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SIX YEAR DATA OF CARBOHYDRATE TOLERANCE IN TURNER SYNDROME TREATED WITH GROWTH HORMONE AND OXANDROLONE. M. Almaguer, P. Saenger, J. Frane, D. Wilson, R.G. Rosenfeld & Genentech Collab. Group, A. Einstein Coll. Med., Bronx, N.Y., Stanford U., Ca., Genentech, So. San Francisco, Ca.

The increased incidence of glucose intolerance in Turner syndrome leads to an abnormal GTT in 30-60% of adult women. Since currently explored therapies to increase adult height may impair carbohydrate metabolism we evaluated the effect of more than 3-6 yrs. of GH alone or in combination with oxandrolone on glucose metabolism. The trial was begun in 1983 (n=71) and study subjects receive currently hGH 0.375 mg/kg/wk either in equal daily doses or tiw (hGH) or hGH in combination with oxandrolone 0.0625 mg/kg/day (combination). Based on Natl. Diabetes Data Group criteria 15% (11/71) had impaired GTT at baseline. Postprandial insulin results before treatment and after 3 to 6 years of therapy are summarized below. Geometric means (U/ml) are used because of inherent skewness.

		Pretreatment			After 3+ Years		
	n	mean	mean-SD	mean+SD	mean	mean-SD	mean+SD
HGH	15	37.6	17.0	82.8	71.9	50.3	102.8
Combination	41	33.9	16.9	68.7	104.5	54.4	200.5

The change from baseline to long-term follow-up was significant in the hGH group ($p<0.015$) and in the combination group ($p<0.0005$). The change in the combination group was significantly greater than in the hGH group ($p<0.0021$) using the unequal variance two sample t-test. There was no difference in Hgb A1C, pre and postprandial glucose, and cholesterol or triglyceride values in the 2 groups. No difference in any of the parameters was seen between hGH tiw or daily.

In summary: Oxandrolone treatment increases insulin resistance in Turner syndrome. The long-term risk from these alterations in glucose tolerance is unclear at present.

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RESPONSE TO TWICE-DAILY DOSING OF MET-GH IN NAIVE GH DEFICIENT PATIENTS. R. Levy, D. Norman, and Colleen Miller, Rush Presbyterian-St. Luke's Med. Cntr., Chicago, IL 60612, USA

Daily dosing of GH has become well accepted and utilized as reflected in the Genentech National Cooperative Growth Study, because of its increased efficacy at no additional cost. We examined whether changing the frequency to BID dosing would yield similar gains in growth velocity. Thirty three naive GH deficient patients (18 Male) were assigned to a BID schedule of GH injections. The results are listed below:

Subset (All NCGS groups are Naive GHD patients)	N	Pre-RX Growth Velocity	1 Yr. Growth Velocity	Delta HA minus Delta BA
All BID Pts	33	4.8	10.8	0.5
All QD NCGS Pts	1,168	4.4	10.3	0.1
Pubertal BID Pts	13	2.9	10.8	0.5
Pubertal QD NCGS Pts	519	4.3	9.8	0.2
Pre-Pubertal BID Pts	20	5.4	10.7	0.5
Pre-Pub. QD NCGS Pts	1,513	4.5	10.5	0
All TIW NCGS Pts	2,057	4.5	9.1	0

The BID regimen was well tolerated and compliance was good. While these results are preliminary they are encouraging. We are also comparing a group of patients who were switched between QD and BID dosing. A randomized controlled study should be undertaken to validate these studies.

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GH PREINCUBATION FAILS TO INDUCE RESISTANCE TO THE MITOGENIC ACTION OF INSULIN IN A PYGMY T-CELL LINE. M. E. Geffner, N. Bersch, R. C. Bailey, and D. W. Golde, Departments of Pediatrics and Anthropology, UCLA, Los Angeles, CA 90024 and Memorial Sloan-Kettering Cancer Center, New York, NY 10021 USA

In T-cell lines from normal individuals transformed by the HTLV-II retrovirus, we have shown that (1) GH preincubation completely inhibits the normal insulin-induced two-peak clonal response, and (2) direct clonal stimulation of GH on T-cells is mediated via local IGF-1. In the current study, we sought to determine (1) if the ability of GH preincubation to induce resistance to the mitogenic action of insulin is also mediated by IGF-1 and (2) if a Pygmy T-cell line (previously shown to be both GH- and IGF-1-resistant in direct stimulation assays) would or would not become insulin-resistant following either GH or IGF-1 preincubation. T-cell lines from 3 normals and 1 Pygmy were studied. Colony formation was assessed in response to insulin (a) alone (1.2-43.2 x 10^3 pmol/L), following 2 hr preincubation with either (b) GH (50 µg/L) or (c) IGF-1 (8 µg/L), and (d) following 1 hr preincubation with αIR3, a monoclonal antibody against the IGF-1 receptor, and then 2 hr with GH. The following mean (±SE) responses (first peak/second peak) were noted (*$p<0.001$ vs insulin alone):

	(a) Insulin alone	(b) Insulin+GH	(c) Insulin+IGF-1	(d) Insulin+αIR3+GH
Normal	177±3.2/179±7.0	93±2.9*/97±1.7*	81±2.2*/91±5.8*	180±7.8/165±11
Pygmy	176±13/181±19	169±28/163±16	173±11/188±14	----------

These data indicate that, in normal T-cell lines, either (b) GH or (c) IGF-1 preincubation induces resistance to insulin-induced clonogenesis. Since, in normals, GH-induced insulin resistance can be blocked by preincubation with αIR3 (d), this action of GH must be mediated through a local IGF-1 loop. Therefore, in the Pygmy T-cell line, since there was no significant reduction in insulin responsiveness following either GH or IGF-1 preincubation, the data suggest that the Pygmy is IGF-1-resistant and support our earlier findings of resistance to the direct stimulatory effects of GH and IGF-1.

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TWO YEAR RESULTS OF GROWTH HORMONE THERAPY IN ADOLESCENTS WITH SHORT STATURE AFTER RENAL TRANSPLANTATION (RTx)

In a double-blind, 2-yr study, 16 pubertal patients with growth retardation after RTx were treated with either Norditropin® 4 (gr A) or 8 (gr B) IU/m^2/day. We studied the effects of growth hormone therapy (GHRx) on: growth (height SDS for age ($HSDS_{CA}$)), bone age (BA), renal graft function (GFR, by I^{125}-Thalamate, in ml/min/1.73m^2) and renal plasma flow (RPF, by I^{131}-Hippuran, in ml/min/1.73m^2), bone mineral content (BMC, in (mm Al eq/mm^2)x 10^2), glucose tolerance test (OGTT), plasma IGF-I and -II levels and -binding proteins (IGFBP's). Growth during GHRx was also compared with the 2-year-growth of matched (paired) control patients. Mean (SD) age and BA at start: gr A 15.6(2.6) and 12.6(2.4) yr; gr B 15.7(1.9) and 12.3(2.1) yr. Results (expressed as mean (SD) or mean):

GROUP	$HSDS_{CA}$		ΔBA	GFR		RPF		BMC	
	Start	2 yr	0 - 2 yr	Start	1 yr	Start	1 yr	Start	2 yr
A (n=9)	-3.5	-1.6*	1.8 yr	66.7	64.0	304	311	372	429*
B (n=7)	-4.4	-2.0*	1.7 yr	67.0	60.1	268	260	368	416*

* $P < 0.0001$ compared to start of study

Growth: $HSDS_{CA}$ improved significantly in both gr A and gr B ($P<0.0001$), without difference between gr A and B. Compared to the matched control patients our study patients showed a significantly better growth during GHRx: mean (SD) height increment during 2 yr was 15.3(5.7) cm in GHRx patients versus 6.4(3.8) cm in control patients ($P<0.002$). BA: Bone maturation was similar for gr A and B and the ΔBA/ΔCA ratio was <1 during GHRx. GFR and RPF: Mean values remained stable during 1 yr of GHRx. However, 5 patients had renal problems during the 2 years: 2 had one episode of acute renal rejection (after 7 and 15 mo,resp.) with restoration of renal function after a course of high dose prednisone, 3 showed chronic vascular rejection with decreasing renal function (after 10, 11, and 21 mo, resp.). OGTT: Glucose levels did not change after 6 mo, but all insuline levels were sign. higher during GHRx ($P<0.04$); no signs of impaired OGTT. BMC: A continuing and sign. increase in BMC was found ($P<0.0001$). IGF-I: Mean IGF-I SDS for BA increased sign. and similarly in gr A (from -0.11 to 1.82) and gr B (from 0.21 to 1.93)($P<0.0001$). Conclusion: GHRx with 4 IU/m^2/day results in a significant and sustained improvement of height and bone mineral content in pubertal patients after RTx. Height improved also in comparison with matched control patients. Bone maturation was unaffected. The higher GH dose of 8 IU/m^2/day had similar results. This strongly indicates that GHRx results in an improved final height.

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MULTIPLE LEVELS OF TGF-α MODULATION DURING RAT LIVER REGENERATION. W.E. Russell, S. Sitaric, A.J. Peck, C.M. Patton, P.J. Dempsey, and R.J. Coffey, Jr. Departments of Pediatrics, Cell Biology, and Medicine; Division of Pediatric Endocrinology, Vanderbilt University and Vanderbilt Children's Hospital, Nashville, TN 37232-2579 U.S.A.

Liver provides insights into the mechanisms that control growth in a differentiated tissue. Rat liver regenerates its lost cells within 4 d of a 70% hepatectomy (PH). Hepatic mRNA for TGF-α, a mitogen for various liver cells, increases after PH. To test the hypothesis liver growth is regulated by increased concentrations of TGF-α peptide, we developed a highly specific RIA for rat TGF-α. Livers from male S.-D. rats (n=5 per group) after a 70% PH or sham hepatectomy (SH) were extracted in a low ionic strength buffer (pH 8) containing detergents and protease inhibitors. An increase in TGF-α peptide ($p<0.02$) in PH animals over SH controls was attained at 17 h, coincident with the onset of DNA synthesis. Two-fold elevations in peptide were maintained until 96 h, then lost by 8 d. Liver extracts were chromatographed on Biogel P-60: In samples from sham-operated as well as DNA replicating (17 h) liver, TGF-α immunoactivity in was present exclusively as 18-40 kD precursors. Not until late proliferative (48 h), and post-regeneration (96 h) liver, did a significant fraction of the TGF-α appear as the mature 5.6 kD protein. Both transcriptional and post-translational controls appear evident in the regulation of TGF-α after PH: only precursor forms are present in quiescent and S-phase liver, while mature TGF-α is detected only *after* the major proliferative response. The functions and/or target cells of precursor and mature forms of TGF-α may differ in liver, and the conversion of TGF-α precursors to mature peptide may be regulated by signals initiated by PH.

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INSULIN-LIKE GROWTH FACTORS (IGFs) STIMULATE VLA-4 INTEGRIN GENE EXPRESSION IN DIFFERENTIATING MOUSE SKELETAL MUSCLE CELLS. S.M. Rosenthal and D.Hsiao, Dept. Ped., UCSF, San Francisco, CA 94143, USA

Muscle cell differentiation is regulated not only by soluble growth factors, but also by cell-extracellular matrix (ECM) and direct cell-cell interactions. VLA-4, a member of the integrin family of cell surface molecules involved in cell-ECM and cell-cell interactions, is expressed in skeletal myocytes and plays an important role in differentiation; antibodies to this integrin inhibit myogenesis. Interactions between growth factor signalling and integrin function, however, are not well understood. To explore potential interactions between the IGFs, known stimulators of myogenesis, and integrin expression in muscle cell differentiation, we examined the effects of IGFs on VLA-4 gene expression in mouse C2C12 and BC3H-1 skeletal muscle cells. Myoblasts were placed in serum-free medium and studied for up to 4 days in the absence or presence of 3-12 nM IGF-I, IGF-II, and [Leu^{27}]IGF-II, an IGF-II analog with markedly reduced affinity for the IGF-I receptor but normal affinity for the IGF-II receptor. IGF-I caused a 3-4 fold increase in VLA-4 mRNA in comparison to untreated cells while IGF-II resulted in a 2-3 fold increase. [Leu^{27}]IGF-II was 25-50% as potent as IGF-II in stimulating VLA-4 mRNA. When comparing the relative temporal expression of IGF-II (the principal IGF peptide expressed by muscle cells) with that of VLA-4 during differentiation, an increase in IGF-II mRNA preceded that of VLA-4 by approximately 24 h. These data suggest that IGFs, acting principally through the IGF-I receptor, stimulate muscle cell differentiation, at least in part, by increasing expression of the VLA-4 integrin.