

CALCITRIOL TREATMENT DECREASES ATHEROSCLEROSIS IN APOE NULL MICE

Dr. Maya Ish-Shalom¹ Dr. Jessica Sack¹ Dr. Michal Vechoropoulos¹ Dr. Aviv Shaish² Dr. Yehuda Kamari² Prof. Naftali Stern¹ Dr. **Karen Tordjman**¹

1. Institute of Endocrinology, Metabolism and Hypertension, Tel Aviv Sourasky Medical Center 2. The Bert W. Strassburger Lipid Center, Sheba Medical Center, Tel-Hashomer

Introduction: Epidemiologic evidence suggests vitamin D is inversely correlated with blood pressure and with cardiovascular morbidity and mortality. Potential mechanisms for an anti-atherogenic effect of calcitriol backed by in vitro data include inhibition of macrophage activation, reduction of proteins that promote arterial calcification, and a decrease in pro-thrombotic factors. Furthermore, calcitriol via the VDR is a negative regulator of renin, the rate-limiting step of the renin-angiotensin system (RAS), thereby limiting the production of angiotensin II (AII), itself a potent pro-atherogenic cytokine. Surprisingly, animal studies addressing the in vivo relevance of these data are scarce. Recent evidence points at the RAS as a contributing factor in the atherosclerosis that develops in the ApoE null mouse, a classic model of hyperlipidemia-related atherosclerosis. We therefore hypothesized that vitamin D treatment might reduce atherosclerosis in ApoE null mice by downregulating tissue RAS.

Methods: At 6 weeks, ApoE null mice were switched to an atherogenic diet. 16 animals received calcitriol as an intraperitoneal injection of 0.25 ng/g body weight every other day for 8 weeks, while control mice (n=20) received the vehicle only. Blood pressure (BP) was measured non invasively. The extent of atherosclerosis at the aortic sinus was assessed by serial sections of the area and by quantification of Oil-Red-O-stained lesions. The expression of renin, and that of the AII type 1 receptor (AT1-R) mRNA was assessed in the aorta. NADPH oxidase activity in the aorta, a reflection of the tissue inflammatory stress caused by AII was also measured.

Results: Calcitriol reduced the extent of atherosclerosis at the aortic sinus by 35.4% (P=0.003), despite worsening the metabolic profile (significant elevation of glucose and cholesterol). Likewise, systolic BP was significantly lower in calcitriol-treated mice 91.5±3.4 vs. 109.4±2.4 mm Hg P=0.0009. Renin mRNA was down by 24% (P=0.026) in the aortas of calcitriol-treated mice. However, treatment had no effect neither on the expression AT1-R nor on aortic NADPH oxidase activity.

Conclusions: Calcitriol treatment has a compelling anti-atherogenic effect in ApoE null mice. Although this is accompanied by downregulation of renin, the lack of effect on NADPH oxidase activity, a major target of AII, suggests that other effectors of the RAS are involved. Alternatively, mechanisms other than suppression of the RAS may be at play.

DT56a (Femarelle), contrary to Estradiol- 17and Diadzein, has a positive stimulatory effect on human derived female cultured bone cells in hyperglycemic conditions.

Prof. **Dalia Somjen**¹ Dr. Sara Katzburg¹ Mrs. Orli Sharon¹ Dr. David Hendel³ Dr. Israel Yoles²

1. Institute of Endocrinology, Tel-Aviv medical center, Tel-Aviv

2. Department of Gynecology, Tel-Hashomer Medical Center, and the Sackler Faculty of Medicine, Tel-Aviv

3. Department of Orthopedic Surgery, Shaarei- Zedek Medical Center, Jerusalem

Introduction: We have previously reported, that cultured female- derived human bone cells (hObs) responded to DT56a (Femarelle). Since the skeletal protective effects of estrogens are not discernible in diabetic women, we sought to test the effects of DT56a on hObs grown in high glucose concentration in the growth medium

Methods: Cells were grown either in normal glucose (NG) (4.5g/L, 22mM) or HG (9.0g/L, 44mM) for 7 days. We used the stimulation of creatine kinase specific activity (CK), as a marker for hormone responsiveness and ³[H] thymidine incorporation into DNA (DNA synthesis), in response to E2, DT56a or D.

Results: Cells were grown either in normal glucose (NG) (4.5g/L, 22mM) or HG (9.0g/L, 44mM) for 7 days. HG slightly increased DNA synthesis in hObs. The stimulation in response to E2, and to D was decreased in HG but not to DT56a in both age groups. In HG the CK activity induced by E2 and D was decreased but not by DT56a. Growing hObs in HG led to increased expression of both ERs mRNA in hObs from pre-menopausal women but not in hObs from post-menopausal women. Cells from both age groups expressed mRNA for 25 hydroxy vitamin D3 1- (1-OHase). This was down-regulated by HG in both age groups.

Conclusions: Whether DT56a acts differentially via the different ERs and/or 1-OHase, is still to be established. Since DT56a, contrary to E2 or D, is active on skeletal cells even in HG environment, it might be used as an effective treatment for the hyperglycemic/diabetic postmenopausal women.

The Effect of Vitamin D on the Response of Keratinocytes to High Temperature Heat Shock

Ms. Noa Slaiter^{1,2} Prof. Ruth Koren^{1,2} Dr. Amiram Ravid^{1,3}

1. Felsenstein Medical Research Center, Sackler faculty of Medicine, Tel Aviv University

2. Department of physiology and Pharmacology, Sackler faculty of Medicine, Tel Aviv University

3. Department of Developmental and Cell Biology, Sackler faculty of Medicine, Tel Aviv

Introduction: The keratinocyte contains the full machinery for the production of the hormonal form of vitamin D, calcitriol, from its parent compound 7-dehydrocholesterol, and a vitamin D response system. We have previously shown that calcitriol protects keratinocytes from cell death induced by a variety of environmental and patho-physiological stresses. Burns are one of the most common accidental skin injuries. However, little is known about the consequences of exposing keratinocytes to short-duration high temperatures (~60°C). Exposure to heat shock results in a transient overall inhibition of protein synthesis, while synthesis of a limited number of proteins is induced. Among those are the heat shock proteins HSP70 and GRP78 the hallmarks of cytosolic stress and endoplasmic reticulum (ER) stress, respectively. The aim of this study was to examine the effect of calcitriol on keratinocytes exposed to heat shock simulating burn injury.

Methods: The non-tumorigenic immortal HaCaT keratinocytes were employed as an experimental model. Confluent cultures in serum-free medium were exposed for 10 seconds to 62°C. Cell death was assessed by propidium iodide staining, protein translation rate by 35Smet/cys incorporation into acid insoluble pools during a 10-minute pulse and the level of heat shock proteins was quantified by Western blotting.

Results: 24-hour treatment with calcitriol before exposure to heat shock (60-64°C) protected keratinocytes from cell death occurring 24 hours later. 30 minutes following heat shock the rate of protein translation declined to ~20% of control and this effect was further accentuated in calcitriol-treated cells. 2-3 hours following heat shock protein translation recovered up to 80% of pre-heat shock levels. The recovery was significantly delayed in calcitriol-treated cells. Recovery was abolished in the presence of the RNA synthesis inhibitor actinomycin D indicating the involvement of de-novo synthesized protein(s). The effects of calcitriol on protein translation did not occur when the hormone was added at the time of heat shock. Heat shock significantly increased levels of both HSP70 and GRP78. We found no effect of calcitriol on HSP70 levels, but observed a hormone dependent increase in GRP78 in control and less in heat shocked cultures.

Conclusions: Assuming that inhibition of translation protects heat shocked cells from the deleterious effect of accumulation of denatured proteins, it is possible that more pronounced attenuation of translation in calcitriol-treated cells is part of the protective effect of the hormone. The increase in GRP78 levels suggests that the effect of the hormone may be partially due to reduction of ER stress.

Vitamin D status among patients referred for evaluation of osteoporosis or low bone mass.

Dr. **Yair Liel**¹ *1. Endocrine Service, Soroka University Medical Center*

Introduction: Osteoporosis is currently a major health economy issue. While some of the major risk factors (advanced age, menopause, genetics) are impossible or difficult to modify, others (calcium intake, vitamin D sufficiency) can be easily corrected. Previous studies have identified certain sub-populations in Israel at risk for vitamin D inadequacy (Bedouin women, ultra-orthodox Jews, immigrants from Ethiopia, elderly hospitalized patients, patients following osteoporotic hip fractures). Based on correlations of multiple outcomes with 25(OH)D serum levels, it is currently widely accepted that adequate serum vitamin D stores should be defined as 25(OH)D >30 ng/ml. A steep physiological deterioration occurs with 25(OH)D levels of less than 20 ng/ml, therefore, lower values are considered as "vitamin D deficiency". The present study was aimed to determine vitamin D sufficiency status in patients referred to our endocrine clinic for evaluation of low bone mineral density (BMD) or established osteoporosis.

Patients: Our endocrine clinic serves as the main consulting facility for osteoporosis for the largest health-services provider in Southern Israel. Our standard work-up for osteoporosis includes hematological and biochemistry routine tests, as well as PTH, 25(OH)D, celiac disease serology and urine collection for calcium phosphorus and creatinine. Qualitative data on the occurrence of vitamin D deficiency (<20 ng/ml) was obtained from the author's personal computerized patients database starting in 1993. Detailed, quantitative, data was collected retrospectively from files of all patients referred to our endocrine clinic for evaluation of osteoporosis or osteopenia throughout the 4-year period between 1 January 2004 and 31 December 2007.

Results: Between January 1993 and December 2008, there was a total of 486 patients referred for evaluation of decreased bone mineral density (BMD). Among these, 25(OH)D levels of less than 20 ng/ml was noted in 99 (20%), any time during their follow-up. There was no difference in the prevalence of vitamin D deficiency before and after 1/1/2004 (21% and 18%, respectively, N.S by chi-square test). Between January 2004 and December 2007 (the period for which detailed quantitative data was collected), 140 patients were seen for evaluation of osteopenia or osteoporosis. 6 patients were excluded due to lack of vitamin D results. The final analysis included 134 patients, 103 women and 31 men of a mean age of 58.6±13 years (range, 19-85). Overall, initial 25(OH)D level was 29±12 ng/ml (mean±SD, median- 26 ng/ml). Vitamin D levels were not different between women and men. Whenever repeat measurements were performed, the mean lowest level of 25(OH)D observed was 27.6±12 ng/ml (median- 25 ng/ml). Vitamin D deficiency (<20 ng/ml) was detected in 26/134 patients (19.4%), with no obvious difference between genders. Vitamin D insufficiency was observed in 79/134 (58.9%) of the patients, with no difference between genders. A significant negative correlation was observed between 25(OH)D level and PTH ($r^2 = 0.96$, $p = 0.002$). Trends, that did not reach statistical significance, were observed between 25(OH)D and age (positive), serum total calcium (negative), z-score of BMD (positive).

Conclusions: Vitamin D inadequacy is extremely common among patients referred for consultation for low BMD and osteoporosis, which presumably reflects on a high prevalence of vitamin D inadequacy in the general population. Low vitamin D levels are adversely associated with bone health and other physiological functions, but can easily -and cheaply- be corrected. While attention should be devoted to adequate vitamin D supplementation of individual patients at risk, the policy regarding vitamin D fortification of food products, on a national scale, should also be reconsidered.

Double-stranded RNA, TNF and interferon gamma upregulate 25-hydroxyvitamin D 1alpha-hydroxylase in epidermal keratinocytes

Mrs. **Eti Ziv**^{1,2} Prof. Ruth Koren^{1,2} Dr. Amiram Ravid^{1,3}

1. felsenstein Medical Research Center, Sackler faculty of Medicine, Tel Aviv University

2. Department of physiology and Pharmacology, Sackler faculty of Medicine, Tel Aviv University

3. Department of Development and Cell Biology, Sackler faculty of Medicine, Tel Aviv University

Introduction: The epidermal keratinocyte contains the full machinery for the production of the hormonal form of vitamin D, calcitriol, from its parent compound 7-dehydrocholesterol, and a vitamin D response system. We have previously shown that calcitriol protects keratinocytes from cell death induced by a variety of environmental and patho-physiological stresses and that it has anti-inflammatory activity in the skin. We postulate that calcitriol takes part in the stress and inflammatory responses in the skin and argue that to fulfill these roles calcitriol synthesis should be unregulated when keratinocytes are exposed to stress or to pro-inflammatory mediators. This notion was assessed by examining the effect of double-stranded RNA, which represents viral infection and the cytokines TNF and interferon gamma that are secreted by immune cells recruited to the site of injury or infection on gene expression of the rate limiting enzyme in calcitriol synthesis, 25-hydroxyvitamin D 1alpha-hydroxylase (1alpha-OHase), CYP27B1

Methods: The non-tumorigenic immortal HaCaT keratinocytes were employed as an experimental model. Cultures were exposed to the viral mimic double-stranded RNA (poly(I:C)), TNF, IL-1 beta or interferon gamma for 4 - 24 hours and 1alpha-OHase mRNA was quantified by real time PCR .

Results: brought,β but not to IL-1, Exposure of to poly(I:C), TNF and interferon gamma about a marked, significant and consistent increase in 1alpha-OHase mRNA levels. mRNA levels increased three fold in response to poly(I:C) and TNF peaking at 12 hours and the increase in response to interferon gamma was twenty fold at 16 hours. By using the transcription inhibitor actinomycin D we found that exposure to TNF enhanced 1alpha-OHase mRNA stability. By employing inhibitors for the ERK cascade (U0126) and for c-Jun N-terminal kinase (SP600125), we established that the activity of both cascades is obligatory for upregulation of 1alpha-OHase.

Conclusions: These findings demonstrate that the capacity of keratinocytes to produce the hormonally active metabolite of vitamin D increases following exposure to stimuli commonly present in the stressed and inflamed epidermis. Taken together with the capacity of the hormone to exert protective and anti-inflammatory actions on keratinocytes, we maintain that the epidermal vitamin D endocrine system may serve as a hormonal stress system in the skin.

Characterization of Vitamin D Status in Maccabi Health Services' Patients

Dr. Shulamit Cidon¹ Dr. Tamar Stam¹ Dr. Moshe Leshno² Dr. Anat Bet-Or³ Prof. Sophia Ish-Shalom⁴

1. Maccabi Health Services, The Northern Laboratory, Haifa

2. Tel Aviv University

3. Maccabi Health Services, Clinical Laboratory Services, Medical Division

4. Bone and Mineral Metabolism Unit, Rambam Campus, Rappaport Faculty of medicine, Technion

Introduction: Vitamin D deficiency is common worldwide. In Israel Vitamin D status was previously reported in sample populations: hip fracture patients, elderly, orthodox religious subjects, pregnant women and functionally challenged individuals. The purpose of our study was to assess vitamin D status in the patients of Maccabi Health Services that were tested during medical workup and to detect variables that may influence vitamin D status.

Patients/ Methods: 25(OH)D was assessed using the LIAISON 25(OH)D Total kit. Data of all patients that have been tested in Israel during 2007 was analyzed. Patients with serum creatinine above the upper limit of the reference range (matched for age and gender) and patients with albumin-corrected calcium above the upper limit of normal were excluded from analysis. The 25(OH)D concentration mean and standard deviation were analyzed according to gender, age, and season. The correlation between vitamin D status and BMI, diabetes, heart disease, hypertension and cancer was assessed. Vitamin D status was defined as follows: vitamin D sufficiency - 25(OH)D level ≥ 32 ng/ml, vitamin D insufficiency- 25(OH)D between 20-32 ng/ml and vitamin D deficiency < 20 ng/ml.

Results: 20,123 serum samples were tested in 2007 for 25(OH)D. After applying the exclusion criteria 15892 samples were included in this analysis. 25(OH)D concentration was: 23.86 \pm 9.57 ng/mL (mean \pm -SD): 23.94 ng/mL \pm 10.01 for men (n=3074) and , 23.82 \pm 9.46 ng/mL for women (n=12818) p=0.88. 36% (n=5712) of the patients had vitamin D < 20ng/ml. There was a low positive correlation between 25(OH)D concentration and age r= 0.03, p=0.021. 25(OH)D concentration was higher during July-September quartile- 26.07ng/mL \pm -8.69 (n=4592) compared to 21.6 ng/mL \pm -10.29 (n=2867) during January-March quartile. Using stepwise logistic regression we found that diabetes(n=2197) and elevated BMI were correlated with vitamin D deficiency.

Conclusions: Impaired vitamin D status is common among Maccabi Health Services' patients, even in the summer, although significant seasonal variation was observed. Diabetic and obese patients were at higher risk of vitamin D deficiency. Due to the beneficial vitamin D effects on glucose metabolism, on body composition and weight reduction, these populations should be diagnostically and therapeutically targeted for improvement of vitamin D status. Slightly higher 25(OH)D serum level in older patients may be due to a more frequent vitamin D replenishment in this group.

Genetic and immunologic involvement of the Calcium Sensing Receptor (CaSR) in a cohort of 15 patients with parathyroid disorders

Dr. Auryan Szalat¹ Dr. Gabriel Munter² Dr. Abdulsalam Abulibdeh³ Dr. Liat de Vries⁴ Prof. Gil Leibowitz¹ Dr. Andreas Buchs⁵ Prof. Leitersdorf Eran⁷ Ms. Sandrine Benhamron⁹ Mr. Hillel Galitzer⁸ Prof. Benjamin Glaser¹ Prof. Vardiella Meiner⁶ Prof. Mevorach Dror⁹

1. Endocrinology and Metabolism Service, Hadassah-Hebrew University Medical Center, Jerusalem. 2. Internal Medicine Department, Shaare Zedek Medical Center, Jerusalem. 3. Pediatric Department, Makassed Islamic Hospital, Jerusalem. 4. Institute of Endocrinology and Diabetes, Schneider Children Medical Center of Israel, Petah Tikva. 5. Internal Medicine Department, Assaf Harofe Medical Center, Zerifin. 6. Department of Human Genetic, Hadassah-Hebrew University Medical Center, Jerusalem. 7. Internal Medicine Department, Hadassah-Hebrew University Medical Center. 8. Minerva Center for Calcium and Bone Metabolism, Hadassah-Hebrew University Medical Center. 9. Laboratory for Cellular and Molecular Immunology, Hadassah-Hebrew University Medical Center.

Introduction: CaSR is a 1078 amino acid protein member of the G coupled protein family. Mechanisms of diseases involving the CaSR include gain of function (hypercalciuric hypocalcemia) or loss of function (Familial hypocalciuric hypercalcemia, FHH) which are due either to mutations in the gene or to autoimmune antibodies against the CaSR. We propose to evaluate the involvement of the CaSR as a cause of disease in patients with parathyroid disorders.

Patients/ Methods: Cases were recruited in endocrinology, nephrology, pediatric and Internal Medicine services throughout Israel. DNA was extracted from peripheral blood lymphocytes and the entire CaSR coding region including flanking sequences were analyzed and compared to the reference sequence NG_009058(CASR). Flow cytometry was used to compare the fixation of a specific mouse monoclonal IgG CaSR receptor antibody (5C10, ADD) to the surface of HEK293 cells expressing the CaSR (kindly provided by Pr Silver and Pr Naveh-Many) with or without the addition of serum from healthy volunteers, patients with other autoimmune diseases and from a patient with suspected antibodies to the CaSR.

Results: Altogether 8 idiopathic hypoparathyroidism patients (originating from 7 families) , and 7 patients (6 families) with suspected FHH were evaluated. Among patients with hypoparathyroidism, none had CaSR mutations, yet 2 patients had CaSR antibodies: 1 associated with celiac disease (previously reported at the IES 2008), the other associated with Hashimoto's thyroiditis. Two patients with suspected FHH harbored novel mutations: a missense heterozygous mutation in the exon 2 (I32V) in a female index case and her father, and a homozygous donor splice mutation IV4+1G>A at exon 4 in a 2 year old child, whose parents were heterozygous for the mutation. Positive antibodies to the CaSR were found in one patient with diabetes mellitus type 1 and idiopathic hyperparathyroidism. Serum of patients with Hashimoto's thyroiditis and/or diabetes mellitus, but without parathyroid disorders were negative for CaSR antibodies.

Conclusions: We performed a combined genetic and immunologic investigation of the CaSR to evaluate patients with idiopathic parathyroid disorders. Disease-causing abnormalities were found in 30% of the patients. Two novel mutations were identified in patients with hyperparathyroidism. In two patients with hypoparathyroidism, and in one patient with hyperparathyroidism, we found antibodies to the CaSR which can explain the disease. Further in-vitro functional evaluation of the mutations and of the CaSR antibodies are ongoing. In patients without mutations neither antibodies to the CaSR, other mechanisms leading to parathyroid disorder should be investigated.

Zoledronic acid use in men with advanced prostatic cancer led to improvement in general well being and performance.

Dr. Elena Segal¹ Dr. Sima Serafimovich¹ Dr. Eliahu Gez²

1. Bone Metabolic Diseases Unit, Rambam Health Care Campus

2. Oncology Department, Rambam Health Care Campus

Introduction: Use of zoledronic acid was shown to slow progression of bone involvement and to cause pain palliation in patients with bone metastases. Aim: to evaluate quality of life (QOL) of patients treated with 4 mg of zoledronic acid monthly during one year, to check relationship between 25OHD levels and QOL.

Patients/ Methods: 14 men suffering of prostate cancer with bone metastases, age 70 ± 10.02 received monthly intravenous infusion of 4 mg of zoledronic acid during one year with daily supplementation with calcium carbonate 600 mg and 1000 IU of vitamin D. Laboratory assessment: Plasma intact PTH by IRMA, 25OHD by 125I- radioimmunoassay, routine chemistry. Blood samples were taken at 0,3,6,9,12 months. QOL was assessed using: brief pain inventory form with scale from 1 to 10 for pain and from 10 to 1 for pain interference with the daily activities and general life, standard analgesic score from 0 to 4, functional assessment of cancer therapy -prostate (FACT-P) questionnaire to assess functional abilities, performance status by Eastern Cooperative Oncology Group (ECOG) scale.

Results: 10 patients completed the one year follow-up, 3 died, one didn't have the drug use approved by local medical authorities. At baseline mean 25OHD was 24.14 ± 9.8 , range 9.6-46.2 ng/ml, mean PTH 50.17 ± 18 , range 28-99.8. None had hypercalcemia. No correlation was observed between baseline 25OHD and any parameter of QOL. The pain decreased during the first 6 mo, and deteriorated after that. In Table 1 are presented the data as changes during continuous one year process using Friedman's test. There was significant positive correlation between strength of pain and sleep, contacts with people, enjoyment of life, ability to work: Pearson's test $p < 0.01$ at 3 and 6 mo, 0.005 at 12, $p = 0.01$ at 3 and 6 mo 0.05 at 12, $p < 0.01$ at 3 and 9 mo, 0.001 at 12, $p < 0.01$ at 3 and 6 mo, 0.025 at 12 mo. There was significant correlation with mood: $p \leq 0.001$ at all time points. Despite deterioration in pain after 6 month, the enjoyment from life was significantly higher in the end of study, compared to the start point. Positive trend was observed in increasing ability to work and improvement in mood. No significant changes were observed in the analgesics scores Study limitations- small sample size

Conclusions: treatment with monthly infusions of zoledronic acid might improve QOL in prostate cancer patients with bone metastases.

HISTOMORPHOMETRIC ASSESSMENT OF BONE LOSS IN OVARIECTOMIZED RATS: A modulating effect of excess gonadotropins on trabecular bone

Dr. V. Rouach¹ Dr. S. Katzburg¹ Prof. Y. Koch² Prof. N. Stern¹ Prof. D. Somjen¹

1. Institute of Endocrinology, Metabolism and Hypertension Tel-Aviv Sourasky Medical Center and the Sackler faculty of medicine, Tel-Aviv University, Tel-Aviv 64239

2. Dept of Neurobiology, Weizmann Institute of Science, Rehovot 76100, ISRAEL.

Introduction: The concept that estrogen deficiency is the sole factor underlying postmenopausal osteoporosis has been recently challenged, since both FSH receptor knockout and FSH β knockout mice were found to be resistant to bone loss despite severe estrogen deficiency, suggesting a detrimental role for FSH. In the present study we assessed whether or not lowering FSH levels by the use of the GnRH analogue Decapeptyl (Ferring) in ovariectomized (OVX) rats might improve bone quality.

Patients/ Methods: Wistar- derived female rats, aged 25 days were OVX, and subsequently injected with estradiol (E2, 0.5ug i.p., 5 days per week) or Decapeptyl (3.75 ug intramuscular , every 10 days) or both estradiol and Decapeptyl, for 10 weeks. Twenty four hours after the last injection the rats were sacrificed and serum and organs were collected.

Results: As expected, FSH and LH serum levels were markedly increased in OVX rats, in association with smaller growth plates and disrupted architecture, heavy infiltration of bone marrow with numerous adipocytes, and reduced thickness of the cortical bone. In OVX rats treated with E2, FSH and LH levels were intermediate and the tibia's morphological appearance was similar to that of intact rats, except for reduced cortical thickness, which remained unmodified by E2 replacement. In Decapeptyl-treated OVX rats, FSH and LH levels were entirely suppressed, in association with disrupted organization of the growth plate and trabecules typical for the OVX state, fewer proliferative and chondroblastic cells and a large adipocyte population in the bone marrow. An outstanding morphometric feature conferred by Decapeptyl compared to OVX alone was a two fold increase in trabecular bone volume. In the combined E2-Decapeptyl treatment group, FSH and LH levels were likewise suppressed. Bone features were intermediate as compared with the OVX group. The growth plate architecture was partially restored, the percentage of trabecular bone volume was significantly improved, but all other parameters did not differ from those of the OVX group.

Conclusions: E2 therapy reversed the damage of ovariectomy in almost all bone histomorphometric parameters, whereas lowering the gonadotropins levels with decapeptyl improved trabecular bone volume with no other effects. Combined E2-Decapeptyl treatment improved only trabecular volume significantly, but failed to affect other parameters. Hence, the addition of decapeptyl to E2 replacement had detrimental effects. In conclusion, E2 deficiency is the dominant factor impairing bone in the OVX state. However, concomitant changes in FSH/ gonadotropin levels achieved by decapeptyl do have some modulating, though complex and selective role in this setting. Clearly, then, the role of high FSH/LH levels in post-menopausal bone loss needs further investigation.

Topogenesis of epithelial sodium channel (ENaC) reconstituted with mutant subunits identified in multi-system pseudohypoaldosteronism patients.

Mr. Oded Edelheit^{1,2} Ms. Yafit Shriki^{1,2} Prof. Nathan Dascal³ Prof. Aaron Hanukoglu^{4,5}
Prof. Israel Hanukoglu¹

*1. Dept. of Molecular Biology, Ariel University Center, Ariel, 2. Tel-Aviv University, Sackler Medical School
3. Dept. of Physiology and Pharmacology, Tel-Aviv University, Sackler Medical School .4. Dept. of Pediatrics,
Tel-Aviv University, Sackler Medical School 5. Div. of Pediatric Endocrinology, E. Wolfson Medical Center,
Holon*

Introduction: Multi-system pseudohypoaldosteronism (PHA) is a syndrome of aldosterone resistance inherited as a recessive autosomal disease. In our previous studies we showed that PHA is caused by missense, nonsense, frameshift and splice site mutations in the genes encoding for one of the three subunits of epithelial sodium channel (ENaC) named as alpha-, beta- and gamma-ENaC. These studies established that all three subunits are essential for normal activity of ENaC. Assay of human ENaC reconstituted in *Xenopus* oocytes by microinjection of cRNAs showed that PHA mutations indeed reduce ENaC activity. Reduced activity of ENaC reconstituted with mutant subunits could be due to several possible defects, including reduced expression of the channel within the oocyte and a problem in protein trafficking from the site of translation to the cell membrane. To distinguish among these possibilities, we constructed a plasmid encoding for gamma-ENaC-YFP (Yellow Fluorescent Protein) fusion protein to track membrane location of the whole channel.

Methods: The cDNAs for the three subunits of human ENaC were subcloned in plasmid pGEM-HJ. The mutated forms were generated by a site-directed mutagenesis method. In vitro transcriptions of the cDNAs were carried out using T7-RNA polymerase. The cRNAs (3 ng for each subunit) were micro-injected into immature *Xenopus* oocytes. ENaC dependent amiloride-sensitive whole-cell inward Na⁺ current was measured 2-3 days after cRNA injection using the two-electrode voltage-clamp method while oocytes were clamped at -80 mV and continuously superfused with ND-96 +10 mcM amiloride and ND-96 alternately at 22-25C. To generate a fluorescently labeled gamma-ENaC, the coding sequence of Yellow Fluorescent Protein (YFP) was inserted in frame after the gamma ENaC coding sequence in PGEM-HJ vector.

Results: The expression of ENaC reconstituted with all three subunits including gamma-ENaC-YFP fused protein yields activity similar to wild type ENaC. Confocal microscopy images of oocytes injected only with cRNA encoding for gamma-ENaC-YFP showed trace amounts of fluorescence in the membrane. In contrast, in oocytes injected with three cRNAs encoding for alpha, beta and gamma-ENaC-YFP there was very strong fluorescence indicating presence of the ENaC in the membrane.

Conclusions: Our results demonstrate that gamma-ENaC-YFP is carried to the membrane together with the other two subunits, but not when it is expressed by itself alone. The new construct can be used to determine if the reconstituted channels reach the membrane. Using this new construct we are currently screening a series of alpha ENaC mutants to examine their intracellular transport and oocyte membrane location.

A novel mutation in the AVPR2 gene in a Palestinian family with nephrogenic diabetes insipidus

Dr. Abdulsalam Abu-Libdeh¹ Dr. Imad Dweikat¹ Dr. Bassam Abu-Libdeh¹

1. Makassed Hospital, Department of Pediatrics, Jerusalem

Introduction: Nephrogenic diabetes insipidus (NDI) is a urinary concentrating defect resulting from resistance of the collecting duct to the antidiuretic action of vasopressin (AVP). NDI is classified into hereditary and acquired. The X-linked recessive form is the most frequent genetic cause of inherited NDI and is caused by mutations in the gene encoding the V2 vasopressin receptor (AVPR2 gene). We describe a novel mutation in the AVPR2 gene in a Palestinian family with NDI.

Patients/ Methods: A Palestinian male infant presented in the neonatal period with FTT, vomiting, irritability, fever, and polyuria of 7 cc/kg/hr. His serum sodium and osmolarity were 170 mEq/L and 330mOsm/kg respectively, while his urine osmolality remained low between 45-135mOsm/kg. The diagnosis of NDI was established based on the clinical picture, a similarly affected older brother suggesting X-linked inheritance of the disease, and absence of response to ddAVP.

Results: Sequencing the AVPR2 gene for the patient and his affected brother revealed a novel missense mutation with replacement of G by A in codon 82 of exon 2 (TGC TAC), predicting Cysteine to Tyrosine substitution (C82Y). Testing of the mother showed that she is a carrier of that mutation, and it was absent in a healthy brother and the father.

Conclusions: To our knowledge, this is the first confirmation of this diagnosis by molecular testing in a Palestinian family allowing genetic counseling and future prenatal diagnosis, in addition to early diagnosis of affected males to prevent severe dehydration and complications.

Congenital IGF-I deficiency effects on dental morphology and histology in patients with Laron syndrome

Dr. Timothy Wright¹ Dr. G.D. Horsay¹ **Prof. Zvi Laron²**

1. School of Dentistry, University of North Carolina, USA 2. Endocrinology & Diabetes Research Unit, Schneider Children's Medical Center

Introduction: Laron Syndrome (LS, OMIM #262500, primary growth hormone insensitivity) is an autosomal recessive form of dwarfism clinically indistinguishable from congenital isolated GH deficiency (cIGHD), however with abnormally high serum hGH and low or undetectable insulin-like growth factor (IGF-I) levels due to molecular defects (exon deletions or mutations) of the GH receptor. The characteristic phenotype of Laron syndrome includes underdevelopment of the facial bones leading to a protruding forehead (Fig. 1). Patients with LS have a retarded skeletal maturation, delayed dentition, many caries (Fig. 2) and at a later age crowding of the teeth (Fig. 3). The adult patients require prosthesis at a younger age than the ageing healthy population. To determine the effect of congenital IGF-I deficiency such as in patients with LS affects dental shape and composition.

Patients: The patients followed by ZL were asked to bring teeth that shaded or were extracted. Thirteen teeth were collected and sent to JTW. Thirteen exfoliated or extracted, dry primary teeth from Laron syndrome patients (aged 4-13 years) were evaluated by photography, morphometry of whole teeth and histology using light microscopy. Teeth from Laron syndrome patients belonging to multiple families were evaluated using light microscopy to assess the tooth morphology, enamel thickness and structure. The teeth from individuals with Laron Syndrome were sectioned with a diamond blade for light microscopy and viewed at variable magnifications. Computerized morphometry was used to measure the tooth width and enamel thickness on mineralized thin sections (approximately 150 μm thick). Tooth width was evaluated at the broadest area of the crown in sections cut perpendicular to the long axis of the tooth in a mesial distal plan in posterior teeth and facial lingually in anterior teeth to provide a consistent orientation. The ratio of the enamel to total crown width was determined thereby providing a means to compare the enamel thickness in teeth of different size.

Results: The teeth showed a normal architecture and morphology but reduced facial-lingual enamel thickness when compared to the total tooth width ($p < 0.05$). This indicated that the enamel is significantly thinner than normal in teeth of LS patients. The facial-lingual width of some teeth was also reduced. The histological examination revealed a normal prismatic structure of the enamel, although some patients had an accentuated neonatal line and Striae of Retzius. The teeth appeared grossly normal with normal crown morphology and lacking visible enamel hypoplasia or enamel discolorations suggestive of gross mineralization defects. Histological evaluation showed several changes that were seen in some but not all teeth. The dentin in some cases showed increased interglobular dentin and the enamel demonstrated accentuated Striae of Retzius. Both of these features are indicative of stress on the cells responsible for mineralization of dentin and enamel respectively. Measurements of the enamel thickness to tooth width ratios indicate that in the teeth of LS patients the enamel is significantly thinner than normal. The enamel thickness when compared directly was not significantly different between tooth types. This suggests that the enamel is thin compared to the total tooth width indicating that growth hormone insensitivity as occurs in Laron Syndrome may cause tooth size changes. Further study of tooth size discrepancies should be evaluated in this population.

Conclusions: Congenital IGF-I deficiency in LS patients, in addition to retarding mandibular bone growth and delayed tooth eruption causes slight tooth changes in tooth size and reduction in enamel thickness. The discrepancy between bone and teeth size and maturation in this disease proves again the lack of close interdependence between the dental and skeletal systems.