# Posters

# Group B

# INTRAMUSCULAR GLUCAGON STIMULATION TEST FOR ASSESSING ADRENAL FUNCTION IN SHORT CHILDREN

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**Background**: The glucagon stimulation test (GST) has been shown to be effective in evaluating growth hormone (GH) secretion in children but there are few data on its use in evaluating the hypothalamic-pituitary axis (HPA).

**Objective**: To investigate the diagnostic value of the GST in evaluating the adrenocortical response in short children.

**Patients and Methods**: Intramuscular glucagon was used to assess the HPA axis in addition to GH in children evaluated for short stature. A total of 194 children aged 7.7±4.4 years were evaluated (158 healthy children; 36 with various disorders). Adrenal function was considered normal if peak cortisol was >550 nmol/l and/or absolute increase of cortisol was >250nnol/l. A 250-µg ACTH test was performed in 31 children with inadequate response to GST.

**Results**: Abnormal adrenal response to GST was found in 25.7% of the cohort. Inadequate cortisol response was significantly more common among males than among females (28.7% vs, 16.4%, $p<0.04$) and among children ≥ 6 years than among younger children (32.7% vs. 18.4%, $p<0.02$). Both mean basal and mean peak cortisol levels were significantly higher in the females than in the males: 381±165 vs. 319±151 nmol/l ($p=0.003$) and 741±102 vs. 595±208 nmol/l ($p<0.001$), respectively. By 180 minutes peak cortisol was achieved in 98% of the patients, with the highest proportion (44%) of patients showing peak cortisol response at 180 minutes. In only 4 of the 31 patients undergoing an ACTH stimulation test was peak cortisol <550 but higher than 500 nmol/l. There were no significant differences in proportions of patients with abnormal cortisol response based on GH secretory status. Analyses including only healthy children yielded the same results.

**Conclusions**: GST may serve as a useful screening tool for adrenal function in both healthy and "abnormal" children with suspected hypopituitarism, especially in children <6 years old and in female girls. The adrenal response to GST is age and gender related. Larger studies are needed for establishing the best cut-off level for adequate cortisol response to the GST.

# EFFECT OF AGE AND AFFECTION STATUS ON BLOOD PRESSURE, SERUM POTASSIUM AND STATURE IN FAMILIAL HYPERKALEMIA AND HYPERTENSION

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**Background**: The rare autosomal dominant genetic disorder familial hyperkalemia and hypertension which is caused by mutations in WNK4 kinase, is characterized by childhood hyperkalemia and hypercalciuria, and appearance of hypertension in the third to fourth decade. Accompanying short stature is often described.

**Methods**: We determined height, blood pressure and blood and urinary biochemical parameters in members of a very large family of FHHt with the WNK4 Q565E mutation.

**Results**: The family has 57 members, 30 of whom (including 14 children) are affected. Prehypertension occurred in 7/11 affected and 1/10 unaffected children (P = 0.024). Serum potassium (SK) was ~0.5 mmol/L higher in affected children vs adults [5.98 ± 0.42 vs 5.46 ± 0.40 mmol/L, respectively (P < 0.0001)] (33 samples from 11 children and 36 samples from eight adults). SK of ≥6.0 mmol/L occurred in 16/33 children's samples and in 3/36 adults' samples (P = 0.0003). Hyperkalaemia in children is currently untreated. Children also had more severe hyperchloraemia and hypercalciuria. The family contains four large subfamilies, and each includes 8–10 siblings. In one subfamily, height Z-score was lower in affected vs unaffected subjects [−2.69 ± 0.36 vs −1.05 ± 0.16, respectively (P < 0.0001)]. In the other three subfamilies, no such difference was found.

**Conclusions**: Short stature is not part of FHHt with the WNK4 Q565E mutation. Children affected with FHHt have a high prevalence of prehypertension, and their hyperkalaemia is more severe than that of affected adults. Children may have a more severe defect in the basic mechanism that produces hyperkalaemia. We suggest that, in affected adults, the attenuation of hyperkalaemia and appearance of hypertension may be the result of a late rise in the activity of renal transporters or channels such as the epithelial sodium channel.

# ETHNIC AND GENDER INEQUITIES IN THE EVALUATION OF REFERRED SHORT CHILDREN

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**Aims**: To examine ethnicity and gender differences in the evaluation of referred children with short stature and to investigate adherence of the primary care evaluation to published guidelines.

**Methods**: Cross-sectional study in a referral center. 371 short patients aged 2 to 18 years were included. Outcome measures were patient's growth characteristics, final diagnosis, and prevalence of pre-referral patient data.

**Results**: The study population was composed of 239 Bedouin children and 132 Jewish children ($P < 0.0001$). More males (61%) than females were evaluated ($P < 0.0001$). There were no significant differences between males and females in age and growth parameters at the time of referral. Bedouins, males and females, were significantly shorter than their Jewish counterparts at the time of referral: Ht SD -2.44±0.73 and -2.62±1.05 versus -2.13±0.55 and -2.21±0.57, respectively ($P < 0.05$). There were no significant ethnic or gender differences in the final diagnosis. Significant deficiencies in the primary care evaluation of referred short children were found.

**Conclusions**: We demonstrated novel ethnic- and gender-based inequities in the evaluation of referred short children. We found that the current evaluation of short stature in our area does not comply with existing guidelines.

# PATIENTS WITH LARON SYNDROME SECRETE INCREASED AMOUNTS OF PROLACTIN

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**Background**: One of the diagnostic characteristics of Laron Syndrome (LS) are high serum growth hormone (GH) levels. GH is secreted by the mammosomatotropic cells in the pituitary, which also secrete PRL.

**Aim**: To find out whether the serum levels of PRL in LS patients are also increased and whether IGF-I administration affects its secretion.

**Subjects**: We studied 31 untreated (14M, 17F) and 20 IGF-I treated LS patients (18M, 12F) followed from childhood into adult age.

**Methods**: Laboratory records of serum PRL determination from childhood to age 55 were collected. Serum PRL was determined by radioimmunoassays. A total of 178 determinations were analyzed in untreated patients and 269 in treated patients.

**Results**: Considering mean normal serum PRL concentrations for young adult men as 5.2 $\pm$ 0.55 ng/ml and 20.9 ng/ml for young women (15-25 years) and 8-10 ng/ml for women aged 55-65 years, patients with Laron syndrome secreted increased amounts of PRL but not as high as GH. Serum PRL levels during IGF-1 treatment did not show a clear effect of IGF-1 in female patients but variations of serum PRL concentrations tended to correlate with those of serum GH as they are secreted from the same mother cell.

**Conclusions**: Untreated LS patients oversecrete PRL but to a lesser extent than GH.

# LONG-TERM hGH ADMINISTRATION TO CHILDREN WITH ISOLATED GH DEFICIENCY (IGHD) AUGMENTS ADIPOSITY AND SERUM CHOLESTEROL

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**Introduction**: The most visible features in hGH deficiency from early childhood on are short stature and obesity. if untreated, there is a progressive increase in the fat mass.

**Background**: hGH has been reported to reduce body fat and blood cholesterol during treatment for 1-2 years in children and adult patients.

**Aim:** To find out whether the body fat loss continues during long-term hGH administration.

**Subjects**: 21 children with congenital IGHD (11 boys, 10 girls) treated with hGH for 2-20 years.

**Methods**: Subscapular skinfolds (SSK) were measured by a Harpenden caliper before initiation of treatment, during treatment and up to 4 years after stopping hGH

**Results**: A mean reduction of 32 ± 15% in SSK was observed during the first 1½ years of hGH treatment. Continuation of treatment resulted in a progressive mean increase in SSK over the previous value of 192 ± 158 (SD) % in boys and of 224 ± 164% in girls. Stopping hGH resulted in a further increase in SSK of 46 to 61% from the treatment value. Cholesterol increased progressively to above normal values in most patients, so did insulin.

**Conclusions**: Long-term hGH therapy causes an increase in the subcutaneous fat tissue, as we previously reported for IGF-1 ; thus hGH and IGF-1 can be considered adipogenic hormones.

# HEAD SIZE AND GROWTH RESPONSE TO hGH IN CHILDREN WITH IGHD

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**Background**: Head circumference (HC) is a measure of brain size and longitudinal measurements in childhood serve as an index of brain growth.

**Objective**: To determine the effects of congenital IGF-I deficiency and treatment on HC in patients with Laron syndrome (LS).

**Patients**: 20 untreated adult LS patients, aged 48.4 ± 11.2 y and 13 LS patients treated between ages of 5.6 ± 4 to 11.3 ± 3 y were studied. 15 patients with congenital (IGHD) treated between 6.1±4.4 to 13±4.5 by Hgh served as controls.

**Methods**: HC was expressed as standard deviation (SD) and Ht as SDS. HC was measured and plotted on Nellhaus charts. Linear height (Ht) was measured by a Harpenden Stadiometer.

**Results**: The mean HC deficit of the adult untreated LS males was -2.9 ± 0.6 SD compared to a Ht deficit of -7.0 ± 1.7 SDS. The HC of the LS adult females was -3.6 ± 1 SD compared to a Ht SDS of -6.9 ± 1.5 ($p<0.001$). IGF-I treatment (150-200 µg/kg once daily) increased the HC from -3.3 ± 0.9 (m ± SD) to normal values (0.87 ± 1.8 SD) ($p<0.001$) in 11/13 children. The Ht SDS deficit decreased only by 1.5 SDS.

**Conclusions**:
a) Untreated children and adults with LS have reduced HC (i.e. brain size) . IGF-I replacement in children induces catch-up growth denoting the role of IGF-I on brain growth.
b) Comparison between IGF-I and hGH revealed a greater potency of hGH only in height stimulation

# COCKAYNE SYNDROME PRESENTING UNIQUELY WITH GH DEFICIENCY IS CAUSED BY A NOVEL SPLICE SITE MUTATION

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**Background**: Cockayne syndrome (CS) is a photosensitive DNA repair progeroid disorder presenting with growth failure, and multisystem progressive degeneration including cutaneous photosensitivity, loss of adipose tissue, mental retardation and neurological abnormalities. Although mice models of CS exhibited suppressed growth hormone(GH) secretion, human CS mutations have not been associated with GH deficiency. Here we describe a novel mutation with unusual presentation of CS.

**Clinical Data**: Twin boys born to consanguineous parents (with cousins that died from a progeroid syndrome) presented at 4.8y of age with photosensitive dermatitis, mild learning difficulties, short stature and low growth velocity. Peak stimulated GH levels and basal IGF-1 serum levels were low (GH peak - 4 and 5 ng/ml, IGF -1 - 5, 6.2 nmol/l) for both children. GH therapy increased the growth rate from 3 to 8 cm/y. Skin derived fibroblasts showed low transcription coupled DNA repair ability in specific (TCR) Transcription Coupled Repair tests.

**Molecular Data**: DNA from peripheral lymphocytes of the affected sibling and other family members was sequenced for the ERCC6 /CS-B gene responsible for CS type B. A splice site mutation was found at the beginning of intron 18- c.3778+2T>A predicting the addition of 70 nucleic acids from intron 18 into the transcript and a stop codon thereafter. Missing the C-terminal causes the failure of the resultant protein to bind ubiquitin essential for transcription coupled DNA repair.

**Conclusion**: A novel splice site mutation in the C-terminal of the CSB gene is associated with a mild phenotype of Cockayne Syndrome firstly described in Palestinian kindred. Interestingly the clinical phenotype includes a unique presentation of GH responsive-GH deficiency, a phenotype found so far primarily in the mice model of CS. Further studies on the C terminal motif of this gene may explain its relevance both to the mild phenotype and to the GH deficiency.

# GROWTH AND WEIGHT-REGULATION DISORDERS IN CHILDREN ARE NOT COMMONLY ASSOCIATED WITH MUTATIONS OF THE GHRELIN AND GH SECRETAGENOUS RECEPTOR (GHSR) GENES

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**Background**: Ghrelin and its receptor, growth hormone secretagenous receptor, GHSR, play a major role in appetite control and growth regulation. To date, only four confirmed mutations in the *GHSR* gene have been identified in children with obesity and short stature, while no such mutations have been found in the *ghrelin* gene.

**Objective and hypothesis**: In the current study, we tested the hypothesis that mutations in *ghrelin* or *GHSR* will result in subjects being either over or underweight, and exhibiting abnormal growth.

**Methods**: Ninety-five subjects (37F/58M) were enrolled with FTT (10 pts), GHD (45 pts), ISS (18 pts) or obesity (22 pts). Both *ghrelin* and *GHSR* genes were sequenced.

**Results**: Seven different sequence changes were identified (66.3%) in *GHSR*, two of them novel and five described previously. None of the sequence changes identified in the *GHSR* gene changed the sequence of the encoded protein. The prevalence of these sequence changes did not differ between the subgroups. One previously described sequence change, Leu72Met, within the *preproghrelin/ghrelin* gene was identified in two patients (2%), one with FTT and the other with obesity and partial GHD. This sequence change, which had been identified previously in obese women, is located in exon 2 outside the coding region of the mature ghrelin.

**Conclusion**: Our results suggest that mutations of the *ghrelin* and *GHSR* genes are not commonly associated with growth and weight-regulation disorders in children.

# PARICALCITOL TREATMENT DECREASES ATHEROSCLEROSIS IN APOE NULL MICE: A PILOT STUDY

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**Background and aim of study**: We previously demonstrated that low dose of calcitriol (0.25 $ng.g^{-1}$ body weight every other day) decreases atherosclerosis in $ApoE^{-/-}$ mice by down regulation of renin, the rate-limiting step of the renin-angiotensin system. At the dose used, the effect did not appear to be mediated by immunologic processes, as there was no difference in T-regs or inflammatory cytokines with treatment. However, even at this relatively low dose, prolonged calcitriol administration resulted in hypercalcemia. Less calcemic analogs are in clinical use in hemodialysis subjects, and they have shown clinical benefit with respect to cardiovascular mortality. In a parallel preliminary study, chronic treatment of Tsukuba Hypertensive Mice with low dose paricalcitol did not induce any rise in serum calcium. We therefore sought to investigate the effect of paricalcitol on the development of atherosclerosis in $ApoE^{-/-}$ mice.

**Methods**: At the age of 7 weeks, $ApoE^{-/-}$ mice were switched to an atherogenic diet. Five animals received paricalcitol as an intraperitoneal injection of 0.25 $ng.g^{-1}$ body weight every other day for 8 weeks, while control mice (n=6) received the vehicle only. The extent of atherosclerosis at the aortic sinus was assessed by quantification of oil-red-O-stained lesions. Biochemical parameters were assessed at the end of the study.

**Results**: Paricalcitol reduced the extent of atherosclerosis at the aortic sinus by 60% (P=0.005). Paricalcitol treatment had no significant effect on any of the metabolic parameters: glucose, cholesterol, triglycerides. Additionally, paricalcitol treatment had no effect on the weight of the mice, nor did it affect the index of myocardial hypertrophy, i.e the heart weight to body weight ratio.

**Summary and conclusions**: In this pilot study, paricalcitol treatment had a significant anti-atherogenic effect in $ApoE^{-/-}$ mice. A larger study with escalating doses likely to have an impact on immunologic mechanisms involved in atherogenesis, will help determine the treatment regimen with the best anti-atherogenic potency.

# CAROTENOID DERIVATIVES PREVENT CANCER AND IMPROVE BONE HEALTH BY INHIBITION OF NFkB AND INDUCTION OF NRF2 TRANSCRIPTION SYSTEMS

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Nrf2 mediates induction of detoxifying and antioxidant enzymes which are important for cancer prevention. In contrast, activation of the NFkB transcription system contributes to cancer progression. NFkB system also has a harmful effect on bone health.

Interestingly, under un-stimulated conditions, both Nrf2 and NFkB transcription factors are retained in the cytoplasm by their inhibitory proteins, Keap1 and IkB, respectively which harbor cysteine thiols. The interaction of electrophyles with these cysteines results in activation of Nrf2 and inhibition of the NFkB system. Intact carotenoids such as lycopene and beta-carotene lack such electrophylic groups and we have recently demonstrated that carotenoid derivatives, but not the intact carotenoids, activate the Nrf2 transcription system.

The aim of the current study was to examine whether carotenoid derivatives prevent cancer and improve bone health by inhibiting the NFkB transcription system in both cancer and bone cells.

To this end, we analyzed the structure-activity relationship of a series of dialdehyde carotenoid derivatives in NFkB inhibition. These compounds inhibited NFkB-driven reporter gene expression in both cancer and bone cells. Moreover, similar to our previous findings regarding the Nrf2 system, the activity of the carotenoid derivatives depended on the relative position of the methyl group to the terminal aldehyde. This position determines the reactivity of the conjugated double bond in reactions such as Michael addition to SH groups in proteins (e.g. Keap1, IKK). Carotenoid derivatives attenuated the NFkB signal at multiple stages: IkB phosphorylation (western blot), and accordingly, NFkB nuclear translocation were inhibited. Moreover, a reduced mRNA level of NFkB target genes such as TNF alpha and Mcp-1 was observed.

Importantly, direct inhibition of IKK activity by carotenoid derivatives was found in an in vitro kinase assay.

In conclusion, we suggest that electrophylic carotenoid derivatives contribute to cancer prevention as well as bone health maintenance by two mechanisms: Nrf2 activation and NFkB inhibition. Both could be mediated by modification of SH groups of upstream proteins.

# THE ROLE OF THE ERK, JNK AND NF-KAPPA B CASCADES IN THE REGULATION OF THE VITAMIN D ENDOCRINE SYSTEM IN EPIDERMAL KERATINOCYTES UNDER INFLAMMATORY CHALLENGE

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**Introduction**: The epidermal keratinocyte contains a complete vitamin D endocrine system that includes the enzymes responsible for the production of the hormonal metabolite of vitamin D calcitriol and the vitamin D receptor, VDR. Exposure to calcitriol rapidly induces the expression of its target gene CYP24A1, responsible for calcitriol catabolism. We have previously shown that the epidermal vitamin D endocrine system is up-regulated when keratinocytes face an inflammatory challenge such as the inflammatory cytokines, TNF$\alpha$ and interferon $\gamma$ (IFN$\gamma$). Whereas the regulation of the systemic vitamin D endocrine system is well-characterized, much less is known about the signaling pathways responsible for the regulation of the epidermal system.

**Objective**: to identify the signaling pathways involved in the regulation of the epidermal vitamin D endocrine system under inflammatory challenge.

**Methods**: HaCaT keratinocytes were exposed to TNF$\alpha$ or IFN$\gamma$for 24 hours and then for 5 hours to vitamin $D_3$, 25-hydroxyvitmain $D_3$, 1$\alpha$- hydroxyvitamin $D_3$ or calcitriol. mRNA levels of CYP24A1, VDR and 25-hydroxyvitamin D 1$\alpha$-hydroxylase (1$\alpha$(OH)ase) were assayed by real-time PCR.

**Results**: TNF$\alpha$ but not IFN$\gamma$ upregulated the expression of VDR. However, both cytokines upregulated the expression and activity of 1$\alpha$(OH)ase, while not affecting vitamin D 25-hydroxylase activity. These effects of the cytokines on the various components of the keratinocyte vitamin D endocrine system are manifested as increased expression of CYP24A1 following exposure of the cells to vitamin $D_3$. By using pharmacological inhibitors of ERK, JNK and NF-$\kappa$B pathways we demonstrated that activation of each of these pathways is necessary for the induction of 1$\alpha$(OH)ase by both cytokines, while only the JNK pathway was obligatory for the induction of VDR by TNF$\alpha$.

**Conclusions**: It seems that signaling pathways known to participate in the inflammatory response of the epidermis, are also responsible for up-regulation of the vitamin D endocrine system known for its anti-inflammatory action.

# THE EFFECTS OF ESTROGEN RECEPTORS α AND β SPECIFIC AGONISTS AND ANTAGONISTS ON CELL PROLIFERATION AND ENERGY METABOLISM IN HUMAN BONE CELL LINE

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We have reported that in cultured human female bone cells estradiol-17β (E2) modulated DNA synthesis, the specific activity of creatine kinase BB (CK), 12 and 15 lipoxygenase (LO) mRNA expression and formation of 12- and 15-hydroxyeicosatetraenoic acid (HETE), the arachidonic acid derived metabolites of these enzymes. We now investigate the response of human bone cell line (SaOS2) to estrogen receptors specific agonists and antagonists. Treatment of SaSO2 with E2, 2,3-bis (4-hydroxyphenyl)-propionitrile (DPN; ERβ specific agonist) and 4,4',4''-[4-propyl-(1H)-pyrazol-1,3,5-triyl] tris-phenol (PPT; ERα specific agonist) showed increased DNA synthesis and stimulated CK. Raloxifene (Ral), an ERα antagonist, inhibited E2 or PPT stimulations, but not DPN. The other ERα specific antagonist methyl-piperidino-pyrazole (MPP) and the ERβ specific antagonist 4-[2-Phenyl- 5, 7-bis (tri-fluoro-methyl) pyrazolo [1, 5-a] pyrimidin-3-yl] phenol (PTHPP) inhibited specifically DNA synthesis, CK and reactive oxygen species (ROS) formation induced by estrogenic compound. The LO inhibitor baicaleine did not affect PPT but inhibited E2 and DPN effects. E2 had no effect on ERα mRNA expression whereas DPN and PPT stimulated them. ERβ mRNA expression was stimulated by all compounds. All estrogenic compounds modulated the expression of 12 and 15LO mRNA and 12 and 15 HETE productions. All hormones stimulated ROS formation which was inhibited by NADPH oxidase inhibitor diphenylene iodonium chloride (DPI). DPI did not significantly affect hormonal induced cell proliferation and energy metabolism. In conclusion, we provide herein evidence for the separation of mediation via ERα and ERβ pathways in the effects of E2 on osteoblasts, but the exact mechanisms and the role of ROS are unclear.

# AT LOW DOSE THE LESS CALCEMIC VITAMIN D ANALOG PARICALCITOL LOWERS BLOOD PRESSURE IN TSUKUBA HYPERTENSIVE MICE (THM) BY AFFECTING RENIN EXPRESSION WITHOUT CAUSING HYPERCALCEMIA

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**Introduction**: We recently showed that calcitriol suppresses renin expression, lowers blood pressure (BP), and reduces atherosclerosis in the Tsukuba Hypertensive Mouse (THM), a model of hypertension and atherosclerosis due to the transgenic expression of the human renin-angiotensin system (RAS). However, even at low dose, prolonged treatment resulted in hypercalcemia and an unfavorable metabolic profile. We sought to investigate the effect of a similar low dose of a less calcemic analog, paricalcitol (P), on BP and metabolic parameters in THM.

**Methods**: At age 8-10 weeks, animals were allocated to either P, given IP at a dose of 0.25 ng/g every other day (n=22), or to the vehicle-C (n=17) for 4 weeks. Plasma renin activity (PRA) was measured by RIA. Expression of aortic endogenous and human transgenic RAS was assessed by real-time PCR.

**Results**: Measured via the tail-cuff method, at the end of the study, BP was significantly lower in P than in C: 116.2±3.8 vs 147.1±3.4 mm Hg ($P$<0.0001). Serum calcium was unchanged 8.8±0.4 mg/dl in P and 9.2±0.3 in C. Likewise, urinary calcium excretion was unaffected and was 488±24 µg/mouse/d in P and 440±45 in C. Despite significant variability, PRA showed a definite trend toward lower values with P, 275+170 vs 428+164 ng/ml/h in C, $P$=0.06. By real-time PCR, P caused a 79% reduction in the human renin mRNA level at the aorta ($P$=0.04), while a 51% reduction in the mouse renin mRNA didn't reach significance. None of the other genes assessed showed any significant alteration.

**Conclusions**: In THM, paracalcitol treatment for 4 weeks was as efficient as calcitriol in reducing BP, but in contrast it caused no derangement in calcium metabolism. If extended to the anti-atherogenic effect of calcitriol previously demonstrated in this model, clinical studies to assess its efficacy in the treatment of hypertension and the prevention of atherosclerosis might be warranted.

# SPHINGOSINE-1-PHOSPHATE [S1P] MAY MINIMIZE THE GONADOTOXIC EFFECT OF CHEMOTHERAPY ON HUMAN LUTEINIZED GRANULOSA CELLS

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**Background**: The increase in malignancy of young women in the recent decades, combined with a significant improvement in long term survival of young patients after gonadotoxic chemotherapy, have brought about a ubiquitous interest in preservation of fertility in these young patients. None of the currently used methods [IVF and cryopreservation of embrya, ova or ovarian tissue] is ideal and none guarantees future fertility. Furthermore, autotransplantation of cryopreserved ovarian tissue may reintroduce malignant cells in leukemia and possibly other malignancies. Cotreatment with GnRH-agonistic analogs is beneficial but does not guarantee future fertility in all cases.

**Objective**: Since Sphingosine-1-Phosphate [S1P] may minimize gonadotoxicity, we have examined its possible anti-gonadotoxic effect on human luteinized granulosa cells [GC]. Methods: Human GC's were donated by women undergoing follicular aspiration and IVF, after informed consent and institutional approval of ethics committee [IRB, Helsinki]. The GC were separated from RBC's by centrifugation on percoll/ficoll and plated on 96 multiwell plates at a density of 20-25,000 cells/well. Each experiment, done between 2-7 days after plating, was conducted at least in quadruplicates and repeated at least three times.

**Results**: Doxorubicin, between 200 nM and 2 μM concentrations was toxic to granulosa cells as evaluated by the XTT method and/or LDH concentration in the medium. Co-incubation with S1P at 1-5 μM concentrations for 2-4 days significantly diminished the gonadotoxic effect of Doxorubicin. Cyclophosphamide, at 2 mg/mL, but not 0.5 mg/mL, was toxic to GC's and S1P at 1 and 5 μM concentrations could inhibit the gonadotoxic effect.

**Conclusion**: Future development of a device which may deliver S1P directly to the gonads may possibly prevent chemotherapy induced gonadotoxicity and enable for fertility preservation.

# IDENTIFICATION OF EPITHELIAL SODIUM CHANNEL (ENaC) EXPRESSION IN THE FEMALE REPRODUCTIVE TRACT USING POLYCLONAL ANTIBODIES AGAINST ENaC SUBUNITS

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The control of the fluid environment of the uterus is essential for key reproductive events, such as sperm and embryo transport and implantation. In mice, interaction between Epithelial Sodium Channels (ENaC) and the cystic fibrosis transmembrane conductance regulator (CFTR) has been proposed as the major mechanism regulating uterine fluid absorption and secretion. In this study we examined the localization of ENaC in the female reproductive tract to understand the role of these channels in the regulation of the fluid environment.

For histochemical localization of ENaC we generated polyclonal antibodies against human ENaC subunits. For this purpose we expressed human ENaC subunits in E. coli cells. We then isolated expressed proteins from inclusion bodies and injected these into rabbits to generate polyclonal antibodies. On Western blot analysis of protein from mouse, bovine and human kidney, lung and uterine samples, the antibodies specifically identify a band that matches the expected molecular weight of the subunit. To verify the identity of the protein recognized by the antibodies we expressed human ENaC subunits also in insect SF9 cells. Under confocal microscopy, using fluorescent secondary antibodies we observed specific localization of expressed ENaC in distinct vesicles associated with cell surface. Pre-immune sera from the same rabbits did not show any specific reaction. For histochemical analysis tissue samples were fixated in formalin, frozen and then cryo-sectioned. The sections were reacted with the primary antibody followed by secondary antibody conjugated to horseradish peroxidase. Control kidney sections showed clear identification of kidney tubules where ENaC is known to be localized. Similar to kidney tubules we also observed specific staining of epithelial cells in a distinct pattern along the female reproductive tract. This is the first study to show expression of ENaC in epithelial cells of the human female reproductive tract. The expression of ENaC in these cells indicates that ENaC plays a role in the regulation of the functions of the reproductive tract including oocyte and sperm transport and implantation.

# GnRH INDUCES VARIOUS HISTONE MODIFICATIONS AT THE GONADOTROPIN GENES TO INDUCE THEIR EXPRESSION

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Luteinizing hormone (LH) and follicle stimulating hormone (FSH), which control reproductive development and function, are heterodimeric glycoproteins made up of a common α- and a hormone specific β-subunit. The synthesis and release of both hormones are regulated by the gonadotropin releasing hormone (GnRH). We previously demonstrated that GnRHcan regulate gonadotropin subunit gene transcription at the level of chromatin, through displacing of histone deacetylases, thereby allowing subsequent histone acetylation. We hypothesize that transcriptional activation of the subunit genes by GnRH involves the induction of a sequence of histone modifications, including monoubiquitination of histone H2B at lysine K120 (H2BK120ub), trimethylation of histone H3 at lysine 4 (H3K4me3) and/or phosphorylation of H3 at serine 10 (H3S10p), modifications previously shown to be implicated in yeast and mammalian transcriptional regulation.

Although nuclear protein levels of H3K4me3 are unaltered by GnRH, ChIP studies normalized against levels of total H3 present at the promoters, demonstrated an increase in H3K4me3 at the FSHβ subunit gene promoter after GnRH treatment, while levels at the LHβ and common α-subunit (GSU) gene promoters remained unchanged.GnRH also increased nuclear levels of H2BK210ub as well as its levels at the β-subunit gene promoters, most notably for FSHβ. Furthermore, our data shows that GnRH increases phosphorylation of MSK1, which is activated by ERK or p38MAPK, and targets H3S10, while the levels of phosphorylated H3S10 in the nucleus were also elevated following GnRH treatment. This modification is enriched on the LHβ and FSHβ promoters following exposure to GnRH, and suggests that GnRH may activate subunit gene transcription through regulating H3S10p. H3S10p may also act as a pre-requisite for downstream H3 acetylation by recruiting GNC5, a histone acetyltransferase which we have found associated with the LHβ promoter. These results suggest that GnRH regulates gonadotropin subunit gene transcription through the induction of various histone modifications.

# CHARACTERIZATION OF THE MOLECULAR PATHWAYS LEADING TO GnRH-INDUCED APOPTOSIS IN MATURE GONADOTROPES

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The gonadotropes are a population of cells in the pituitary that play a pivotal role in the mammalian reproductive system. When exposed to gonadotropin-releasing hormone (GnRH), these cells undergo several intracellular modifications leading to production and secretion of the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH).GnRH is also involved in the gonadotrope development, and we have already reported thatitinduces cell proliferation in immature gonadotropes, while leading to apoptosis in mature gonadotropes.Several MAPK cascades are activated by GnRH in the gonadotropes,but the downstream mechanismsresponsible for mediating the GnRH-induced cell proliferation and death,have not been elucidated yet. We have previously reported that the protein levels of prohibitin, a protein involved in cell proliferation regulation, are higher in the nuclei of mature gonadotropes, when compared to immature gonadotropes. We hypothesize that prohibitin, as well as theBcl-2 family proteins, Bax and Hrk, are at least partially responsible for mediating the effects of GnRH on cell death in mature gonadotropes, and that this may involve interaction of prohibitin with Bcl2.

Here, we show that GnRH increases the levels of Bax and Hrk in mature gonadotropes. Additionally, it increases the transcript levels of prohibitin both in a mature gonadotrope cell line, and in primary gonadotropes.Our data also shows that prohibitin is found both in the nucleus and cytoplasm, and indicates GnRH-induced nuclear export of prohibitin. Our knockdown and over expression experiments indicate a role for prohibitin in GnRH-induced cell death in mature gonadotropes. We also report here that prohibitin is able to interact with the cytoplasmic protein Bcl2, and that this interaction seems to be induced by GnRH. Collectively our findings suggest that Bax, Hrk and prohibitin play a role in GnRH-induced cell death in mature gonadotropes, and that prohibitin may prevent the anti-apoptotic actions of Bcl2 by interacting with it, therefore allowing apoptosis.

# DIFFERENTIAL SIGNALING OF THE GnRH RECEPTOR IN PITUITARY GONADOTROPES AND PROSTATE CANCER CELLS

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The type I GnRH receptor (GnRHR) mediates the pituitary functions of GnRH, as well as its anti-proliferative effects in sex hormone-dependent cancer cells. Here we examine and compare the signaling of GnRHR in pituitary gonadotrope vs. prostate cancer (CaP) cells. We first noticed that the expression level of PKCα, PKCβII and PKCε is much higher in αT3-1 and LβT2 gonadotrope vs. LNCaP and DU-145 cells, while the opposite is seen for PKCδ. Activation of PKCα, PKCβII and PKCε by GnRH is transient in αT3-1 and LβT2 gonadotrope cells and prolonged in LNCaP and DU-145 cells. On the otherhand, the activation and redistribution of the above PKCs by PMA was similar for both gonadotrope and CaP cells. Activation of ERK1/2 by GnRH and PMA was robust in pituitary cells, with a smaller effect observed in the CaP cells. The $Ca^{2+}$ ionophore A23187 stimulated ERK1/2 in gonadotrope but not in CaP cells. GnRH, PMA and A23187 stimulated JNK activity in gonadotropes, with a more sustained effect in CaP cells. Sustained activation of p38 was observed for PMA and A23187 in Du-145 cells, while p38 activation by GnRH, PMA and A23187 in LβT2 cells was transient. Thus, differential expression and redistribution of PKCs by GnRH and the transient vs. the more sustained nature of the activation of the PKC-MAPK cascade by GnRH in gonadotrope vs. CaP cells respectively, may provide the mechanistic basis for the cell context-dependent differential biological responses observed in GnRH interaction with pituitary gonadotropes vs. prostate cancer cells.

# NORM PERSISTENT HYPERTHYROTROPINEMIA IN CONGENITAL HYPOTHYROIDISM; SUCCESSFUL COMBINATION TREATMENT WITH LEVOTHYROXINE AND LIOTHYRONINE

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**Context**: Recommended therapy for congenital hypothyroidism (CH) is based on levothyroxine. Some children develop persistent hyperthyrotropinemia despite good compliance. It is considered important to normalize TSH but this might require increasing thyroxine to above the upper normal limit which may be harmful.

**Objective**: To evaluate the utility of combining liothyronine (= T3, cytomel ) with levothyroxine in order to achieve normal TSH levels in CH.

**Design and Patients**: Data from patients' files were collected retrospectively. Eight female patients diagnosed by neonatal screening had persistently high levels of TSH. All 8 patients had persistent levels of FT4 in the upper range of normal. Average levothyroxine dose was 3.1± 0.8 mcg/kg/day. Good compliance was assumed by the repeated presence of high normal serum FT4 levels and by parents' testimony.

**Intervention**: Patients were given either 6.25 or 12 mcg liothyronine and the thyroxine dose was reduced appropriately.

**Main outcome measure**: Hormone levels and final combined drug doses.

**Results**: TSH values on the combined regimen decreased in all patients and normalized in 6/8 patients. FT4 and FT3 remained within the normal range. The levothyroxine-equivalent final dose on the combined regimen was 5.0±0.3 mcg/kg/d in infants and 3.4 ± 0.4 mcg/kg/day in children above 2.5 years. The dose difference was caused by higher liothyronine requirements in infants compared with older children (0.66 ± 0.01 versus 0.3 ± 0.05 mcg/kg/day).

**Conclusions**: In CH, combined therapy with liothyronine and thyroxine can achieve normal TSH levels without abnormal elevation of FT4 or FT3. This may improve neurodevelopmental outcome.