

## ULTRA-SENSITIVE ASSAY OF GROWTH HORMONE IN SERUM.

T. Torresani, E. Alig

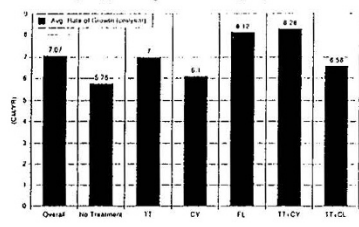
Department of Pediatrics, University of Zürich, Zürich Switzerland

In the last few years the immunological methods for measurement of serum GH have been subject to criticism because of discordance between results obtained from different laboratories and poor sensitivity of most assay systems. Aim of our study was to develop an ultra-sensitive assay for GH and to evaluate its utility for the assessment of the GH secretory status. For this purpose we adapted an ELISA-test for the measurement of GH in urine to the measurement of serum GH. Due to the high concentration of serum GH versus urinary GH, a dilution of the serum samples was required. The assay is performed in microtiter plates coated with a monoclonal GH antibody. Sample size is 100 µl serum diluted 1:100 or 1:400. The assay is completed within 24 hours. The characteristics of this ultra-sensitive GH assay are as follows: minimum detectable concentration: 0.514 ng/L; intraassay coefficients of variation: 10.9% (1.80 ng/L) and 4.4% (9.74 ng/L); interassay coefficients of variation: 17.5% and 13.8% at the same concentrations. Serial dilutions of serum samples were linear and parallel to the standard curve. For the latter r-hGH was used. To assess the utility of the assay, we measured a) samples from 24 hour studies in healthy adult volunteers; b) samples drawn before and after stimulation of GH in children operated for craniopharyngioma. A concentration of GH could be measured in every single serum sample, the lowest found so far being 0.97 ng/L, a value which is statistically different from the minimum detectable concentration for the assay. This ultra-sensitive assay for GH should open new perspectives for the investigation of GH secretion.

## VOLITIONAL ADJUNCTIVE THERAPY FOR THE SHORT BOY Gary G.

Carpenter and Rovinder S. Sandhu, Dept. Pediatrics, Thomas Jefferson University, 1025 Walnut Street, Philadelphia, PA 19107. . .

Short boys, referred without pathologic etiology but with social maladjustment present or anticipated, were offered a choice of ameliorative therapy. 168 boys, over a period of more than 15 years, ranging in age from less than 10 to 16 were originally referred; 37 were seen twice or less and 23 were less than 11 years old, leaving 108. Of the 108, 35 chose no treatment; 6 grew well enough to cross growth lines and 29 continued to grow within growth lines. 58 of the treated boys received only testosterone enanthate at 50 mg/month or less, considered to be less than the dose that would induce pubescence. 30 of the 58 crossed growth lines; 28 did not. A total of 72 boys were treated with combined therapies, receiving cyproheptadine (CY), clonidine (CL), fluoxymesterone (FL) and oxandrolone, singly or in combination with testosterone (TT). About 1/2 of this group also crossed growth lines. Neurosecretory growth hormone dysfunction was not absolutely ruled out in either group although none was truly growth hormone deficient. The results of such choice therapy with testosterone in low dose for the treatment of delayed (or anticipated delayed) adolescence and short stature in boys, may recommend its selected use.



R. Badolato\*, H. Bond\*, G. Valerio\*, N. Gasparini\*, A. Petrella\*, M.J. Waters\*, S. Venutal\*, and A. Tenore. Dept of Pediatrics\* and Dept of Biochemistry\*, University of Naples; Dept of Physiology, University of Queensland; Dept of Experimental Medicine, Medical School of Catanzaro; and Dept of Pediatrics, University of Udine. GROWTH HORMONE RECEPTOR (GH-R) ON PERIPHERAL BLOOD LYMPHOCYTES (PBL): HIGH LEVEL OF EXPRESSION ON B LYMPHOCYTES (L).

Although the GH-R is known to be expressed on a variety of cells, among the immune cells it has been detected on IM9 (human plasmacytoma cell line) and PBL by binding studies with <sup>125</sup>I-GH. This method does not allow to accurately determine which L subset expresses the receptor. Incomplete knowledge of the GH-R distribution among L subsets makes an understanding of the mechanism of action of GH on the immune system difficult. Using a fluorescein-conjugated, GH-R specific monoclonal antibody (mAb 263) in a direct immunofluorescence assay, it was observed that the antibody binds to all L. However, a small subset of PBL binds the anti GH-R mAb with high affinity. Using the conjugated mAb 263 along with different phycoerythrin-conjugated mAbs specific for T, T4, T8, Natural Killer and B cells, this small subset was identified as B cells. The binding of mAb 263 to the different L subsets was quantified in 7 donors. The number of GH-R/cell in each subset was expressed relative to T cells (CD2+ cells). B cells express GH-R 2.58±0.51 fold higher than T cells (p<0.05). The GH-R in the other subsets did not differ from that of T cells. Scatchard analysis of the binding to T and B cells showed that B cells express approximately 4000 GH-R/cell vs. the 1500 GH-R/cell found in T cells. The dissociation constant was identical.

Although the significance of this finding is uncertain, it has been hypothesized that high concentrations of GH result in a suppressive effect of the hormone by disunion of GH-R dimers into monomers. GH may have differential effects on specific cell subsets based not only on its concentration, but on GH-R density as well. GH seems to have specific targets among L and the regulation of their activation and function could be important in the child during phases of rapid growth.

Salerno MC, Argenziano A, Gasparini N, Sandomeni MI, Di Maio S, Tenore A\*

Department of Pediatrics University of Naples and Udine\* Italy

## OVERWEIGHT IN TURNER SYNDROME (TS): INFLUENCE ON GROWTH HORMONE (GH) SECRETION AND ON RESPONSE TO GH THERAPY

The role of GH in the growth failure of TS is still unclear. Recently it has been reported that overweight in TS can decrease GH response to GRF like in adults with idiopathic obesity. The degree of obesity in 30 TS girls was correlated with the response to various stimulation tests (GRF, Arginine, L-DOPA, Clonidine and propranolol-glucagon[PGST]) and with linear growth rate during GH therapy. Chronological age ranged from 3.1 to 14.4 yrs (mean 9.9 yrs), bone age from 2.0 to 12.5 yrs (mean 8.7 yrs). GH secretion was studied in each patient with at least two stimulation tests. GH response was evaluated as peak (P) and as area under curve (AUC). Growth velocity was expressed as standard deviation score (SDS-GV) for TS. The degree of adiposity was expressed as percentage of ideal body weight (%IBW) and as body mass index (BMI). 26 girls received GH therapy (0.6 U/Kg/week) for a period of at least 6 months.

GH secretion after GRF was negatively correlated as peak and as AUC with %IBW (P: r=-0.52 p<0.03; AUC: r=-0.58 p<0.01) and with BMI (P: r=-0.54 p<0.02; AUC: r=-0.60 p<0.01); no correlation was found between the other tests and overweight. Furthermore GH response to GRF resulted significantly reduced in obese (%IBW>120) vs non obese (%IBW<120) patients as P (p<0.04) and as AUC (p<0.04). This trend was observed also after PGST (p<0.05) but not after the other tests. After GH treatment SDS-GV increased from -0.23±0.89 to 2.63±1.63 (p<0.001), but no correlation was found with the GH response as P or AUC to single tests and with BMI or %IBW. Infact the increase of SDS-GV in 11 obese patients (3.2±1.2) was the same as that observed in 15 non obese (2.5±1.9). No karyotype-dependent difference was found in the GH secretion, in the overweight trend or in the GV response after GH therapy. Our data confirm that obesity can influence GH secretion in TS not only after GRF but also after PGST; however it does not affect the response to GH treatment.

## PREDICTION OF ADULT HEIGHT IN TURNER SYNDROME

DA Price\*, MB Ranke, K Albertsson-Wikland, LC Karlsson, on behalf of the KabiPharmacia International Growth Study Board.

\*Royal Manchester Children's Hospital, Manchester, M27 1HA, UK.

Predictions of adult height in girls with Turner Syndrome using established methods, projected height (PROJ), predicted height (PRED), and index of potential height (IPH), were compared to a novel method (KIGS):

KIGS (as ht SDS) = ht SDS (using Ranke standards) - BA SDS for CA. BA SDS for CA was calculated from Greulich and Pyle BAs of 640 untreated girls with Turner Syndrome entered into the KabiPharmacia International Growth Study. Adult height predictions for 245 girls at onset of GH treatment (mean ± SD) were 145.5 cm ± 6.0 (PROJ), 144.9 cm ± 6.4 (PRED), 153.7 cm ± 7.2 (IPH), and 143.5 cm ± 6.4 (KIGS). 26 girls treated for 3 years with growth hormone alone had increases in height prediction of 8.7 cm ± 4.3 (PROJ), 5.6 cm ± 5.1 (PRED), 7.2 cm ± 6.0 (IPH), and 3.3 cm ± 6.4 (KIGS). Height predictions for 15 girls treated with combinations of growth hormone, oestrogen and oxandrolone were then compared to their own achieved adult heights: at 2 years into treatment, PROJ, PRED, and IPH already overestimated adult height by 1.8 cm ± 2.5, 2.5 cm ± 1.3, and 13.8 cm ± 1.6, respectively, and KIGS underpredicted by 2.5 ± 1.5 cm. We conclude that the established methods, especially IPH, tend to overpredict adult height in Turner Syndrome, and that the KIGS method is more conservative.

SE Gargosky, KF Wilson, II Anhalt, M MacGillivray\*, RG Rosenfeld

Department of Pediatrics at Stanford University Medical School, Stanford, CA and at \*Buffalo Childrens Hospital, Buffalo NY.

## IS IMMUNOREACTIVE IGFBP-3 IN THE URINE AN INDICATOR OF GROWTH HORMONE DEFICIENCY (GHD)?

The insulin-like growth factors (IGF) are potent polypeptide mitogens that are carried in the blood, primarily by a GH-dependent protein, IGF binding protein-3 (IGFBP-3). This IGFBP can be fragmented by IGFBP-proteases, subsequently altering the affinity for IGFs. We have characterized the serum and urine IGFBP profiles of children both pre- and post GH treatment. Techniques used were an IGFBP-3 RIA, Western ligand blot (WLB) and an IGFBP-3 protease assay. In the sera of pretreatment GHD children, IGFBP-3 levels were low by both RIA and WLB analysis, and there was no protease activity. Serum concentrations of IGFBP-3 were corrected following GH administration. In the urine, we have established a normal range of IGFBP-3 and IGFBP-3 protease activity in subjects between 5-44 years. Urinary IGFBP-3 (uIGFBP-3) is age-dependent, increasing from 40 ng/mg Cr to 60 ng/mg Cr at age 11-15, after which levels decline to 18 ng/mg Cr by 25 years. Furthermore, little uIGFBP-3 protease activity is detected. In pretreatment GHD subjects, immunoreactive uIGFBP-3 as determined by RIA were generally higher than age-matched controls, although there was some overlap with the normal range. Following GH therapy, uIGFBP-3 levels declined to within the normal range. This was in striking contrast to serum, where serum IGFBP-3 levels increased with GH administration. Although immunoreactive uIGFBP-3 were high, IGFBP-3 was undetectable by WLB. This difference was due to significant protease activity in these subjects, especially in the younger age groups. Following GH therapy, uIGFBP-3 became detectable by WLB and the protease activity diminished. Thus, in the urine of GHD children, an IGFBP-3 protease activity was detected that was apparently GH dependent. Furthermore, the urinary IGFBP-3 protease activity is a serine-type protease which is not metal ion dependent. The source of the IGFBP-3 protease is uncertain, although its appearance solely in the urine of GHD patients is a unique finding. Further assessment of this proteolytic activity may provide insight as to the renal function and clearance in GHD patients.