

THE IMPACT OF DOSAGE ON THE EFFECT OF GROWTH HORMONE THERAPY IN CHILDREN WITH IDIOPATHIC SHORT STATURE. J.M.Wil, H.E.Nienhuis, B.J.Oiten, S.M.P.F. de Muinck Keizer-Schrama, N.M.Drayer, H.A.Delemarre-Van de Waal, W.Oostdijk, T.Vulsma, and G.A.Kamp (Dutch Growth Hormone Working Group). Departments of Pediatrics of the Universities of Utrecht, Nijmegen, Rotterdam, Groningen, Amsterdam Free University, Leiden and Amsterdam, The Netherlands.

The growth response to growth hormone (GH) therapy in children with idiopathic short stature (ISS) is generally characterized by a rapid height velocity (HV) increment followed by a progressive decline. There are no data about the dose-response relationship in such children. We studied 24 children (19 M, 5 F) with ISS and a HV < P25, who, after two years of observation, underwent GH therapy over 3 years in three regimens: gr 1 and 2 received 3 and 4.5 IU/m<sup>2</sup> b.s. 6 times per week, and gr 3 received 3 IU/m<sup>2</sup> in the 1st year and 4.5 IU thereafter. The median HV SDS was -1.8 in the preinclusion year, and 0.3 in the pretreatment year (p < 0.05). In the 1st year on GH the mean (SD) HV SDS rose from 0.4 (1.5) to 5.7 (2.4) on 3 IU/m<sup>2</sup> (gr 1+3) and from 0.1 (0.7) to 7.1 (2.1) on 4.5 IU/m<sup>2</sup>. There was no statistical difference between the groups. In the 2nd and 3rd year the prepubertal children from all groups showed a similar decrease. Mean height SDS increased by 1.2 in all groups, compared to 0.3 in an untreated control group (n=40). Mean bone age (BA) in gr 1, 2 and 3 increased by 3.4, 3.0 and 3.9 years (NS between groups). Predicted adult height SDS (Bayley Pinneau) increased by 1.6 (0.8) in group 2 (p=0.01), but not in groups 1 and 3 (p=0.31 and p=0.13). In conclusion: 1) short children selected on the basis of a low HV show a normal mean velocity in the next year; 2) the three dosage regimens lead to a similar initial growth acceleration followed by a similar decrease; 3) the higher dosage appears more effective in terms of predicted adult height, although attained final heights have to be awaited for definitive conclusions.

THE SECRETORY DYNAMICS OF GROWTH HORMONE IN PATIENTS WITH CUSHING DISEASE. M.A.Magiakou<sup>1</sup>, M.T.Gomez<sup>1</sup>, G.Mastorakos<sup>1</sup>, S.R.Rose<sup>2</sup>, G.P.Chrousos<sup>1</sup>, DEB, NICHD, NIH<sup>1</sup>, Bethesda, MD and Department of Pediatrics, University of Tennessee<sup>2</sup>, Memphis TN.

Growth retardation to complete growth arrest is the hallmark of Cushing syndrome in children. The major mechanism for this has been considered the glucocorticoid-induced resistance of target-tissues to insulin-like growth factor 1 (IGF-1) and other growth factors. The purpose of this study was to examine the growth hormone (GH) secretory dynamics of patients with Cushing disease before and up to 12 months after their cure by transphenoidal adenomectomy. In fourteen patients, every 20 minutes blood sampling during 24h for determination of plasma GH was performed. These patients also underwent arginine infusion and L-Dopa stimulation tests. Fourteen sex- and pubertal stage- matched normal volunteers were used as controls. Prior to therapy, the patient group had an increased BMI (31.5 ± 5 kg/m<sup>2</sup>) and markedly decreased plasma 24h GH mean, peak amplitude and peak area values, with pulse frequency similar to that of the controls. GH values after arginine and L-Dopa stimulation were also subnormal in many of these patients with 2 out of 8, and 8 out of 10 failing to show GH responses greater than 7 ng/ml in the respective test. Surprisingly, the same pattern of GH suppression was observed in the patients who were studied 10-11 days, 3 months and 6 to 12 months after their cure, when their BMIs were respectively, relatively stable, 26.9 ± 3.8 kg/m<sup>2</sup>, and 24.8 ± 3.3 kg/m<sup>2</sup>. These findings suggest that patients with Cushing disease have marked GH suppression during their illness, which continues for at least a year during their convalescence. We conclude that frank GH deficiency may be another important mechanism for the growth retardation observed in children with Cushing syndrome. In view of the persistent GH suppression after curative removal of the pituitary adenomas, patients should be clinically evaluated for the potential need for GH replacement.

FIBROBLAST GROWTH FACTORS 1 AND 2 ACT ON THE IMMATURE HUMAN FETAL ISLET CELL BUT NOT ON THE MATURE β-CELL. T. Otonkoski, G. M. Beattie, M. I. Mally, and A. Hayek, Lucy Thorne Whittier Children's Center, The Whittier Institute, LA Jolla, CA 92037, USA.

Fibroblast growth factors (FGFs) play an important role in the embryogenesis of many organs. In order to elucidate the regulation of pancreatic islet development, we have used tissue culture of human fetal pancreas to study the effects of FGFs. Our method is based on the formation of islet-like cell clusters (ICCs), consisting of 87% undifferentiated cells and 13% islet hormone-positive cells. Supplementation of the culture medium with FGF-2 (basic FGF) more than doubled the formation of ICCs, stimulated their DNA synthesis, inhibited the insulin release (ED<sub>50</sub> ~ 1 ng/ml), but did not affect the insulin content. This activity was shared by FGF-1 (acidic FGF) but not by FGF-6 or FGF-7 (KGF). When ICCs were treated for 72h with bFGF-Saporin (F-SAP), a mitotoxin targeted against FGF-receptor expressing cells, a distinct population (45%) of cells within the ICCs were killed, whereas some strongly hormone-positive (mature) islet cells were left intact. F-SAP treatment also blocked the endocrine differentiation induced by nicotinamide. Similar experiments with isolated adult human islets showed no activity of FGF-2 or F-SAP, supporting a role for FGF in the early development of human islet cells.

SHORT- AND LONG-TERM EFFECTS OF GH IN PATIENTS WITH NORMAL GROWTH DESPITE GH-DEFICIENCY AFTER REMOVAL OF CRANIOPHARYNGIOMA. E.J. Schoenle<sup>1</sup>, T. Torresani<sup>1</sup>, J. Zapf<sup>2</sup>, A. Prader<sup>1</sup>, E. Werder<sup>3</sup> and M. Zachmann<sup>1</sup>, Dept. of <sup>1</sup>Pediatrics and <sup>2</sup>Medicine, University of Zurich, Zurich, and <sup>3</sup>Kinderspital, St. Gallen, Switzerland

Removal of craniopharyngioma usually results in panhypopituitarism. Nevertheless, a number of children grow normally or even excessively after extirpation of the tumor despite the proven lack of GH. These children did not undergo GH therapy. We studied the effects of short- and long-term administration of GH on growth and metabolism in 6 patients under regular hormonal replacement therapy. During the short-term GH <sup>15</sup>N retention was not significantly stimulated (115.4 ± 9.6 % of basal balance, mean ± SEM) and not different from controls. In contrast, <sup>15</sup>N retention was 210.3 ± 20.7 % in children with GH-deficiency of other causes. Long-term administration of GH (2 IU/m<sup>2</sup> s.c. per day during 12 months) did not influence growth velocity, but increased calf circumference and decreased body mass index and skinfold thickness in prepubertal patients. General well-being and strength improved impressively. IGF-I, IGFBP-3 and the 150 kD IGFBP-complex were decreased before and restored to normal during GH treatment. The reverse was observed for the 50 kD IGFBP-complex. Thus, growth velocity in these patients is not related to any of the usual indicators of the growth status and remains unexplained. Although GH therapy does not affect growth, it has other beneficial effects and is recommended for this group of patients with normal growth velocity in the absence of GH.

ATTENUATED EFFECT OF GROWTH HORMONE(GH) IN ACIDOSIS- AND METHYLPREDNISONE(MP)-INDUCED GROWTH RETARDATION(GR) IN WEANLING RATS. D.B.Allen, M.C.Chobanian, A.L.Friedman. Dept. of Pediatrics, Univ. of Wisconsin, Madison, 53792, USA.

Acidosis and glucocorticoid excess retard linear growth. GH reverses MP-induced GR and stimulates urinary net acid excretion in mid-sized uremic rats. However, the efficacy of GH in reversing acidosis- and MP-induced GR in early postnatal life is unknown. We gave GH (0.5mg s.c. 5d/wk x 2wk) to NH<sub>4</sub>Cl-treated(0.28M), MP-treated (6mg/kg/day), and control(H<sub>2</sub>O) weanling Sprague-Dawley rats (mean wt. 50gm) and to adult (A)(mean wt. 250gm) NH<sub>4</sub>Cl-treated and control rats. Acidosis was confirmed by a mean venous pH of 7.26 ± 0.03 in NH<sub>4</sub>Cl animals. Pair feeding (PF), matching food intake to NH<sub>4</sub>Cl rats, was examined in a subset of weanling- and A-H<sub>2</sub>O rats. Compared with non-PF weanling H<sub>2</sub>O rats, who had change in length (d-lt[cm]) of 5.68 ± 0.07, linear growth was retarded (p < 0.05) by NH<sub>4</sub>Cl (d-lt 4.32 ± 0.8), MP (d-lt 4.62 ± 0.1), and PF (d-lt 4.63 ± 0.05). PF markedly reduced weight gain (d-wt[gm]) in H<sub>2</sub>O rats (38 ± 4 PF vs. 91 ± 12 non-PF). In A rats, NH<sub>4</sub>Cl diminished d-lt (2.64 ± 0.25 vs. [A-H<sub>2</sub>O] 3.17 ± 0.2) but PF did not. GH treatment did not accelerate growth in weanling NH<sub>4</sub>Cl, MP, or H<sub>2</sub>O rats. In contrast, GH did increase d-lt in A-NH<sub>4</sub>Cl rats (2.65 ± 0.25 vs. 2.12 ± 0.1; p < 0.05). We conclude: 1) GH ameliorates acidosis-induced GR in A rats; 2) poor caloric intake accounts for much of acidosis-induced GR in weanling rats; 3) the efficacy of GH in reversing GR in acidosis- or MP-induced GR is limited in very young animals. These results suggest that rapid early linear growth in weanling rats is not primarily GH-dependent, and that neonatal GR due to acidosis or glucocorticoid excess results predominantly from mechanisms other than impaired GH secretion and action.

Gene knockout mice demonstrate that IGF-I is essential for normal muscle development.

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Insulin like growth factor (IGF-I) is a pleiotropic hormone synthesized by a wide variety of cell types and with effects on a diverse set of target tissues. IGF-I has been implicated in linear growth, glucose metabolism, organ homeostasis and the development and function of the immune and nervous systems. As an approach to the definition of the role of IGF-I in a whole animal we have generated mice with an inactivating mutation in the gene encoding IGF-I. This was accomplished by replacing one endogenous IGF-I allele in embryonic stem cells with a copy that has a neomycin resistance gene inserted into the gene region that encodes the mature IGF-I protein. These es cells were then used to generate mice. Heterozygotes (one normal and one mutant IGF-I allele) are healthy and fertile but are 10-20% smaller than their wild-type littermates. This difference is primarily due to a decreased muscle mass. In contrast, mice which are homozygous for the mutant allele die at birth and are half the weight of their wild-type littermates. The cause of this peri-natal death is being investigated.