 ± 87 UI/L at the end of GHRH therapy (paired t test, $p < 0.05$). Although mean levels of serum creatinine were similar before and after GHRH treatment two children on conservative treatment showed an increase in serum creatinine from 2.0 and 3.0 mg/dl to 3.0 and 3.6 mg/dl respectively (patients 1 and 2).

Discussion

This study documents the efficacy of twice daily GHRH treatment in patients with CRF. Five of our nine patients increased their height velocity to 8.0 ± 1.2 cm/year.

GHRH has been used to stimulate the growth of GH deficient children. Our velocity data are similar to those reported by Rochiccioli et al (8.6 ± 1.2 cm/year)[17], Ross et al (7.2 ± 2.5 cm/year)[18] and Thorner et al (7.9 ± 2.4 cm/year)[19] in GH deficient children treated with either once or twice daily sc injections of GHRH.

Ross et al[18] have shown that the twice-daily subcutaneous administration of GHRH (1-29) NH_2 promoted linear growth of more than 2 cm/year in 8 out of 18 GH-deficient children, and in these children it was as effective as the established hGH regimens. We chose to use the same shorter 29 residue analogue because its action is identical to that of the native peptide when it is given intravenously[20].

Our patients with preterminal CRF responded best to GHRH therapy: 9.3 cm/year, versus 4.8 cm/year in patients on dialysis and 4.7 cm/year in rTx patients. A parallel situation has been observed in patients with CRF who were treated with hGH[6, 7]. The lower response in patients on dialysis may be related to the greater degree of uremia while in rTx patients corticosteroid therapy may contribute to it.

In our patients, the mean 12-hour GH levels during the night were within the range values of normal children as reported by Rose et al[21]. Patients who have undergone transplantation, showed the lowest endogenous GH secretion probably related to immunosuppressive treatment with corticosteroids. In fact, Schaefer et al[22, 23] found an inverse relationship between the daily steroid dose and mean levels of GH.

GH response to the acute GHRH test was similar to that reported in the literature for renal patients[24] and slightly higher than the mean normal value of 45.8 ± 4.8 ng/ml[25]. Perhaps an altered somatostatinergic tone in patients with CRF can explain the higher GH response to GHRH in these patients.

We found that when peak pretreatment serum GH response to GHRH was lower than 43 ng/ml, GHRH treatment was effective in increasing growth velocity. It is not clear why the patients who did not grow released more GH in response to a bolus of GHRH. It is possible that peripheral resistance to the biological action of GH and/or IGF-1 may be increased in these subjects. It could be that larger GHRH doses would be necessary in our growth non-responders, as previous studies in GH-deficient children have shown a clear dose response correlation with growth velocity[19].

Two children with conservative treatment showed a slight increase in serum creatinine which either reflect the normal progression of their renal disease or the anabolic effect of GH. Studies in uremic adults[26] and in uremic children[27] receiving GH do not demonstrate any impact in glomerular filtration rate.

Patients in the transplant group maintained stable kidney function during GHRH treatment.

The goal of both, GH and GHRH treatment of children with renal disease is to facilitate achievement of their genetic height potential. Although, it has been shown that growth velocity may decline after 6 to 12 months of GH therapy, Tonshoff et al[6] and Van Es et al[7] found improvement of 1.5 and 1.0 in height SDS after 2 years of treatment with GH.

Similarly, in children with GH deficiency treated with GHRH, Duck et al[28] and Rochiccioli et al[17] reported that growth velocity slows after 6 to 12

months of GHRH therapy. On the other hand, Lanes et al[29] recently reported that 11 children with GH deficiency were able to maintain a sustained increase in growth velocity during 12 to 24 months of GHRH treatment.

Possible advantages of GHRH treatment over GH might be that, as GHRH is a smaller molecule than GH, it can be manufactured by direct chemical synthesis. Circulating IGF-1 appears to modulate the effects of GHRH on pituitary GH secretion, and so, GHRH therapy preserves feedback at pituitary level. Moreover, it appears that GHRH can be effective when given subcutaneously once daily[17, 29]. Other methods of administration, in particular the intranasal route, need to be asessed.

In conclusion, 5 out of 9 short children with CRF, 3 on conservative treatment, 1 on dialysis and 1 post rTx, showed improved growth in response to GHRH therapy. No consistent effect on renal function was detected, but this merits further investigation. GHRH may be an alternative therapy to increase growth velocity in patients with CRF.

Acknowledgement

This work was supported by Serono Laboratories and by a grant from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas).

We thank Dres Marta Balzaretti and Andrea Kozak for technical assistance.

Resumen

Aumento de crecimiento en niños con insuficiencia renal crónica tratados con el factor liberador de la hormona de crecimiento (GHRH).

El retardo de crecimiento es frecuente en niños con insuficiencia renal crónica (IRC). Nueve niños con IRC (3 en tratamiento conservador, 3 en diálisis y 3 post transplante renal) cuyas edades variaron entre 1,6-14,0 ($\bar{x}$ ± SE: 8,1 ± 1,4) años, recibieron 2 dosis subcutáneas diarias de 26 ± 2,4 µg/kg/día de factor liberador de hormona de crecimiento, [GHRH (1-29) NH_2 Serono (Geref)] durante 3-6 meses. Los niveles medios de creatinina y urea séricas permanecieron estables, aunque 2 pacientes en tratamiento conservador tuvieron un leve aumento. Al inicio del estudio el SDS de talla fue - 2,2 ± 0,2 ($\bar{x}$ ± SE), la velocidad de crecimiento 4,5 ± 1,0 cm/año (- 2,3 ± 0,6 DS para edad cronológica) y la respuesta de hormona de crecimiento al test agudo con GHRH (1 µg/kg IV) fue 62 ± 17,5 ng/ml. Cinco pacientes aumentaron la velocidad de crecimiento de 3,8 ± 0,7 a 8,0 ± 1,2 cm/año (test de t apareado, $p < 0,05$). En conclusión, 5 de 9 pacientes con IRC, 3 de ellos en tratamiento conservador, 1 en diálisis y 1 post transplante renal, mejoraron su crecimiento con GHRH. No se detectaron efectos deletéreos sobre la función renal. GHRH podría ser una terapia alternativa para aumentar la velocidad de crecimiento en niños con IRC.

References

1. Van-Diemen-Steenvoorde R, Donckerwolcke RA, Brackel H, Wolff ED, de Jong MCJW. Growth and sexual maturation in children after renal transplantation. *J Pediatr* 1987; 110: 351-6.
2. Ferraris JR, Ramírez JA, Lejarraga H. Crecimiento en pacientes con transplante renal. *Bol Med Hosp Infant Mex* 1988; 45: 485-90.
3. Rees L, Rigden SPA, Ward GM, Preece MA. Treatment of short stature by recombinant human growth hormone in children with renal disease. *Arch Dis Child* 1990; 65: 856-60.
4. Koch VH, Lippe BM, Nelson PA, Boechat MI, Sherman BM, Fine RN. Accelerated growth after recombinant human growth hormone treatment of children with chronic renal failure. *J Pediatr* 1989; 115: 365-71.
5. Tonshoff B, Mehls O, Heinrich U, Blum WF, Ranke MB, Schauer A. Growth-stimulating effects of recombinant growth hormone in children with end-stage renal disease. *J Pediatr* 1990; 116: 561-6.
6. Tonshoff B, Dietz M, haffner D, Tonshoff C, Stover B, Mehls O and members of the German Study Group for Growth Hormone Treatment in Chronic Renal Failure. Effects of two years of growth hormone treatment in short children with renal disease. *Acta Pediatr Scand* (Suppl) 1991; 379: 33-41.
7. Van Es A on behalf of the European Study Group. Growth hormone treatment in short children with chronic renal failure and after renal transplantation: combined data from European clinical trials. *Acta Pediatr Scand* (Suppl) 1991; 379: 42-8.
8. Fine RN, Kohaut EC, Brown D, Perlman AJ for the Genentech Cooperative Study Group. Growth after recombinant human growth hormone treatment in children with chronic renal failure: report of a multicenter randomized double-blind placebo-controlled study. *J Pediatr* 1994; 124: 374-82.
9. Hokken-Koelega ACS, Stijnen T, de Ridder MAJ. et al. Growth hormone treatment in growth-retarded adolescents after renal transplant. *Lancet* 1994, 343: 1313-7.

10. Thorner MO, Spiess J, Vance ML, et al. Human pancreatic GHRH selectively stimulates GH secretion in man. *Lancet* 1983; 1: 24-8.
11. Gelato MC, Pescovitz O, Cassorla F, Loriaux DL, Merriam GR. Effects of a growth hormone releasing factor in man. *J Clin Endocrinol Metab* 1983; 57: 674-6.
12. Pertzelan A, Keret R, Bauman B, et al. Plasma growth hormone response to synthetic GHRH 1-44 in 52 children and adults with growth hormone deficiency of various etiologies. *Horm Res* 1985; 22: 24-31.
13. Gelato MC, Malozowski S, Skerda M, Cassorla F, Merriam GR. Effects of pulsatile administration of growth hormone (GH)-releasing hormone on short term linear growth in children with GH deficiency (GHD). *J Clin Endocrinol Metab* 1985; 61: 444-50.
14. Lejarraga H, Orfila G. Estándares de peso y estatura para niños y niñas argentinos después del nacimiento hasta la madurez. *Arch Arg Pediatr* 1987; 85: 209-22.
15. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist. 2nd ed, Stanford, CA Stanford University Press, 1959.
16. Virasoro E, Copiuschi G, Bruno OD, Leclercq R. Radioimmunoassay of human growth hormone using a charcoal-dextran separation procedure. *Clin Chim Acta* 1971; 31: 294.
17. Rochiccioli PE, Tauber MT, Coude FX, et al. Results of 1-year growth hormone (GH)-releasing hormone (1-44) treatment on growth, somatomedin-C, and 24-hour GH secretion in six children with partial GH deficiency. *J Clin Endocrinol Metab* 1987; 65: 268-74.
18. Ross RJM, Rodda C, Tsagarakis S, et al. Treatment of growth-hormone deficiency with growth-hormone-releasing hormone. *Lancet* 1987; 1: 5-8.
19. Thorner MO, Rogol AD, Blizzard RM, et al. Acceleration fo growth rate in growth hormone-deficient children treated with human growth hormone-releasing hormone. *Pediatr Res* 1988; 24: 145-51.
20. Grossman A, Savage MO, Lytras N, et al. Responses to analogues of growth-hormone-releasing hormone in normal subjects, and in growth-hormone deficient children and young adults. *Clin Endocrinol* 1984; 21: 321-30.
21. Rose SR, Levine Ross J, Uriarte M, Barnes KM, Cassorla FG, Cutler GB. The advantage of measuring stimulated as compared with spontaneous growth hormone levels in the diagnosis of growth hormone deficiency. *N Engl J Med* 1988; 319: 201-7.
22. Schaefer F, Hamill G, Stanhope R, Preece MA, Scharer K, and the Cooperative Study Group on Pubertal Development in chronic renal failure. Pulsatile growth hormone secretion in peripubertal patients with chronic renal failure. *J Pediatr* 1991; 119. 568-77.
23. Schaefer F, Veldhuis JD, Stanhope R, Jones J, Scharer K, and the cooperative study Group on Pubertal Development in Chronic Renal Failure. Alterations in growth hormone secretion and clearance in peripubertal boys with chronic renal failure and after renal transplantation. *J Clin Endocrinol Metab* 1994, 78: 1298-306.
24. Bessarione D, Perfumo F, Giusti M, et al. Growth hormone response to growth hormone-releasing hormone in normal and uraemic children. Comparison with hypoglycaemia following insulin administration. *Acta Endocrinol* 1987; 114: 5-11.
25. The GHRH European Multicenter Study Group, Chatelain P, Alamercery Y, Blanchard J, Boissel JP, Evain-Brion D, Morre M, Olivier M, Sizonenko P, Van Vliet G. Growth hormone (GH) response to a single intravenous injection of synthetic GH-Releasing Hormone in prepubertal children with growth failure. *J Clin Endocrinol Metab* 1987; 65: 387-94.
26. Haffner D, Zacharewicz S, Mehls O, et al. The acute effect of growth hormone on GFR is obliterated in chronic renal failure. *Clin Nephrol* 1989; 32: 266-9.
27. Tonshoff B, Tonshoff C, Mehls O, et al. Growth hormone treatment in children with preterminal chronic renal failure: no adverse effect on glomerular filtration rate. *Eur J Pediatr* 1992; 151: 601-7.
28. Duck SC, Schwarz HP, Costin G, et al. Subcutaneous growth hormone-releasing hormone therapy in growth hormone-deficient children: first year of therapy. *J Clin Endocrinol Metab* 1992; 75: 1115-20.
29. Lanes R, Carrillo E and the Venezuelan Collaborative Study Group. Long term therapy with a single daily subcutaneous dose of growth hormone releasing hormone (1-29) in prepubertal growth hormone deficient children. *J Pediatr Endocrinol* 1994; 7: 303-8.