

A total of 81/145 S- (55%) and 21/54 S+ (39%) pts were treated. Within all 3 groups, Ht SDS, IGF-1 SDS, GV and GH dose (0.25–0.29 mg/kg/wk) were not different between S- and S+ at 6, 12 and 24 months.

**Conclusions:** GH and IGF-1 characteristics were not different between S- and S+ pts. Response to GH was similar between S- and S+ pts for up to two years of treatment.

## P100

### Prevalence of IGF-1 deficiency (IGFD) among ISS patients

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**Background:** ISS represents children with heterogeneous reasons for short stature. Primary IGFD may be more prevalent in this group than once thought. Selection of potential responders for rIGF-1 therapy remains controversial.

**Objective:** To identify the prevalence of patients with IGFD in the ISS group.

**Methods:** We reviewed charts of 78 patients with short stature [age 8.75±3.2 years, height (-2.65)±0.71 SD]. All patients underwent GHRH stimulation testing and 48 underwent IGF-1 generation test. 68 patients were diagnosed with ISS, and 10 patients had growth hormone deficiency (GHD).

68 patients with ISS were divided into 2 groups: low IGF-1 [ $<-2$  SD, n = 37 (54%)] and normal IGF-1 [ $>-2$  SD, n = 31 (46%)].

**Results:** 54% (37/68) of ISS children had IGFD, defined as normal peak GH levels ( $>10$  ng/ml) and an IGF-1 level  $<2.0$  SD. 11 out of 48 had low  $\Delta$ IGF-1  $<100$  response to IGF-1 generation test and were treated with rIGF-1 (Increlex) from the beginning. All of them had growth velocity (GV)  $>-1$  SD (mean GV 11.7±4 cm/year) within next 6 months. Other 37 patients, who has passed IGF-1 generation test were treated with rGH. In GH treated group 12 children had GV  $<-1$  SD, and 25 had GV  $>-1$  SD.

According to GV on rGH therapy and the results of IGF-1 generation test 23/48 patients (48%) have primary IGF-1 deficiency, and 25/48 (52%) were classified as neuro-secretory GH dysfunction.

**Conclusions:** In contrast to previously done studies (Clayton PE et al. – 25%), prevalence of primary IGF-1 deficiency is as high as 48–54% in ISS patients, which confirms data previously reported by Ranke MB et al.

We propose that primary IGFD patients can be identified by combination of GH stimulation test and IGF-1 generation test at diagnosis.

## P101

### Regulation of osteoclast differentiation by the IGF binding protein IGFBP-2

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IGFBP-2 knockout mice (IGFBP-2<sup>-/-</sup>) have significantly impaired bone turnover, reflected in reduced cortical bone area and a reduction in the trabecular bone volume due to thinner trabeculae, compared with wild-type mice (IGFBP-2<sup>+/+</sup>).

In vitro studies demonstrated a dysfunction in both osteoblast and osteoclast maturation. The aim of this study was to determine whether IGFBP-2 exerts its effects on osteoclastogenesis in an IGF dependent or independent manner.

Bone marrow cells (BMC) were isolated from IGFBP-2<sup>+/+</sup> and <sup>-/-</sup> male mice at 8 weeks of age. BMCs were cultured in medium

containing rmM-CSF (25 ng/ml) and rhRANKL (50 ng/ml) for 7–10 days to induce osteoclast differentiation and fusion. Cells were either fixed in 10% formalin and stained for tartrate resistant acid phosphatase (TRAP) or lysed and proteins visualized by western immunoblotting.

BMC from IGFBP-2<sup>-/-</sup> formed 84% fewer osteoclasts than IGFBP-2<sup>+/+</sup> BMC. Using a bone resorption “pit” assay there was an 80% reduction in bone resorption by RANKL stimulated BMC from the IGFBP-2<sup>-/-</sup> mice compared with the wild-type mice BMC. To determine whether IGFBP-2 exerted its effects on osteoclast maturation in an IGF dependent or independent manner we used the lentivirus system to express wild-type and two mutant forms of IGFBP-2 in the bone marrow cells from the knockout mice. The first mutant incorporated substitutions in the amino acids required for IGF binding. The second mutant incorporated substitutions in the heparin binding domain (HBD) in the linker region of IGFBP-2 that is unique to this binding protein. While expression of wild-type IGFBP-2 was sufficient to confer the ability of the BMC to become osteoclasts equivalent to the cells from the wild-type mice, expression of neither mutant was sufficient. In order to further determine whether the HBD played a distinct role in osteoclastogenesis we examined whether addition a small synthetic peptide homologous to the HBD was sufficient to permit osteoclastogenesis in the BMC from the knock-out mice. In the presence of this peptide the ability of the BMC to become osteoclasts was significantly increased however the peptide was less effective than the addition of full-length recombinant IGFBP-2.

Previous studies have shown in various cell types that there is an inverse relationship between IGFBP-2 levels and PTEN (phosphatase and tensin homolog) levels. Consistent with the significant increase in PTEN there was a significant reduction in the amount of phosphorylated AKT that could be detected in the IGFBP-2 knockout cultures compared with the wild-type cultures but the addition of IGFBP-2 was sufficient to restore AKT phosphorylation. The PTEN in the IGFBP-2<sup>-/-</sup> mice was highly phosphorylated. To determine whether the phosphorylation of PTEN was related to the inhibition of osteoclastogenesis we used an inhibitor of casein kinase 2 (CK2), a kinase implicated in PTEN phosphorylation. The addition of the CK2 inhibitors was sufficient to induce osteoclast maturation in the BMC from the knockout mice.

Taken together our data suggest that IGFBP-2 acts in both an IGF dependent and independent manner to regulate osteoclast maturation mediated in part by regulation of PTEN.

## P102

### Growth hormone-induced oscillations in cytosolic free Ca<sup>2+</sup> in single rat hepatocytes, modulation by different agonists

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Oscillations in cytosolic free Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>c</sub>) are a fundamental mechanism of intracellular signalling in hepatocytes. Single rat hepatocytes microinjected with the photoprotein aequorin generate oscillations in the cytosolic free Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>c</sub>) when stimulated with agonists acting through the phosphoinositide signalling pathway.

In single rat hepatocytes, bovine growth hormone (bGH) is able to induce [Ca<sup>2+</sup>]<sub>i</sub> oscillations which display similarities with oscillations induced by phenylephrine. Thus the rate of rise of intracellular Ca<sup>2+</sup> in each oscillation closely resembles that induced by Ins(1,4,5)P<sub>3</sub>-mediated agonists. However, the duration of bGH-induced oscillations increases with agonist concentration, in contrast to phenylephrine-induced oscillations, which undergo an increase in frequency as the agonist concentration is raised, without any increase in the duration of individual oscillations. In

the absence of extracellular  $Ca^{2+}$  these transients persist indicating a requirement for intracellular  $Ca^{2+}$ . bGH rapidly increases the levels of  $Ins(1,4,5)P_3$  and treatment of the hepatocyte with the phosphatidylinositol-specific phospholipase C (PI-PLC) inhibitor U-73122 removed the oscillations. These results suggest bGH-induced oscillations are due to PLC activation and generation of  $Ins(1,4,5)P_3$ .

Under physiological conditions, cells are exposed to a variety of hormones activating different signalling pathways at the same time. We examine the ability of pancreatic hormones (glucagon, insulin and somatostatin) to induce changes in the  $[Ca^{2+}]_c$  or to modify bGH effect in  $[Ca^{2+}]_c$  oscillations in rat hepatocytes.

Glucagon, insulin and somatostatin did not themselves produce modifications in  $[Ca^{2+}]_c$ . However, glucagon enhanced oscillatory response induced by agonists acting via the phosphoinositide signalling pathway. Elevation of intracellular cyclic adenosine monophosphate (AMP) concentration, by the co-application of either dibutyryl cyclic AMP or 7 B-desacetyl-7 b-[g-(N-methylpiperazino)butyryl]-forskolin (L858051), produced similar response than this generated by glucagon. In contrast, insulin attenuated cytosolic  $Ca^{2+}$  oscillations through a decrease in their frequency. Somatostatin had no effect on Phe-induced oscillations however inhibited GH-induced  $Ca^{2+}$  oscillations, suggesting a selective inhibitory action of somatostatin on GH.

These findings provide support for opposite roles for the pancreatic hormones, insulin and glucagon on the GH-induced calcium oscillations and demonstrate that besides inhibiting pituitary GH secretion, somatostatin exerts inhibitory (peripheral) effects on GH-stimulated oscillations of  $[Ca^{2+}]_c$  in rat hepatocytes. Our data provide a possible acute mechanism for regulation of GH hepatic action by pancreatic hormones more related to an interactive control of hormones involved in metabolic homeostasis (insulin dominates in the absorptive state and glucagon in the postabsorptive state). These polypeptides may mediate the effects of GH in hepatic protein anabolism and glucose homeostasis by directly influencing GH transduction. Also the data provide another example of receptor-specific information being retained in the oscillator mechanism.

### P103

#### Anti-Müllerian Hormone (AMH) in short girls born Small for Gestational Age (SGA) and the effect of growth hormone (GH) treatment

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**Context:** Restricted fetal growth is thought to negatively influence reproductive function in later life. Serum Anti-Müllerian hormone (AMH) is a marker of the follicle pool.

**Hypothesis:** We hypothesized that short girls born Small for Gestational Age (SGA) have lower serum AMH levels, but we expected that growth hormone (GH) treatment would not affect serum AMH levels in these girls.

**Patients and Methods:** Serum AMH levels were assessed in 249 prepubertal girls aged 3 to 10 years (119 untreated short SGA and 127 healthy controls). The associations between AMH levels and clinical characteristics were analyzed using multiple regression analyses. In addition, we investigated the effect of GH on serum AMH levels in short SGA girls.

**Results:** Mean serum AMH levels were similar in short SGA and healthy control girls ( $p=0.95$ ). In short SGA girls, birth weight SDS, birth length SDS and gestational age were not significantly correlated with the serum AMH levels, even after adjustment for age, height SDS and BMI SDS at sampling, socioeconomic status (SES) and maternal smoking. Serum AMH levels did not change during 1 and 4 years of GH treatment in short SGA girls.

**Conclusion:** Serum AMH levels in short SGA girls were similar to age-matched, healthy controls, indicating that the follicle pool is not comprised due to SGA birth. GH treatment has no effect on AMH levels in short SGA girls.

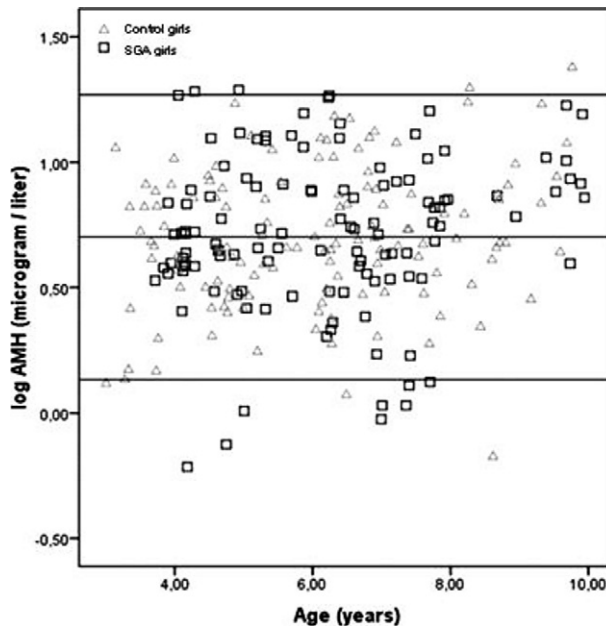


Figure 1. Serum AMH levels in untreated, short SGA and control girls. Serum AMH levels (mcg/liter) in 119 untreated SGA and 127 control girls ( $p=0.95$ ). Lines represent SD-scores of control group: +2 SDS, 0 SDS, -2 SDS.

### P104

#### Identification of a novel cell death receptor (IGFBP-3R) mediating IGFBP-3-induced antitumor effects in human cancer

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The insulin-like growth factor (IGF) system is a critical regulator of the growth and differentiation of many tissues and organ systems. The IGF system consists of the IGF-I and IGF-II ligands, IGF-I and IGF-II receptors and IGF binding proteins (IGFBPs). Recently, the importance of the IGF system in a variety of human cancer has been addressed in large prospective studies by demonstrating a strong correlation between high IGF-I/low IGFBP-3 levels in the circulation and increased risk of cancer. Recent epidemiologic studies suggest that IGFBP-3 contributes to cancer risk protection in a variety of cancers and polymorphic variation of IGFBP-3 influences cancer risk, although other studies vary in their conclusions. Moreover, the involvement of IGFBP-3 in cell growth inhibition and induction of apoptosis in breast, prostate, colon and lung cancer has been demonstrated, although the specific mechanisms involved are unclear.

Some antiproliferative actions of IGFBP-3 have been reported to be independent of IGFs, but the precise biochemical/molecular mechanisms of IGF-independent, antiproliferative actions of IGFBP-3 are largely unknown. Our previous studies demonstrated that IGFBP-3 binding to a cell surface protein is indispensable for its antiproliferative action in human breast cancer cells and the mid region of IGFBP-3, which is the least conserved region among IGFBPs 1–6 protein, is responsible for cell surface binding. Moreover, IGFBP-3 induced apoptosis through the activation of specific caspases that are involved in the death receptor-mediated apoptotic pathways in breast cancer cells. These findings strongly suggest the existence of an IGFBP-3-specific receptor participating in the direct proapoptotic effect of IGFBP-3 in cancer cells.