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PROGRESSIVE NORMALIZATION OF GH BINDING PROTEIN AND IGF_I PLASMA LEVELS DURING THE FIRST YEAR OF GH THERAPY IN GH-DEFICIENT CHILDREN. M.C. Postel-Vinay, M. Noël, P. Czernichow, J. Léger. Pediatric Endocrinology, Hôpital Robert Debré and Endocrinologie Moléculaire INSERM U. 344, Hôpital Necker, Paris, France.

The short- and long-term effects of GH treatment on GH binding protein (GHBP) were examined in GH deficient children. Ten prepubertal children (6 boys, 4 girls), aged 2-10 yr, with isolated GH deficiency and short stature (SD from normal height = 2.4 ± 1.2) were studied before treatment and at regular intervals during GH therapy (0.50 IU/Kg/week). GHBP was measured by HPLC-gel filtration and correction was made for GH levels > 6 ng/ml. Results of plasma GHBP (% of radioactivity) and IGF_I levels (ng/ml) are expressed as mean ± SEM. Normal values for age are: GHBP = 24.8 ± 1.7%, IGF_I = 105 ± 10 ng/ml.

Duration of GH treatment :	0	6h	24h	48h	month 1	month 6	month 12
GHBP	12.1 ±1.3	8.0±1.3	12.1±1.3	10.8±1.1	11.5±2.1	15.1±2.2	21.8±1.5
IGF_I	43±9	49±14	53±15	59±13	69±11	115±18	108±22

The basal GHBP level is low. A significant decrease in GHBP is found 6 h after the first injection of GH. The time of induction of GHBP by GH appears variable: in half of the patients GHBP values are normalized during the first 6 months of treatment. 100% of the GHBP values are normal after 12 months. Mean IGF_I levels are significantly increased 48 h following the first injection of GH and IGF_I levels are normal after 6 months. No correlation was found between the plasma levels of GHBP and IGF_I nor between their increment under GH treatment. In conclusion, low GHBP levels are found in GH-deficient children. During GH therapy, the progressive increase in IGF_I levels occurs before the increase in GHBP levels.

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ANTI-ECP & ANTI-GH ANTIBODIES IN PATIENTS TREATED WITH MAMMALIAN vs E.COLI-DERIVED hGH: A SINGLE BLIND CONTROL STUDY. A.Cohen, A.Lavagetto, A.Morchio & C.Romano. University Department of Pediatrics, Gaslini Institute, Genoa, Italy.

Anti-ECP (E.Coli Polypeptide) and anti-GH antibodies (Ab) were analyzed on a total of 88 blood samples withdrawn from 73 patients. 24 samples belonged to 22 children who were never treated with hGH (Control Group). The remaining 64 samples were obtained from 51 patients treated with GH for at least 12 months. The 64 samples belonged to 4 groups according to the type of GH used (Table). A code number was assigned to each one of the 88 samples, thereby fulfilling the conditions for a *single-blind-control* study. The determination of the anti-GH Ab was performed with a radioimmuno-precipitation assay while the determination of antibodies to ECP was performed using an ELISA method.

Results: *Anti-GH Ab* were positive in 3 samples of 2 patients: 1 of the 2 patients was treated with Met-GH who stopped growing. The same child, 6 months after transfer to mammalian-derived GH, had reduced but still detectable Ab but resumed growth. The second patient of the mammalian-derived hGH group had a positive anti-GH titer but a normal growth-rate. The results of the *Anti-ECP Ab* are shown in the table.

GROUP	PATIENTS		ANTI-ECP No.	Pos %
Control	22	(24)	10 (11)	45.55
Non E.coli-der.	25+1*	(32)	11 (13)	42.3
- Mam.-hGH	19+1*	(25)	9 (11)	
- Pit-hGH	6	(8)	2 (2)	
E.coli-Derived	26	(31)	14 (18)	53.8
- Met-GH	2	(2)	0 (0)	
- Non Met-GH	24	(29)	14 (18)	

( ) Number of samples
* Patient transferred from Met-hGH to mammalian-derived hGH
§ Chi-square distribution p=.256 not significant

Conclusion: Of the 2 patients (3 samples) with positive anti-GH Ab in the GH treated groups, only one had a reduction in growth rate (the Met-GH patient); following transfer to a mammalian-derived GH, resumption of growth rate was observed although anti-GH antibody titer was not completely reduced. The presence of anti-ECP Ab in all groups including the control group suggests that this determination is of no value in the GH treated child. Since in the one patient who developed neutralizing anti-GH Ab to the Met-GH no antibodies to ECP were found, ECP contamination is apparently not the cause for the immunogenicity of this particular GH molecule.

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LOW VITAMINE A INTAKE IN CHILDREN WITH SHORT STATURE.
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We have recently demonstrated a correlation (r= 0.64; p<0.001) between nocturnal GH secretion and plasma vitamine A levels in 68 french short prepubertal children (aged 4 to 12) without organic disease. 25 of these children had a vitaminA/ Retinol Binding Protein ratio <0.6, pointing to a relative vitamine A deficiency. In order to assess the possibility of an inadequate dietary vitamine A, vitamin A supply was estimated as mean dialy intake over a one year period in 56 short children compared to a group of age matched normal children (n=56).Vitamine A intake was significantly lower(p<0.001) in children with short stature(mean±SD 659±600 uG/day) as compared with normal children. (1305 ± 999). Interestingly vitamin A intake was significantly (p<0.01)lower (mean ± SD: 459± 192 ug/day) in 10 children with neurosecretory dysfunction, i.e. impaired nocturnal GH secretion and normal GH peaks to 2 stimulation test than in 17 short children with normal physiological and stimulated GH secretion (mean± SD: 886± 583 ug/day).This suggests that a relative vitamin A deficiency due to inadequate dietary intake might be involved in the GH neurosecretory dysfunction in some children with short stature in industrialized countries.

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LOW PROCONVERTIN (FACTOR VII) AND IMPAIRED BLOOD CLOTTING DUE TO GROWTH HORMONE DEFICIENCY IN THE RAT. L.S. Sävendahl, K.G. Engström+ and K. Grankvist*, Dept of Pediatrics, Dept of Histology and Cell Biology+ and Dept of Clinical Chemistry*, University of Umeå, S-901 87 Umeå, Sweden.

The vitamin K-dependent coagulation factors II (prothrombin), VII (proconvertin), IX (Christmas-factor), and X (Stuart-factor) are all synthesized in the liver as proenzymes. The synthesis of other liver enzymes are affected by growth hormone (GH). To investigate whether GH affects the synthesis or metabolism of coagulation factors, hypophysectomized (hypox) male rats were treated with GH (mini-osmotic pumps) or daily injections of cortisone, thyroxine, vitamin K or saline (n=7-10). At day 11, all rats were cardiopunctured and the prothrombin complex (measures the activity of vitamin K-dependent coagulation factors), and the factors II, VII, IX, and X were determined. The prothrombin complex was 52.9±1.2% for shamoperated rats and 39.1±0.8% for hypox rats receiving saline injections (mean±SEM; p<0.001). All vitamin K-dependent coagulation factors were decreased after hypophysectomy. However, this was significant only for factor VII decreasing from 264±23 to 131±9% (p<0.001) and factor IX decreasing from 28.4±2.2 to 17.1±2.5% (p<0.01). When hypox rats were treated with GH, the prothrombin complex increased to 50.9±1.0 % (p<0.001, compared to 39.1±0.8%) and the factor VII increased to 299±10% (p<0.001, compared to 131±9%). All the other factors were normalized after GH-treatment (data not shown). The injection of cortisone, thyroxine, or vitamin K to hypox rats had no effect. It can be concluded that GH is of great importance for normal blood coagulation in the male rat. GH-deficiency causes a decrease in the levels of vitamin K-dependent coagulation factors, especially factor VII, and the prothrombin complex.

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GLOMERULAR FILTRATION RATE (GFR) DETERMINES METABOLIC CLEARANCE RATE (MCR) OF HGH
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Supraphysiological doses of rhGH are used for treatment of renal growth failure. Because of reduced renal MCR of rhGH this may result in accumulation of rhGH. To determine the quantity of renal and extrarenal MCR of rhGH in chronic renal failure (CRF) we performed stepwise steady-state infusion studies of rhGH in 12 patients (pts) with CRF (6 adults, 6 children; GFR range 8 to 44 ml/min x 1.73 m^2) and 24 healthy adult controls (Co). Endogenous GH secretion was suppressed by i.v. infusion of octreotide (2 ug/1.73 m^2 x h). HGH was infused at three different rates, resulting in mean plasma levels of 5 (I), 30 (II) and 57 (III) ug/l. GFR was measured by simultaneous inulin clearance. Plasma and urin hGH concentrations were measured by monoclonal IRMA. - MCR of rhGH was significantly reduced in pts compared to Co at each infusion rate (I: 139 ± 65 vs 207 ± 61, p < .01; II: 76 ± 35 vs 124 ± 22, p < .001; III: 58 ± 15 vs 113 ± 17, p < .001 ml/min x m^2). In both pts and Co MCR decreased significantly with increasing GH plasma concentrations (p < .01). MCR was correlated to GFR (r = 0.86, p < .001). Calculated extrarenal MCR did not differ between pts and Co at any given GH plasma concentration. T 1/2 was significantly prolonged in pts compared to Co (I: 22 ± 9 vs 13 ± 4, p < .01; II: 27 ± 6 vs 18 ± 5, p < .001; III: 30 ± 6 vs 21 ± 4, p < .001 min). In Co urinary GH excretion was dose dependent but minimal and urinary excretion fraction (0.1 x 10^{-4}) remained constant. - Conclusions: (I) In Co renal MCR and rhGH amounts to about 50 % of total MCR. (II) Renal and total MCR is correlated to GFR. (III) Extrarenal MCR does not increase if renal MCR is low. (IV) In Co and pts MCR is inversely correlated to GH plasma concentration, pointing to extrarenal saturation mechanisms. (V) Adjustment of rhGH doses to GFR should be considered in CRF.

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URINARY GROWTH HORMONE, its value in diagnosis and therapy survey of disorders of GH secretion
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GH-excretion in urine has formerly been shown to correlate with mean plasma levels.A positive correlation was found between the mean of 5-10 night (first morning void) and 24 hour samlpes.Inter- and intraindividual variation in young healthy adults.:100 timed urine samples (4 hours) with an overall mean of 830 pg/ 4 hrs (range 78-2613)had a variation of the mean of 10 samples of 25% between the individuals .The intra-individual variation of the 10 samples was 40%.Injection or infusion (over 60 minutes) of 1 mg of biosynthetic GH resulted in a mean plasma level over 90 minutes of 35 and 75 ug/l respectively. Collecting urine over 240 minutes = approximately 12 x T/2 gave a urine excretion of 0.0042 % and 0.0024% of the mean plasma level for infusion and bolus respectively. 0.0006% and 0.0008% of the total amount injected was found in the 4 hour urine sample.A reference range of urine GH was established with a 25 percentile value of 1.13 / 1.87 and 2.3 ng/24 hours and 0.4 / 0.62 and 1.0 ng/night for the age groups < 7 , 7-10/12.5 and 10/12.5 - 18/19 years respectively . 1-3 years GH- therapy improved height expressed as delta height SDS by + 1.63 and 2.0 in patients with pretreatment urine GH levels < p 50 and < p 25 respectively. Related to mean plasma night profiles < or > 3 ug/l delta height SDS was + 1.61 and + 2.2. Mean urinary gh excretion correlated with r = 0.56 with IGF 1 levels during treatment. In treated patients 24 hr gh excretion on / off therapy were in ng/24 hr :

percentiles	10	25	50	75	90
controls	1.27	2.3	4.11	7.1	14
on HGH	.8	2.4	5	7.7	10.5
off HGH	.3	1.2	1.7	2.6	5.6

Urine GH excretion reflects the large intraindividual variation of GH secretion. A mean of several (first morning) urines must be collected.Intact renal tubular function must be assessed by simulataneous assay of beta 2 microglobulin.The diagnostic value of urinary GH is at least as reliable as plasma profiles or stimulation tests.In spite of a large intra and interindividual variation the bioavailability of injected GH is reflected in urine excretion under therapy.