Abstract Code: A1

STRUCTURE-ACTIVITY RELATIONSHIP OF CAROTENOID DERIVATIVES IN ACTIVATION OF THE ANTIOXIDANT RESPONSE ELEMENT TRANSCRIPTION SYSTEM

(1) MRS. LINNEWIEL KARIN (1) MRS. SALMAN HAGAR (2) DR. ERNST HANSGEORG (3) DR. CARIS-VEYRAT CATHERINE (1) DR. KAMPF ARIE (1) MRS. AMOSI YAARA (1) MR. SUKENIK DROR (1) PROF. LEVY JOSEPH (1) PROF. SHARONI YOAV

(1) CLINICAL BIOCHEMISTRY, FACULTY OF HEALTH SCIENCES, BEN-GURION UNIVERSITY AND SOROKA MEDICAL CENTER OF KUPAT HOLIM, BEER-SHEVA (2) BASF AKTIENGESELLSCHAFT, FINE CHEMICALS, AND BIOCATALYSIS RESEARCH, LUDWIGSHAFEN, GERMANY (3) UMR SAFETY AND QUALITY OF PLANT PRODUCTS, INRA, DOMAINE SAINT PAUL, SITE AGROPARC, AVIGNON, FRANCE

Introduction: Activation of the Electrophile/Antioxidant response element (EpRE/ARE) and the Nrf2 transcription factor induces expression of phase II enzymes. This activation is a major cellular strategy for reducing risk of cancer, inflammation and chronic degenerative diseases. Under unstimulated conditions, the transcription factor Nrf2 is bound to Keap1 which represses Nrf2 activity. Although inducers of this system are diversified in their chemical structure, they are all chemically reactive electrophiles which can interact with SH groups in proteins. Hydrophobic carotenoids such as lycopene and β-carotene, which lack any electrophilic group, were recently found by us to be potent activators of the EpRE/ARE transcription system. Moreover, ARE was transactivated by ethanolic extract of partially oxidized lycopene containing unidentified hydrophilic derivatives but not by the intact carotenoid.

Methods/ Patients: We hypothesized that oxidation products of carotenoids are the active mediators in the activation of the EpRE/ARE transcription system. To identify the putative activators, various carotenoid derivatives were synthesized and their activity to stimulate the Nrf2/ARE transcription system and to inhibit breast and prostate cancer cell proliferation was determined.

Results: The most active molecule found thus far in the series of carotenoid derivatives, is 10,10’-diapocarotene-10,10’-dial. By comparing the activity of several mono-apo-lycopenals and diapocarotenedials we found that their activity depends on the nature of the ending groups of the molecule and, more particularly on the relative position of the methyl group to the terminal aldehyde group. Molecules in which the methyl group is in a gamma position are the most active, which may imply that the reactivity of the carbon-carbon double bond adjacent to the terminal aldehyde group is a major factor in the activation of Nrf2.

Conclusions: A possible explanation of these results is that the reactive double bond is involved in adduct formation with Keap1. Formation of adducts between some food derived electrophiles and keap1 through specific cysteine residues was recently reported. The resulting high molecular weight structures of keap1 leads to its degradation and thus to activation of Nrf2. In support of this hypothesis we detected high molecular weight forms of keap1 after treatment of cells with the 10,10’ derivative or tBHQ, a known activator of Nrf2.
Abstract Code: A2

ENDOGENOUS PLATELET TYPE 12[S]LIPOXYGENASE HAS AN ANTI APOPTOTIC ROLE IN H295R CELLS

(1) DR. JAFFE ANAT (1) MRS. KARBY SHULAMIT (2) DR. WEISINGER GARY (2) PROF. STERN NAFTALI

(1) ENDOCRINE UNIT, HILLEL YAFFE MC, HADERA 38100, ISRAEL. (2) INSTITUTE OF ENDOCRINOLOGY, METABOLISM AND HYPERTENSION, SOURASKY MC TEL AVIV 64239, ISRAEL

**Introduction:** Adrenal cortex carcinoma is a highly malignant tumor for which no effective medical treatment is currently available. Because the secretion of aldosterone from adrenal cortex depends on activation of 12 Lipoxygenase [12LOX] and the production of 12[S] hydroxyeicosatetraenoic acid [12HETE], and since 12LOX appears to be over expressed in a number of human malignancies such as prostate, breast, and testicular cancer, we examined the potential role of 12LOX in human adrenocortical carcinoma.

**Patients / Methods:** The steroid hormone producing human adrenocortical carcinoma - H295R cell line- serves as the study model.

**Results:** We found expression of the human platelets type 12LOX [12LOX-p] by RT-PRC. Using an immunopurified antibody directed to exon 4 of the 12LOX-p sequence, which has the least homology to other 12LOX isoforms, we detected protein products of this enzyme by Western Blot, which could be eliminated in the presence of the blocking peptide. The activity of this enzyme was confirmed by the measuring 12HETE content in H295R by HPLC. Pharmacological inhibition of 12LOX by 4 structurally different inhibitors, baicalein, esculetin, N-propyl-galate and phenidone decreased cell number and caused a stable increase in Sub-G0/G1 fraction by FACS analysis. Induction of programmed cell death [PCD] by pharmacological inhibition of 12LOX was further validated by [DAPI] 4,6,Diamido-2-phenylindole staining and DNA fragmentation kit [Roche Cell Death ELISA]. To differentiate autophagic from apoptotic PCD we used double staining Annexin-V and Propidium-Iodide and observed that pharmacological inhibition of 12LOX resulted in typical membrane-phosphatidyl-serine characteristic of apoptotic changes. 12 LOX inhibition also resulted in the activation of caspase 3. To further assess the role of 12 LOX in H295R cell survival, we used 2 different-promoters anti-sense constructs to knockdown 12LOX-p gene expression. Following a selection period with G418 the knockdown groups had fewer colonies Vs control; 45±9, 23±5 Vs 302±12.5, 299±17, and fewer cells 2×103, 6×103 Vs 5×105, 4.5×105 for ENK and RSV promoters respectively. In this work we showed that 12LOX-p is expressed in H295R and has a key role in the prevention of apoptotic cell death.

**Conclusions:** This new knowledge opens an avenue for putative treatment of adrenal cortex carcinoma.
Abstract Code: A3

MECHANISMS FOR CANCER PREVENTION BY CAROTENOIDS: THE ROLE OF ANTIOXIDANT RESPONSE ELEMENT AND THE NRF2 TRANSCRIPTION FACTOR

(1) MRS. SKLADNIK ANDREA (1) MRS. LINNEWIEL KARIN (1) DR. HIRSCH KEREN (1) MRS. SALMAN HAGAR (1) MRS. KANTOROVICH LIA (1) MR. SUKENIK DROR (1) DR. DANILENKO MICHAEL (1) PROF. SHARONI YOAV (1) PROF. LEVY JOSEPH

(1) DEPARTMENT OF CLINICAL BIOCHEMISTRY, FACULTY OF HEALTH SCIENCES, BEN-GURION UNIVERSITY OF THE NEGEV AND SOROKA MEDICAL CENTER OF KUPAT HOLIM, BEER-SHEVA

Introduction: In hormone dependent malignancies, estrogens and androgens are important risk factors. We previously demonstrated that carotenoids inhibit estrogen- and androgen-induced cancer cell growth. In addition we reported that carotenoids induce phase II enzymes by activation of the Electrophile/Antioxidant response element (EpRE/ARE) and the Nrf2 transcription factor, and that this induction was abolished by dominant negative Nrf2. The aim of the study was to determine whether Nrf2 transcription system is involved in the inhibition of estrogen and androgen signaling in hormone responsive mammary, endometrial and prostate cancer cells.

Patients / Methods: 

Results: Ectopic expression of Nrf2 or activation of endogenous Nrf2 by tert-butyl-hydroquinone (tBHQ, a classical activator of Nrf2) resulted in the inhibition of the estrogen- and androgen-induced reporter genes activity similar to that achieved by incubating the cells with carotenoids. Using Western blotting and real time RT-PCR we found that carotenoids, tBHQ or Nrf2 over-expression inhibited the expression of the estrogen-induced genes, c-myc, progesterone receptor (PgR) and pS2, the androgen induced gene PSA and, as expected, stimulated the expression of NQO1, a classical Nrf2-induced phase II enzyme. Moreover, reduction of Nrf2 level by siRNA attenuated the lycopene-induced decrease in PgR expression, confirming that Nrf2 is involved in the inhibition of estrogen signaling by lycopene. Experiments with dominant negative Nrf2 suggest that the inhibition of estrogenic activity by Nrf2 is not due to ARE activation but rather to direct interaction between Nrf2 and components of the estrogen-receptor transcription complexes. Chromatin immuno-precipitation (ChIP) assays show that Nrf2 is introduced by lycopene and tBHQ treatment into the estrogen receptor complex which is bound to the promoters of pS2 and PgR.

Conclusions: Carotenoids can prevent cancer by two mechanisms, both related to the activation of the Nrf2 transcription factor. Primarily the activated Nrf2 stimulates Phase II detoxifying enzymes by binding to EpRE/ARE sequences present in their promoters. In addition Nrf2 can interact directly with estrogen receptor transcriptional complexes to inhibit the induction of multiple cancer-related genes.
REGULATION OF INSULIN–LIKE GROWTH FACTOR I RECEPTOR GENE EXPRESSION BY FOLIC ACID: POTENTIAL IMPLICATIONS IN COLON CANCER

(1) MRS. ATTIAS ZOHAR (2) DR. VAISMAN NAHUM (1) PROF. WERNER HAIM

(1) DEPARTMENT OF HUMAN MOLECULAR GENETICS AND BIOCHEMISTRY, SACKLER SCHOOL OF MEDICINE, TEL AVIV UNIVERSITY (2) UNIT ON CLINICAL NUTRITION, TEL AVIV SOURASKY MEDICAL CENTER, TEL AVIV

**Introduction:** The insulin–like growth factor (IGF) system plays a critical role in the regulation of cell growth and transformation. Overexpression of the IGF-IR in colorectal cancer has been shown to enhance invasion and resistance to apoptosis. Folic acid (FA), a member of the vitamin B family, has emerged in recent years as an important nutritional factor with potentially significant roles in the prevention of colorectal cancer. Consistently, previous studies found an inverse correlation between dietary FA intake and the risk of colorectal cancer. The aim of this work was to evaluate the hypothesis that the mechanism of action of folic acid and its metabolites involves regulation of IGF-IR gene expression.

**Patients / Methods:** The colorectal cancer-derived HCT+/+, HCT-/- and CaCO2 cell lines were employed. The effect of FA on endogenous IGF-IR levels was assessed by Western blotting. IGF-IR mRNA levels were measured by RT-PCR. Transient transfections were performed using an IGF-IR promoter-luciferase reporter plasmid. To examine the functional interaction between FA and transcription factor Sp1, Chromatin Immunoprecipitation (ChIP) experiments were performed. Apoptosis was demonstrated by PARP (Poly ADP-ribose polymerase) and Annexin V-FITC assays.

**Results:** Our results show that FA treatment induced a dose-dependent decrease in IGF-IR protein and mRNA levels in the HCT +/+ colon cancer cell line. Furthermore, results of transient transfection experiments showed that FA and its metabolites dihydrofolic acid and tetrahydrofolic acid induced a significant decrease in IGF-IR promoter activity, suggesting that the effect of FA was mediated at the level of transcription. Results of ChIP assays demonstrated that FA induced a decrease in Sp1 binding to the IGF-IR promoter. In addition, FA induced a decrease in IGF-I-stimulated signal transduction mediators. Finally, FA exhibited a pro-apoptotic activity, as demonstrated by PARP cleavage and Annexin V-FITC staining.

**Conclusions:** Our results suggest that the tumor preventive role of FA in colon cancer may result, at least in part, from suppression of IGF-IR gene expression. Furthermore, the finding that FA was unable to down-regulate IGF-IR levels in p53-depleted cells, suggests that p53 is required to elicit the FA effect.
Abstract Code: A5

VITAMIN D SENSITIZES COLON CANCER CELLS TO H2O2-INDUCED CYTOTOXICITY WHILE INHIBITING CASPASE ACTIVATION

(1) DR. RAVID AMIRAM (2) MRS. WACKSBERG SHIRI (2) DR. WEITSMAN GREGORY (2) PROF. KOREN RUTH

(1) DEPARTMENT OF CELLULAR AND DEVELOPMENTAL BIOLOGY, AND THE FELSENSTEIN MEDICAL RESEARCH CENTER, SACKLER FACULTY OF MEDICINE, TEL-AVIV UNIVERSITY (2) DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY, AND THE FELSENSTEIN MEDICAL RESEARCH CENTER, SACKLER FACULTY OF MEDICINE, TEL-AVIV

Introduction: The preventive and therapeutic anti-cancer effects of calcitriol, the active metabolite of vitamin D, on colon cancer is usually attributed to its anti-proliferative and pro-differentiative actions. We have previously shown that calcitriol sensitizes breast cancer cells to the cytotoxic action of reactive oxygen species (ROS). The level of ROS is high in colon carcinomas due to increased aerobic metabolism and exposure to immune cells and various anti-cancer modalities. We examined whether calcitriol modulates the response of colon cancer cells to the cytotoxic action of the common mediator of ROS injury, H2O2.

Patients / Methods: HT-29 colon carcinoma cells were used as the experimental model. Cell death was monitored by the Crystal Violet and Hoechst/PI staining methods and executioner caspase activity by cleavage of the fluorogenic substrate ac-DEVD-AMC.

Results: Pretreatment with calcitriol (100 nM, 48 hours) sensitized HT-29 colon cancer cells to cell death induced by acute exposure to H2O2 (5-20 mM) or chronic exposure to the H2O2 generating system, glucose/glucose-oxidase. The morphological features of H2O2-induced HT-29 cell death, chromatin condensation preceding PI nuclear staining, are consistent with programmed cell death. However, we detected no executioner caspase activation in response to cytotoxic concentrations of H2O2. Moreover, treatment with a pan-caspase inhibitor did not affect cytotoxicity induced by acute or chronic exposure to H2O2 nor its enhancement by calcitriol indicating that H2O2-induced programmed cell death in HT-29 cells is caspase independent. Conversely, exposure of HT-29 cells to sub-toxic concentrations of H2O2 (1 mM) resulted in low executioner caspase activation that was inhibited by pretreatment with calcitriol.

Conclusions: The sensitization of colon cancer cells to ROS-induced cytotoxicity by calcitriol may underlie its putative action as a chemopreventive agent and may increase its therapeutic potential as a single agent or in combination with ROS-generating anti-cancer modalities such as radiotherapy, photodynamic therapy, hyperthermia and chemotherapy.
Abstract Code: A6

MECHANISM FOR COMBINATION DIFFERENTIATION THERAPY WITH PLANT POLYPHENOLIC ANTIOXIDANTS AND VITAMIN D: ACTIVATION OF THE AP-1 TRANSCRIPTION SYSTEM

(1) MRS. ROSSOVA VICTORIA (1) MRS. SALMAN HAGAR (1) PROF. LEVY JOSEPH (1) PROF. SHARONI YOAV (1) DR. DANILENKO MICHAEL

(1) CLINICAL BIOCHEMISTRY, FACULTY OF HEALTH SCIENCES, BEN-GURION UNIVERSITY AND SOROKA MEDICAL

Introduction: Acute myeloid leukemia (AML) is an aggressive cancer without effective treatment. Differentiation therapy is a promising approach to treat AML. 1α,25-dihydroxyvitamin D3 [1,25D3] is a powerful differentiation agent, but it is highly toxic at pharmacologic doses. We have recently shown that plant polyphenols greatly enhance the antileukemic activity of low, non-toxic concentrations of 1,25D3, however, the molecular mechanisms of such an enhancement remain unknown.

Patients / Methods: The expression of surface differentiation markers (CD11b and CD14) was determined by flow cytometry. The functional differentiation was measured by the levels of superoxide production using cytochrome C reduction assay. Protein levels were estimated by Western blotting. AP-1 binding to DNA was measured by Electrophoretic Mobility Shift Assay. Transcriptional activity of AP-1 was measured by reporter gene assay.

Results: Combined treatment of human AML cell line (HL-60) with a combination of 1,25D3 and a plant polyphenol (curcumin, carnosic acid or silibinin) reduced cell growth and enhanced differentiation in a greater extent than either compound alone. To investigate the molecular mechanism of these combined effects, we correlated the extent of differentiation with the expression levels of vitamin D receptor (VDR) and retinoid X receptor (RXR) proteins, which form the VDR/RXR transcription complex. Curcumin alone increased the expression of VDR and RXR as well as c-Jun and c-Fos, and these effects were synergistically enhanced by 1,25D3. Curcumin also markedly increased AP-1 binding to DNA and 1,25D3 further potentiated this action. Further, curcumin and 1,25D3 synergistically increased transactivation of the AP-1 driven reporter gene. In all assays, the effects of curcumin/1,25D3 combinations were time-dependent and were similar or even greater than those induced by a high concentration of 1,25D3 alone. The enhancing activity of other plant polyphenols (silibinin and carnosic acid) was similar to that of curcumin.

Conclusions: The ability of polyphenols to enhance 1,25D3-induced differentiation may directly result from elevation of cellular VDR content. AP-1 response elements are found in the promoter of the VDR gene. Therefore, AP-1 activation by polyphenol/1,25D3 combinations may, at least in part, be responsible for the increase in VDR protein levels, as seen in this study, and thus for the promotion of leukemic cell differentiation.
Abstract Code: A7

DIAGNOSIS OF STEROID ENZYMATIC DEFECTS BY GAS CHROMATOGRAPHY – MASS SPECTROMETRY (GCMS)

(1) DR. KNOPF CARLOS (1) DR. IDIN ANNA (1) MRS. BARUCH OSHRAT (2) DR. ZUCKERMAN LEVIN NEHAMA (2) DR. TIOSANO DOV (2) PROF. HOCHBERG ZE'EV

(1) DEPARTMENT OF CLINICAL BIOCHEMISTRY-RAMBAM MEDICAL CENTER, HAIFA, ISRAEL (2) DEPARTMENT OF PEDIATRIC ENDOCRINOLOGY- RAMBAM MEDICAL CENTER, FACULTY OF MEDICINE, TECHNION- ISRAEL INSTITUTE OF TECHNOLOGY, HAIFA, ISRAEL

Introduction: GCMS demonstrates better specificity for identifying and quantifying steroids and the capacity to analyze many more steroids than RIA and immunoassays. We currently determine quantitatively 39 urinary steroids in a single assay. We now demonstrate the possibility to diagnose every enzymatic derangement of steroid metabolism, including conditions that cannot be diagnosed by any other biochemical mean, showing the use of this system in 4 cases of specific steroid enzyme deficiencies.

Patients / Methods: Pt 1 was born as a normal girl, found to have testes in both groins, which were removed, and a provisional diagnosis of androgen insensitivity syndrome was made in infancy. Twenty-five years later, she asked for a more definitive diagnosis of her condition. Pt 2 was born with clitoral enlargement and a urogenital sinus. Urgent diagnosis was essential and GC-MS was requested. Pt 3 presented with hypospadias, micropenis, and pubertal gynecomastia. His diagnosis was enigmatic until the GCMS analysis. Pt 4 was a 6 y.o. with gynecomastia, and no other clinical signs. The method requires 5 ml morning urine; steroids are extracted by solid-phase, and the conjugates are enzymatically hydrolyzed, doubly derivatized, purified by gel chromatography and analyzed by GC-MS both in Scan and Selected Ion Monitoring modes.

Results: The GC-MS Profiling of Steroids in these patients revealed: Pt 1: decreased ratios of 5α/5β metabolites in the four pairs of 5α/5β isomers (An & Et; 11-OH-An & 11-OH-Et; THB & αTHB; THF & αTHF, -reductase deficiency was diagnosed in this agonadal woman; Pt 2: αand 5 increased urinary 17HP, PT, decreased cortisol and cortisone metabolites and the pathognomonic Ptone (11-Oxo-pregnanetriol), derived from 11OHase activity on 17OHP, all of which is typical for 21-hydroxylase deficiency; Pt 3: increased THB, αTHB and THA, a decrease of THF, αTHF, and THE, and absent An and Et, all characteristic of combined 17OHase/17,20-lyase deficiency; Pt 4: rise in THS, THDOC, and 6-OH-THS and low THF, αTHF, and THE, diagnostic of 11-Hydroxylase deficiency.

Conclusions: The profile provides an at-glimpse analysis of all steroidal pathways, and the results we show illustrate that GC-MS profile of steroids is a very powerful tool for the diagnosis of steroid enzymatic defects.
INCIDENTAL ADRENAL TUMOR: CLINICAL, BIOCHEMICAL AND ANATOMICAL
CHARACTERISTICS IN 100 PATIENTS FOLLOWED DURING 1995-2005

(1) DR. TZVETOV GLORIA (1) DR. SHIMON ILAN (1) DR. BENBASSAT CARLOS

(1) ENDOCRINE INSTITUTE, RABIN MEDICAL CENTER, CAMPUS BEILINSON

Introduction: Adrenal incidentaloma (AI) is a mass serendipitously discovered by radiological examination, not always considered a true incidental finding when discovered in oncologic patients. We studied the clinical and biochemical behavior of AI for a short-term period and compared results between oncologic and non-oncologic patients.

Patients / Methods: We reviewed medical records of 100 patients with AI (55% female, age 62.3±11.5 yr), followed in our clinic in 1995-2005 for a median of 24 month. At least one CT was performed in 96 patients, one US in 44 patients and one MRI in 22 patients, with a mean of 3.2 (1-10) procedures per patient during follow-up. Endocrine evaluation was done in all patients (DST 1 mg, UFC, urine catecholamines and plasma renin, aldosterone).

Results: Associated morbidities included hypertension 57%, obesity 39%, and diabetes mellitus 26% and osteoporosis 60%. Indications for imaging were follow-up of malignancy 23%, GI evaluation 25%, urological evaluation 15%, lung disease 10%, and hypertension 4%. Mean tumor diameter was 24±10 mm. A mass of ≥3 cm was found in 27%, bilateral tumors in 23%, unilateral right in 34% and unilateral left in 43%. Functional abnormalities were found in 23%: subclinical Cushing 20%, Cushing syndrome 1.1%, hyperaldosteronism 1.4%, pheochromocytoma 4%. History of malignancy was present in 32 patients (double malignancy in 7, metastatic disease in 3).

Comparison between oncologic and non-oncologic patients showed: mean age 67.5 ± 9.6 vs 59.4 ± 11.3 yr (p=0.001), mean size 22 ± 9 vs 25 ± 10 mm (p=0.112), bilateral tumors 25% vs 22.6% (p=0.548), functional abnormalities 22.5% vs 25% (p=ns), respectively. During follow-up tumor growth was found in 14% patients (24% of oncologic and 8% of non-oncologic). FNA was done in 3 patients (2 oncologic), revealing metastasis in one. No changes in function evolved during follow-up. Seven patients were surgically treated (2/32 oncologic, 5/68 non oncologic): 1 pheochromocytoma, 1 Cushing syndrome, 2 subclinical Cushing, 1 myelolipoma, and 2 because tumor size and clinical suspicion (both, adenomas). Three oncologic patients died without evidence of growth or hyperfunction.

Conclusions: In our highly selected oncologic group, clinical behavior of AIs was comparable to that of non-oncologic patients.
LOW ESTRIOL LEVELS IN THE MATERNAL TRIPLE–MARKER SCREEN AS A PREDICTOR OF ISOLATED ACTH DEFICIENCY CAUSED BY A NEW MUTATION IN THE TPIT GENE

(1) DR. WEINTROB NAOMI (2) DR. DROUIN JACQUES (3) DR. VALLETTE-KASIC SOPHIE (4) DR. MAROM DAPHNA (1) DR. LEBENTHAL YAEL (4) DR. KLINGER GIL (5) DR. BRON-HARLEV EFRAT (3) PROF. SHOHAT MORDECHAI

(1) INSTITUTE FOR ENDOCRINOLOGY AND DIABETES, SCHNEIDER CHILDREN’S MEDICAL CENTER OF ISRAEL, PETAH TIKVA, ISRAEL (2) INSTITUT DE RECHERCHES CLINIQUES DE MONTREAL, LABORATOIRE DE GENETIQUE MOLECULAIRE, MONTREAL, CANADA (3) INSTITUTE FOR MEDICAL GENETICS, RABIN MEDICAL CENTER, BELINSON CAMPUS, PETACH TIKVAH, ISRAEL (4) NEONATAL INTENSIVE CARE UNIT, SCHNEIDER CHILDREN’S MEDICAL CENTER OF ISRAEL, PETACH TIKVAH, ISRAEL (5) PEDIATRIC INTENSIVE CARE UNIT, 6SACKLER FACULTY OF MEDICINE, TEL AVIV UNIVERSITY, ISRAEL

Introduction: Adrenal insufficiency is a life-threatening condition. However, when diagnosed on time, it can be easily treated.

Patients / Methods: We describe two brothers with adrenal insufficiency due to isolated ACTH deficiency. The parents are first cousins of Jewish-Indian origin whose first-born son was healthy and aged 5 years. Estriol level during the first pregnancy was normal (1.03 MOM). Their second-born son died at the age of 7 weeks of a presumed cardiomyopathy. During that pregnancy, a low estriol level of 0.09 MOM (normal >0.15) was noted on the triple-marker screen test (TMST).

Results: During the third pregnancy, a low estriol level (0.14 MOM, normal >0.15) was found again, and the parents were referred for genetic consultation. Ultrasonography revealed no fetal abnormalities, and amniocentesis demonstrated a normal 46XY karyotype. The level of steroid sulfatase activity in amniotic cell culture ruled out the diagnosis of X-linked ichthyosis, and the normal amniotic fluid levels of 7-dehydrocholesterol ruled out Smith-Lemli-Opitz syndrome. Postnatal evaluation yielded low basal and stimulated cortisol levels (<27.6 and 58 nmol/L, respectively) and undetectable ACTH and DHEAS levels (<2.2 pmol/L and <0.41 mcmol/L, respectively). Thyroid function tests and levels of growth hormone, gonadotropins, and testosterone were all within normal range for age, consistent with the diagnosis of isolated ACTH deficiency. Molecular analysis of the TPIT gene revealed a new mutation (IVS4+1G>A) affecting the first nucleotide (G>A) of the splice site at the 5’-end of the fourth intron.

Conclusions: Low estriol levels in the maternal TMST should alert physicians to the possibility of secondary adrenal insufficiency. Prompt postnatal evaluation and therapy can prevent neonatal death.
Abstract Code: A10

HYPOTHALAMIC -PITUITARY -ADRENAL AXIS IN INTERRUPTED PITUITARY STALK SYNDROME

(1) DR. ZUCKERMAN-LEVIN NEHAMA (2) PROF. CHROUSOS GP (1) PROF. HOCHBERG ZE'EV

(1) MEYER CHILDREN'S HOSPITAL AND FACULTY OF MEDICINE,TECHNION,HAIFA,ISRAEL (2) REPRODUCTIVE BIOLOGY AND MEDICINE BRANCH,NICHD,BETHESDA,MD,USA

Introduction: In interrupted pituitary stalk syndrome hypopituitarism results from disrupted venous portal blood supply from the hypothalamus to the pituitary. Most patients have multiple anterior pituitary hormone deficiencies however ACTH-stimulated cortisol is frequently normal. Our working hypothesis was that ACTH stimulation test does not reflect the complexity of the HPA axis, and that more detailed study would identify the mechanism of this paradox.

Patients / Methods: To address this issue we studied the HPA axis in 4 boys and 7 girls with interrupted pituitary stalk syndrome, age 3 - 17 years, using CRH stimulation (1 microgr/kg), ACTH (250 microgr/m²) and metyrapone (30mg/kg) tests. Patients were treated as needed with replacement hGH, LT4 and estrogen. Twenty one age- and sex-matched children comprised the control group.

Results: Peak ACTH-stimulated cortisol was very low in one, and normal in the other 10 children at 724 ± 74 nmol/l (mean ± SEM). Peak CRH-stimulated cortisol was extremely low in 3 (<50 nmol/l), low in 4 (166-285 nmol/l) and normal (>350nmol/l) in 4 pts, as compared to 460 ± 23 nmol/l in controls. CRH-stimulated ACTH was normal in 8/11 (>10 pmol/l vs. control 18.2 ± 2.1 pmol/l). In all patients, but one, metyrapone gave a low response of 11-deoxycortisol at 82 ± 15 nmol/l (normal >200 nmol/l), and of ACTH at 3.2 ± 0.5 pmol/l (normal >17 pmol/l), while cortisol was suppressed to 15 ± 2.5 nmol/l.

Conclusions: We concluded that 1) In interrupted pituitary stalk syndrome most cases show insufficient response of the HPA axis. 2) The discrepancy between low ACTH response to metyrapone (endogenous CRH) and normal response to exogenous CRH suggests a dysfunctional hypothalamic association that applies to the HPA axis as well. 3) The ACTH stimulation test does not reflect subtle changes in the HPA axis; the metyrapone test detects subtle dysfunction in this condition. 4) These patients need cortisol replacement during stress.
SEVENTEEN YEARS EXPERIENCE WITH MITOTANE AS ADJUVANT THERAPY FOR ADRENOCORTICAL CARCINOMA

(1) DR. DICKSTEIN GABRIEL (1) DR. SHECHNER CARMELA (1) DR. GERSHINSKI MICHAL (1) DR. REUT MARIA

Abstract Code: A11

INTRODUCTION: Adrenocortical carcinoma is a rare and lethal disease. Five year survival is considered to be about 30% the most. Mitotane treatment is used for many years in high doses (up to 12 gr) as treatment in advanced stages of the disease, with very limited success rates. Given in high doses it has severe gastrointestinal side effects. We have previously published promising results with low doses (1.5 - 2.5 gr) of mitotane as adjuvant treatment, started shortly after surgical resection of the tumor, for adrenocortical carcinoma. We hereby present our experience with this treatment modality, which we use now for 17 years.

METHODS / PATIENTS: Over the years 1988-2005 we encountered 16 patients with adrenocortical carcinoma who were operated in our institute. On surgery, tumor size ranged 7.5 - 25 cm, mean 14, and weights of 153 - 2615 gr, mean 739. One patient died on surgery, and is therefore not included. The other 15 patients were started on mitotane shortly after - and lately even before - surgery. The dose was raised to the highest easy tolerated one, no more than 2.5 gr. Medication was continued up to 10 years after surgery. Recurrent metastasis, when encountered, were resected.

RESULTS: Out of 15 patients, two died after long follow up of more than 5 years, from unrelated causes. Five patients died from the disease, 12-87 months after surgery. Three of these were put on a combined chemotherapy treatment because of an advanced disease from the start. Ten patients (including the two dying from unrelated causes) were free of disease on long follow up - mean 47.5, range 6 - 132 months. Six patients were followed for longer than 5 years (62-132 months). Adverse effects included addisonian crisis - 5, diarrhea - 3, gennicomastia - 2, elevated liver enzymes -2, depression -1, hypercolesterolemia - 5.

CONCLUSIONS: Patients treated with low dose mitotane as adjuvant therapy for adrenocortical carcinoma, have a better overall and disease free survival then expected, suggesting effectiveness in preventing disease progression.
A SINGLE INTRAVENOUS BOLUS OF DEXAMETHASONE FOR THE DIAGNOSIS OF CUSHING'S SYNDROME

(1) DR. MUNTER GABRIEL (2) DR. KIRSHNER MORIAH (1) DR. SHILO SHMUEL (2) PROF. ROSLER ARIEL (2) DR. LEIBOWITZ GIL (2) PROF. GLASER BENJAMIN

(1) INTERNAL MEDICINE DPT, ENDOCRINOLOGY UNIT, SHAARE ZEDEK MEDICAL CENTER (2) ENDOCRINOLOGY SERVICE, HADASSAH HEBREW UNIVERSITY HOSPITAL

Introduction: The diagnosis of Cushing's syndrome (CS) is based primarily on diagnostic tests evaluating the cortisol response to dexamethasone suppression. Tests based on oral administration of dexamethasone may be compromised by variable absorption or poor compliance. We evaluated the diagnostic accuracy of a novel intravenous dexamethasone suppression test (IDST)

Methods/ Patients: The test is performed by intravenous bolus injection of 8 mg dexamethasone, with blood cortisol determinations made before injection, then hourly during the first 6h and finally at 24h. ACTH is measured prior to dexamethasone injection and at 6 and 24 h following injection. We performed a retrospective analysis of patients studied for suspected CS in the Endocrinology Outpatient Unit at Hadassah, between 1994-2004. The study included 101 patients: 54 patients with pituitary CS, 22 with adrenal CS, 4 with ectopic ACTH CS (EAS) and 24 in whom the diagnosis of CS was excluded.

Results: Patients without CS showed rapid suppression of cortisol and ACTH that persisted for 24 hours. Patients with pituitary CS showed suppression of cortisol and ACTH levels at 6 hours with subsequent escape at 24 hours. Patients with adrenal CS or with EAS failed to suppress cortisol or ACTH levels. Using 60% suppression of blood cortisol at 24 h as the cutoff for the diagnosis of CS, IDST had 94% sensitivity, 95% specificity and 98% positive predictive value (PPV) for the diagnosis of CS. Similar results were obtained by using a cortisol level of 200 nmol/l at 24 hours as the cutoff for the diagnosis of CS. Adding the criteria of ACTH levels >4 pmol/l at 24 hours, the PPV of the IDST increased to 100%.

Conclusions: IDST is a reliable, simple and accurate test for diagnosing hypercortisolism. Measuring cortisol levels before and 24h after 8 mg intravenous dexamethasone administration is required to adequately diagnose patients with CS. Taking into account plasma ACTH at 24 h may increase the test’s PPV. The sensitivity, specificity and PPV of the IDST are higher than those reported for other commonly used non-invasive tests. Further studies are required to determine if IDST can discriminate effectively between pituitary disease and EAS.
Abstract Code: A13

PROTEIN KINASE C DELTA IS INVOLVED IN STIMULATION OF GLYCOGENESIS BY INSULIN IN HEPATOCYTES

(1) MRS. BRUTMAN-BARAZANI TAMAR (2) DR. ROSA JAGODA (1) PROF. SAMPSON SANFORD R.

(1) FACULTY OF LIFE SCIENCES, BAR-ILAN UNIVERSITY, ISRAEL (2) DEPARTMENT OF PHYSIOLOGY, ZAGREB UNIVERSITY SCHOOL OF DENTISTRY, ZAGREB, CROATIA

Introduction: The liver is a major insulin-responsive tissue responsible for glucose regulation. One important mechanism in this phenomenon is insulin-induced glycogen synthesis. The binding of insulin to its receptor (IR) induces a cascade of events leading to activation of downstream signaling elements followed by GSK3 phosphorylation and inactivation and subsequent glycogen synthesis. Important components of this cascade are Protein Kinase C (PKC) and Akt/PKB. Studies in our laboratory have shown that PKC delta is important in upstream insulin signaling in skeletal muscle, but its role in liver is unclear. In this study we investigated the importance of PKC delta in mediation of insulin-induced phosphorylation of GSK hepatocytes.

Patients / Methods: Studies were performed on primary cultures of rat hepatocytes and on the AML-12 (Alpha Mouse Liver) cell line in culture. PKC delta protein levels were determined by Western blotting, and RNA levels were determined by RT PCR. PKC activity was measured on PKC delta immunoprecipitated from lysates of control and insulin-stimulated cells.

Results: PKC delta was found to be constitutively associated with IR and this association was increased by insulin stimulation. Insulin stimulation increased tyrosine phosphorylation and activity of PKC delta within 5 min. Inhibition of PKC delta either by treatment with rottlerin, or and suppression of PKC delta expression by transfection with RNAi, reduced both the activation of PKB and the phosphorylation of GSK3 induced by insulin. Treatment of primary rat hepatocytes with rottlerin abrogated insulin-induced increase in glycogen synthesis. In contrast to PMA, which down regulates PKC delta protein levels, insulin caused a rapid increase in total PKC delta protein and a decrease in total PKC alpha protein. Moreover, insulin stimulation increased PKC delta RNA expression. Effects of insulin on both protein level and RNA expression of PKC delta were abrogated by the transcription inhibitor Actinomycin D.

Conclusions: We conclude that PKC delta plays an essential role in insulin-induced glycogenesis in hepatocytes. This work was supported by the Russell Berrie Foundation and D-Cure, Diabetes Care in Israel.
Abstract Code: A14

ACTIVATION/TRANSLOCATION OF PKC ISOENZYMES IN PANCREATIC &SZLIC-CELLS AND ALTERATIONS IN GK RAT, T2DM MODEL

(1) MR. WARWAR NASIM (2) PROF. EFENDIC SUAD (2) PROF. &OUML;STENSON CLAES-G&OUML;RAN (1) PROF. CERASI EROL (1) DR. NESHER RAFAEL

(1) ENDOCRINOLOGY AND METABOLISM SERVICE, DEPARTMENT OF MEDICINE, THE HEBREW UNIVERSITY - HADASSAH MEDICAL CENTER, (2) DEPARTMENT OF MOLECULAR MEDICINE, ENDOCRINE AND DIABETES UNIT, KAROLINSKA INSTITUTE AND HOSPITAL, STOCKHOLM, SWEDEN.

Introduction: Glucose metabolism affects most major signal pathways in pancreatic beta-cells. Multiple protein kinases are functioning downstream, the role of most is poorly defined.

Patients / Methods: We examined the dynamics of glucose dependent activation and translocation of PKC isoenzymes in beta-cells of perfused wistar rat pancreas and compared with that of the GK rat, model for type 2 diabetes displaying diminished insulin response to glucose. Role in exocytosis was investigated for PKCalpha and PKCepsilon in isolated rat islets.

Results: PKCalpha was localized to beta-cell membrane during early 1st phase insulin response and associated with insulin granules. The signal declined prior to the onset of 2nd phase, reappearing as foci, associated with insulin granules, during and following, 2nd phase insulin release. Inhibition of PKCalpha translocation diminished exocytosis. Minimal or no localization was observed in GK rat. PKCdelta was partially associated with insulin granules, but no glucose-dependent translocation was observed. In the GK minimal staining was observed, increasing exclusively during early 1st phase. PKCepsilon concentrated in a biphasic manner in areas near the nucleus, associated with insulin granules, persisting for 15 min after cessation of release. Inhibition of PKCepsilon translocation diminished calcium-independent exocytosis. In GK rats, PKCepsilonon exhibited no glucose-dependent changes or association with insulin displayed bimodal dynamics in control beta-cells: During early granules. PKCzeta 1st phase, accumulation near the cell membrane was observed, dispersing thereafter. This was followed by a gradual accumulation near the nucleus; 15 min after glucose stimulus was halted, clear PKCzeta staining was observed within the nucleus. In GK rat, a similar dynamic was rarely observed. In control beta-cells, glucose stimulus leads to a transient recruitment of PKCtheta, the function of which awaits definition. The GK rat exhibited no beta-cell glucose-dependent changes in PKCtheta.

Conclusions: PKCalpha, PKCepsilon, PKCzeta and PKCtheta are activated and translocated in pancreatic beta-cells by glucose stimulus, each to a unique site and with a unique dynamics. Furthermore, our results suggest that diminished levels and/or activation of PKCalpha, PKCepsilon, PKCtheta and PKCzeta may be part of the defective signals, downstream to glucose metabolism, responsible for the deranged insulin response to the sugar in the GK animal model of T2DM.
DISRUPTION OF THE INSULIN RECEPTOR REVEALS A CRITICAL ROLE OF INSULIN IN EPITHELIAL CARCINOGENESIS

(1) MRS. SIROTA JENNY (2) DR. WEINGARTEN GALINA (3) DR. TAKEDA JUNJI (4) DR. KAHN C. RONALD (5) DR. WERTHEIMER EFRAT

(1) DEPARTMENT OF PATHOLOGY, SACKLER SCHOOL OF MEDICINE, TEL AVIV UNIVERSITY, TEL AVIV, ISRAEL (2) DEPARTMENT OF PATHOLOGY, SACKLER SCHOOL OF MEDICINE, TEL AVIV UNIVERSITY, TEL AVIV, ISRAEL (3) DEPARTMENT OF SOCIAL ENVIRONMENTAL MEDICINE, OSAKA UNIVERSITY GRADUATE SCHOOL OF MEDICINE, SUITA, OSAKA, JAPAN (4) RESEARCH DIVISION, JOSLIN DIABETES CENTER, DEPARTMENT OF MEDICINE, HARVARD MEDICAL SCHOOL, BOSTON, MASSACHUSETTS, USA (5) DEPARTMENT OF PATHOLOGY, SACKLER SCHOOL OF MEDICINE, TEL AVIV UNIVERSITY, TEL AVIV, ISRAEL

**Introduction:** Insulin and insulin receptor (IR) are directly involved in regulating skin proliferation, differentiation and programmed cell death. Thus, abnormal insulin signaling might lead to an imbalance between these processes, resulting in tumorigenesis.

**Patients / Methods:** To elucidate the role of the IR in skin tumorigenesis, we utilized two unique research models, in vitro and in vivo. For in vitro model we used cultured primary epidermal keratinocytes, isolated from wild type (WT) and IR knockout (IR-KO) transgenic mice. Cellular transformation of the cultured cells was induced by overexpression of the Ras oncogene (Ha-Ras). Ha-Ras is known to initiate cellular transformation in mouse skin cells. For in vivo study we used skin-specific insulin receptor knockout mice (SIRKO), developed using the Cre-Lox system. Skin tumors were induced following the two-step experimental skin carcinogenesis protocol.

**Results:** In vitro, we have found that lack of IR expression resulted in a marked increase in the endogenous levels of activated Ras protein (Ras-GTP). However, insulin stimulation up-regulated Ras-GTP levels in WT cells, while in IR-KO cells Ras-GTP levels remain unchanged. These results demonstrate that insulin regulates Ras activity via the IR. Next, we followed the IR-Ras cooperation in cellular transformation by overexpressing Ha-Ras protein in the IR-KO cells. Ha-Ras transformed IR-KO cells exhibited different morphology and markedly decreased proliferation and migration rates compared to the Ha-Ras transformed WT cells. Thus, activated Ras signaling failed to induce complete transformation of IR-KO primary skin cells. In vivo, SIRKO mice exhibited a significant decrease in skin tumor induction compared to the control group.

**Conclusions:** In conclusion, our data provide evidence that IR expression is required for skin transformation, both in vitro and in vivo.
Abstract Code: A16

DISTINCT REGULATION OF INSULIN SECRETION AND PROINSULIN BIOSYNTHESIS BY SUCCINATE AND NADPH

(1) MRS. ATTALI VERONIQUE (1) MRS. PARNES MARCELA (1) MRS. ARIAV YAFA (1) PROF. CERASI EROL (1) PROF. KAISER NURIT (1) DR. LEIBOWITZ GIL

(1) ENDOCRINOLOGY AND METABOLISM SERVICE, DEPARTMENT OF MEDICINE, HADASSAH-HEBREW UNIVERSITY MEDICAL CENTER, JERUSALEM 91120

Introduction: Succinate is a potent stimulus for insulin secretion and proinsulin biosynthesis in rat pancreatic islets. We aimed to define the role of succinate in the β-cell dysfunction of the type 2 diabetes model, P. obesus.

Patients / Methods: The insulin secretion and proinsulin biosynthesis response to succinate ester (SAM) and to other secretagogues were studied in isolated rat and P. obesus islets. Insulin secretion was evaluated by static incubations and proinsulin biosynthesis by metabolic labeling with 3H-leucine followed by immunoprecipitation with anti-insulin serum.

Results: In rat islets, SAM doubled insulin secretion under glucose-free conditions. In contrast, insulin secretion was not stimulated in P. obesus islets, whereas proinsulin biosynthesis increased 5-fold in the absence of glucose. Moreover, SAM lacked K-ATP independent amplifying effect on insulin release, and raising cAMP with IBMX failed to augment succinate-stimulated insulin secretion. In marked contrast, SAM doubled insulin secretion from P. obesus islets under stimulating glucose concentrations. These data indicate that succinate responsiveness in P. obesus islets is glucose dependent. Pyruvate ester mimicked the effect of glucose on succinate-stimulated insulin secretion, suggesting that TCA cycle-derived signals are sufficient to induce insulin secretion in response to succinate. We hypothesized that anaplerotic NADPH production is required for succinate stimulation of insulin secretion. Disruption of the anaplerotic pyruvate/malate shuttle by phenylacetic acid in P. obesus islets inhibited glucose- and succinate-stimulated insulin secretion in a dose-dependent manner, without affecting glucose-stimulated proinsulin biosynthesis. Inhibition of the NADPH-consuming enzyme nitric oxide with L-NAME induced ~50% increase of succinate-stimulated insulin secretion in P. obesus islets. In rat islets, SAM and L-NAME together augmented insulin secretion by ~10-fold, suggesting that succinate and NADPH synergistically interact to amplify insulin secretion. In contrast, L-NAME had no effect on succinate-stimulated proinsulin biosynthesis in rat and P. obesus islets.

Conclusions: 1. Succinate is a potent stimulus for proinsulin biosynthesis in different species, 2. In P. obesus, permissive effect of glucose is required for succinate-stimulated insulin secretion, but not for proinsulin biosynthesis, 3. Anaplerotic NADPH production is important for glucose- and succinate stimulated insulin secretion, and 4. Nitric oxide signaling is a negative regulator of insulin secretion, probably via modulation of islet NADPH.
Abstract Code: A17

DISSECTION OF ADIPONECTIN RECEPTORS SIGNAL TRANSDUCTION PATHWAYS AND THEIR REGULATION UNDER PATHOLOGICAL CONDITIONS

(1) MRS. ASHWAL REUT (2) DR. HEMI RINA (1) MRS. BIRENBOIM ELEANOR (2) PROF. KARASIK AVRAHAM (2) DR. KANETY HANNAH

(1) FACULTY OF LIFE SCIENCES, BAR-ILAN UNIVERSITY, RAMAT GAN, ISRAEL (2) INSTITUTE OF ENDOCRINOLOGY, SHEBA MEDICAL CENTER, TEL-HASHOMER, ISRAEL

**Introduction:** Adiponectin, an adipocyte derived abundant plasma protein, gained recognition as a potential mechanistic link between obesity and its related morbidities. The insulin-sensitizing and anti-atherogenic properties of the hormone are transmitted through two distinct receptors, AdipoR1 and AdipoR2, which were recently identified. Several studies suggest that reduced expression of the receptors, induced by genetic or environmental factors (i.e. obesity), may lead to the development of adiponectin resistance and thereby may accelerate the development of diabetes and cardiovascular diseases. The aim of this study was to characterize adiponectin receptors and signalling pathways in human liver cells and to evaluate the impact of hyperinsulinemia on these pathways.

**Patients / Methods:** Studies were conducted on HepG2 hepatoma cells. The relative abundance of AdipoR1&2 was evaluated by real time PCR and the specific contribution of each receptor to adiponectin's biological effects was assessed by their selective knockdown using siRNA. Adiponectin signalling pathways were determined by Western blotting.

**Results:** Adiponectin induced the phosphorylation of several key pathways in HepG2 cells including AMPK and its major substrate acetyl CoA carboxylase (ACC), Erk1/2, p38MAPK and JNK. Except for JNK, phosphorylation of these signal molecules was also observed in cells treated with AICAR, an AMPK activator. Suppression of AdipoR1 or AdipoR2 with siRNA attenuated specifically some of these pathways, indicating that Erk1/2 activation is linked to AdipoR1, JNK activation to AdipoR2, and both receptors are involved in the phosphorylation of ACC and p38MAPK. Noteworthy, the knockdown of AdipoR1 led to increased expression of AdipoR2, and vice versa, suggesting the existence of a compensatory mechanism. To evaluate adiponectin signaling under pathological conditions like obesity and type II diabetes, HepG2 cells were incubated with high insulin levels. Long-term incubation with 100 nM insulin did not alter the expression AdipoR1&2, however, it attenuated the adiponectin-induced ACC and p38MAPK phosphorylation.

**Conclusions:** These findings indicate that AdipoR1&2 mediate distinct activation of various signalling pathways in liver cells. In addition, they demonstrate that hyperinsulinemia induces adiponectin resistance by attenuating its signal transduction, but not its receptors expression.
Abstract Code: A18

LONG-TERM NEURODEVELOPMENTAL OUTCOME IN CONSERVATIVELY TREATED CONGENITAL HYPERINSULINISM

(1) DR. MAZOR-ARONOYITCH KINNERET (2) DR. LOBEL DANIELLE (3) DR. GILLIS DAVID
(1) DR. PINHAS-HAMIEL ORIT (1) DR. MODAN DALIT (3) PROF. GLASER BENJAMIN (1) PROF. LANDAU HEDDY

(1) SAFRA CHILDREN’S HOSPITAL, CHAIM SHEBA MEDICAL CENTER, PEDIATRIC ENDOCRINE UNIT (2) SCHNEIDER’S CHILDREN MEDICAL CENTER, DEPARTMENT OF PEDIATRIC NEUROLOGY (3) HADASSAH-HEBREW UNIVERSITY HOSPITAL, PEDIATRIC ENDOCRINE AND METABOLIC ENDOCRINE UNITS

Introduction: Congenital Hyperinsulinism (CH) describes a group of genetic disorders characterized by inadequate suppression of insulin secretion in the presence of recurrent hypoglycemia. The treatment of choice in most centers worldwide is surgical, either near-total or partial pancreatectomy for diffuse and focal disease respectively. Most patients treated with near-total pancreatectomy developed diabetes during childhood/puberty. CH patients are at increased risk of developmental disorders, some severe, which are reported to occur in 14 - 44% of patients from very heterogenous cohorts.

Patients / Methods: We describe the outcome of 21 Ashkenazi patients, with early-onset CH, treated medically/intensively since birth. All have known mutations in the beta-cell KATP channel. Eleven are homozygotes, and thus have diffuse disease, 9 are heterozygotes with mutations on the paternal allele and thus most probably have focal disease. All have been in clinical remission for at least 3 years. Data were collected, by telephone interviews with a parent or guardian, regarding the patients (aged 8-23 years, mean 13.7y), and the siblings closest in age as controls.

Results: Ten CH patients had perinatal seizures, most of short duration. Four had post-neonatal seizures, which remitted entirely. During infancy/early childhood, 4 patients (19%) had hypotonia, 8 (38%) had fine motor problems, 7 (33%) had gross motor problems (clumsiness) and one has mild Cerebral Palsy (CP). Three (14%) had speech problems. Eight patients required developmental therapy, compared to one in the control group. Remarkably, the majority of these problems resolved by age 4-5 years. At school age, none had mental retardation, some excelled in their studies, 6/21 patients (29%) had learning problems (2/21 controls), but all enrolled in regular education classes. Four parents reported that their child with CH had some functional problems (learning disorder, clumsiness, obesity, emotional problems) that affected their quality of life. None has overt diabetes.

Conclusions: CH patients treated with early, intensive, conservative treatment show a good long-term neurodevelopmental prognosis compared to that reported in the literature. None has diabetes. We must caution, however, that conservative treatment requires a very cooperative and supportive family, and thus is not feasible in all patients.
MONTHLY ADMINISTRATION OF VITAMIN D – A SAFE AND EFFICIENT THERAPEUTIC OPTION FOR VITAMIN D REPLENISHMENT IN HIP FRACTURE PATIENTS

(1) DR. ISH-SHALOM SOPHIA (1) DR. SALGANIK TINA (1) DR. SEGAL ELENA (2) MRS. RAZ BATIA (3) DR. VIETH REINHOLD

(1) METABOLIC BONE DISEASES UNIT, RAMBAM HEALTH CARE CAMPUS (2) ENDOCRINE LABORATORY, RAMBAM HEALTH CARE CAMPUS (3) UNIVERSITY OF TORONTO

Introduction: Hip fracture rate increases yearly by 1-3% in developed countries. Improvement of vitamin D status decreases hip fracture risk by 30%. Adherence of elderly hip fracture patients to daily calcium and vitamin D supplements is low. A recent consensus about the role of vitamin D3 in the prevention of hip fractures in the elderly concludes that serum 25-hydroxyvitamin D (25(OH)D) concentrations should be higher than 30 ng/mL (75 nmol/L). Administration of monthly dose of vitamin D during post fracture hospitalization may provide a window of opportunity for vitamin D replenishment. We wanted to find out (A) whether the daily, weekly or monthly use of the same total dose would produce the same response, and (B) whether 1500 IU/day of vitamin D would ensure that elderly Israeli hip fracture patients achieve 25(OH)D levels higher than 30 nmol/L.

Patients / Methods: We randomly allocated 48 patients (age 81 ±8 SD yrs) to the same cumulated dose of vitamin D but given as 1500 IU daily, or 10500 IU once weekly, or 45000 IU once monthly for 8 weeks.

Results: Starting, mean serum 25(OH)D was 19 ±9 SD ng/mL. By the end of the 8 wk protocol the mean increase in 25(OH)D was 17 ng/mL. Dose of 1500 IU/day was given to 16 patients, mean increase was 18 ng/ml, lower 95% CI 14, upper 95% CI 23. With the dose of 10500 IU (17 patients) the main increase was 13 ng/ml, 95% CI 7-20. With the dose of 45000 IU (15 patients) the main increase was 21 ng/ml, 95% CI 17-25. No hypercalcemia or elevation of 1,25(OH)2D3 serum level was observed. Patients treated with monthly dose of vitamin D displayed faster increase in 25(OH)D3 serum level.

Conclusions: We conclude that monthly administration of 45 000 units of vitamin D is a safe and convenient option for vitamin D replenishment in hip fracture patients. One third of the patients did not reach the target concentration of 25(OH)D3 - 30 ng/ml. Higher loading dose is needed for these patients.
IGF-I MODULATES GLUCOSE TRANSPORTER 4 (GLUT4)-MEDIATED SUGAR METABOLISM IN SKELETAL GROWTH CENTERS

(1) MRS. HASAN-BRIL RONI (2) MRS. VASILIVER GAYA (3) PROF. KARIELEI EDDI (4) DR. MAOR GILA

(1) DEPARTMENT OF ANATOMY AND CELL BIOLOGY (2) DEPARTMENT OF ANATOMY AND CELL BIOLOGY (3) DEPARTMENT OF DIABETES AND METABOLISM (4) DEPARTMENT OF ANATOMY AND CELL BIOLOGY

Introduction: Skeletal growth process requires high glucose utilization rates. Somatic growth retardation that improves upon adequate therapy is a characteristic of poorly controlled type I diabetes mellitus (IDDM) in children. While glucose transport is known as a rate-limiting step in adipose and muscle glucose metabolism, the information exists about the function and the regulating mechanism of GLUT4 in skeletal growth is still limited. We have shown that insulin-sensitive glucose transporters (GLUT4) exist in the growing bone and that under experimental IDDM mice it is spatially co-regulated with insulin like growth factor-I receptors (IGF-IR). In the current work we further explore the interrelations between IGF-I and insulin and their effects on cartilage growth and metabolism.

Patients / Methods: Neonatal mice-derived mandibular condyles (MC) were either cultured as an organ or served as a source for chondrocytes primary tissue culture. Cultures were incubated in the presence of IGF-I or insulin, with or without neutralizing Ab of IGF-I R. Effects on growth and metabolism were studied by following the expression of PCNA, type II collagen, cartilage proteoglycans and GLUT4; by detecting the uptake of 2-deoxy glucose and by studying GLUT4 translocation. GLUT4 transfected primary MC-derived chondrocytes (MCDC) served for determining the membrane translocation of GLUT4 using confocal microscopy. GLUT4 translocation was also determined using ultramicroscopy of MC chondrocytes detected by immunogold analysis.

Results: Both insulin and IGF-I increased proliferation, differentiation and the levels and activity of GLUT4 in Mandibular condyles. However, IGF-I exerted higher effects than those of insulin and, opposed to the latter, did induce translocation of GLUT4 to the plasma membrane. Moreover, immunoinhibition of IGF-IR abolished insulin-induced increase of condylar growth and levels of GLUT4. Using insulin receptor knockout mice, we could also show that insulin increased both GLUT4 expression and condylar growth.

Conclusions: Our results indicate that in skeletal growth centers IGF-I and not insulin is the major regulator of both cartilage growth and inducible glucose metabolism. Moreover, insulin-induced increase in skeletal growth and GLUT4 expression and activity, studied in cartilaginous growth centers, are mediated through IGF-I receptors.
Abstract Code: A21

VITAMIN D STATUS IN ORTHODOX MALES STUDYING IN DIFFERENT TYPES OF YESHIVAS

(1) DR. TSUR ANAT (1) MRS. MEIR RACHEL (1) MRS. RAVER HANA (2) DR. GUR HAMUTAL
(3) DR. DRESNER-POLLAK RIVKA

(1) DIABETES AND ENDOCRINE CLINIC, CLALIT HEALTH SERVICES, JERUSALEM (2)
DEPARTMENT OF MEDICINE, HADASSAH-HEBREW UNIVERSITY MEDICAL CENTER
(3) ENDOCRINOLOGY AND METABOLISM, HADASSAH HEBREW UNIVERSITY
MEDICAL CENTER

Introduction: Vitamin D deficiency and insufficiency are not uncommon in the sunny country of Israel. Dress codes and lack of sun exposure were previously described as risk factors for vitamin D deficiency in high risk populations: elderly people, orthodox women of child-bearing age and Bedwins. Ultra orthodox yeshiva males are also “at risk” as they spend most of their time indoors, wearing traditional clothing with very limited sun exposure. In this study we aimed to determine the prevalence of vitamin D deficiency among orthodox yeshiva males and to compare it to vitamin D status in yeshiva students who spend a significant part of their time outdoors (Hesder).

Patients / Methods: 74 males from 3 different yeshivas were studied in the area of Jerusalem in March 2005. Yeshiva 1 includes young males spending most of their time indoors and wearing clothing that limit sunlight exposure. Yeshiva 2 includes older males who wear similar clothing but spend some time outdoors. Yeshiva 3 includes young males spending equal time learning indoors yet clothing and outdoor activities allow sun exposure. Serum samples were collected for Ca, P, 25OH-vitamin D3 and PTH. Data regarding the amount of time spent outdoors with short sleeves was obtained by a clinical questionnaire.

Results: There were 26, 23 and 25 subjects in yeshivas 1, 2 and 3 respectively. Mean ages were 20.1±0.6, 33±4.2 and 19.8±2.02 years respectively. Mean serum vitamin D levels were significantly lower in orthodox males spending their time indoors compared to Hesder yeshiva boys (8.9±3.6, 10.2±5.7, 21.7±10.4 ng/mL, respectively, p<0.001). PTH levels were 54.9±22.7, 84.6±32.1 and 59.1±37 pg/mL. All subjects in yeshiva 1 had 25OH vitD3 ≤ 15ng/mL, while it was found in 85% and 32% of subjects in yeshivas 2 and 3. Serum vitamin D levels correlated with the times spent outdoors with short sleeves (r=0.43, p<0.001).

Conclusions: Vitamin D deficiency is extremely prevalent in Orthodox males spending most of their time studying indoors and wearing traditional clothing. Orthodox yeshiva scholars represent an important previously unrecognized high risk group for osteomalacia, metabolic bone disease and future fractures. Screening programs and preventive measures are urgently needed for this high risk population.
Abstract Code: A22

PERIPHERAL CB2 CANNABINOID RECEPTOR REGULATES BONE MASS

(1) MR. OFEK ORR (2) DR. KARSAK MELIHA (3) DR. LECLERC NATHALIE (1) MRS. FOGEL MEIRAV (3) PROF. FRENKEL BARUCH (4) PROF. WRIGHT KAREN (1) DR. TAM JOSEPH (1) MRS. ATTAR-NAMDAR MALKA (1) MRS. KRAM VARDIT (5) PROF. SHOHAMI ESTHER (6) PROF. MECHOULAM RAPHAEL (2) PROF. ZIMMER ANDREAS (1) PROF. BAB ITAI

(1) BONE LABORATORY, THE HEBREW UNIVERSITY OF JERUSALEM, JERUSALEM 91120 (2) LABORATORY OF MOLECULAR NEUROBIOLOGY, DEPARTMENT OF PSYCHIATRY, UNIVERSITY OF BONN, 53105 BONN, GERMANY (3) DEPARTMENT OF ORTHOPAEDIC SURGERY, INSTITUTE FOR GENETIC MEDICINE, KECK SCHOOL OF MEDICINE OF THE UNIVERSITY OF SOUTHERN CALIFORNIA, LOS ANGELES, CA 90033, USA (4) DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF BATH, BATH BA2 7AY, UK (5) DEPARTMENT OF PHARMACOLOGY, THE HEBREW UNIVERSITY OF JERUSALEM, JERUSALEM 91120 (6) DEPARTMENT OF MEDICINAL CHEMISTRY AND NATURAL PRODUCTS, THE HEBREW UNIVERSITY OF JERUSALEM, JERUSALEM 91120

Introduction: The endogenous cannabinoids bind to and activate two G-protein coupled receptors, the predominantly central CB1 and peripheral CB2. CB1 mediates the cannabinoid psychotropic, analgesic and orectic effects. CB2 has been recently implicated in the regulation of liver fibrosis and atherosclerosis. The purpose of the present work was to assess the involvement of CB2 in the regulation of bone mass.

Patients / Methods: Mice: we used male and female CB2-deficient mice (CB2-/- mice) and C57BL/6J WT controls. The effect of CB2 signalling on ovariectomy (OVX)-induced bone loss was analysed in C3H mice. Materials: the synthetic CB2 specific agonist HU-308 was injected intraperitoneally to OVX and control mice once daily for one month. Micro-computed tomography: whole femora were subjected to qualitative and quantitative analysis by µCT. Scans were performed at a 20 µm resolution. Histomorphometry: undecalcified, unstained sections were used for dynamic measurements. Consecutive sections were stained with tartrate resistant acid phosphatase to identify osteoclasts. Cultured bone cells were analyzed for gene expression, proliferation and activity.

Results: The CB2-/- mice show a markedly accelerated age-related trabecular bone loss and cortical expansion, reminiscent of human osteoporosis. The CB2-/- phenotype is also characterized by increased trabecular osteoblast (bone forming cells) activity, increased osteoclast (bone resorbing cells) number and a markedly decreased number of diaphyseal osteoblast precursors. CB2 is expressed in osteoblasts, osteocytes and osteoclasts. HU308, a CB2 specific agonist, that does not have any psychotropic or other central effects, enhances endocortical osteoblast number and activity and restrains trabecular osteoclastogenesis, by inhibiting proliferation of osteoclast precursors and RANK ligand expression in bone marrow-derived osteoblasts. The same agonist attenuates OVX-induced bone loss and markedly stimulates cortical thickness through the respective suppression of osteoclast number and stimulation of endocortical bone formation.

Conclusions: The endocannabinoid system is essential for the maintenance of normal bone mass via osteoblastic and osteoclastic CB2 signalling.
DIFFERENTIAL EFFECTS OF NUCLEAR AND MEMBRANE ESTROGEN RECEPTORS ON ACCELERATED OSTEOGENESIS IN SKELETAL GROWTH CENTERS

(1) MRS. GURMAN MARINA (2) PROF. PHILLIP MOSHE (3) DR. MAOR GILA

(1) DEPARTMENT OF ANATOMY AND CELL BIOLOGY (2) INSTITUTE OF ENDOCRINOLOGY AND DIABETES (3) DEPARTMENT OF ANATOMY AND CELL BIOLOGY

Introduction: As puberty skeletal growth terminates, the width of the EGP is gradually diminishes until its final closure at which point there is a cessation of skeletal growth. In both genders, the growth restraining process is estrogen regulated. Estrogen exerts its biological effects through two receptors isotypes: ER-alpha and ER-beta which act at both and ER-beta, act as nuclear and membrane levels. As nuclear receptors, ER-alpha transcriptional factors and as membrane receptors activate cellular pathways-mainly PKC and p38. The current study was aimed at elucidating the proper mechanisms that mediate estrogen-induced accelerated osteogenesis on the expense of chondrogenesis, and at determining whether these two activities are interrelated.

Patients / Methods: Two experimental models of endochondral ossification were used; a murine derived Mandibular condyle (MC) organ culture (MCOC), and MC derived primary tissue culture (MCDC). The ER isotypes present in the cartilage cells were determined using immunoblotting (IB) and immunohistochemistry (IHC). The cellular localization of ER was determined using immunoprecipitation (IP). The biological activity of the membrane receptor was determined using the cell impermeable derivative E2-BSA. The effects on chondrogenesis and osteogenesis were estimated by following the expression of PCNA, type II, X collagens, aggrecan and osteocalcin.

Results: We have shown that both ER-alpha and ER-beta are present in cartilage cells of growth centers, and that these receptors are distributed in both membrane and cytoplasmic fractions. Using organ and tissue culture models we showed that these reagents interfered in the differentiation and maturation and not in proliferation activities of skeletal growth centers. However, while E2-BSA had more profound effects on accelerated chondrogenesis reflected in higher type II collagen, E2 enhanced the expression of type X collagen. These effects were accompanied by modulation of PKC and IGF-I levels.

Conclusions: We have shown that estrogen modulates skeletal growth rate leading to its final closure, by inducing uncoupling between proliferation and differentiation. These effects are mediated by both membrane and cytoplasmic receptor. However, different activities are modulated by each receptor: accelerated chondrogenesis by the former and, osteogenesis by the nuclear receptors.
IGF-I DEFICIENT PATIENTS SEEM PROTECTED FROM CANCER NOT SO THEIR HETEROZYGOTES FIRST-DEGREE FAMILY MEMBERS

(1) MRS. SHEVAH ORIT (1) PROF. LARON ZVI

(1) ENDOCRINOLOGY AND DIABETES RESEARCH UNIT, SCHNEIDER CHILDREN'S MEDICAL CENTER, PETAH-TIKVA

Introduction: The development and progress of many types of cancer, such as colon, breast, ovary and prostate, have been linked to the presence or elevated levels of IGF-I. Malignant cells have also been found to have an increased number of IGF-I receptors (1,2). Laron Syndrome is a recessively inherited disease caused by molecular defects in the GH receptor and is a unique human model for the lack of IGF-I generation.

Patients / Methods: We have collected by personal interview and/or a prestructured questionnaire data from 129 patients with Laron Syndrome (LS; primary GH insensitivity) from our cohort of patients (n=40) and from abroad (n=89) as well as from 130 first degree family members (66 documented as heterozygotes for the disease).

Results: ISRAEL • Patients: N=40 (18M:22F; age range: 3-75), the molecular defect is known in 34 patients. No malignancies were registered. • Family members: N=94 (45M:49F; age range: 15-85), 39 are documented heterozygotes. 23 family members had a malignant disease. OTHER COUNTRIES • Patients: N=89 (51M:38F; age range: 3-46), the molecular defect is known in 14. No malignancies were registered. • Family members: N=36 (16M:20F; age range: 25-76), 27 are documented heterozygotes. 5 family members had a malignant disease. The distribution of the malignancies was as follows: breast-4, uterus-1, prostate-5, testis-1, colon-4, lung-7, gastric-1, thyroid-1, brain tumors-2, leukemia-1 and squamous cell ca-1.

Conclusions: It is evident that none of the LS patients had cancer whereas the heterozygotes for the disease had a high prevalence of malignancies mainly breast, prostate, colon and lung. Our study, though preliminary, supports the evidence that IGF-I is an important and necessary factor in cancer development. We continue this study by including other diseases with congenital isolated hGH/IGF-I deficiency. REFERENCES: (1) Werner H and LeRoith D. Crit Rev Oncog 1997;8:71-92. (2) Schoen RE et al. Gastroenterology 2005;129:464-475.
Abstract Code: A25

EXPRESSION AND FUNCTION OF HEPARANASE IN TUMORS OF THE ANTERIOR PITUITARY

(1) DR. RUBINFELD HADARA (1) MRS. MEISEL SHILHAV (2) PROF. HADANI MOSHE (1) DR. SHIMON ILAN

(1) INSTITUTE OF ENDOCRINOLOGY AND METABOLISM AND FELSENSTEIN MEDICAL RESEARCH CENTER, RABIN MEDICAL CENTER, BEILINSON CAMPUS, PETACH TIKVA (2) DEPARTMENT OF NEUROSURGERY, CHAIM SHEBA MEDICAL CENTER, TEL HASHOMER, AND SACKLER SCHOOL OF MEDICINE, TEL AVIV UNIVERSITY, TEL AVIV, ISRAEL

Introduction: Tumors of the anterior pituitary arise from adenohypophyseal cell types expressing trophic hormone gene products. Although aggressive local growth may occur, pituitary tumors are benign and do not proceed to true malignancy. Except for gsp, no other oncogene mutations have been identified in pituitary tumors. Yet, altered expression and function of intrapituitary factors influencing tumor progression have been extensively reported. Heparanase is an endoglycosidase capable of degrading heparan sulfate, the main polysaccharide constituent of the extracellular matrix and basement membrane. Heparanase was found to play a role in diverse physiological and pathological processes associated with extracellular matrix remodeling and cell migration, such as development and morphogenesis, angiogenesis, and cancer metastasis.

Patients / Methods: We have studied the expression of heparanase in dispersed human fetal and adult pituitaries and cultured non-functional and hormone-secreting adenomas.

Results: Heparanase mRNA expression was undetectable in fetal pituitaries and in 3 out of 5 normal adult pituitaries, the two others were showing faint bands. The majority of GH-secreting adenomas (15/19) and half of PRL-secreting adenomas (7/13) and non-functioning (4/10) adenomas expressed heparanase. Western blot analysis was performed to detect and distinguish between the latent 65 kDa heparanase precursor and the 50 kDa active enzyme. Both forms were detected in four GH-secreting adenomas and two non-functioning adenomas, tested so far. A weak immunoreactivity of 65 kDa protein was detected in PRL-secreting adenoma and normal pituitary extracts. In addition, we employed an immunofluorescence technique using anti heparanase specific antibodies. Heparanase expression was detected in cultured GH-secreting adenoma cells, identified as GH-producing cells when tested by double immunostaining with anti GH specific antibodies.

Conclusions: This is the first report of heparanase expression in pituitary adenomas. Heparanase may play a role in the angiogenesis of pituitary tumors and may therefore contribute to tumor progression.
Abstract Code: A26

MODULATION OF GHRELIN BY ACUTE PSYCHOLOGICAL CHALLENGE: CORRELATION WITH POST-STRESS SUBJECTIVE URGE FOR UNCONTROLLED EATING

(1) DR. ROUACH VANESSA (2) DR. BLOCH MIKI (2) DR. ROSENBERG NOA (3) DR. GILAD SUSAN (3) DR. LIMOR RONA (2) PROF. STERN NAFTALI (3) DR. GREENMAN YONA

(1) PNIMIT B, TEL AVIV-SOURASKY MEDICAL CENTER (2) DEPARTMENT OF PSYCHIATRY, TEL AVIV-SOURASKY MEDICAL CENTER (3) INSTITUTE OF ENDOCRINOLOGY AND METABOLISM, TEL AVIV-SOURASKY MEDICAL CENTER

Introduction: Ghrelin is a GH secretagogue that plays an important role in appetite and weight regulation. Observations that patients suffering from anorexia or bulimia nervosa have higher basal ghrelin levels than weight matched controls, suggest that psychological factors may affect ghrelin levels. The aim of this study was to examine the effects of psychologically-induced acute stress on plasma ghrelin levels in patients with binge eating disorder (BED) and in healthy subjects of normal or increased body mass index (BMI).

Patients / Methods: Eight BED patients, 8 obese (BMI>30 kg/m2), and 8 lean individuals (BMI<25 kg/m2) were subjected to the standardized Trier Social Stress Test (TSST). Heart rate, blood pressure and serum cortisol, prolactin and ghrelin were measured at 6 different time points during the test. Eating related psychopathology was evaluated by the Eating Disorder Inventory-2 (EDI-2). Subjects were also requested to rate their feelings of anxiety, tension and desire to binge (DB) by means of a visual analog scale before and after the TSST.

Results: There was a significant and similar rise in cortisol (p = 0.0005) and systolic blood pressure (p = 0.0002) in all groups, reflecting induction of physiological changes by the psychological challenge. Basal ghrelin levels were higher in thin (385 ± 79 pg/ml) than in healthy obese (170 ± 15 pg/ml) subjects (p < 0.05). Nine subjects responded to the psychological stress with an elevation of more than 20% in plasma ghrelin levels, which was not accompanied by an increased desire to eat (DB score 6.6±5.5 pre-and post-stress). In contrast, non-responding subjects reported a significant increase in the urge to eat after the psychological stress (DB score 9.6±4.6 before and 22±7.3 after stress, p = 0.01). Subjects with increased post-stress DB scores had higher values of ghrelin AUC (p = 0.053), but showed no change in ghrelin levels throughout TSST. Age, BMI, and EDI-2 score and the increase in the anxiety and tension scores were similar in all groups.

Conclusions: Psychologically induced uncontrolled eating is not modulated by stress related elevations in ghrelin levels, but is more prevalent in subjects that have overall higher ghrelin levels throughout a psychological challenge.
CHARACTERIZATION OF RECOMBINANT 22 K AND 20K PLACENTAL AND 20K PITUITARY HUMAN GROWTH HORMONES INDICATE THAT PLACENTAL GROWTH HORMONES HAVE NO LACTOGENIC ACTIVITY IN HUMANS

(1) MRS. SALOMON GILI (2) PROF. GERTLER ARIEH

(1) PROTEIN LABORATORIES REHOVOT (PLR), LTD (2) FACULTY OF AGRICULTURAL, FOOD AND ENVIRONMENTAL QUALITY SCIENCES, THE HEBREW UNIVERSITY OF JERUSALEM.

Introduction: Two genes encode human growth hormone (hGH): GH-N is expressed in pituitary and GH-V in placenta. They are 22K single chain proteins. Both mRNAs undergo alternative splicing into 20K isoforms.

Patients/Methods: Human pituitary growth hormone (hGH-N 20K) and placental (hGH-V 20 and 22K) were expressed in bacteria, refolded and purified to homogeneity.

Results: All 4 hGhs formed a 1:2 complex with hGHR-ECD (hGH binding protein) and binding experiments revealed similar EC50 values. The somatogenic activity was tested in vitro using FDC-P1 cell lines. Whereas the bioactivity of both 22K hGhs and of hGH-N 20K was almost equal and 2-3 fold higher than hGH-V 20K in FDC-P1 9D11 cells stably transfected with hGHR, the activity of both 20K analogues was significantly 5-9 fold lesser in 3B9 cells stably transfected with rbGHR. The lactogenic activity measured in heterologous assays using the rat lymphoma Nb2-11C cells and Baf/3 cells stably transfected with rabbit prolactin receptor (PRLR) revealed that the activity of the 20K hGH-N was close to that of hGH-N 22K in Baf/3 cells stably transfected with the long form of rbPRL receptor but 4.5-fold lesser in Nb2 cells. The activity of hGH-V 22K was 9-fold lesser in Nb2 cells and 55-fold lesser in Baf/3 cells, whereas hGH-V 20K had no lactogenic activity in both bioassays. In contrast, the homologous lactogenic assay using Baf/3 LP cells stably transfected with hPRLR the activity of the both placental hGhs was nil and the activity of hGH-N 20K was 4.3 fold lesser than that of hGH-N 22K.

Conclusions: In contrast to heterologous bioassys, in the homologous lactogenic assay the activity of the both placental hGhs was nil and the activity of hGH-N 20K was 4.3 fold lesser than that of hGH-N 22K. Those findings raises a question whether the lack of intrinsic lactogenic activity in placental hGhs dominating during pregnancy have any physiological relevance. Our results emphasize the importance of using homologous bioassays.
Abstract Code: A28

ENDOCRINE DYSFUNCTION AND PARAMETERS OF THE METABOLIC SYNDROME AFTER BONE MARROW TRANSPLANTATION DURING CHILDHOOD AND ADOLESCENCE

(1) DR. SHALITIN SHLOMIT (2) DR. YANIV ISAAC (2) DR. STEIN JERRY (2) DR. GOSHEN YACOB (1) DR. CARMI DORON (1) PROF. PHILLIP MOSHE

(1) INSTITUTE FOR ENDOCRINOLOGY AND DIABETES, SCHNEIDER CHILDREN’S MEDICAL CENTER OF ISRAEL (2) BONE MARROW TRANSPLANTATION UNIT, DEPARTMENT OF HEMATOLOGY AND ONCOLOGY, SCHNEIDER CHILDREN’S MEDICAL CENTER OF ISRAEL

Introduction: An increasing number of long-term surviving bone marrow transplantation (BMT) recipients have recovered from their primary disease and short-term transplant-related complications, but are at risk of developing endocrine dysfunction. The aim of the present long-term analysis was to assess endocrine function and parameters of the metabolic syndrome and their determinants in survivors receiving BMT during childhood and adolescence at our center in Israel.

Patients/Methods: Endocrine dysfunction and parameters of the metabolic syndrome were assessed in 91 patients aged 4.3-32.5 years who underwent BMT in childhood. Median follow-up duration was 5.7 years.

Results: Final short stature, found in 5 of the 35 patients who attained final height, was associated with the underlying disease (p = 0.0013), previous cranial irradiation (p= 0.0007), irradiation as part of the conditioning (p<0.05), but not with type of chemotherapy, presence of GVHD or age at transplantation. Growth hormone deficiency, found in 10 patients, was associated with previous cranial irradiation (p<0.005) and TBI conditioning (p<0.001), but not with age at transplantation or time since transplantation. Twelve patients had primary hypothyroidism, one had hyperthyroidism, and one, papillary thyroid carcinoma. Hypothyroidism was associated with neck/mediastinal (p<0.005) and conditioning irradiation (p< 0.05). Primary gonadal failure was found in 24 of the mature patients (62.5% females). In both sexes, hypogonadism was associated with underlying disease (p<0.05), pretransplant treatment (p<0.05), irradiation as part of the conditioning (p<0.001), older age (p<0.005), and advanced pubertal stage at BMT (P<0.05), but not with type of chemotherapy. Body mass index-SDS was >2 in only 4.4% of the patients. Type 2 diabetes and IGT were diagnosed in 3 patients each at a mean age of 21.5± 6.1 yr. Dyslipidemia was found in 27.9 % of the 43 patients tested.

Conclusions: These findings emphasize the need for long-term endocrine follow-up of young patients after BMT in order to offer proper treatment to improve their quality of life.
A NOVEL INSIDE-OUT PROSTANOID SIGNALLING PATHWAY THAT MEDIATES GNRH RECEPTOR AUTO-REGULATION

Introduction: The asynchronous phased secretion of luteinising hormone (LH) and follicle stimulating hormone (FSH) from the gonadotrope in response to the single hypothalamic gonadotropin-releasing hormone (GnRH) during the female reproductive cycle is a central dogma of reproduction, the mechanism of which remains unresolved. GnRH regulates its own receptors to enhance gonadotrope responsiveness during the reproductive cycle, but the mechanism of the ensuing LH refractoriness during continued FSH secretion is not clarified.

Patients / Methods: Biochemistry, cell biology

Results: We now demonstrate that GnRH stimulates arachidonic acid (AA) release from the gonadotrope LbetaT2 cells via the Ca2+-independent cytosolic phospholipase A2 (iPLA2) and not via the more common cPLA2alpha. AA release was followed by a marked induction of COX-1 and COX-2 by GnRH, which was mediated by the PKC/c-Src/PI3K/MAPK pathway and iPLA2 but not via cPLA2alpha, or transactivation of the EGF receptor. COX1/2 act on AA to produce prostaglandins (PGs) and GnRH stimulates PGE2, PGI2 and PGF2alpha production. These PGs may act in an autocrine manner to regulate gonadotrope function and we demonstrated that rat pituitary gonadotropes express the prostanoids receptors EP1, EP2, FP and IP while EP3 and EP4 were localized to the prolactin and growth hormone producing cells, respectively. PGF2alpha and PGI2, but not PGE2, inhibit GnRH receptor expression through FP and IP receptors. The inhibitory effect of PGF2alpha and PGI2 seems to be mediated by inhibition of GnRH-stimulated phosphoinositide turnover. PGF2alpha but not PGE2 or PGI2, reduced GnRH-induction of LHbeta, but like PGE2 and PGI2 had no effect on the induction of common alpha, or FSHbeta. PGF2alpha, or the COX1/2 inhibitor, indomethacin, inhibited and enhanced GnRH-induced LH secretion, respectively from rat pituitaries, but both had no effect on FSH secretion. Therefore, reduction of both GnRH receptor and LHbeta expression are required to observe the differential inhibition of LH release. Hence, a novel inside-out signalling pathway mediated by PGF2alpha-FP and PGI2-IP, limits GnRH regulation of the GnRH receptor, while PGF2alpha inhibits also GnRH stimulation of LH but not FSH release.

Conclusions: This mechanism may provide a means for the cyclical responsiveness of pituitary gonadotropes and the asynchronous LH and FSH release during the female reproductive cycle.
Abstract Code: A30

VITAMIN D ATTENUATES THE INDUCTION OF MATRIX METALLOPROTEINASE 9 BY TNF IN KERATINOCYTES: THE ROLE OF EGF RECEPTOR AND MAP KINASE CASCADES

(1) MRS. BAHAR SHANY KEREN (2) DR. RAVID AMIRAM (1) PROF. KOREN RUTH

(1) DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY AND FELSENSTEIN MEDICAL RESEARCH CENTER, SACKLER FACULTY OF MEDICINE, TEL AVIV UNIVERSITY (2) DEPARTMENT OF DEVELOPMENTAL AND CELLULAR BIOLOGY AND FELSENSTEIN MEDICAL RESEARCH CENTER, SACKLER FACULTY OF MEDICINE, TEL AVIV UNIVERSITY

Introduction: Matrix metalloproteinases (MMPs) participate in the degradation of extracellular matrix constituents during the response of the skin to injury and stress. Excessive MMP activity during tissue remodeling may result in impaired wound healing and exacerbated damage. The epidermis has a self-contained vitamin D endocrine system. Calcitriol, the hormonal form of vitamin D, is beneficial in excessive cutaneous inflammatory conditions and was shown to enhance wound healing in animal models. We aimed to examine whether the beneficial effects of vitamin D in the skin are associated with down regulation of MMP activity.

Patients / Methods: HaCaT keratinocytes that represent the mitotic population of basal keratinocytes were exposed to the inflammatory cytokine TNF known to play a crucial role in the cutaneous response to injury. MMP-9 activity was quantified in culture media by gelatin zymography. mRNA levels were quantified by real-time PCR.

Results: Exposure of HaCaT cells to TNF (20 ng/ml, 24 hours) resulted in marked induction of MMP-9 activity and mRNA. Pretreatment with calcitriol (100 nM, 48 hours) reduced MMP-9 induction by ~50%. The effect of calcitriol was dose and time dependent, detectable at 0.1 nM, significant after 24 hours but more pronounced after a 48-hour incubation. MMP-9 mRNA levels were elevated 3 and 7 fold after 6 and 24 hour treatment with TNF, respectively. TIMP-1 is considered to be the major endogenous inhibitor of MMP-9 but neither TNF nor calcitriol had a significant effect on TIMP-1 gene expression. By the use of specific inhibitors we established that the induction of MMP-9 by TNF was entirely dependent upon the activity of the EGF receptor (EGFR) and the ERK pathway, while p38 MAPK inhibited this process. Induction of MMP-9 mRNA by TNF was dependent upon de-novo protein synthesis. Similarly to TNF, exposure of HaCaT cells to EGF (24 h, 10 ng/ml) induced MMP-9 and this activity was also inhibited by calcitriol.

Conclusions: Calcitriol inhibits the induction of MMP-9 gene expression by TNF in keratinocytes probably by acting downstream to the convergence of the EGFR and the TNF signaling pathways. This effect may contribute to the anti inflammatory and wound healing activity of the hormone.
LEPTIN MEDIATES CHONDROGENESIS IN ATDC5 CELLS THROUGH THE MAPK PATHWAY

(1) MRS. BEN ELIEZER MIRI (1) DR. GAT-YABLONSKI GALIA (1) PROF. PHILLIP MOSHE

(1) INSTITUTE FOR ENDOCRINOLOGY AND DIABETES, NATIONAL CENTER FOR CHILDHOOD DIABETES, SCHNEIDER CHILDREN'S MEDICAL CENTER

Introduction: Leptin, known mainly as a regulator of food intake and energy expenditure via its central effects in the hypothalamus, has other peripheral effects. We have previously shown, for the first time, that leptin participates in growth-plate cartilage growth, using an ex vivo model of mandibular condyle. The aims of the present study were to evaluate the effects of leptin on chondrogenesis in the ATDC5 murine chondrocytic cell line, and to investigate the signal transduction pathways involved, specifically the ERK1/2 pathway.

Patients / Methods: Western blot analysis and immunocytochemistry with specific anti general or anti phospho- ERK1/2 antibodies were used to study signal transduction. Real-time PCR was used to follow the effect of leptin on gene expression.

Results: ATDC5 cells express the Ob-Rb functional isoform, as well as Ob-Ra and Ob-Re, suggesting that leptin can interact with these cells. Indeed, we found that stimulation with 50ng/ml leptin elevated the levels of collagen type X mRNA and decreased the levels of collagen type II mRNA, suggesting that leptin is involved in the process of differentiation. Similar results were obtained in primary culture of the mandibular condyle cells, as well as in in vivo experiments. Activation of the ERK pathway involves phosphorylation and translocation to the nucleus. Administration of leptin increased tyrosine phosphorylation of ERK1/2 by 2.4 fold (P=0.02, n=4) with a peak at 10 min. MEK1/2 inhibitor (U0126) inhibited ERK1/2 activation by leptin. In addition, a dramatic decrease of cytoplasmic ERK1/2 was noted following addition of leptin. The decrease was time dependent. Using immunofluorescence, we could show that ERK1/2 migrates from the cytoplasm to the nucleus.

Conclusions: In conclusion, leptin apparently regulates differentiation of ATDC5. Leptin can activate phosphorylation in ERK1/2, as well as nuclear translocation, which indicates a full activation of the MAPK pathway in the chondrocytes of ATDC5 cell line.
Abstract Code: A32

MOLECULAR MECHANISMS UNDERLYING THE NEUROPROTECTIVE EFFECT OF AURINTRICARBOXYLIC ACID

(1) MRS. KRISHER TAMAR (2) DR. BEERY RACHEL (1) PROF. NIR URI (2) PROF. KARASIK AVRAHAM (2) DR. KANETY HANNAH

(1) FACULTY OF LIFE SCIENCES,BAR-ILAN UNIVERSITY,RAMAT-GAN (2) INSTITUTE OF ENDOCRINOLOGY, SHEBA MEDICAL CENTER, TEL-HASHOMER

Introduction: Neurodegenerative disorders, such as Alzheimer's and Parkinson's disease (PD), are thought to involve excessive apoptotic cell death. In the recent years, there is a great interest in the use of neurotrophic factors, such as insulin-like growth factor-1 (IGF-1), as therapeutic agents for these devastating diseases. We have previously demonstrated that the aromatic polyanion aurintricarboxylic acid (ATA) promotes survival of different cell types, via activation of the IGF-1R signaling pathway. In the present study we investigated the molecular mechanisms underlying the antiapoptotic effect of ATA in SH-SY5Y cells.

Patients / Methods: SH-SY5Y human dopaminergic neuroblastoma cells, were exposed to Salsolinol, a dopamine-derived neurotoxin that has been suggested to be involved in the etiology of PD. Activation of IGF-1R signaling pathways and the effect of ATA on stress kinases, caspase-3 cleavage and JAK/STAT were evaluated with specific antibodies.

Results: We found that ATA activated the IGF-1R for a longer time and enhanced the phosphorylation of Shc proteins and ERK1/2 kinases to a higher extent, compared with IGF-1. ATA, but not IGF-1, reduced the phosphorylation of ERK1/2 and p38 and prevented the activation of caspase-3, which were induced by long term exposure to Salsolinol (12h), while JNK phosphorylation was unchanged. Addition of NAC, a specific inhibitor of ROS, inhibited caspase-3 cleavage and the phosphorylation of p38 and ERK1/2. In addition, ATA but not IGF-1, increased the tyrosine phosphorylation of a 130 kDa membrane-bound glycoprotein. As previous reports indicated that JAK2/STAT5 was activated by ATA, we examined whether JAK2 can be a candidate for this protein. Our results show that ATA, but not IGF-1, induced a remarkable increase in JAK2 phosphorylation. However, the basal phosphorylation of STAT5 remained almost unchanged.

Conclusions: Our results suggest that the cytoprotective effect of ATA in SH-SY5Y cells can be mediated via different molecular mechanisms: a distinct activation of the IGF-1R signaling pathway, inhibition of stress kinases and caspase-3 cleavage, and a presumable activation of the JAK/STAT pathway. Further understanding the molecular mechanisms by which the nonpeptide synthetic compound ATA protects cell viability, may be useful in designing a novel class of orally available drugs for treatment of neurodegenerative diseases.
ACTIVATION OF PROTEIN KINASE C ALPHA POSITIVELY REGULATES INSULIN RECEPTOR SIGNALING VIA IRS1 IN SKELETAL MUSCLE

(1) MRS. CIPOK MICHAL (1) MRS. BAK ASIA (1) PROF. BRODIE CHAYA (1) PROF. SAMPSON SANFORD R.

(1) FACULTY OF LIFE SCIENCES, BAR-ILAN UNIVERSITY, ISRAEL

Introduction: The first step in the insulin signaling cascade is activation of the insulin receptor (IR), which phosphorylates endogenous substrate proteins, primarily members of the Insulin Receptor Substrate (IRS) family. Certain members of the Protein Kinase C (PKC) family of serine-threonine kinases have been found to be involved in upstream insulin signaling. Thus, PKC delta has been shown to interact with IR to regulate in part its phosphorylation and internalization, while PKC alpha may regulate IRS activity in response to insulin. The role of PKC alpha in downstream insulin signaling is less clear. One of the major downstream signaling elements is Akt/PKB. In this study we investigated the possibility that PKC alpha may be involved in insulin-induced IRS-1 and Akt/PKB activation.

Patients / Methods: Studies were conducted on skeletal muscle in adult mice and on L6 skeletal cells. PKC alpha protein levels were determined by Western blotting. PKC activity was measured on PKC alpha immunoprecipitated from lysates of control and insulin-stimulated cells.

Results: PKC alpha is constitutively associated with IRS1, but not IR, and insulin causes the 2 proteins to disassociate within 5 min. Blockade of PKC alpha by GO6976 or by DN PKC alpha inhibited insulin-induced disassociation of PKC alpha from IRS1. Akt/PKB was activated by insulin stimulation within 5 min and reached a peak by 15-30 min. Insulin-induced threonine phosphorylation of Akt/PKB increased on blockade of PKC alpha with GO6976. Insulin tolerance tests on control and PKC alpha -inhibited mice indicated that blood glucose lowering effects of insulin were greater in GO6976-treated mice than in control. Insulin increased PKC alpha-Akt/PKB association and this could be accounted for by an increase in binding of PKC alpha to the GLUT4 Vesicle Complex (G4VC).

Conclusions: We conclude that PKC alpha serves as a physiological down regulator of IRS-1 and in response to insulin stimulation dissociates from IRS-1 and exerts a permissive effect on insulin signaling. In addition, PKC alpha may regulate insulin-induced activation of Akt/PKB in skeletal muscle through its association with the G4VC. This work was supported by the Russell Berrie Foundation and D-Cure, Diabetes Care in Israel.
Abstract Code: A34

ANDROGEN REPLACEMENT THERAPY IN TURNER SYNDROME

(1) DR. ZUCKERMAN-LEVIN NEHAMA (2) MRS. FROLOVA-BISHARA TATIANA (3) DR. MILITIANU DANIELLA (4) MRS. ROSEN GILA (1) DR. LEVIN MOSHE (2) DR. AHARON JUDITH (1) PROF. HOCHBERG ZE'EV

(1) PEDIATRIC ENDOCRINOLOGY, MEYER CHILDREN'S HOSPITAL, RAMBAM MEDICAL CENTER (2) COGNITIVE NEUROLOGY, RAMBAM MEDICAL CENTER (3) RADIOLOGY, RAMBAM MEDICAL CENTER (4) CLINICAL NUTRITION, RAMBAM MEDICAL CENTER

Introduction: Women with Turner syndrome (TS) have reduced levels of androgens due to ovarian failure. Our working hypothesis was that morbidity associated with TS, such as reduced bone mineral density, obesity, metabolic changes, sexual problems and specific neurocognitive profile may improve on androgen replacement therapy (ART).

Patients / Methods: To address the effect of ART, 14 TS patients (age 17 - 27 y) participated in a randomized, double-blind, placebo-controlled study in a cross-over design of either oral 1.5 mg methyl testosterone or placebo for one year and then the alternative for another year. TS patients were fully pubertal, and on low-dose estrogen/progesterone replacement therapy. The study compared physiological measures, biochemistry, body composition by total body DXA, visceral fat by abdominal CT, caloric intake, performance on a comprehensive neuropsychological battery, and quality of life (QOL) questionnaire.

Results: Treatment with testosterone lowered total cholesterol (p=0.036), triglycerides (p<0.001), SHBG (p<0.0001), LH (p<0.001), FSH (p=0.017), Alk Pase (p<0.005), urinary DPD (p<0.032) and WBC (p=0.031). It improved bone mineral content and density in total body (p=0.020), spine (p=0.049), and femur (p=0.004), and trunk bone mineral content increased (p=0.028). Lean body mass increased (p=0.009) and visceral fat decreased (p=0.024). There was a rise in albumin (p=0.035), DHEAS (p=0.023), and HDL (p=0.021). Cognitive tests revealed that ART speeded motor performance (p=0.008) and extended digit span forwards (p=0.004). Testing QOL, on ART TS patients felt healthier (p=0.033), happier (p<0.001), and stronger in daily activity (p<0.001), dealt better with stressful events (p<0.001), their relationships with friends improved (p=0.031) and their sexual desire increased (p<0.001). No drug-related side effects were recorded. There was no change in liver function tests, the degree of hirsutism or acne.

Conclusions: It is concluded that ART for one year in TS has beneficial effects on body composition, bone health, lipid profile, attention and QOL. ART is recommended for TS patients, but long-term follow-up is still to be recorded.
UNUSUAL PRESENTATION OF FAMILIAL GLUCOCORTICOID DEFICIENCY (FGD) WITH A NOVEL MRAP MUTATION

(1) DR. MODAN DALIT (2) DR. BEN-ZE'EV BRURIA (3) DR. HOFFMANN CHEN (1) DR. PINHAS-HAMIEL ORIT (4) DR. ANIKSTER YAIR

(1) PEDIATRIC ENDOCRINOLOGY UNIT, THE EDMOND AND LILY SAFRA CHILDREN'S HOSPITAL, TEL-HASHOMER (2) PEDIATRIC NEUROLOGY UNIT, THE EDMOND AND LILY SAFRA CHILDREN'S HOSPITAL, TEL-HASHOMER (3) DEPARTMENT OF DIAGNOSTIC IMAGING, SHEBA MEDICAL CENTER, TEL-HASHOMER (4) METABOLIC DISEASES UNIT, THE EDMOND AND LILY SAFRA CHILDREN'S HOSPITAL, TEL-HASHOMER

Introduction: Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disease characterized by resistance to the effect of ACTH. Mutations in the ACTH receptor gene account for about 25% of the cases, but the etiology in the remaining FGD cases has remained unknown. Recently, mutations in MRAP, an interacting partner of the ACTH receptor, have been shown to cause FGD in about 20% of kindreds with confirmed FGD and no ACTH receptor mutations. The current report is the first description of MRAP mutation following the discovery of the gene.

Patients / Methods: We describe a male born to non-consanguineous Jewish-Ethiopian parents who presented at the age of 19 months with severe psychomotor retardation, myoclonic seizures, spastic quadripareisis and microcephaly. Extensive investigation including metabolic and endocrine work-up as well as brain imaging was consistent with FGD. Of note, before the diagnosis was made, a female sibling was born in another hospital and succumbed during the neonatal period due to septic shock and severe hypoglycemia. DNA was extracted from peripheral blood samples from the boy and his mother, and the DAX-1, MRAP and ACTH receptor (MC2R) genes directly sequenced by an automated genetic analyzer.

Results: No DAX-1 and ACTH (MC2R) gene mutations were detected. The patient was found to be homozygous for a novel MRAP mutation – a seven base deletion in exon 1 of the coding region of the MRAP gene. This deletion causes a frame shift resulting in a stop codon after 23 aminoacids (L31X), predicting no functional protein. The patient's mother was found to be heterozygous for the same mutation.

Conclusions: Our patient presented with particularly severe phenotype including profound psychomotor retardation, seizures, and spastic quadripareisis, and had sister who succumbed in the neonatal period with septic shock and hypoglycemia, suggesting that the novel MRAP mutation described in this family may be associated with a devastating outcome. The novel MRAP mutation described is the 8th MRAP mutation to be described. The identification of the mutation will enable genetic counseling and screening for the ethiopian-jewish community.
Introduction: Patients with aromatase deficiency have delayed bone maturation and tall stature. The working hypothesis was that aromatase inhibition during puberty of short children will slow bone maturation and allow for attainment of taller adult height.

Patients / Methods: An open-label match-controlled study of anastrozole (Arimidex) in pubertal boys with idiopathic short stature (height <5th centile) and bone age <14 ‘years’. Twenty boys were to receive and 20 to not receive anastrozol, 1 mg/day for a period of 3-5 years, with interim evaluation at 2 years. Endpoints included safety and efficacy in delaying bone maturation and promoting final adult height. Safety panel were surveyed at 1, 3, 12 m and yearly thereafter, and auxology, physical examination and bone age were monitored 6-monthly. The treatment group age was 14 ± 1.1 year (mean ± SD), their pubertal stage was P 2.7 ± 0.8, G 3.0 ± 0.6, and their bone age was 11.8 ± 1.3 ‘y’, or 2.0 ± 1.4 ‘y’ behind chronological age.

Results: On anastrozole therapy serum testosterone increased from 14.0 ± 8.0 to 27.5 ± 7.7 nmol/l, p<0.001, and estradiol decreased from 107± 68 to 66 ± 37 pmol/l p<0.05. The drug was well tolerated with no side effects or other biochemical changes. Serum LH was 2.3 ± 6.2 before and 4.6 ± 5.7 IU/L on therapy, and FSH was 2.7 ± 5.8 and 4.6 ± 2.1 IU/L, resp. Over 2 years of therapy, the height SDS remained unchanged, at –2.2 ± 0.6 before treatment and –2.0 ± 0.6 two years later(NS). The growth velocity was 7.1 ± 3.0 and 3.4 ± 2.0 cm/y, resp., and the growth velocity SDS was 0.8 ± 2.0 and –0.3 ± 2.1, resp. There was a bone age advancement of 3.3 ± 0.2 ‘y’ from 11.7 ± 1.3 to 15 ± 1.3 ‘y’. The adult height prediction decreased from pretreatment to 2 years on treatment by 2.8 ± 7.8 cm.

Conclusions: Aromatase inhibition was ineffective in slowing down bone maturation and increasing predicted adult height, and therefore unlikely to promote final adult height in pubertal boys with short stature. The study was interrupted 2 years after initiation.
ISOLATED HYPOGONADOTROPIC HYPOGONADISM DUE TO MUTATIONS IN TWO DIFFERENT GENES: HOMOZYGOUS MUTATION IN THE GONADOTROPIN-RELEASING HORMONE RECEPTOR AND HETEROZYGOUS MUTATION IN GPR54

(1) DR. ADMONI OSNAT (2) DR. DE ROUX NICOLAS (1) DR. TENENBAUM-RAKOVER YARDENA

(1) PEDIATRIC ENDOCRINE DEPARTMENT, HA’ EMEK MEDICAL CENTER, AFULA, ISRAEL (2) MOLECULAR BIOLOGY LABORATORY, INSERM, HOPITAL NECKER, PARIS, FRANCE.

Introduction: Mutations in the gonadotropin-releasing hormone receptor (GnRHR) are associated with autosomal recessive inheritance of isolated hypogonadotropic hypogonadism (IHH). A functional GnRHR in the anterior pituitary is critical for normal gonadotropins secretion, pubertal development and reproduction. Recently, loss-of-function mutations in a gene termed GPR54 (G-protein-coupled receptor 54) were identified as causing familial IHH. GPR54 is involved in the control of GnRH secretion and the regulation of pubertal onset.

Patients / Methods: Here we describe a 20-yr-old male from a consanguineous family, who presented at the age of 9 yr with micro-phallus and retractile testis. He exhibited delayed and incomplete puberty, under-virilization, tall stature and eunuchoidal body proportion with normal testicular volume and without anosmia.

Results: Endocrine evaluations at the age of 16 yr revealed low basal testosterone levels (0.9 ng/mL) with normal human chorionic-gonadotropin stimulated levels (7.25 ng/mL), and normal peak LH (18.6 mIU/mL) with subnormal peak FSH levels (5.2 mIU/mL) following GnRH stimulation. Sequencing of the GnRHR gene revealed a homozygous mutation, Arg262Gln, in the third exon. This mutation resulted in loss of function of the GnRHR which decreased signal transduction by diminishing the activity of phospholipase C. Sequencing of the GPR54 gene revealed a heterozygous mutation, Leu102Pro, resulting in loss of function of the GPR54 receptor. Screening for both mutations in the family revealed that one pre-pubertal sister carried both mutations and the mother carried the Arg262Gln mutation in the GnRHR gene and displayed irregular menses, infertility and premature menopause. The propositus presented with incomplete IHH and showed a good response to hCG therapy. The contribution of the heterozygous Leu102Pro mutation in GPR54 to the phenotype of the proband has yet to be elucidated.

Conclusions: To the best of our knowledge, this is the first case of IHH caused by two different mutations in two key genes that are responsible for reproduction. Our findings emphasize the importance of genetic study in patients with IHH, as an aid for the clinical approach on the one hand, and as a contribution to our understanding of the physiology of pubertal development and normal reproduction on the other.
CORD BLOOD ADIPONECTIN AND INFANT GROWTH AT ONE YEAR

(1) DR. MAZAKI-TOVI TOVI (1) DR. SIVAN EYAL (2) MRS. PARIENTE CLARA (2) DR. HEMI RINA (1) PROF. SCHIFF EYAL (2) DR. KANETY HANNAH

(1) DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, SHEBA MEDICAL CENTER, TEL-HASHOMER, ISRAEL 52621 (2) INSTITUTE OF ENDOCRINOLOGY, SHEBA MEDICAL CENTER, TEL-HASHOMER, ISRAEL 52621

Introduction: Adiponectin is a novel adipocytokine produced abundantly and exclusively in adipose tissue and has an important role in regulation of glucose metabolism. In adults and children there is a negative correlation between adiponectin levels and body weight, as opposed to a positive correlation in newborns. The aim of this prospective study was to explore the correlation between cord blood adiponectin and early infant growth.

Patients / Methods: Seventy one newborns (mean gestational age 38.9 ± 2.1 weeks, and mean birth weight 2959.3 ± 641.4g) were included in the study and their cord blood adiponectin and leptin were measured. At one year of age, the height and weight of these newborns were measured. The correlations between these parameters and cord blood adiponectin and leptin were evaluated.

Results: Mean weight and height at one year were 9159.1 ± 125.4 g, and 72.7 ± 3.6 cm, respectively. Cord blood adiponectin was positively correlated with birth weight (r = - 0.4, P < 0.02) but negatively correlated with the weight at one year of age (r = - 0.4, P < 0.001) and with average weight gain after one year (r = - 0.6, P < 0.001). No correlation was found between adiponectin and height. Cord blood leptin was positively correlated with both weight and height at one year of age (r = 0.2, P < 0.02 and r = 0.2, P < 0.04, respectively). As expected, average weight gain after one year was positively correlated with weight and height at one year (r = 0.8, P < 0.001 and r = 0.3, P < 0.005, respectively).

Conclusions: As previously shown adiponectin levels were positively correlated with birth weight. However, at one year of age the correlation between cord blood adiponectin and weight became negative. This intriguing finding can be explained by a relatively higher increase in weight during the first year of life (“catch up growth”) in newborns with a low birth weight known to have low cord blood adiponectin levels.
LOCAL PRODUCTION OF THE GONADOTROPIC HORMONES IN THE RAT OVARY

(1) DR. SCHIRMAN-HILDESHEIM TAMAR (2) MR. GERSHON ERAN (1) MRS. LITICHEVER NAOMI (2) MRS. GALIANI DALIA (1) MRS. BEN-AROYA NURIT (2) PROF. DEKEL NAVA (1) PROF. KOCH YITZHAK

(1) NEUROBIOLOGY - WEIZMANN INSTITUTE OF SCIENCE, REHOVOT (2) BIOLOGICAL REGULATION - WEIZMANN INSTITUTE OF SCIENCE, REHOVOT

Introduction: The gonadotropic hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are heterodimeric glycoproteins, produced by the gonadotropes of the anterior pituitary in response to the hypothalamic gonadotropin-releasing hormone (GnRH) signaling. In the female, LH and FSH affect folliculogenesis, ovarian steroid production, oocyte maturation, ovulation and corpus luteum formation.

Patients / Methods: We have recently studied the expression of GnRH and its receptor in rat ovaries throughout the estrus cycle. Using real-time PCR, we found differential, organ-specific and estrous cycle-dependent, regulation of the expression of GnRH and of its receptor in the ovary as compared to those of the pituitary or the hypothalamus. Subsequently, we wished to determine whether rat ovaries also express gonadotropic hormones.

Results: Using RT-PCR, we detected LHβ, FSHβ and the common α-subunit mRNA's in intact follicles, theca cells, corpora lutea and in meiotically-competent and incompetent oocytes. Granulosa cells, express mRNA's for LHβ,α-subunit, but not for FSHβ. Follicles and oocytes contain LH as determined by radioimmunoassay. We cloned and sequenced the ovarian LHβ transcript and found it to be longer (2.3 kb) than the one produced by pituitary gonadotropes (0.8 kb), due to a longer 5'-UTR. We studied the regulation of ovarian LHβ mRNA in sexually immature female rats administered with pregnant mare serum gonadotropin (PMSG) and in adult cyclic rats. PMSG administration caused a significant decrease in LHβ mRNA expression, detected by real-time PCR. Similarly, LHβ mRNA levels were lower on estrus morning versus proestrus evening. It thus seems probable that the ovarian LH is heterologously/homologously regulated by pituitary - and possibly also local - gonadotropins.

Conclusions: Although the reproductive system, by and large, is regulated by the hypothalamus-pituitary axis, it is possible that ovarian functions may possess a greater degree of autonomy than it is currently conceived. Indeed, the ovary produces the entire set of hormones and their receptors that are known to regulate reproduction: GnRH and its receptor, the gonadotropic hormones and their receptors, prolactin, activin, inhibin, follistatin and their receptors, etc. Thus, our data provide evidence for the existence of a local GnRH-gonadotropin axis in the mammalian ovary.
Abstract Code: A40

INVERSE RELATIONSHIP BETWEEN NITRIC OXIDE SYNTHASE AND ENDOTHELIN-1 IN BOVINE CORPUS LUTEUM: INTERACTIONS AT THE LEVEL OF THE LUTEAL ENDOTHELIAL CELL

(1) MRS. ROSIANSKY MAYA (1) MRS. KISLIOUK TATIANA (1) DR. KLIPPER EYAL (1) PROF. MEIDAN RINA

(1) HEBREW UNIVERSITY, FACULTY OF AGRICULTURE

Introduction: Endothelin-1 (ET-1) and Nitric Oxide (NO) are known to play pivotal roles in corpus luteum (CL) function. These two vasoactive compounds act as mutual antagonists in numerous physiological processes such as maintenance of vascular tone, platelet activity and leukocyte chemotaxis. The present study was designed to examine the interplay between NO and ET-1 synthesis in the bovine CL.

Patients/Methods: mRNA levels of tissue or cells were determined by quantitative real-time PCR. Protein levels quantified by either western blot analysis (eNOS) or Elisa kit (ET-1).

Results: The expression of the inducible and constitutive-endothelial NO synthase (iNOS and eNOS, respectively) in CL tissue during the luteal phase was determined. Young CL (days 1-5) expressed the highest mRNA levels of both eNOS and iNOS (30.42±6.07; 3.13±0.58, respectively). These values later declined to 10.57±2.48; 2.15±0.54, respectively, at mid cycle and remained low at days 16-18. Luteolysis, initiated by PGF2alpha, further reduced NOS mRNA and 24h later their values dropped to 10%-20% of those observed at mid cycle. eNOS protein levels were also reduced on days 16-18 and were further decreased after luteolysis. The profile of luteal ET-1 was the mirror image of NOS, where levels were lowest at early luteal stage reaching a peak during luteolysis. These findings suggest that ET-1 and NO may modulate the production of each other. As luteal endothelial cells (LEC) are the main site of ET-1 and NO production within the CL, we examined the direct effect of NO donor, NONOate on ET-1 levels in LEC. Elevated NO dose-dependently decreased ppET1 and ET-1 secretion in cultured LEC. Inhibition of NOS activity by L-NAME resulted in increased ppET-1 levels. Interestingly, together with ET-1 inhibition, NO up-regulated PGF2alpha receptor mRNA in LEC.

Conclusions: These findings suggest that the inverse relationship between NOS and ET-1 throughout the CL lifespan is a consequence of these two compounds interacting at the level of the resident LEC. Luteal NOS levels are regulated in a physiologically meaningful manner; as elevated NO during early luteal phase supports angiogenesis while reduced levels during final stages of CL lifespan permit the rise in ET-1 and enable the process of luteolysis.
DISRUPTION OF GAP JUNCTIONAL COMMUNICATION WITHIN THE OVARIAN FOLLICLE INDUCES OOCYTE MATURATION

(1) MRS. SELA-ABRAMOVICH SAGIT (1) MRS. EDRY IRIS (1) MRS. GALIANI DALIA (1) DR. NEVO NAVA (1) PROF. DEKEL NAVA

(1) BIOLOGICAL REGULATION

Introduction: Meiotically arrested mammalian oocytes are stimulated to resume meiosis by LH. This effect is associated with termination of gap junctional communication (GJC) in the ovarian follicle and can be reversed by elevation of intraoocyte cAMP. We have recently reported that LH-induced oocyte maturation and breakdown of GJC are mediated by MAPK. In the present study, we examined the hypothesis that termination of cellular communication in the ovarian follicle is sufficient for induction of oocyte maturation.

Patients / Methods: To test our hypothesis we used carbenoxolone (CBX), a potent blocker of GJC. Either rat follicle-enclosed oocytes (FEO) or a primary culture of granulosa cells served as our experimental model. In addition, we performed in-vivo experiments in which we used injections into the ovarian sac (bursa) of rats.

Results: We found that similar to LH, addition of CBX to the culture medium results in 90% oocyte maturation; this response was also obtained by a transient (two hour) exposure to this agent. CBX-induced oocyte maturation was accompanied by a substantial decrease in intraoocyte concentrations of cAMP that was not associated with elevated activity of type 3A phosphodiesterase (PDE3A). The effect of CBX on reinitiation of meiosis was blocked by isobutylmethylxanthine (IBMX), a phosphodiesterase inhibitor. Unlike LH, CBX did not activate MAPK in the follicular cells, and inhibition of the MAPK signaling pathway by means of UO126, did not prevent the resumption of meiosis. Injection of CBX into the ovarian bursa of intact animals stimulated maturation in 30% of the oocytes, whereas no maturation was observed in the contralateral ovary injected with PBS.

Conclusions: We conclude that since experimentally-induced breakdown of communication within the ovarian follicle is associated with a drop in intraoocyte cAMP concentrations and results in resumption of meiosis, this could be the physiological mechanism employed by LH to stimulate oocyte maturation.
Abstract Code: A42

TRANSCRIPTIONAL FLEXIBILITY OF THE STEROIDOGENIC ACUTE REGULATORY (STAR) PROTEIN DURING FOLLICULAR DEVELOPMENT

(1) MRS. YIVGI-OHANA NATALIE (1) PROF. ORLY JOSEPH

(1) DEPARTMENT OF BIOLOGICAL CHEMISTRY, THE ALEXANDER SILBERMAN INSTITUTE OF LIFE SCIENCES, THE HEBREW UNIVERSITY OF JERUSALEM

Introduction: The steroidogenic acute regulatory protein (StAR) is a hormone/cAMP regulated protein essential for steroid hormone synthesis in the gonads and the adrenal. However, no consensus cAMP responsive elements (CRE) exist in the StAR promoter. Yet, our former studies of StAR expression in the rat ovary showed that 21 cis-regulatory nucleotides are sufficient for transcriptional activation mediated by binding of GATA-4 and CCAAT/enhancer-binding protein β (C/EBPβ) to -66/-61 and -81/-71 motifs, respectively. The present study re-considers the possibility that b-ZIP factors of the CREB/CREM family might regulate StAR expression during various phases of follicular development.

Patients / Methods: We used reporter-promoter analyses including functional activity tests of wild-type and mutated promoter regions examined in primary rat ovarian granulosa cells and heterologous human embryonic kidney 293 (HEK293) cells, DNA binding assays (EMSA) and protein-protein co-immuno-precipitation approaches.

Results: Promoter activity and EMSAs revealed that a single promoter element located at -81/-70 has dual binding characteristics; on one hand it binds CREB-1 upon onset of follicular development toward ovulation in hormone induced ovaries, while later-on it associates with C/EBPβ in post hCG/LH-surge ovaries. Granulosa cells expressing dominant negative proteins of either CREB (A-CREB) or C/EBPβ (LIP) showed 75% and 45% decrease in promoter activity, respectively. In heterologous cell model, CREB or C/EBPβ alone can activate transcription, as well as strongly synergize with GATA-4. Interestingly, co-transfection with cAMP-dependent kinase (PKA) tremendously upregulated CREB-dependent transcription while the presence of C/EBPβ markedly suppressed the promoter activity under similar circumstances.

Conclusions: Altogether, this work observed a functional switch of trans-factors that upregulate StAR expression in the ovary; whereas during cAMP-dependent phases of follicular growth CREB-1 binding and action mediates StAR induction, C/EBPβ probably replaces CREB-1 during a functional transition to cAMP-independent transcription known to occur following LH-induced luteinization. These results make us suggest that during evolution the STAR gene acquired a non-consensus C/EBPβ site (TGACTGATGAC) that nests two CRE half-sites (TGAC) that evolved to allow modular accommodation of different b-ZIP proteins receptive to both cAMP-dependent and independent signaling cues.
A NOVEL LINK BETWEEN STEROID PRODUCTION AND CASPASE ACTIVATION: INHIBITING ONE ATTENUATES THE OTHER IN CULTURED RAT PREOVLATORY FOLLICLES

(1) YACOBI KEREN (1) PROF. TSAFRIRI ALEX (1) DR. GROSS ATAN

(1) DEPARTMENT OF BIOLOGICAL REGULATION

**Introduction:** Atresia is a well-documented process, in which most of the growing ovarian follicles are eliminated by apoptosis. Caspases form a family of cystein proteases which are major executioners of apoptosis. We have previously reported that LH induces caspase-3 and -7 activation in cultured follicles, and therefore we next wished to elucidate the mechanism of this activation.

**Patients / Methods:** Follicle development was stimulated in immature rats by eCG treatment. For in-vitro studies preovulatory follicles isolated 2 days after eCG were used and treated with LH in culture. For in-vivo studies hCG was administered 2 days after eCG to induce ovulation and luteinization of preovulatory follicles.

**Results:** One of the main effects of LH is the induction of steroid production. Thus, we examined whether LH-induced steroid production might be related to its ability to activate caspases in cultured follicles. In these studies, we used two inhibitors of steroid production: aminoglutethimide, an inhibitor of p450scc located in the mitochondria, and epostane, an inhibitor of 3βHSD located in the endoplasmic reticulum (ER). We found that addition of aminoglutethimide, but not of epostane, significantly reduced LH-induced caspase activation and apoptosis. Thus, inhibition of the early stages of steroidogenesis in the mitochondria, but not in the ER, inhibits LH-induced caspase activation. To examine whether caspase activity, in turn, might affect progesterone production, we used two broad caspase inhibitors and found that inhibition of caspases attenuated LH-induced progesterone production. Thus, a functional link exists between steroid production and caspase activation in cultured follicles. A similar functional link might also exist in-vivo since we found that the hCG-induced increase in progesterone production was accompanied by an increase in caspase activity.

**Conclusions:** This study reveals a novel linkage between two seemingly distinct processes in which steroid production is coupled to caspase activation in cultured rat preovulatory follicles.
THYROXINE-TRIIODOTHYRONINE COMBINATION THERAPY VERSUS THYROXINE MONOTHERAPY FOR CLINICAL HYPOTHYROIDISM - SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED TRIALS

(1) DR. GROZINSKY-GLASBERG SIMONA (2) MRS. FRASER ABIGAIL (3) DR. NAHSHONI EITHAN (4) PROF. WEIZMAN ABRAHAM (5) PROF. LEIBOVICI LEONARD

(1) ENDOCRINE INSTITUTE AND DEPT. OF MEDICINE B, BEILINSON CAMPUS, RABIN MEDICAL CENTER, PETACH-TIQVA 49100, ISRAEL. (2) HEAD OF RESEARCH UNIT, RABIN MEDICAL CENTER, BEILINSON CAMPUS, DEPT. OF MEDICINE E, PETACH-TIQVA 49100, ISRAEL. (3) CONSULTATION LIAISON PSYCHIATRIST, GEHA PSYCHIATRIC HOSPITAL, PETACH-TIQVA 49100, ISRAEL. (4) DIRECTOR, FELSENSTEIN MEDICAL RESEARCH CENTER, RABIN MEDICAL CENTER, PETAT-TIQVA 49100, ISRAEL. (5) HEAD OF DEPARTMENT, RABIN MEDICAL CENTER, BEILINSON CAMPUS, DEPT. OF MEDICINE E, PETAH-TIQVA 49100, ISRAEL

**Introduction:** Objective: To compare the effectiveness of thyroxine-triiodothyronine (T4-T3) combination therapy versus thyroxine (T4) monotherapy for the treatment of clinical hypothyroidism in adults. Design: Systematic review and meta-analysis. Data sources: Pub Med, EMBASE, LILACS, and The Cochrane Central Register of Controlled Trials (CENTRAL) databases were searched in September 2005. References of all included trials were scanned for additional studies. We put no restrictions on language, year of publication, or publication status.

**Patients / Methods:** Review methods: All randomized or quasi-randomized trials that compared the effectiveness of thyroxine-triiodothyronine combination therapy versus thyroxine monotherapy for the treatment of clinical hypothyroidism in adults were included.

**Results:** No difference was found in the effectiveness of combination versus monotherapy in any of the following symptoms: bodily pain (standardized mean difference = 0.00, 95% confidence interval -0.34, 0.35), depression (standardized mean difference = 0.04; 95% confidence interval -0.25, 0.34), and anxiety (standardized mean difference = 0.00, 95% confidence interval -0.12, 0.11). A slight advantage in favor of combination therapy was found when assessing the degree of fatigue (standardized mean difference = -0.24, 95% confidence interval -0.47, -0.01). No difference was found in the reported quality of life between patients receiving combination therapy or monotherapy (standardized mean difference = 0.03, 95% confidence interval -0.09, 0.15), in weight, total serum cholesterol, triglyceride levels, LDL and HDL. Adverse events were mildly more frequent in patients receiving T4 monotherapy (Relative Risk = 1.13, 95% confidence interval - 0.59, 2.17).

**Conclusions:** T4 monotherapy should remain the treatment of choice for clinical hypothyroidism and further trials are unwarranted.
Abstract Code: A45

NOVEL MUTATIONS IN THE THYROTROPIN RECEPTOR AS A MAJOR GENETIC DETERMINANT OF SUBCLINICAL HYPOTHYROIDISM IN AN ISRAELI ARAB COMMUNITY

(1) DR. TENENBAUM-RAKOVER YARDENA (2) DR. GRASBERGER HELMUT (2) DR. MAMANASIRI SUNEE (2) DR. RINGKANANONT USANEE (3) DR. MAHAMEED- HAGDAHOOD AHMAD (2) DR. MONTANELLI LUCIA (2) PROF. REFETOFF SAMUEL

(1) PEDIATRIC ENDOCRINE DEPARTMENT, HA’ EMEK MEDICAL CENTER, AFULA, AFFILIATED TO THE TECHNION FACULTY OF MEDICINE, HAIFA, ISRAEL (2) DEPARTMENT OF MEDICINE, THE UNIVERSITY OF CHICAGO, ILLINOIS, USA (3) CLALIT HEALTH SERVICE, ISRAEL

Introduction: Resistance to thyrotropin (RTSH) is a congenital condition of impaired responsiveness of the thyroid gland to biologically active TSH, clinically characterized by elevated serum levels of TSH, normal or low free thyroid hormone levels and absence of goiter.

Patients / Methods: Clinical and genetic investigation of 76 members of families with RTSH phenotype from the Israeli Arab community from one village.

Results: In the extended kindred originally investigated, 13 members had nonautoimmune hyperthyrotropinemia. We identified two novel mutations in the TSH receptor (TSHR) gene (P68S and L653V), segregating independently in this highly consanguineous family. Both mutations were found in genealogically unlinked families from the same community. Haplotype analysis suggested that the mutations were likely introduced in the population by the local founders, 6-7 generations ago. With the exception of one homozygous for L653V, affected individuals were not identified by the T4-based newborn screening. TSH levels were significantly higher in the 5 homozygotes (46.2 ± 6.2) and 21 heterozygotes (3.6 ± 1.3) for L653V vs. the 42 individuals with wild type TSHR (2.3 ± 1.0). The cross-sectional study in this genetically homogenous population indicated that, in untreated L653V heterozygotes, the elevated TSH levels do not spontaneously normalize with increasing age. Mean TSH levels of the 4 P68S heterozygotes and individuals homozygous for wild type TSHR were not different, but the impact of P68S on pituitary-thyroid feedback regulation was clearly evident in the 4 P68S/L653V compound heterozygotes, which had TSH levels (11.8 ± 3.3) intermediate to those in L653V hetero- and homozygotes. As expected, L653V produced a more severe defect than P68S in TSH-stimulated cAMP generation in vitro.

Conclusions: Our study provides an example of compound heterozygosity in an inbred population, a phenomenon repeatedly observed in Israeli Arab communities. TSHR mutation may be the major genetic cause of abnormal thyroid function tests in this community. Association studies with biomarkers in this large cohort of RTSH patients may clarify whether elevated TSH levels in RTSH reflect a compensated euthyroid condition with an appropriately adjusted set point of pituitary-thyroid feedback regulation or mild tissue hypothyroidism sensu strictu with potential benefits of T4 replacement therapy.
TROPONIN T IS NOT ELEVATED IN PATIENTS WITH SEVERE HYPOTHYROIDISM

(1) DR. NESS-ABRAMOF ROSANE (1) DR. NABRISKI DAN (2) MR. WEISS ELIAHU (3) MR. KATZ BERNARD (4) DR. TRIPTO-SHKOLNIK LIANA (1) PROF. SHAPIRO MENACHEM (5) PROF. SHENKMAN LOUIS

(1) ENDOCRINE UNIT, SAPIR MEDICAL CENTER (2) ENDOCRINE LABORATORY, SAPIR MEDICAL CENTER (3) CLINICAL CHEMISTRY LABORATORY, SAPIR MEDICAL CENTER (4) DEPARTMENT OF MEDICINE A, SAPIR MEDICAL CENTER (5) DEPARTMENT OF MEDICINE C, SAPIR MEDICAL CENTER

Introduction: Patients with hypothyroidism often have increased creatine kinase levels (CK). It is possible that there is increased production of CK, but other mechanisms such as an increased enzyme permeability or decreased enzyme clearance are possible. In our previous work we found that 21% of patients with hypothyroidism had concomitant elevation of CK and CK MB without clinical signs of cardiovascular disease (unpublished data). Recently, troponin T and I were extensively studied due to their cardiac specificity. Cardiac troponins are sensitive and specific markers of cardiac injury. We therefore decided to examine troponin T levels in patients with hypothyroidism.

Patients / Methods: Twenty five patients with primary hypothyroidism were evaluated. The etiology of hypothyroidism was thyroidectomy in 24 patients and I-131 therapy for Graves disease in one. There were 3 men and 22 women with a mean age of 47.5 ± 12.4 y. None of the patients had clinical evidence of heart disease.

Results: Mean TSH and FT4 levels were 61 ± 12 mU/L and 0.4 ± 0.1 ng/dl, respectively. CK levels ranged between 86-1221 U/L (nl< 170 women, <195 men) with a mean of 322 U/L ±279. CK MB was elevated in 17 patients. Absolute MB was elevated in 3 patients (12%), MB percentage was elevated in 12 patients (48%) and both were elevated in 2 patients (10%). All 25 patients had normal troponin levels < 0.01 ng/mL (nl: 0-0.1 ng/mL).

Conclusions: Elevation of CK and its MB fraction are common in patients with severe hypothyroidism. Troponin T levels were not elevated in our patients with severe hypothyroidism. In view of the frequent elevation of CK and its MB fraction, assessment of troponin T levels is a reliable tool in evaluating the hypothyroid patient with possible cardiac symptoms.
Abstract Code: A47

SCANNING ELECTRON MICROSCOPY OF THYROID CELLS UNDER FULLY HYDRATED CONDITIONS—NOVEL TECHNIQUE FOR A SEASONED PROCEDURE

(1) DR. COHEN OHAD (1) DR. BEERY RACHEL (1) DR. ILANY JACOB (2) DR. ANABY DEBBIE (2) DR. CHAJUT AYELET (1) DR. LEVIT SHMUEL (BORIS) (1) DR. SCHWARTZ IGNAT (3) PROF. COHEN DAVID (1) PROF. SHABTAI MOSHE (4) PROF. ALFICI RICARDO (5) DR. CZERNIAK ABRAHAM (1) PROF. KARASIK AVRAHAM

(1) INSTITUTES OF ENDOCRINOLOGY, SURGERY B AND PATHOLOGY, CHAIM SHEBA MEDICAL CENTER, TEL-HASHOMER, SACKLER SCHOOL OF MEDICINE TEL-AVIV UNIVERSITY (2) QUANTOMIX, LTD. WEIZMANN SCIENCE PARK, REHOVOT (3) HERZELIA MEDICAL CENTER (4) HYLLEL YAFFE MED. CTR. HADERA (5) WOLFSON MED. CTR. HOLON

**Introduction:** Fine-needle aspiration (FNA) is a central diagnostic modality in the interpretation of thyroid lesions. Although this test has long-established high sensitivity and specificity, it includes several limitations. Interpretation of follicular lesions are hindered by the lack of tissue structure, and nondiagnostic or “gray zone” specimens, which may appear in up to 30% of cases. Use of electron microscope (EM) imaging of thyroid FNA samples was hampered by the inability to preserve and analyze structures at a state most closely approximating the native state. The study applied a novel method, WETSEM™, that allows, for the first time, complete insulation of the sample from the vacuum in the EM, thus allowing direct EM imaging of fully hydrated, thyroid samples obtained by FNA.

**Patients / Methods:**

**Results:** Presented are illustrative cytological images of thyroid samples obtained from patients with diverse thyroid disorders, shown for the first time. The unique subcellular characteristics of the different cells, as well as the presumed changes occurring during malignant transformation, will be discussed.

**Conclusions:** In conclusion, the novel WETSEM technique allows the visualization of organelles undemonstrated by the current thyroid cytological methods. The contribution of this imaging modality for the clinical practice awaits further clinical studies.
CHARACTERIZATION OF PATIENTS WITH REPEATED THYROTROPIN DETERMINATIONS BY PRIMARY CARE PHYSICIANS: A THREE-YEAR FOLLOW-UP OF 567,817 PATIENTS

(1) DR. ROTMAN-PIKIELNY PNINA (2) DR. SHERF MICHAEL (2) MR. BATTAT EREZ (1) DR. LEVY YAIR (2) DR. MEYEROVITCH JOSEPH

(1) DEPARTMENT OF MEDICINE E, MEIR MEDICAL CENTER, KFAR-SABA (2) HEALTH PLANNING AND POLICY WING, CLALIT HEALTH SERVICES, TEL-AVIV

Introduction: The Thyrotropin (TSH) blood test is one of the most frequently performed screening tests by primary care physicians (PCPs), however, screening recommendations in the general population are controversial. In this study we used a centralized computerized database of 3.75 million insured persons to analyze screening habits of PCPs in Clalit Health Services (CHS), focusing on the clinical outcome of these tests. Objectives: To study the current utilization of TSH blood levels as a screening tool by PCPs and to define populations at greater risk for developing a significant thyroid disease based on their initial TSH levels or for recurrent normal TSH measurements.

Patients / Methods: Patients with one TSH level determination in 2002, for whom follow-up data was available until 2004 were included. Patients with a known thyroid disease and patients treated with lithium or amiodarone throughout the study period were excluded. Positive thyroid events were defined by initiation of thyroid therapy. Logistic regression analyzed the effect of patients' characteristics on the probability to have a positive thyroid event.

Results: 567,817 patients fulfilled the above inclusion criteria, for which 679,239, 282,609 and 321,877 TSH level determinations were performed in 2002, 2003 and 2004, respectively. 93.9% of the 567,817 initial TSH levels were within normal limits, while 1.4%, 4.1% and 0.7% were <0.3mU/L, between 5.5 and 10mU/L, and >10mU/L, respectively. 97% of repeated TSH levels were normal when the initial values were normal. Notably, only 0.97% of patients with normal initial TSH levels were placed on thyroid therapy during a 3-year follow-up period. Patients’ characteristics that were associated with at least three TSH measurements without initiation of a thyroid medications were: diabetes, hyperlipidemia and psychiatric disorders (3, 2.96, 2.9 odds ratio, respectively).

Conclusions: Our results suggest that when the 1st TSH performed as a screening test is normal, the likelihood of developing a significant thyroid disease within 3 years is low. Patients with a greater likelihood to have repeated TSH measurements without a clinical benefit are patients with diabetes, hyperlipidemia and psychiatric disorders. Educated utilization of TSH screening tests by PCPs should be encouraged.
UNILATERAL ADRENALECTOMY -BASED ON SCINTISCAN UPTAKE RESULTS - FOR ACTH INDEPENDENT MACRONODULAR ADRENAL HYPERPLASIA (AIMAH)

(1) DR. GERSHINSKI MICHAL (1) DR. SHECHNER CARMELA (2) DR. BEJAR JACOB (1) DR. REUT MARIA (1) DR. DICKSTEIN GABRIEL

(1) DIVISION OF ENDOCRINOLOGY, BNAI ZION MEDICAL CENTER (2) DEPARTMENT OF PATHOLOGY, BNAI ZION MEDICAL CENTER

Introduction: ACTH independent macronodular adrenal hyperplasia (AIMAH) is a rare form of primary adrenal hypercortisolism, histologically characterized by bilateral adrenal enlargement due to adrenocortical macronodules with hyperplastic internodular cortex. The disease is caused by the existence of aberrant receptors to different hormones in the adrenocortical tissue. Specific therapy for long periods is unavailable yet, and bilateral adrenalectomy is generally used as treatment, with consequent adrenal insufficiency. We hereby present 3 patients with AIMAH, who underwent only unilateral adrenalectomy, and on long term follow up regained - after a period of hypoadrenalism - normal adrenal function.

Patients / Methods: Three female patients presented with Cushing's syndrome, which was found to be ACTH independent (suppressed ACTH levels and no cortisol suppression on dexamethasone). Two patients had marked cushingoid stigmata, while in the third severe osteoporosis with vertebral fractures was the main presenting symptom. CT scans in all three revealed bilateral adrenal nodular enlargement. However, adrenal scans in all showed only unilateral isotope uptake. One patient needed resection of adrenal lesion on the side of the adrenal lesion which was scan positive, and therefore it was decided to perform adrenalectomy in the same procedure. In the two others, laparoscopic unilateral adrenalectomy to the scan positive side was performed, with the understanding that should this fail to cure the disease, a second, contralateral adrenalectomy will be needed.

Results: In all three patients the resected adrenal showed features characteristic to AIMAH - nodules with hyperplasia. All 3 patients dropped cortisol levels to zero after surgery. All 3 needed cortisol replacement for different times, and regained normal adrenal function afterwards. On follow -up CT scans, the remaining adrenal did not show further enlargement, yet is still nodularly- hyperplastic. After more than two years in all cases, adrenal function remains normal.

Conclusions: Based on these three cases, and a few other suggestions from the literature, we suggest performing adrenal scans in all patients with AIMAH prior to surgery. This may be crucial for deciding whether to perform bilateral adrenalectomy, with consequent hypoadrenalism, on unilateral adrenalectomy only. In the later case, euadrenalism for an unknown, yet significant time, can be the result.
Abstract Code: P50

LOW DOSE KETOCONAZOLE FOR RAPID CONTROL OF HYPERCORTISOLAEMIA - A CASE REPORT

(1) DR. TRIPTO SHKOLNIK LIANA (1) DR. TOLEDANO YOEL (1) DR. AGBARIA ZUHDI (1) DR. JAFFE ANAT

(1) ENDOCRINOLOGY AND DIABETES UNIT, HILLEL YAFFE MEDICAL CENTER

Introduction: There is a relative paucity of information concerning time course of pharmacological cortisol synthesis inhibition. Etomidate, the anesthetic medication, is an imidazole derivative that inhibits cholesterol side-chain cleavage and 11β-hydroxylase. Etomidate causes a rapid (24 hours) and massive reduction of cortisol synthesis according to a few reports, but intravenous route of administration limits its use in the outpatient setting. Metyrapone alone, or combined with aminoglutethimide, is effective in normalizing cortisol over period of weeks to months, but data concerning more rapid reduction of cortisol synthesis is lacking. Moreover, metyrapone, by inhibiting 11β-hydroxylase, causes mineralcorticoid and androgen effect and, thus, numerous side effects. In addition, the drug is not readily available. Ketoconazole, an imidazole derivative, inhibits cytochrome P450 enzymes, including side-chain cleavage, 17,20-lyase and 17α-hydroxylase. The prime use of the medication is related to its antifungal properties, and ketoconazole is easily accessible. Ketoconazole efficacy in reducing endogenous cortisol production is well documented, but super acute (hours and days) effects are not extensively described. We found only one reference – reporting three patients treated with high dose ketoconazole resulting in about 50% reduction of UFC in two days. We present the efficacy of low dose ketoconazole for acute cortisol reduction in our patient.

Patients / Methods: A twenty-nine year old female presented with a rapidly evolving clinical picture of Cushing syndrome due to adrenocortical carcinoma. The laboratory values showed extremely high endogenous cortisol load. Ketoconazole was administered in 400 mg/day in two divided doses for 5 days prior to surgery. Urinary free cortisol had fallen from 1180 microgram/day to 313 microgram/day after 48 hours and to 130 microgram/day after 4 days of therapy. Morning cortisol decreased from 26 microgram/dl prior to treatment to 21.3 microgram/dl after one day and to 18.5 microgram/dl after 48 hours. The patient tolerated the drug well and liver function tests remained normal.

Conclusions: Treatment with low dose ketoconazole resulted in rapid decrease in endogenous cortisol production and had no side effects in our patient. It might be a feasible treatment option when rapid decline is needed.
A COMPARISON BETWEEN THREE COMMERCIAL KITS FOR MEASUREMENT OF URINARY FREE CORTISOL

(1) DR. LIMOR RONA (1) MRS. GILAD SUSAN (1) MRS. GLODFARB HAYA (1) PROF. STERN NAFTALI

(1) INSTITUTE OF ENDOCRINOLOGY, METABOLISM AND HYPERTENSION, TEL AVIV SOURASKY MEDICAL CENTER AND SACKLER FACULTY OF MEDICINE, TEL AVIV UNIVERSITY, ISRAEL

Introduction: The measurement of free urinary cortisol (UFC) is one of the most useful screening tests for Cushing’s syndrome. In recent years, changes in the kits distributed in Israel and elsewhere have been hardly examined independent of the manufacturers’ claims. Yet, it well known that immunoassays currently employed by most clinical laboratories present limitations, especially concerning specificity and steroid / steroid interference. To assess how the type of assay used affects actual outcome we compared UFC measurement obtained by three different commercially available kits.

Patients / Methods: UFC was measured with the following commercial kits, each performed according to the manufacturers’ recommendations: active Cortisol RIA, DSL-2100, Diagnostic System Laboratories, Inc, Texas, USA; Cortisol RIA, Immunotech, Beckman Coulter Company, Praque, Czech Republic; Coat-A-Count Cortisol, Diagnostic Products Corporation, Los Angeles, USA. Free cortisol was measured in 24-hours urine specimen collected from 78 patients attending the endocrinology out-patients clinic.

Results: While the mean values derived by all three methods were relatively close, the absolute ranges appeared vastly different and not necessarily related to the calculated means: 14.5-952 ug/24 hours (average 96.33ug/24 hours), undetectable –412 ug/24 hours (average 69 ug/24 hours), 54-279 ug/24 hours (75ug/24 hours) for DPC, Immunotech and DSL respectively. According to the urine normal range provided by the manufacturer for each kit, 19 out of the 78 patients would be classified differently, depending on the kit used. Using the DPC kit, 3 out of these 19 subjects would be defined as “normal” whereas 16 could be suspected for Cushing disease/ syndrome. Using the Immunotech kit, 12/19 would be defined as normal subjects and only 7 would have been defined as hypercortisolism. Finally, based on the DSL kit 16/19 passes, as “normal”, and only three would be labeled as suspected for Cushing disease.

Conclusions: Immunoassay methods currently used for measurement of urinary cortisol yield unacceptably different, which might lead to erroneous clinical decisions or to unduly excessive further clinical investigations. Because the diagnosis of Cushing disease in its current clinical spectrum is often a challenging task, UFC immunoassays should be supplemented by additional testing such as salivary cortisol, UFC by HPLC and dexamethasone suppression tests.
Abstract Code: P52

VASCULAR FUNCTION IN PRIMARY HYPERPARATHYROIDISM DOES NOT DIFFER FROM THAT OF AN ADEQUATELY MATCHED CONTROL POPULATION: RESULTS OF NONINVASIVE VASCULAR STUDIES

(1) DR. TORDJMAN KAREN (1) DR. YARON MARIANNA (1) PROF. STERN NAFTALI

(1) INSTITUTE OF ENDOCRINOLOGY, METABOLISM AND HYPERTENSION, TEL AVIV SOURASKY MEDICAL CENTER

Introduction: Primary hyperparathyroidism (PHPT) is commonly associated with hypertension, dyslipidemia, and impaired glucose metabolism. Epidemiologic studies have suggested it confers an inherent risk of cardiovascular mortality. Using noninvasive measurements, vascular dysfunction, particularly increased arterial stiffness, has been reported in patients with PHPT. As these parameters are critically dependent on the subjects' age and blood pressure, selecting an adequately matched control group is essential when undertaking such a comparison. The aim of the present study was to noninvasively assess vascular function in PHPT patients, and to relate the findings to those of a control group matched for age, and the presence and severity of cardiovascular risk factors.

Patients / Methods: Twenty-four subjects (20 women/4 men, age 67±1.8 y) comprised the PHPT cohort: 12 had normocalcemic PHPT while 12 had hypercalcemic PHPT. The control group consisted of 15 subjects (11 women/4 men, age 64.4±2.5 y), matched with PHPT patients for age, sex, and cardiovascular risk factors. All subjects underwent a battery of noninvasive vascular function studies based on applanation tonometry and pulse wave analysis technology. These studies yielded large (C1) and small (C2) vessel compliance measures; augmentation index (AI), an assessment of systemic arterial stiffness; and pulse wave velocity (PWV) determined between the carotid and radial arteries, yet another index of large artery stiffness.

Results: There were no differences between the two subgroups of PHPT subjects in any of the assessed vascular parameters. Although some degree of vascular function impairment was evident in the PHPT patients when compared to our young and normotensive reference data (not shown), subjects with PHPT did not differ from adequately matched subjects. In particular, we found no difference in any of the indices of arterial stiffness. AI was 29.8±2.4% in PHPT vs 32±2.2% in controls, P:NS. PWV was 8.4±0.3 m/s and 7.7±0.3 m/s, respectively, P:NS.

Conclusions: Although cardiovascular risk factors are common in PHPT, when these subjects are compared to an adequately matched control group, no differences in noninvasively assessed vascular function can be detected. These data do not support the notion that PHPT is associated with vascular dysfunction which is in excess of what can be ascribed to age or other traditional risk factors.
ADHERENCE TO TREATMENT AND CHANGES IN VITAMIN D STATUS IN HIP FRACTURE PATIENTS – PARTICIPANTS OF POST-SURGICAL TREATMENT PROGRAM

(1) DR. SEGAL ELENA (2) DR. ZINMAN CHAIM (3) MRS. RAZ BATIA (4) DR. ISH-SHALOM SOPHIA

(1) METABOLIC BONE DISEASES UNIT (2) ORTHOPEDIC SURGERY DEPARTMENT (3) ENDOCRINE LABORATORY (4) METABOLIC BONE DISEASES UNIT

Introduction: Hip fracture rate increases yearly by 1-3% in developed countries. Improvement of vitamin D status may decrease hip fracture risk by 30%, treatment with alendronate leads to 50% fracture risk reduction. Aims: To evaluate adherence to treatment in elderly participants of one year Post-Surgical Treatment Program (PSTP); to assess changes in the vitamin 25(OH)D3 and bone mineral density (BMD) in this group; to assess treatment status and fracture rate 3 years after discontinuation of participation in PSTP.

Patients / Methods: 125 consecutive elderly patients after surgical hip fracture correction were enrolled in PTSP that consisted of quarterly physical examination and laboratory evaluation. All the patients received 1500 mg of calcium carbonate and 800 IU of vitamin D3 daily. After improving vitamin D status alendronate 70 mg/wk was administered for one year within the program. Laboratory evaluation: 25(OH)D3 by 125I-radioimmunoassay, routine biochemical tests. BMD was assessed with Lunar DEXA at baseline and after one year of antiresorbing therapy

Results: Results: 91(73%) women and 34(27%) men aged 72.68±9.5 were enrolled in PSTP. At baseline 124(99%) had inadequate 25(OH)D3 serum level; 27(21.6%)<10 ng/ml. Initial patients' adherence was 29(23%). The time till improvement of vitamin D status (25(OH)D3 >18 ng/ml) and initiation of treatment with alendronate was 18±6 months. 65(52%) discontinued participation in the PSTP before starting alendronate due to noncompliance. Alendronate was started in 54(43%); 46(36.8%) completed PTSP. Mean 25(OH)D3 level increased by 5.6ng/ml (34%). BMD increased in LS by 5.2%, in FN by 4.8%, in TH by 2.7%, p<0.001. Three years later 32 patients died, 23 were lost to follow up; 71 patients were interviewed about their current treatment and new fractures since PSTP. Completed the PSTP had 0 new hip fractures vs 3(8%) of non-completed, DEXa was performed in 9(25%) vs 4(11%), Antiresorbing treatment received 28(78%) vs 7(20%), p< 0.01, Calcium and vit d supplements received 27(75%) vs 18(51%), p<0.001

Conclusions: Majority of elderly hip fracture patients had inadequate vitamin D status; adherence to the prescribed calcium and vitamin D supplements and bisphosphonates was unsatisfactory even in hospital bound outpatient program. Existing treatment options are underused in the public medical service setting.
COGNITIVE RESEMBLANCE IN PERSONS SUFFERING FROM OSTEOPOROSIS AND THEIR FIRST DEGREE RELATIVES

(1) MRS. KAFRI NAAMA (1) PROF. WERNER PERLA (2) DR. VERED IRIS

(1) DEPARTMENT OF GERONTOLOGY, UNIVERSITY OF HAIFA (2) INSTITUTE OF ENDOCRINOLOGY, SHEBA MEDICAL CENTER, AND SACKLER FACULTY OF MEDICINE, TEL AVIV UNIVERSITY

Introduction: The importance of family history of low bone mineral density as a risk factor for osteoporosis has been widely documented. First-degree relatives of persons suffering from osteoporosis are defined as a high-risk group for the development of the disease. Therefore, encouraging them to engage in preventive behaviors aimed at reducing the risk of the disease is of utmost importance. Studies assessing other chronic diseases demonstrated that rates of adherence to preventive and screening behaviors among first-degree relatives are associated to their illness representations of the disease, i.e., to their health beliefs and cognitive representations regarding the illness. The aim of the present study was to assess and compare the illness representations of women suffering from osteoporosis and their first degree relatives

Patients / Methods: Fifty two dyads of women suffering from osteoporosis (mean age = 67.8; 98% menopausal), and their daughters (mean age = 40.9; 10% menopausal) participated in the study. Illness representations were assessed using an adapted version of the Illness Perception Questionnaire (Weiman, Petrie, Moss-Morris & Horne, 1996).

Results: Overall, although the daughters perceived the symptoms of the disease as more severe than the mothers – especially for the bending of the back, weakness, and loss of height), the mothers perceived the disease as more permanent than the daughters. Similarly, the mothers perceived the outcomes of the disease as more serious than the daughters (especially regarding the influence of the disease on their lives, and the financial outcomes of the disease). Compared to their daughters, the mothers perceived the disease as caused mainly by internal reasons (such as inadequate diet, genetic reasons, life habits, and lack of physical activity).

Conclusions: The findings of this study suggest that osteoporotic women and their daughters do not share similar beliefs regarding the disease of the mother. These data also provide the foundation for future studies aimed at the development of interventions to increase adequate knowledge and awareness of the disease among relatives of individuals with osteoporosis.
PREVALENT AND UNDERRECOGNIZED: VITAMIN D DEFICIENCY IN ISRAELI ARAB WOMEN

(1) DR. TRIPTO SHKOLNIK LIANA (1) DR. TOLEDANO YOEL (1) DR. AGBARIA ZUHDI (2) DR. ROSENBLUM K. JOSEPH (1) DR. JAFFE ANAT

(1) ENDOCRINOLOGY AND DIABETES UNIT, HILLEL YAFFE MEDICAL CENTER (2) MEDICAL WING, KUPAT HOLIM MEUHEDET

Introduction: In Israel, a country of abundant sunlight, deficiency of the UV radiation dependent vitamin D hormone seems unlikely. Nevertheless, such vitamin D deficiency was described in several unique Israeli groups: Ethiopian immigrants, Bedouin women of the Negev and ultra-Orthodox Jewish women. Worldwide, there are reports of vitamin D insufficiency in Arab women with tight relation to limited sunlight exposure due to modest dress code. The condition is particularly noteworthy in pregnant and lactating women. Arab women in Israel are predisposed to vitamin D deficiency due to adherence to traditional dress and a diet poor in dairy products. Moreover, number of pregnancies and average lactation period in comparison to Jewish non-observant women is relatively high. Despite that, vitamin D status of Arab Israeli female has never been described.

Patients / Methods: Women attending endocrinology and diabetes clinic in Kfar Kara underwent nutritional anamnesis. Blood was drawn from patients dressed traditionally and with reported low calcium intake. Blood samples were checked for 25(OH)D and parameters of bone metabolism. The study group was compared to Jewish female patients with anamnestic clues to vitamin D deficiency treated in several endocrinology and diabetes clinics in the same geographic area.

Results: Forty one vitamin D deficient Arab women were compared to 48 vitamin D deficient Jewish women. The control group consisted of 68 Jewish women. Median age was 45, 58 and 62 years, respectively. Average vitamin D level was 6.7+3.2, 11.5+2.8 and 23.1+7.7 ng/ml in the three groups, respectively. Based on the anamnesis, daily calcium intake was much lower in the Arab vitamin D deficient group: 300+190, 500+264 and 640+230 mg/day respectively. The degree of secondary hyperparathyroidism was more pronounced in the Arab vitamin D deficient group, as was the alkaline phosphatase elevation. Total average number of lactation months per woman in the Arab vitamin D deficient group was 61 – significantly higher compared to both Jewish groups.

Conclusions: These results point out to a serious health issue in Israeli Arab women – a large population group in Israel. A multidisciplinary approach to enhance awareness among the patients and their care givers is required. Moreover, screening measures might be justified.
Abstract Code: P56

HASHIMOTO THYROIDITIS IN CHILDREN AND ADOLESCENTS – LONG-TERM FOLLOW UP

(1) DR. DE VRIES LIAT (1) DR. BULVIK SHMUEL (1) PROF. PHILLIP MOSHE

(1) INSTITUTE FOR ENDOCRINOLOGY AND DIABETES

Introduction: Hashimoto thyroiditis (HT) is the most common cause of goiter and hypothyroidism in children older than 6 years. Spontaneous remission may occur in 30-50% of adolescent patients, though long-term reports on HT in children and adolescents are scarce.

Patients / Methods: We investigated the clinical manifestations at presentation, clinical course, and long-term outcome of HT in children. We reviewed charts of 93 patients (F=77, M=16; 41 prepubertal, 52 pubertal) with HT. Mean age at presentation was 12.2 years, and mean follow-up duration was 5.6 years.

Results: The common complaints leading to referral were goiter, either isolated (30%) or associated with other complaints (7.6%), growth retardation (11.8%), fatigue (7.6%), irregular menses (6.5%), weight gain (6.5%) and increased appetite (3.3%). Of the 71 patients who had goiter at presentation, only 35 noticed thyroid enlargement before admission. Although the prevalence of goiter was similar in males and females, it accounted for significantly more referrals in females (46.3% vs 16.7%). At referral, more males complained of growth retardation (37.5%) than females (6.5%), although height–SDS was actually similar. Hypothyroid patients had lower levels of alkaline phosphatase and higher levels of total cholesterol than euthyroid patients (136±76 vs. 205.4±96.6 U/L and 196.3±51.6 vs. 162.7±36.4, respectively). Euthyroid patients with family history of thyroid disease had a lower likelihood of remaining euthyroid than those without 9% vs. 26%, p<0.04. Fourty-four patients were treated by LT4 for hypothyroidism and 40 were treated for other indications. Nine patients remained euthyroid without treatment. Therapy was discontinued in 10 patients: In 5 hypothyroidism recurred and 5 (5.3%)remained euthyroid for at least 18 months.

Conclusions: Although goiter is the most common complaint at presentation in HT, it goes unnoticed in half the children, especially in males. Growth retardation is more common in males than in females. A positive family history of thyroid disease is associated with a low likelihood of remaining euthyroid. Spontaneous remission may occur in a lower percentage of children and adolescents than previously reported.
SUBACUTE THYROIDITIS: A RETROSPECTIVE ANALYSIS OF FIFTY-TWO CONSECUTIVE PATIENTS DIAGNOSED BETWEEN 1999-2005

(1) DR. BENBASSAT CARLOS (2) DR. OLCHOVSKY DAVID (1) DR. SHIMON ILAN

(1) ENDOCRINE INSTITUTE, RABIN MEDICAL CENTER, CAMPUS BEILINSON (2) ENDOCRINE INSTITUTE, SHEBA MEDICAL CENTER

Introduction: Subacute thyroiditis (SAT) is a transient condition of unclear etiology and variable clinical presentations.

Patients / Methods: We retrospectively reviewed the medical records of 52 consecutive patients treated in 3 outpatient clinics between 1999-2003, aiming to identify prognostic factors of clinical outcome.

Results: The mean age was 48.5 ± 12 yr (71% females) and the seasonal distribution as follows: autumn 38%, winter and summer 23.5% each and spring 15%. Twenty six percent had antithyroid antibodies (ATA) and 10% recurrent disease. Mean duration of disease was 96 ± 67 days. A second phase of hypothyroidism evolved in 27 patients (including all ATA positive patients) and remained permanent in 8 of them. There were no correlations between the severity of disease (clinical score, ESR, max FT4) and max TSH or disease duration. Ten patients received no treatment and data was missing in 4 patients. The other 38 patients received NSAID (23) or steroids (15). There were non-significant differences between the 2 two treatment groups in length of hyperthyroidism, max FT4 or max TSH, but a trend for a shorter overall disease duration and less permanent hypothyroidism was observed in the steroid group. Although no predictive factors of permanent hypothyroidism were recognized, the max TSH was higher in the permanent compared to the transient hypothyroid group (median 49.5 vs 15.8 mIU, respectively, p=0.08).

Conclusions: We conclude that SAT follows an unpredictable clinical course that is hardly affected by its treatment.
Abstract Code: P58

IMPROVED SURVIVAL IN PATIENTS WITH METASTATIC DIFFERENTIATED THYROID CANCER

(1) DR. BENBASSAT CARLOS (1) DR. HIRSCH DANIA (2) DR. MECHLIS-FRISH SARA

(1) ENDOCRINE INSTITUTE, RABIN MEDICAL CENTER, CAMPUS BEILINSON (2) DEPT OF NUCLEAR MEDICINE, RABIN MEDICAL CENTER, CAMPUS BEILINSON

Introduction: Distant metastases (DM) are seen in a minority of patients with differentiated thyroid carcinoma (DTC) but account for most of its disease-specific mortality. Studies on the long-term outcome of patients with DM are controversial.

Patients / Methods: We retrospectively reviewed medical records of 660 patients with DTC followed at our institution from 1994 to 2004. The Chi-square test and Student’s t test were used for comparison between groups The product-limit estimate method (Kaplan-Meier) was used to estimate survival, and the log-rank test to compare survival curves.

Results: Forty-four patients (6.7%) had DM, with a prevalence of 4.8% for PTC, 21% for FTC and 10% for HCC. Primary near-total thyroidectomy followed by I-131 radiation was performed in 97% of metastatic patients (86% operated on in 1980-2003). The mean age at thyroidectomy was 49±19 years, and the female-to-male ratio was 1.9:1. Distant metastasis occurred synchronously to the primary tumor in 45.5% and after a median follow-up of 9 years in the others. Affected sites were lungs (n=24), bones (n=11), lungs and bones (n=9), brain (n=3), and uterus (n=1). Median duration of follow-up was 12 years (range 1-42) from thyroidectomy and 5.5 years (range 1-24) from diagnosis of distant metastases. The 5- and 10-year survival rates (all causes) after diagnosis of DM were 88% and 77%, respectively. No significant differences in survival curves were found by age, sex, metastasis site, histopathology or interval to DM.

Conclusions: We conclude that complete resection of the thyroid gland at diagnosis and high-dose adjuvant radioactive iodine is associated with improved survival in patients with metastatic DTC.
GESTATIONAL EXPOSURE TO HIGH PERCHLORATE CONCENTRATIONS IN DRINKING WATER DID NOT AFFECT NEONATAL THYROXINE LEVELS

(1) PROF. SACK JOSEPH (2) PROF. AMITAI YONA (3) PROF. WINSTON GARY (2) PROF. WASSER JANICE (4) PROF. LEWIS MATTHEW (5) PROF. GOLDBERGER SHALOM (5) PROF. LEVENTHAL ALEX (6) PROF. ISRAELI AVI

(1) SACKLER SCHOOL OF MEDICINE, TEL AVIV UNIVERSITY (2) DEPARTMENT OF MOTHER CHILD AND ADOLESCENT HEALTH, SACKLER SCHOOL OF MEDICINE, TEL AVIV UNIVERSITY (3) DEPARTMENT OF ENVIRONMENTAL HEALTH, SACKLER SCHOOL OF MEDICINE, TEL AVIV UNIVERSITY (4) HEALTH DISTRICT OFFICE (5) THE PUBLIC HEALTH SERVICE (6) MINISTRY OF HEALTH; ISRAEL

**Introduction:** Knowledge of the effect of various levels of exposure to perchlorate during pregnancy on neonatal thyroxine is critical for its regulation in drinking water. The commonly accepted sequelae to the adverse outcomes of perchlorate is that its inhibition of iodide uptake into the thyroid for fetuses, infants and children leads to lower thyroid hormone levels, which in turn can cause neurodevelopmental impairment. This effect was studied in neonates from Ramat Hasharon, Israel, following exposure to high concentrations of perchlorate in drinking water.

**Patients / Methods:** Thyroxine (T4) values obtained from the National Newborn Screening Program for congenital hypothyroidism were compared between newborns whose mothers resided in suburbs with perchlorate concentrations in drinking water of ≥340 µg/L (Group A, n=88), 42-94 µg/L (Group B, n=209) and 3 µg/L (Group C, n=805). In groups A and B, values of T4 were further compared between newborns whose mothers drank tap water (group A1, B1) and whose mothers drank bottled water during pregnancy (groups A2, B2).

**Results:** Mean ± SD T4 values in groups A, B, and C were 14.1±3.7 µg/dL, 14.0±3.4 µg/dL and 14.1±3.5 µg/dL, respectively (P=NS). None of the newborns had the abnormally low T4 (<7.0 µg/dL). There were no significant differences between T4 values in groups A1 and A2, and between B1 and B2.

**Conclusions:** T4 levels in newborns were not affected by gestational exposure to perchlorate in drinking water, in the range of 42-94 µg/L, and probably ≥340 µg/L. The threshold effect of perchlorate in drinking water in pregnancy on T4 in newborns in this study was ≥42 µg/L. A more liberal regulation of perchlorate in drinking water is suggested. Nevertheless, in addition to the lack of adverse effects of such exposure on the biochemical marker of neonatal thyroid function (T4), adequate evaluation of neurodevelopmental behavior of these children is required to establish a safe threshold of gestational perchlorate exposure.
EFFECT OF TREATMENT WITH RADIOACTIVE I 131 ON SALIVARY ACTIVITY, COMPOSITION AND OXIDATIVE STRESS RELATED PROFILE

(1) DR. ISH-SHALOM SOPHIA (1) DR. DURLECHTER LENA (1) DR. SEGAL ELENA (2) DR. NAGLER RAFAEL

(1) METABOLIC BONE DISEASES UNIT, RAMBAM HEALTH CARE CAMPUS (2) ORAL AND MAXILLOFACIAL SURGERY DEPARTMENT, RAMBAM HEALTH CARE CAMPUS

Introduction: Radioactive I131 treatment is commonly used in patients with thyroid carcinoma after total thyroidectomy, even in the presence of a scarce residual thyroid tissue. Salivary glands are able to take up I131. Aims: To assess salivary composition and oral functioning in radioactive I 131 treated patients following total thyroidectomy, focusing on free radical- related effects and antioxidants and to compare it to patients that were treated with surgery only.

Patients / Methods: Forty consenting, age matched, patients who underwent total thyroidectomy for thyroid carcinoma were 13.6 years) were also enrolled in the current study: 23 patients (aged 50 13.2 years ) were not. In treated with I 131, while the other 17 (aged 46.6 all subjects whole saliva samples were analyzed for antioxidant and biochemical composition and oral complaints monitored.

Results: The salivary flow rates of both groups were similar while that the composition substantially altered. The rate of complaints of dry mouth in the I 131 treated patients was higher (33% vs 18%). Five of the treated patients complained of difficulty in swallowing and 3 of taste disturbances. In the non-treated patients there were no such complaints. The salivary superoxide dismutase enzyme (SOD) and total protein concentrations were significantly reduced in the treated patients by 40% and 25% respectively (P<0.01). Other salivary components were also reduced substantially, either in a nearly significant manner, as the LDH (by 30%) and calcium (by 26%), (P=0.07) or in a statistically not significant manner, including total antioxidant status (by 22%) or albumin, amylase, phosphate and magnesium. The salivary pH of the treated patients was increased from 6.5 to 6.8 (p=0.07).

Conclusions: In spite of the relatively small sample size, the results obtained clearly point at an I 131 dependent damage to the salivary glands. A broad spectrum of compositional alterations may be of a clinical impact. The reduction in the salivary antioxidant status and SOD enzyme leaves the oral cavity less protected against oxidative stress. This is especially important as the administration of I 131 for the treatment of thyroid carcinoma was never reported to be harmful for the salivary glands previously and should be further explored.
Abstract Code: P61

BETTER ALIGNMENT OF FT3 THAN TT3 TO TSH AND FT4 RESULTS

(1) DR. CIDON SHULAMIT (1) DR. STAM TAMAR (1) DR. BECK RUTH (2) DR. NADLER
VARDA (2) DR. SHAINBERG BRACHA

(1)MACCABI HEALTHCARE SERVICES REGIONAL LABORATORY OF NORTH ISRAEL,
HAIFA (2) MACCABI HEALTHCARE SERVICES CENTRAL LABORATORY, REHOVOT

Introduction: The free fraction of triiodothyronine (FT3), which represents only 0.3% of total triiodothyronine (TT3), is considered to be the physiologically active fraction. TT3 assays may produce misleading results in various clinical circumstances including patients with non thyroidal illness (NTI), pregnant women, women on oral contraceptives or estrogen therapy, and patients with inherited abnormal forms or concentrations of serum binding proteins. Advia/Centaur FT3 assay demonstrated high diagnostic performance and robustness to interference in clinical evaluations. The FT3 assay replaced TT3 assay in Maccabi laboratories since 2003. The aim of the current study was to evaluate the alignment of FT3 to TSH and FT4 in comparison to TT3.

Patients / Methods: Patients' results were obtained from Maccabi laboratories database. Results of TSH, FT4, and TT3 or FT3 were collected from two periods: the last quarter of 2002 (when TT3 was assayed) and the last quarter of 2005 (when FT3 was assayed). Patients with TSH and FT4 within the reference ranges were selected and defined as "euthyroid". The rate of "euthyroid" patients with elevated TT3 was compared to those having elevated FT3 in 3 selected groups of adults (age>20): pregnant women, non pregnant women and men. The assays for TSH, FT4, TT3 and FT3 were performed using the Advia Centaur analyzer (Bayer Diagnostics).

Results: Among the "euthyroid" patients in 2002, 76% of the pregnant women (n=368), 16.6% of the non pregnant women (n=6880), and 13% of the men (n=1903) had elevated TT3 (>2.8nmol/L). Among the" euthyroid" patients in 2005, none of the pregnant women (n=558), 0.2% of the non pregnant women (n=7353), and 0.6% of the men (n=2046) had elevated FT3 (>6.5pmol/L).

Conclusions: Our data demonstrates a better alignment of FT3 to TSH and FT4 results than TT3. This observation is expected in conditions where thyroid binding globulin are elevated (pregnancy or oral contraceptives use) .The observation that 13% of the "euthyroid" men had elevated TT3 (2002) and within reference range FT3 (2005) further supports the superiority of the FT3 assay.
Abstract Code: P62

C-REACTIVE PROTEIN IN PATIENTS WITH AUTOIMMUNE THYROID DISEASE

(1) DR. MANSUR OMAR (2) DR. ROTMAN-PIKIELNY PNINA (3) DR. NESS-ABRAMOF ROSANE

(1) DEPARTMENT OF MEDICINE B, SAPIR MEDICAL CENTER (2) DEPARTMENT OF MEDICINE E, SAPIR MEDICAL CENTER (3) ENDOCRINE UNIT, SAPIR MEDICAL CENTER

Introduction: C-reactive protein (CRP), a marker of subclinical inflammation is associated with cardiovascular (CVD) disease in healthy subjects. Hypothyroidism, subclinical or overt has been associated with an increased risk for CVD. It is not clear whether the increased risk for CVD in these patients is due to elevated cholesterol or homocysteine levels, impaired fibrinolysis and moreover, if it may be prevented by thyroxine therapy. It is well known that CRP is increased in patients with inflammatory diseases. There is scarce data concerning CRP levels in patients with autoimmune thyroid disease. CRP levels in these patients potentially may be due to the inflammatory autoimmune process and not a marker of subclinical atherosclerosis. We therefore decided to evaluate CRP levels in patients with autoimmune thyroid disease.

Patients / Methods: Retrospective chart review of patients diagnosed with thyroid disease in an endocrine outpatient clinic. Data extracted from the charts included age, sex, diagnosis, medical therapy, CRP, TSH, FT4, T3T, B12, FA, thyroid peroxidase antibodies (TPO AB) and thyroglobulin antibodies (TG AB). Patients diagnosed with thyroid cancer and pregnant women were not included in the study.

Results: Two hundred and three charts were reviewed. Relevant data concerning diagnosis, TSH, FT4, T3T and CRP levels were found in 154 charts. Eighty eight patients were diagnosed with hypothyroidism, 11 with Graves disease, 20 with multinodular goiter, 10 with toxic nodular goiter, 10 with thyroiditis (lymphocytic) and 15 were normal controls. Log CRP was not statistically different between groups (P= 0.138), while TSH, FT4, T3T, TPOAB and TGAB were significantly different (P<0.01, P<0.01, P<0.06, P<0.01, P<0.01). LCRP levels were similar in the autoimmune group (hashimoto’s thyroiditis and Graves) and nonautoimmune group (Nodular goiter toxic and nontoxic and normal controls) (P= 0.137). LCRP levels were positively correlated with age (r=0.156, P=0.039) but not with TSH, FT4 or T3T.

Conclusions: CRP levels are not elevated in patients with autoimmune thyroid disease compared to patients with multinodular goiter and normal controls. CRP levels were correlated with age but not with TSH, FT4 or T3T levels.
Abstract Code: P63

A RELIABLE FT3 ASSAY FROM BAYER HEALTHCARE

(1) DR. LEVENE LAWRENCE

(1) BAYER HEALTHCARE - TAYCO DIAGNOSTICS

Introduction: Historically, the routine clinical measurement of free tri-iodothyronine (FT3) was plagued with inaccuracies. This test, for the biologically active hormone, was virtually abandoned in favour of T Uptake, Total T3 and equilibrium dialysis methods. The reasons for the failure of the early FT3 tests lie mainly in the assay architecture and in particular the inability of many of these assays to remain uninfluenced by varying concentrations of binding proteins and exogenous competitors, e.g. salicylate.

Patients / Methods: The Bayer HealthCare ADVIA Centaur FT3 assay uses a unique, solid-phase linked T2-bovine gamma globulin analog which competes equally with free T3 in the sample for the labelled monoclonal anti-T3 antibodies.

Results: The Centaur FT3 assay shows negligible interference due to varying concentrations of TBG (up to 80 mg/L) or albumin (up to 75g/L) and is not affected by RF, heterophilic antibodies or therapeutic drugs. For example, low albumin or high NEFA produce a spurious decrease in Total T3. High albumin (as in FDH) or high TBG (as in pregnancy) can produce a spurious increase in Total T3. These changes are less obvious when using a reliable FT3 method. The Centaur FT3 exhibits true linearity on dilution, a laboratory exercise to test anomalies in the free/protein bound distribution. In addition, a good correlation exists between the Bayer FT3 assay and equilibrium dialysis FT3 (the "gold standard"). UK NEQAS and US CAP Quality Assurance data show that the Centaur FT3 displays extremely good precision and is the most reported analyzer for this test. Data generated by Macabi Health Services in Israel comparing Centaur FT3 and Total T3 in sera from children, pregnant and non-pregnant women clearly demonstrate how several "pathological" TT3 samples are actually euthyroid when FT3 is employed as the marker.

Conclusions: Most manufacturers of FT3 kits have greatly improved their assays compared to what was available in the 80's and early 90's. The performance of the Bayer HealthCare kit meets the requirements for a true free T3 assay.
Abstract Code: P64

PREVALENCE OF SUBCLINICAL HYPOTHYROIDISM IN WOMEN WITH TYPE 2 DIABETES

(1) DR. ISHAY AVRAHAM (1) DR. SHERTOCK-SHAHAM ELENA (2) MRS. LAVI IDIT (1) PROF. LUBOSHITZKY RAFAEL

(1) ENDOCRINE INSTITUTE, HAEHEK MEDICAL CENTER, AFULA. (2) DEPARTMENT OF COMMUNITY MEDICINE AND EPIDEMIOLOGY, CARMEL MEDICAL CENTER, HAIFA.

Introduction: Subclinical hypothyroidism (SH) is a common disorder with a prevalence ranging from 4-10% in general population surveys. The association between diabetes and thyroid disorders has long been recognized and the importance of subclinical thyroid dysfunction is in recent years largely accepted. Previous studies reported a prevalence of 4.6-8.6% of SH in diabetes. Nevertheless most published data included both type1 and type 2 diabetes, both genders and are not controlled studies. Thus, we investigate the prevalence of SH in a cohort of women with type 2 diabetes and compared them with a group of non-diabetic women. Relationships between SH, lipid profiles and thyroid autoimmunity status were also examined.

Patients / Methods: 410 women (aged 59.6±10.9 years) with type 2 diabetes and 125 women (aged 51.6±16.2 years) without diabetes were studied. All participants with previous thyroid disease were identified and excluded. SH was defined as TSH level above the upper normal limit (4.6 miU/L) along with normal FT4 level (10-27 pmol/L). Antibodies to thyroperoxydase (anti TPO), thyroglobulin (anti Tg), lipids levels and HbA1c were also measured.

Results: 18% of the diabetic women reported thyroid disease. Among the final sample of 335 patients, the prevalence of SH was 5% vs. 7.1% in the control group (p=0.5). One diabetic woman had overt hypothyroidism, and none overt hyperthyroidism. 3.8% and 8%, respectively in the diabetes and control group had subclinical hyperthyroidism (p=0.06). Both TPO and Tg antibodies were more prevalent in non diabetic women (p<0.001). Positivity for TPO and Tg antibodies was significantly more prevalent in diabetic subjects with SH (p=0.001 and p=0.002, respectively). As a whole, TSH levels were significantly higher in subjects with positive TPO or Tg antibodies than those with negative testing (p=0.01 and p=0.03, respectively). There was no significant association between SH and any serum lipid parameter, even after adjusting for lipid lowering therapy. Among diabetic patients, no significant correlation was observed between TSH and HbA1c.

Conclusions: In women with type 2 diabetes without known thyroid disease, SH is an incidental finding . The routine annual screening of thyroid function recommended in type 1 diabetes is not justified in type 2 diabetes.
Abstract Code: P65

OSTEOPROTEGERIN (OPG) EXERTS DIRECT ANABOLIC EFFECTS ON GROWING CARTILAGE

(1) MRS. GOLDBERG RUTH (2) PROF. KARIN NATAN (3) DR. MAOR GILA

(1) DEPARTMENT OF ANATOMY AND CELL BIOLOGY (2) DEPARTMENT OF IMMUNOLOGY (3) DEPARTMENT OF ANATOMY AND CELL BIOLOGY

Introduction: Joints are very vulnerable to damages caused by injuries, aging and inflammatory diseases. Most of the degenerative effects caused in the joints are attributed to the B Ligand (RANKL)/Osteoprotegerin disturbance in the local Receptor Activated NF (OPG) ratio. The therapeutic effects of OPG have been attributed mainly to its anti-RANKL activity. However, our studies have shown that OPG also acts directly on articular cartilage modulating its development, expressed in proliferation and differentiation of the cartilage cells. Consequently, in the current study, we determined the existence of OPG specific binding sites in cartilage cells, and studied the cellular pathways mediating OPG's activities on chondrocytes.

Patients / Methods: Primary chondrocytes cultures with or, α(MCDC cells) were treated with 20% (v/v) RASF or with IL-1 and TNF without 200ng/ml rOPG. The presence of specific binding sites for OPG was studied by immunoprecipitation (IP) with anti OPG Ab of pre-OPG cross-linked chondrocytes. Effects of OPG on tyrosine phosphorylation of its binding sites were also studied. The relevance of PI3K and MAPK cellular pathways to the protective effects of OPG was determined by following Erk and PKB phosphorylation, and the effects of specifically blocking MAP kinase and PI 3-kinase pathways, using PD98059 and LY294002, respectively and also Genistein, as a phosphotyrosine inhibitor.

Results: Using IP we found a ~150 kDa OPG tyrosine kinase specific binding site. In addition, OPG increased both ERK and AKT phosphorylation. Specific inhibition of MAPK, PI 3-kinase and phospho-tyrosylation, blunted the OPG-induced stimulatory effects on viability (MTT), proliferation (PCNA) and differentiation activities (expression of type II collagen and proteoglycans) of MCDC cells. Mediated by both pathways, OPG also transmodulates the activity of IGFI. We succeeded in transiently transfecting MDCD cells with OPG and in transplanting non-transfected chondrocytes in RA- joints. The transplanted chondrocytes differentiated into new cartilage and replenished the articular lesions, thus serving as cartilage grafting.

Conclusions: Our results indicate that OPG acts as a local growth factor mediated by its tyrosine kinase -specific binding sites. OPG's protective effects on articular cartilage are mediated by MAPK and PI3K cellular pathways. We also found therapeutic effects of primary chondrocytes transplanted into RA-induced damaged joints.
Abstract Code: P66

LESS-CALCEMIC VITAMIN D ANALOGS ENHANCE CREATINE KINASE SPECIFIC ACTIVITY AND MODULATE RESPONSIVENESS TO GONADAL STEROIDS IN THE VASCULATURE

(1) DR. SOMJEN DALIA (2) PROF. POSNER GARY H (1) PROF. STERN NAFTALI

(1) INSTITUTE OF ENDOCRINOLOGY, METABOLISM AND HYPERTENSION, TEL-AVIV SOURASKY MEDICAL CENTER, AND THE SACKLER FACULTY OF MEDICINE, TEL-AVIV UNIVERSITY, TEL-AVIV, ISRAEL (2) DEPARTMENT OF CHEMISTRY, THE JOHNS HOPKINS UNIVERSITY, BALTIMORE, MD. USA.

Introduction: Vitamin D receptors are widely expressed in the cardiovascular system and Vitamin D metabolites exert a variety of vascular biological effects. In this presentation we studied the role of vitamin D on energy metabolism in the rat heart and aorta in vivo.

Patients / Methods: Wistar-derived female or male rats were used at 25 days either intact or after ovariectomy (Ovx) or castration respectively, and treatments started 2 weeks post surgery. Vitamin D depletion was elucidated by vitamin D-deficient diet supplemented with calcium and phosphate and dark growth environment. Rats were injected daily for 1, 2 or 8 weeks with the less calcemic vitamin D analogs CB, JKF or QW and 24 hours after the last analog injection, rats were injected with E2, raloxifene (Ral) or tamoxifen (TAM), both or dihydrotestosterone (DHT). Rat organs were collected for the brain type creatine kinase (CK) measurements and western blot analysis for estradiol receptor a (ERa) 24 hours after the last injection.

Results: In vitamin D- depleted rats CK was lower in aorta (Ao) and left ventricle of the heart (Lv) than in vitamin D-replete rats. Moreover, neither E2 nor DHT, which increases CK in Ao and Lv of intact rats, stimulated CK in vitamin D- depleted rats. Treatment of intact female rats for 2 or 8 weeks with JKF or QW, up regulated the E2-induced response of CK, without affecting enzymic constituent levels. All vitamin D analogs enhanced the in vivo CK response to raloxifene (Ral) and tamoxifen (TAM) in these organs but the inhibitory effect of Ral or TAM on E2-induced CK was lost. The non-calcemic analog CB 1093 induced significantly ERa protein in both Ao and Lv from intact and from Ovx female rats.

Conclusions: Vitamin D modulates cell energy homeostasis in vascular tissues through induction of CK and up regulation of the response and sensitivity of CK to E2 and to SERMs, possibly via an increase in ERa protein. These results corroborate our previous in vitro studies in human vascular cells and provide the first evidence that vitamin D is crucial to maintain normal cell energy reservoir in the vasculature.
CALCITRIOL AND THE NONCALCEMIC POTENT ANALOG QW-1624F2-2, LOWER BLOOD PRESSURE IN THE TSUKUBA HYPERTENSIVE MOUSE

(1) DR. TORDJMAN KAREN (1) DR. SACK JESSICA (1) DR. SOMJEN DALIA (2) PROF. POSNER GARY H. (1) PROF. STERN NAFTALI

(1) INSTITUTE OF ENDOCRINOLOGY, METABOLISM AND HYPERTENSION, TEL AVIV SOURASKY MEDICAL CENTER (2) DEPARTMENT OF CHEMISTRY, JOHNS HOPKINS UNIVERSITY, BALTIMORE, MARYLAND

Introduction: Primarily known for its role in calcium homeostasis, vitamin D is gradually emerging as a pleiotropic hormone involved in various human pathologies such as cancer and autoimmune diseases. Epidemiologic studies have also suggested a relationship between vitamin D levels and blood pressure. Furthermore, it was recently demonstrated that 1,25-dihydroxyvitamin D (calcitriol) downregulates the expression of the renin gene through a vitamin D receptor-dependent mechanism. The purpose of the present study was to test the hypothesis that calcitriol, and its noncalcemic potent analog QW-1624-F2-2, could attenuate the hypertension seen in the Tsukuba Hypertensive Mouse (THM), a transgenic model of hypertension due to overexpression of the human renin-angiotensin system (RAS).

Patients / Methods: Starting one week following weaning, THM mice received intraperitoneal injections of either calcitriol or QW-1624F2-2 (0.5ng/g body weight), every other day for 3 weeks. Control animals received the vehicle only (0.1% ethanol in saline). In a separate experiment, treatment with the analog was initiated in older, frankly hypertensive, 6 weeks old animals. Blood pressure was assessed noninvasively through a computerized tail-cuff system.

Results: Three weeks of calcitriol treatment in 18 THM mice, resulted in significantly lower blood pressure than that measured in 13 control animals. Systolic pressure was 122.8±3.9 mm Hg (P=0.0006) and diastolic pressure 70±1.95 vs. 86.1±3.9 mm Hg (P=0.0017) The blood pressure lowering effect seen in 4 analog-treated mice was at least as pronounced as that of calcitriol. Hence, systolic blood pressure was down to 111.8±4.6 mm Hg (P=0.015 ), and diastolic blood pressure to 68.5±1 (P=0.04). Moreover, just one week of analog treatment of older animals with established hypertension was sufficient to lower the systolic blood pressure from 149.4±7.4 to 127.1±4.4 mm Hg (P=0.03) and the diastolic pressure from 92.8±3.1 to 76.0±3.5 mm Hg (P=0.01).

Conclusions: As renin is the rate limiting step of the RAS, our data support the notion that vitamin D modulates this system in vivo. Still underway, determination of renin levels in these animals should provide a more definite answer. If confirmed, these results suggest a therapeutic potential for calcitriol and particularly for its noncalcemic analogs in the treatment of hypertension and cardiovascular diseases.
CENTRAL INTERLEUKIN-1 RECEPTOR SIGNALING REGulates Bone Growth and Mass

(1) MR. BAJAYO ALON (2) MRS. GOSHEN INBAL (3) MRS. FELDMAN SHARON (4) PROF. CSERNUS VALER (5) PROF. IVERFELDT KERSTIN (3) PROF. SHOHAMI ESTHER (2) PROF. YIRMIA RAZ (1) PROF. BAB ITAI

(1) BONE LABORATORY, THE HEBREW UNIVERSITY OF JERUSALEM (2) PSYCHOLOGY, THE HEBREW UNIVERSITY OF JERUSALEM (3) PHARMACOLOGY, THE HEBREW UNIVERSITY OF JERUSALEM (4) ANATOMY, UNIVERSITY OF PECS MEDICAL SCHOOL (5) NEUROCHEMISTRY AND NEUROTOXICOLOGY, STOCKHOLM UNIVERSITY

Introduction: The pro-inflammatory cytokine IL-1, acting via the hypothalamic IL-1 receptor type 1 (IL-1RI), activates pathways known to suppress bone formation such as the HPA axis and the sympathetic nervous system. In addition, peripheral IL-1 has been implicated as a mediator of the bone loss induced by sex hormone depletion and TNF.

Patients / Methods: Mice: Male, 5- and 15-week old (i) IL-1RI deficient mice (IL-1rKO); and (ii) transgenic mice expressing the human IL-1 receptor antagonist targeted to the central nervous system using the murine glial fibrillary acidic protein promoter (IL-1raTG mice). Micro-computed tomography (µCT): Whole femora and bodies of the third lumbar vertebra (L3) were subjected to qualitative and quantitative analysis by µCT. Scans were performed at a 20 µm resolution in all three spatial dimensions. Histomorphometry: Undeplasticized, unstained longitudinal 5 µm sections were used for dynamic measurements. To identify osteoclasts, consecutive sections were deplasticized and stained for tartrate resistant acid phosphatase (TRAP) using an acid phosphatase kit and counterstained with Mayer's hematoxylin. A conventional nomenclature was used to determine the dynamic parameters and osteoclast counts. RT-PCR analysis was performed on RNA extracted from hypothalami and isolated trabecular bone sites using routine procedures. Serum corticosterone and testosterone measurements were performed using radioimmunoassay.

Results: Normal mouse bone expressed low levels of endogenous IL-1ra. Unexpectedly, the IL-1rKO mice exhibited a low bone mass (LBM) phenotype, including impairment of bone growth. The IL-1raTG mice demonstrated a similar phenotype, implying that central IL-1RI silencing induces the LBM in both instances. The bone remodeling analysis indicates that the main process leading to the LBM in both IL-1rKO and IL-1raTG is characterized mainly by doubling the osteoclast number. Either genetic modification does not decrease testosterone or increase corticosterone serum levels suggesting that systems other than the gonads and HPA axis mediate the central IL-1RI effect on bone.

Conclusions: Low levels of IL-1 are present in both brain and bone. Our results suggest that its skeletal activity is normally suppressed by IL-1ra, whereas central IL-1 produces a constant physiologic stimulation of IL-1RI signaling. The outburst of osteoclastogenesis in its absence suggests that normally it controls bone growth and mass by tonically restraining bone resorption.
Introduction: Locally produced growth factors in the bovine mammary gland are believed to mediate the adipocyte-epithelial interactions and alter the actions of several steroid and peptide hormones during lactation. Prolactin, a hormone secreted from the pituitary acidophil cells has an important role in the morphological and biochemical differentiation of the epithelial cells during pregnancy, and regulates milk protein synthesis during lactation. Leptin, a protein hormone produced and secreted predominantly by white adipose tissue, has a critical role in the regulation and coordination of energy metabolism. The identification of leptin in the milk of several mammals, including humans, led us to investigate its presence and regulatory effect during lactation in the cow mammary gland. In the present study we demonstrate that prolactin regulates the magnitude of lactation via leptin at the mammary gland level.

Patients / Methods: Mammary tissue was obtained from cows in the slaughterhouse. Explants/primary culture were prepared and cultured at 37°C, 5% CO2 in medium M-199/DMEM-F12 supplemented with Insulin alone or Insulin and Prolactin and leptin. Primary culture was incubated with or without fat explants and different concentrations of prolactin. At the end of the experiments the explants and medium were harvested and analyzed.

Results: In our study we found that leptin is secreted from the mammary fat pad, and is regulated by prolactin. Using leptin antagonist, we found that it abolished the effect that leptin had on prolactin action in the mammary gland explants. We examined the expression of alpha-casein gene in the primary culture of mammary epithelial cells with or without mammary fat explants. We found that epithelial cell in culture express alpha-casein, but in the presence of fat explants they rich their full capacity of alpha-casein gene expression potential.

Conclusions: All of the above led us to the conclusion that prolactin alter leptin secretion from the mammary fat pad and adipocytes. Since the level of leptin secretion is in a direct relation to the size and amount of the adipocytes, the secretion of local leptin in response to prolactin will determine the magnitude of the lactation potential.
GIANT PROLACTINOMAS IN ELEVEN MEN – EFFICACY OF LONG-TERM CABERGOLINE TREATMENT

(1) DR. SHIMON ILAN (2) PROF. HADANI MOSHE

(1) ENDOCRINOLOGY, RABIN MEDICAL CENTER (2) NEUROSURGERY, SHEBA MEDICAL CENTER

**Introduction:** Giant prolactinoma is a rare large and invasive hormone-secreting pituitary tumor larger than 4 cm in diameter with massive extrasellar extension, associated with very high levels of serum prolactin.

**Patients / Methods:** From 1997 to 2004 eleven men affected by giant prolactinomas were referred to our department. They were 24-52 yr old (mean, 38.1 yr) at diagnosis and all had a pituitary adenoma diameter of at least 4 cm. Two patients had previously undergone a transsphenoidal tumor resection because of visual injury, with poor surgical results.

**Results:** Altogether eight men had visual field defects at presentation, five had erectile dysfunction or libido failure, and two complained of headaches. Before treatment, serum prolactin was extremely high in all patients (range, 2,638-55,033 ng/ml, mean, 15,060 ng/ml). Two patients had secondary hypoadrenalism and hypothyroidism that required replacement. Cabergoline treatment was started at a low dose (0.5 mg, three times a week), and progressively increased to 2 mg/week in 3 patients, 3 mg/week (one patient), 3.5 mg/week (4 men) and 7 mg/week in one patient, without any side effects. Prolactin normalization was achieved in 7 men after 1-84 months of treatment (mean, 21.6 months), in two others prolactin is still marginally elevated (after 30-48 months), and two patients currently have elevated prolactin 2-3 times above normal. Visual fields returned to normal in 3 (out of 8 affected men), improved in 4, and one men showed no improvement. A significant reduction in tumor volume was observed, usually beginning from 6 months of treatment. All men had low testosterone levels before treatment, 7 of them normalized testosterone during cabergoline treatment. Three of the four men with persistent low testosterone have not normalized yet the prolactin levels.

**Conclusions:** Cabergoline was effective and well-tolerated in all eleven male patients affected by giant prolactinomas. Cabergoline should be the first-line therapy for aggressive prolactinomas, even when visual field defects are presented.
Abstract Code: P71

MEDULLARY THYROID CARCINOMA: PRELIMINARY RESULTS FROM A RETROSPECTIVE ANALYSIS IN A PATIENT COHORT TREATED AT A TERTIARY CARE CENTER IN ISRAEL

(1) DR. GROZINSKY-GLASBERG SIMONA (1) DR. SHIMON ILAN (1) DR. BENBASSAT CARLOS (2) PROF. FEINMESSER RAFAEL (1) DR. LAPIDOT MORDECHAI

(1) ENDOCRINE INSTITUTE, BEILINSON CAMPUS, RABIN MEDICAL CENTER, PETAH-TIKVA (2) DEPARTMENT OF ENT, BEILINSON CAMPUS, RABIN MEDICAL CENTER, PETAH-TIKVA

Introduction: Medullary thyroid carcinoma (MTC) represents 5-10% of all thyroid cancers and presents as sporadic or familial forms. We aimed to assess the clinical and biochemical behavior of MTC, the relation between calcitonin and extent of the disease, and differences between sporadic and familial forms.

Patients / Methods: We conducted a retrospective analysis of 55 patients (mean age 46.2 yr, female 53%) with MTC followed at our clinic. Preliminary data for 30 patients (sporadic 19, familial 11) are presented for median follow-up of 8 yr (range, 3 mo-36 yr).

Results: At presentation the disease was defined as “local” in 22 patients (74%), “regional” in 6 patients (20%) and disseminated in 2 patients (6%). Presenting findings were thyroid enlargement 14 patients (47%), elevated CEA 3 patients (10%) and familial history 8 patients (27%). Two patients (6%) presented with a paraneoplastic syndrome (e.g., Cushing’s syndrome). Calcitonin was elevated at diagnosis in 12/13 patients (92%) and CEA in 9/13 patients (69%). RET mutations were identified in 8/16 patients studied. Familial cases were younger (p<0.001) with more multicentric tumors (p<0.02) compared to sporadic cases. Total thyroidectomy was performed in 29/30 patients (6 completion, 2 preventive). Adrenalectomy due to pheochromocytoma was performed in 3 patients and pituitary excision for Cushing’s syndrome in one (revealed later to be ectopic). Long-term remission after primary therapy was achieved in 15 patients, based on normalization of calcitonin and radiology. Persistent disease was associated with larger tumors (31±15 mm vs 19±13 mm, p=0.04), regional dissemination (77% vs 0%, p<0.001) and higher calcitonin (7020 ng/ml, range 400-66,000 vs 723 ng/l, range 42-6,300). Remission rate was higher in sporadic than familial disease (66% vs 33%). Doubling time of calcitonin was >2 years in 8 patients (62%) and < 6 months in 2 patients (15%); in three patients, levels were persistently high, regardless of initial therapy (23%). Analysis of survival is ongoing.

Conclusions: MTC is a disease of variable prognosis with a non-negligible number of patients having persistent or recurrent disease. In our series, persistent disease was associated with larger tumors and disseminated disease at diagnosis.
Abstract Code: P72

CLINICAL AND BIOCHEMICAL STABILIZATION OF NELSON’S SYNDROME WITH LONG-TERM CABERGOLINE TREATMENT

(1) DR. SHRAGA-SLUTZKY ILANA (2) DR. WEINSHTEIN RUTH (3) DR. SHIMON ILAN

(1) INSTITUTE OF ENDOCRINOLOGY, RABIN MEDICAL CENTER, PETAH-TIQWA (2) INSTITUTE OF ENDOCRINOLOGY, RABIN MEDICAL CENTER, PETAH-TIQWAR, (3) INSTITUTE OF ENDOCRINOLOGY, RABIN MEDICAL CENTER, PETAH-TIQWA

Introduction: We report on the results of long-term (4 years) treatment with the dopamine agonist cabergoline in a patient affected by Nelson’s syndrome occurring after bilateral adrenalectomy for Cushing disease. A female patient underwent bilateral adrenalectomy and sellar irradiation because of Cushing disease when she was 29 years old. Thereafter, she received glucocorticoid replacement therapy. At the age of 55 she was referred to us with signs of Nelson’s syndrome - skin and mucosal hyperpigmentation accompanied by elevated ACTH plasma levels (315-350 pmol/l; normal, 2.2-17 pmol/l). Magnetic resonance imaging (MRI) of the pituitary demonstrated sellar enlargement with a 15 mm macroadenoma. The patient was initially treated with bromocriptine (10 mg/day) that did not affect ACTH level or the tumor mass. Because of visual loss, transsphenoidal surgery was performed with partial excision of the adenoma and chiasmal decompression, following by radiosurgery. The surgery achieved only temporary normalization of plasma ACTH level, that soon increased to previous levels. Therefore, cabergoline treatment (1.5 mg/week) was initiated.

Patients / Methods: Case-report

Results: Under cabergoline treatment ACTH levels dramatically decreased from 1050 to 132 pmol/l and remained in this range, accompanied by clinical improvement. Repeated MRI demonstrated stable residual pituitary tumor.

Conclusions: This case demonstrated that long-term cabergoline treatment may be efficient in patients with Nelson’s syndrome.
DID THE SMALL-BODIED HOMININS FROM FLORES (INDONESIA) SUFFER FROM LARON SYNDROME?

(1) PROF. LARON ZVI (2) DR. KORNREICH LIORA (3) PROF. HERSHKOWITZ ISRAEL

(1) ENDOCRINOLOGY AND DIABETES RESEARCH UNIT, SCHNEIDER CHILDREN’S MEDICAL CENTER, SACKLER FACULTY OF MEDICINE, TEL-AVIV UNIVERSITY (2) DEPARTMENT OF RADIOLOGY, SCHNEIDER CHILDREN’S MEDICAL CENTER, SACKLER FACULTY OF MEDICINE, TEL-AVIV UNIVERSITY (3) DEPARTMENT OF ANATOMY AND ANTHROPOLOGY, SACKLER FACULTY OF MEDICINE, TEL-AVIV UNIVERSITY

Introduction: In 2004 an Australian-Indonesian scientific team announced the discovery of a new species within the genus Homo on the island of Flores, namely Homo floresiensis (1). The findings agitated the scientific world: not just that there was another species coexisting with us until very recent (ca. 18,000 yrs BC), but that these people had diminutive body (106 cm in height) with an extremely small head and brain. The origin of the species was unclear and it was postulated that it was a dwarfed descendant of Javanese H. erectus. Further archeological excavations revealed more findings and permitted the characterization of this species (2).

Methods / Patients: Thorough examination of the unique morphological features of Homo floresiensis shows great resemblance with adult patients possessing Laron syndrome (LS; primary GH insensitivity OMIM#262500) (3), to wit: similar stature (LS height range 95-136 cm); small brain (in LS head circumference is below 2 SD or more from the norm); similar facial features (short face, rounded supraorbital rims with pronounced supraorbital ridges and absent of frontal sinuses); absence of chin (which is considered part of the characteristic acromicria typical for GH/IGF-1 deficiency); abnormal body proportion both in regard to upper/lower segment ratio and upper/lower limb proportion; long bones having a thickened cortex relative to their length; limited degree of humeral torsion, among others.

Conclusions: As Homo floresiensis replicates most of the diagnostic features of Laron syndrome patients, it can be assumed that the findings from the island of Flores represents a local highly inbred Homo sapiens (or Homo erectus) population in whom a mutation for the GH-R had occurred. LS has been diagnosed in populations residing or originating from the Mediterranean, Mid-Eastern and Asian regions and thus it can be speculated that the syndrome may have its origins in Flores.

ENDOCRINE EFFECTS OF VALPROATE IN GIRLS WITH EPILEPSY

(1) DR. DE VRIES LIAT (2) DR. GOLDBERG-STERN HADAASSA (1) DR. KARASIK ANNA (3) DR. LANDAU ZOHAR (1) PROF. PHILLIP MOSHE

(1) INSTITUTE FOR ENDOCRINOLOGY AND DIABETES, SCHNEIDER'S CHILDREN MEDICAL CENTER OF ISRAEL (2) NEUROLOGY DEPARTEMENT, SCHNEIDER'S HOSPITAL (3) PEDIATRIC DEPARTMENT, WOLFSON MEDICAL CENTER

Introduction: Valproate therapy has been associated with weight gain, hyperandrogenism, polycystic ovaries and negative effect on growth and pubertal development. There is no conclusive evidence that epilepsy or valproate therapy causes adverse endocrinological effect. Previous studies compared epileptic girls treated with valproate to girls treated by other anti epileptic drugs and to healthy controls. We compared non-treated to valproate treated girls with epilepsy.

Patients / Methods: We investigated the effect of epilepsy and/or valproate on physical growth, weight gain, pubertal development and hormonal status of girls with epilepsy between ages 6-20 years. Eighty-eight girls with epilepsy were evaluated: 45 treated with valproate for 3.5 years (range 1-9.5) and 43 without treatment (14 prepubertal, 11 pubertal and 61 postpubertal). Anthropometric measurements, puberty staging, waist-hip ratio, and bioelectrical impedance analysis were assessed, as well as fasting insulin levels, androgens, sex hormone-binding globulin and thyroid functions. All patients had transabdominal pelvic ultrasound.

Results: Treated girls had higher levels of TSH and lower free T4 levels (all within normal range) compared to non-treated girls. Postpubertal treated girls showed higher testosterone levels compared with controls (1.69 vs. 0.88 nmol/L). However, no significant differences were found between the groups in biochemical, hormonal, anthropometric or ultrasound parameters, when comparing the two groups as a whole, or according to pubertal stage. BMI-SDS was 0.75 in the treated group, and 0.63 in the non-treated, with obesity in 16.3% and 15.5% respectively. No difference was found between the two groups in rate of menses irregularities, hirsutism or acne. No correlation was found between duration of treatment or dosage and BMI-SDS, height SDS or androgens levels.

Conclusions: Long-term treatment with valproate in girls is associated with increased testosterone levels in the postpubertal period, without clinical hyperandrogenism, and no increase in BMI-SDS. Careful endocrine observation of girls taking valproate for epilepsy, is therefore recommended especially in postpubertal girls.
Abstract Code: P75

NUTRITION-INDUCED CATCH UP GROWTH CAUSES AN INCREASE IN THE EXPRESSION OF GHR, IGF-IR AND PTHRP WITHIN THE GROWTH PLATE

(1) DR. GAT-YABLONSKI GALIA (1) MRS. ABRAHAN EFRAT (1) MRS. SHTAIF Biana (1)
PROF. PHILLIP MOSHE

(1) INSTITUTE FOR ENDOCRINOLGY AND DIABETES, NATIONAL CENTER FOR CHILDHOOD DIABETES, SCHNEIDER CHILDREN'S MEDICAL CENTER

Introduction: Catch-up growth is defined as a period of accelerated growth that occurs after correction of a temporary growth attenuation. The aim of the present study was to follow immediate changes in the growth plate during the process of nutrition-induced catch-up growth using a mouse model.

Patients / Methods: Twenty-one-day-old ICR mice were housed individually and fed measured amounts of regular chow, as follows: Group 1: caloric restriction (60% of normal amount of chow) for the duration of the experiment; Group 2: caloric restriction (60%) for 10 days, followed by normal amount of chow ad libitum; Group 3: fed ad libitum throughout the experiment. Animals were sacrificed on day 10, 12 or 17 of the experiment, and the tibias were measured and processed for immunohistochemistry and in situ hybridization.

Results: The calorie-restricted animals did not gain weight, and tibial length, EGP width, and proliferation and differentiation of the growth plate chondrocytes were greatly reduced compared to controls. When food was replenished, catch-up growth occurred very rapidly: weight increased instantaneously, followed by a dramatic increase in EGP width. The correction in tibial length occurred more slowly. Immunohistochemistry study showed dramatic effects on chondrocyte proliferation and differentiation, as well as on the expression of GHR, IGF-IR, PTHrP and Ihh. The expression of GHR and PTHrP, which was reduced, increased at 2 days of catch up growth, whereas an increase in IGF-IR and Ihh expression was detected only after 7 days.

Conclusions: Caloric restriction of 40% is sufficient to lead to growth attenuation in mice. During catch-up growth, the expression of GHR and PTHrP increases quickly, but Ihh and IGF-IR normalization is slower. The reduction in GHR and IGF-IR expression is suggestive of resistance at the level of the EGP, and strengthens the importance of using combined measures to improve growth.
STUNTING OF GROWTH IN FEMALE ADOLESCENTS DIAGNOSED WITH ANOREXIA NERVOSA

(1) DR. MODAN DALIT (2) DR. YAROSLAVSKY AMIT (2) MRS. KOCHAVI B BRIGIT (2) DR. STEIN DANIEL

(1) PEDIATRIC ENDOCRINOLGY UNIT, THE EDMOND AND LILY SAFRA CHILDREN'S HOSPITAL, TEL-HASHOMER (2) THE PEDIATRIC PSYCHOSOMATIC DEPARTMENT, THE EDMOND AND LILY SAFRA CHILDREN'S HOSPITAL, TEL-HASHOMER

Introduction: Growth retardation is a known complication of anorexia nervosa (AN). However, there are yet no consistent findings concerning the influence of AN on final height. The aim of the present study was to assess these phenomena in female adolescent inpatients diagnosed with AN.

Patients / Methods: We reviewed the medical charts of all female adolescent AN patients (n=211) hospitalized in the Pediatric Psychosomatic Department at the Chaim Sheba Medical Center from 1/1/1987 to 31/12/99. Height and weight measurements were assessed at admission and thereafter routinely during hospitalization and follow-up. Final height was defined as the height achieved at 18 years of age. Standard deviation scores (SDS), or z scores, for height and weight in our sample were calculated on the basis of data on growth in a reference population from the US National Center for Health Statistics.

Results: Mean body mass index increased significantly from admission to discharge. The height SDS of the at admission (-.285±1.0), discharge (-.250±1.1), and follow-up (-.230±1.00) were all significantly lower than the expected height in normal adolescents. Assessment of catch-up growth during the inpatient weight restoration phase and ambulatory follow-up revealed greater improvement in AN patients with primary, rather than secondary amenorrhea.

Conclusions: Our findings suggest that linear growth retardation is a prominent feature in female adolescent AN patients. Weight restoration, particularly in the case of primary amenorrhea, may be associated with significant catch-up growth, but complete catch-up growth may not be achieved.
LOW ADIPONECTIN MAY LINK INTRAUTERINE GROWTH RETARDATION WITH ADULTHOOD METABOLIC SYNDROME-A STUDY IN DISCORDANT TWINS

(1) DR. MAZAKI-TOVI SHALI  (2) MRS. PARIENTE CLARA  (1) DR. HEMI RINA  (1) PROF. SCHIFF EYAL  (1) DR. SIVAN EYAL  (2) DR. KANETY HANNAH

(1) DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, SHEBA MEDICAL CENTER, TEL-HASHOMER, ISRAEL (2) INSTITUTE OF ENDOCRINOLOGY, SHEBA MEDICAL CENTER, TEL-HASHOMER, ISRAEL

Introduction: Intrauterine growth retardation (IUGR) is associated with an increased risk of developing insulin resistance, type 2 diabetes and cardiovascular disease. It is hypothesized that fetal adaptation to an adverse intrauterine environment determines an altered programming of endocrine pathways, including insulin resistance (Barker hypothesis). Adiponectin, a novel adipocyte-derived hormone, is negatively correlated with insulin resistance and cardiovascular disease in adults. In addition, recent studies found an association between this hormone and fetal growth. Hence, adiponectin may be a potential link between IUGR and future development of the metabolic syndrome. Severely discordant twins are a unique model which enables the investigation of aberrant fetal growth and intrauterine effect on fetal metabolism. Thus, the aim of this study was to evaluate adiponectin and leptin levels in twins with and without growth discordance.

Patients / Methods: Cord blood samples were obtained from 29 couples of bichorionic, biamniotic twins. Of them, 14 were with severe growth discordancy (range 25-52%) - in all of them the smaller twin was severely growth restricted (mean percentage 3.5 ± 0.9) and the co-twin was appropriate for gestational age (AGA) (mean percentage 35.7 ± 18.2). The additional 15 control twins were accordant (range 0.8-13%, both AGA) and matched for gestational age and maternal age, weight, gravity and parity.

Results: A positive correlation was found between adiponectin and both birth weight and gestational age in all twins. Mean adiponectin was significantly lower in IUGR fetuses of the discordant twins as compared with their co-twin (27.1 vs. 38.2 µg/ml, p < 0.03). Leptin levels were also lower in the IUGR twins but the difference did not reach a statistical significance (2.5 vs. 3.7 ng/ml, p < 0.09). Conversely, in the control group there were no differences in mean adiponectin (29.6 vs. 32.0 µg/ml) and leptin levels (3.1 vs. 3.6 ng/ml).

Conclusions: This paradigm of discordance twins may point to a key role of adiponectin in intrauterine fetal growth. Moreover, since adiponectin has profound insulin-sensitizing and anti-atherogenic effects, the low adiponectin levels found in the IUGR twins may provide a link between abnormal growth in utero and the high prevalence of adulthood metabolic syndrome in IUGR newborns.
INTERACTIONS BETWEEN BRCA1 AND ESTROGEN RECEPTOR IN REGULATION OF IGF-I RECEPTOR GENE EXPRESSION

(1) MRS. MAOR SHARON (2) DR. MAYER DORIS (3) DR. YARDEN RONIT (4) PROF. PAPA MOAHE (5) PROF. WERNER HAIM

(1) DEPARTMENT OF HUMAN MOLECULAR GENETICS AND BIOCHEMISTRY, SACKLER SCHOOL OF MEDICINE, TEL AVIV UNIVERSITY, RAMAT AVIV, TEL AVIV, ISRAEL (2) GERMAN CANCER RESEARCH INSTITUTE, HEIDELBERG, GERMANY (3) DEPARTMENT OF ONCOLOGICAL SURGERY, SHEBA MEDICAL CENTER, TEL HASHOMER, ISRAEL. (4) DEPARTMENT OF ONCOLOGICAL SURGERY, SHEBA MEDICAL CENTER, TEL HASHOMER, ISRAEL. (5) DEPARTMENT OF HUMAN MOLECULAR GENETICS AND BIOCHEMISTRY, SACKLER SCHOOL OF MEDICINE, TEL AVIV UNIVERSITY, RAMAT AVIV, TEL AVIV, ISRAEL

Introduction: The Insulin-like growth factors, IGF-I and IGF-II, are a family of mitogenic polypeptides with important role in growth and differentiation. The biological actions of the IGFs are mediated by the IGF-I receptor (IGF-IR), a cell-surface tyrosine kinase whose activation by serum IGF-I seems to be a key step in breast cancer initiation. Inactivating germ line mutations within the BRCA1 gene account for a large proportion of families with inherited breast and/or ovarian cancer. We have recently shown that the IGF-IR promoter is negatively regulated by BRCA1. In addition, evidence accumulated indicating that estrogens stimulate the IGF axis, and also increase BRCA1 expression which, in turn, inhibits estrogen receptor (ER)-mediated signaling pathways involved in cell proliferation. The aim of our study was to examine the transcriptional mechanisms involved in regulation of IGF-IR gene expression by ER and BRCA1.

Patients / Methods: Transient cotransfections were performed in breast cancer-derived MCF-7 and isogenic ER-negative C4 cells using an IGF-IR promoter-luciferase reporter plasmid, together with a BRCA1 expression vector. To examine the involvement of zinc-finger nuclear proteins in the transactivating effect of estrogens, Chromatin Immunoprecipitation experiments were performed in the MCF7 cell line, using an Sp1 antibody, along with the Sp-family members binding inhibitor MithramycinA.

Results: The results obtained indicated that basal IGF-IR promoter activity was 5.6-fold higher in MCF7 than in C4 cells. Estradiol treatment activated IGF-IR promoter in MCF7, but not in C4 cells. Furthermore, the estrogen responsive region in the IGF-IR promoter was mapped to a fragment located between nucleotides –40 and –476 in the 5’ flanking region. In contrast, BRCA1 decreased both basal and estradiol-stimulated IGF-IR promoter activity by 30 %. Furthermore, at least part of the estrogen activities were mediated through the Sp1 transcription factor as seen in ChIP and MithramycinA experiments.

Conclusions: Our results indicate that IGF-IR gene transcription involves complex interactions between BRCA1, Sp1 and ER. Mutation and/or inactivation of BRCA1 may lead to aberrant expression of the IGF-IR gene, an event usually associated with breast cancer initiation and/or progression.
THE WT1 WILMS' TUMOR SUPPRESSOR GENE PRODUCT IS A DOWNSTREAM TARGET FOR IGF-I ACTION IN PC12 CELLS

(1) DR. SARFSTEIN RIVE (2) PROF. WERNER HAIM

(1) DEPARTMENT OF HUMAN MOLECULAR GENETICS AND BIOCHEMISTRY (2) DEPARTMENT OF HUMAN MOLECULAR GENETICS AND BIOCHEMISTRY

Introduction: The biological actions of the insulin-like growth factors, IGF-I and IGF-II, are mediated by the IGF-I receptor (IGF-IR), a transmembrane heterotetramer linked to the RAS-RAF-MAPK and PI3-PKB/AKT signal transduction cascades. The Wilms' tumor suppressor gene, encodes a zinc finger transcription factor, WT1, that has been implicated in various cellular processes including proliferation, differentiation, and apoptosis. WT1 is also involved in the molecular pathology of several malignancies, including prostate, breast, and brain tumors. In light of the important roles of IGF-I and WT1 in neuronal activity, we examined the hypothesis that at least part of the effects of IGF-I may be mediated via modulation of WT1 gene expression and action.

Patients / Methods: Pheochromocytoma-derived PC12 cells were lysed and electrophoresed through 10% SDS-PAGE, followed by blotting of the proteins onto nitrocellulose membranes. Blots were incubated with rabbit polyclonal anti-human IGF-IR, followed by a horseradish peroxidase-conjugated secondary antibody. In addition, blots were probed with antibodies against WT1, PARP, Bax, pERK1/2, total ERK1/2, pAkt, total Akt and tubulin. Total RNA was prepared from IGF-I-treated and non-treated cultures and WT1 mRNA levels were measured by semiquantitative PCR. Transient cotransfections were performed using a WT1-galactosidase plasmid, β-promoter-luciferase reporter plasmid, along with of a using the LipofectamineTM 2000 transfection reagent.

Results: The results obtained demonstrated that IGF-I had a differential effect on WT1 expression in neurally-derived PC12 cells. Thus, at 1-2 h IGF-I enhanced WT1 levels, whereas at 24 h IGF-I reduced WT1 levels. This effect was mediated through the MAPK and PI3-Kinase signaling pathways, as shown by the ability of the specific inhibitors UO126 and LY294002 to abrogate IGF-I action. Moreover, RT-PCR assays demonstrated corresponding changes in WT1mRNA levels. Furthermore, results of transient transfections assays showed that the IGF-I effect was associated with corresponding changes in WT1 promoter activity. In addition, high WT1 levels were associated with enhanced apoptosis whereas low WT1 levels inhibited apoptosis, as demonstrated by Poly ADP ribose polymerase (PARP) cleavage, Bax expression and Annexin V-FITC staining.

Conclusions: In summary, our results show that the wt1 gene is a novel target for IGF-I action in neurally-derived cells.
Abstract Code: P80

THE EFFECT OF DIFFERENT ANTIDEPRESSANTS ON THE IGF SYSTEM IN THE RAT BRAIN: RELEVANCE TO BRAIN GROWTH

(1) MRS. NOVAK NURIT (1) DR. TALER MICHAL (2) PROF. WEIZMAN RONIT (1) MR. ZILBERMAN REFAEL (1) PROF. WEIZMAN ABRAHAM (1) DR. GIL-AD IRIT

(1) LAB OF BIOLOGICAL PSYCHIATRY, FELSENSTEIN MEDICAL RESEARCH CENTER, RABIN MEDICAL CENTER AND SACKLER FACULTY OF MEDICINE, TEL-AVIV UNIVERSITY (2) 2BRULL COMMUNITY MENTAL HEALTH CENTER, SACKLER FACULTY OF MEDICINE, TEL-AVIV UNIVERSITY

Introduction: Recent evidence suggests that depression is associated with decreased neural plasticity and disrupted information processing. Antidepressants were found to facilitate synaptic connections and neuroplasticity, as evidenced by their induced stimulation of trophic factors such as BDNF. Insulin-like-growth-factors (IGFs) are potent neurotrophic and survival factors in the brain, through activation of specific receptors. Previous studies demonstrated that IGF-I plays a role in brain growth, neural plasticity and cognition. IGF-I is also known to be a growth hormone dependent hormone, and therefore is regulated by different neurotransmitters such as norepinephrine (NE) and serotonin (5-HT). The aim of our study was to evaluate the differential effect on the central IGF-I system, of various antidepressants such as fluoxetine, clomipramine, reboxetine and duloxetine, which act differently on the 5-HT and the NE neurotransmitters in the brain.

Patients / Methods: The antidepressants (15mg/kg) were orally administered daily (3 days) to Wistar male rats. In another experiment animals were given (po) the drugs (15mg/kg x 3/week) for 3 weeks. 24hr after last drug administration, rats were sacrificed by CO2 cell. Brains were removed and frontal cortex, hippocampus and hypothalamus were dissected. IGF-I receptor (IGF-IR) expression was determined by western blot analysis. IGF-I mRNA levels were assessed by semi quantitative PCR reaction.

Results: In the frontal cortex, fluoxetine, duloxetine and reboxetine increased IGF-IR levels after both experiments. In the hippocampus, duloxetine elevated IGF-IR levels after 3 days, and clomipramine increased the levels of IGF-IR after 21 days. In the hypothalamus, 3 days administration of clomipramine, duloxetine and fluoxetine, decreased the IGF-IR levels, while, 21 days of clomipramine and reboxetine treatment increased IGF-IR levels. Fluoxetine, reboxetine and clomipramine decreased mRNA levels of IGF-I in the hippocampus, and increased IGF-I mRNA levels in the frontal cortex after 3 days treatment.

Conclusions: Conclusions: Different antidepressants affect the IGF-I system in the brain. Most antidepressants increased the levels of IGF-IR and IGF-I mRNA levels in the frontal cortex, while a differential effect was noted in the hippocampus and the hypothalamus. These data suggest that some antidepressants mainly 5-HT and NE modulators can affect brain growth and possibly cognition by upregulating the IGF-I system in the cortex.
RECOMBINANT CHICKEN GROWTH HORMONE (CHGH) AND ITS CHGH G119R ANALOGUE - A PUTATIVE ANTAGONIST

(1) DR. PACZOSKA-ELIASIEWICZ ELISABETH (2) MRS. SALOMON GILI (3) MR. REICHER SHAY (4) DR. GUSSAKOVSKY EUGENE (5) DR. HRABIA ANNA (6) PROF. GERTLER ARIEH

(1) DEPARTMENT OF ANIMAL PHYSIOLOGY, UNIVERSITY OF AGRICULTURE, KRAKOW, POLAND (2) FACULTY OF AGRICULTURAL, FOOD AND ENVIRONMENTAL QUALITY SCIENCES, THE HEBREW UNIVERSITY OF JERUSALEM, ISRAEL (3) FACULTY OF AGRICULTURAL, FOOD AND ENVIRONMENTAL QUALITY SCIENCES, THE HEBREW UNIVERSITY OF JERUSALEM, ISRAEL (4) DEPARTMENT OF BOTANY, UNIVERSITY OF MANITOBA, WINNIPEG, CANADA (5) DEPARTMENT OF ANIMAL PHYSIOLOGY, UNIVERSITY OF AGRICULTURE, KRAKOW, POLAND (6) FACULTY OF AGRICULTURAL, FOOD AND ENVIRONMENTAL QUALITY SCIENCES, THE HEBREW UNIVERSITY OF JERUSALEM, ISRAEL

**Abstract**

**Introduction:** Chicken pituitary growth hormone (chGH) is involved in both somatogenic and metabolic activity. In order to provide tools for in vivo research a method for large-scale preparation of recombinant chGH and its putative antagonist (G119R mutein) was developed.

**Patients / Methods:** Synthetic cDNA of chicken GH (chGH) and its G119R mutein was synthesized according to published sequence but optimized for expression in E. coli. The respective cDNAs were inserted into pMON expression vector and transformed into Mon 105 E. coli strain. The proteins expressed upon induction with nalidixic acid were found almost entirely in the insoluble inclusion bodies (IBs). The IBs were isolated, the proteins solubilized in 4.5 M urea, at pH 11.3 in presence of cysteine, refolded and purified to homogeneity by anion-exchange chromatography on Q-Sepharose. The overall yields were 400 to 500 mg from 5 liters of fermentation.

**Results:** Both recombinant proteins were > 98% pure as evidenced by SDS-PAGE and contained at least 95% monomers as documented by gel-filtration chromatography on a Superdex75 column under not denaturing conditions. Circular dichroism analysis revealed that both proteins have identical secondary structure characteristic of cytokines, namely > 50% of alpha helix content. Chicken GH was capable of forming a 1:2 complex with recombinant oGHR-ECD (oGH receptor extracellular domain) though its affinity to ECD as determined by RRA was 11-fold lower than that of ovine GH (oGH). Correspondingly, its bioactivity in vitro, assessed using PDF-P1 3B9 cells stably transfected with rabbit GHR was 20-25 fold lower, whereas chGH G119R mutant did not bind to oGHR-ECD and was devoid any biological activity in PDF-P1 3B9 cells. In contrast, in binding experiments carried-out using chicken liver membranes both oGH and chGH showed similar EC50 values in competition with 125I-oGH. These EC50 and were 5-9 fold lower than that of G119R mutein.

**Conclusions:** Our results provide a method for large-scale preparation of chGH and its G119R mutein, emphasize the importance of species specificity and indicate the possibility of antagonistic activity of chGH G119R.
GLUCAGON STIMULATION TEST TO ASSESS GROWTH HORMONE RESERVE – CAN WE SHORTEN THE TEST?

(1) DR. STRICH DAVID (2) DR. TERESPOLSKY DOV (3) MRS. RIFTIN SIMA (4) MRS. RUBACHA NIRA (5) MRS. VAKNIN TSIP (6) DR. ANCSELOVITS BOAZ

(1) ENDOCRINOLOGY AND DIABETES UNIT, PEDIATRIC SPECIALISTS CLINIC, JERUSALEM, ISRAEL. (2) ENDOCRINOLOGY AND DIABETES UNIT, PEDIATRIC SPECIALISTS CLINIC, JERUSALEM, ISRAEL. (3) ENDOCRINOLOGY AND DIABETES UNIT, PEDIATRIC SPECIALISTS CLINIC, JERUSALEM, ISRAEL. (4) ENDOCRINOLOGY AND DIABETES UNIT, PEDIATRIC SPECIALISTS CLINIC, JERUSALEM, ISRAEL. (5) ENDOCRINOLOGY AND DIABETES UNIT, PEDIATRIC SPECIALISTS CLINIC, JERUSALEM, ISRAEL. (6) ENDOCRINOLOGY AND DIABETES UNIT, PEDIATRIC SPECIALISTS CLINIC, JERUSALEM, ISRAEL.

Introduction: GLUCAGON stimulation test (GST) is a standard tool to determine the growth hormone (GH) reserve in the pediatric population. The aim of our work was to find the most relevant period to sample blood to determine growth hormone levels during GST, and the predictive value of each time in establishing GH deficiency.

Methods/ Patients: A retrospective evaluation of 225 consecutive patients (mean age 9.75±4.0, range 0.2-18.8 yr) with short stature who underwent a GST to assess GH reserve. GH concentration was measured at baseline and at 30, 60, 90, 120, 150 and 180 min during the GST. Patients in whom the GH peak was lower than 10 mcg/l underwent a second stimulation test (CLONIDINE or INRINEARINE). If both peaks were lower than 10, or the child had another pituitary hormone deficiency, he was defined as GH deficient. We analyzed the predictive value of each peak time to determine the most relevant time period to measure GH.

Results:

<table>
<thead>
<tr>
<th>TIME(min)</th>
<th>GST 10&lt;GH mcg/l samples</th>
<th>GST 10&lt;GH mcg/l</th>
<th>GST 10&gt;GH mcg/l</th>
<th>test nd2 10&lt;GH mcg/l</th>
<th>test nd2 10&gt;GH mcg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>30</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>60</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>90</td>
<td>25</td>
<td>32</td>
<td>11</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>120</td>
<td>42</td>
<td>34</td>
<td>22</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>150</td>
<td>10</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>180</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

Conclusions: Those who have a peak GH <10 mcg/l at 150 or 180 min have a high risk (9/10) to be classified as GH deficient, and those who have a peak at 90 or 120 min are at the lowest risk (13/33). If the 180 min sample is eliminated 1/1 child who only achieve GH>10 mcg/l at 180 min, would require a second test. If the 150 min sample is eliminated, 5/15 children who had a GH peak >10 mcg/l only at 150 min would require a second test. In conclusion there is no need to determine GH levels at 180 min, since only 1/225 children would have require a second test. We do not recommend elimination of the 150 min sample since 5/225 would require a second test.
Abstract Code: P83

INSULIN ASSAYS-ARE THEY APPROPRIATE FOR ESTIMATION OF INSULIN RESISTANCE?

(1) DR. NADLER VARDÁ (1) MRS. BEN-BECHAR ESTER (1) MRS. NEHIMOVSKI JENNY (1) DR. SHAINBERG BRACHA

(1) MACCABI HEALTHCARE SERVICES, CENTRAL LABORATORY, REHOVOT

Introduction: Serum insulin concentration is often tested for estimation of insulin resistance in patients with polycystic ovary syndrome, obesity etc. Over 500 publications refer to the option of using fasting insulin and glucose concentrations to evaluate insulin resistance using models as Homeostatic Model Assessment, Quantitative Insulin Sensitivity Check Index or glucose to insulin ratio. However, variations in insulin results measured by different test methods might influence the assessment of insulin resistance. Maccabi laboratories have recently replaced the RIA insulin test with a higher specificity kit. We investigated the effect of replacing insulin testing method on the measured concentrations and on the clinical interpretation.

Patients / Methods: a. Data were obtained from Maccabi's Central Laboratory database. We compared measured insulin concentrations from 2 time periods: Jun-Dec 2003 (assayed using DiaSorin RIA kit) and Jun-Dec 2004 (assayed by DPC Immulite 2000 kit). b. Insulin concentrations were measured in 36 routine samples using 5 different commercial kits.

Results: Replacing insulin testing method with a higher specificity kit caused a significant decrease in measured insulin concentrations: the mean value decreased from 31 mIU/L in 2003 to 11 mIU/L in 2004. Insulin mean concentrations of each patient group (adult females, adult males, children, obese adult and diabetics) decreased in 2004 in comparison to 2003, but nevertheless, the highest results were found for both time periods among obese and diabetic patients, and the lowest results were found in young women. The decrease in results is probably due to the different testing method. To verify this assumption we measured insulin in 36 routine samples using 5 different commercial kits. The results show substantial variability of insulin measurements with the various kits. This variability is strange in light of the facts that all 5 kits are calibrated against the same WHO 66/304 reference material, and the reference ranges cited by the 5 manufacturers are similar.

Conclusions: No "golden standard" for insulin assay exists and it is difficult to determine which assay is the most proper for insulin concentration measurement. Nevertheless, the clinician should bear in mind the variability caused by different kit methodologies when assessing insulin resistance using fasting insulin concentrations.
PREPARATION OF LEPTIN ANTAGONISTS BY SITE-DIRECTED MUTAGENESIS OF HUMAN, OVINE, RAT AND MOUSE LEPTIN'S SITE III: IMPLICATIONS ON BLOCKING UNDESIRED LEPTIN ACTION IN VIVO

(1) MRS. NIV-SPECTOR LEONORA (2) MRS. SALOMON GILI (3) MRS. GONEN-BERGER DANA (4) DR. CALLEBAUT ISABELLE (5) MRS. DJIANE JEAN (6) PROF. GERTLER ARIEH


Introduction: Several recent reports indicate that leptin exhibit undesired effects in auto immune diseases, heart failure and possibly in several types of cancers. Therefore preparations of reagents capable of abolishing leptin action is timely. As no structural information of the 3D structure of leptin receptor (LEPR) is available the model of interleukin 6 (IL6) was applied. In that model, a hexameric complex is formed gradually, first by IL6 molecule which interacts with the IL6R-alpha, then with gp130 forming an inactive trimer which subsequently dimerizes forming an active hexamer, whose formation is achieved due to interaction of IL6 bound in one trimer (through its site III) with the immunoglobulin domain (IGD) of gp130 of the other trimer.

Methods / Patients: We have identified the putative leptin's binding site III by modeling LEPR, on the basis of its alignment with gp130, and fitting leptin on IL6 in the IL6/gp130 complex as leptin's amino acids 39-42 (LDFI), which are preserved in all leptin species. To verify this hypothesis and to test its generality we have prepared and purified to homogeneity human, ovine, rat and mouse triple (L39A/D40A/F41A) and quadruple (L39A/D40A/F41A/I42A, human and ovine only) leptin muteins.

Results: All six muteins had typical cytokine secondary structure, acted as true antagonists, namely they interacted with LEPR with affinity similar to the wild type hormone (as evidenced by SRP and RRA), were devoid of biological activity in several leptin-responsive bioassays and specifically inhibited leptin action in vitro and in vivo. Those muteins can be prepared in gram amounts and thus serve as a novel tool of studying leptin function in vitro and in vivo. To prolong their life in circulation some muteins were pegylated using 40 kDa polyethylene glycol. Although pegylation decreases the affinity, increasing circulation half-life can recompensate this deficit so pegylated antagonists are expected to be more potent in vivo.

Conclusions: Antagonizing leptin has been suggested as a possible therapy in auto-immune diseases and heart failure. Thus leptin antagonists not only offer a novel tool to elucidate the role of leptin in mammalian physiology and pathology but have a potential of becoming a drug.
Abstract Code: P85

DIABETIC STATE MODULATES THE EFFECTS OF ESTRADIOL-17B BUT NOT THAT OF RALOXIFENE ON CREATINE KINASE SPECIFIC ACTIVITY IN RAT ORGANS

(1) DR. SOMJEN DALIA (2) MRS. SHEN MICHAL (1) PROF. STERN NAFTALI (2) DR. MIRSKY NITSA

(1) INSTITUTE OF ENDOCRINOLEGY, METABOLISM AND HYPERTENSION, TEL-AVIV SOURASKY MEDICAL CENTER AND THE SACKLER FACULTY OF MEDICINE, TEL-AVIV UNIVERSITY; TEL-AVIV 64239, ISRAEL (2) FACULTY OF SCIENCE, HAIFA UNIVERSITY HAR- HACARMEL, HAIFA 31905, ISRAEL

Introduction: Diabetes mellitus increases the risk for CVD in women resulting in increased oxidative stress, higher free radical concentration in the organs, and reduced activity of antioxidant defense enzymes. There is evidence suggesting beneficial effects of estrogen on decreasing lipid peroxidation, atherosclerotic processes and cardiovascular diseases, diabetes negates most estrogen protective effects, with increased risk for cardiovascular events. Also the skeletal protective effects of estrogens are not discernable in diabetic women. In the present study we examined the in vivo effects of estradiol-17b (E2), on creatine kinase (CK) specific activity, in rat organs from healthy and diabetic female rats.

Patients / Methods: Sprague Dawley female rats at the age of 5 weeks were injected subcutaneously with a single dose of Streptozotocin. Rats were used either as intact or 2 weeks post ovariectomy (OVX). E2 or raloxifene (Ral) were injected at different doses for 24 h, followed by harvesting the different organs indicated for creatine kinase specific activity (CK) assay.

Results: Healthy or diabetic female rats were injected with either E2 or vehicle, and CK in different organs [Left ventricle of heart (Lv), uterus (Ut), aorta (Ao), para uterine adipose tissue (Ad), epiphyseal cartilage (Ep) and diaphyseal bone (Di)] from healthy animals was assayed. CK in different organs was stimulated with increased doses of E2. Age-matched diabetic female rats exhibited a remarkable decreased response to E2 in all organs examined except in Ut. However, the response to Ral was not affected in diabetic rats. Similar results on the effects of E2 and Ral in the different organs were observed also in OVX rats, healthy or diabetic.

Conclusions: These results support our previous in vitro findings, demonstrating that hyperglycemia decreases CK response to E2 but not to Ral in cultured human vascular and bone cells. In summary, diabetes mellitus decreases CK response to E2 in skeletal and vascular tissues, whereas the response to Ral was not attenuated by diabetes. The decreased response to E2 detected in organs derived from diabetic rats might be due to changes in nuclear and/or membrane estrogen receptors and /or other genomic and non-genomic pathways, as was shown in vitro models.
Abstract Code: P86

LOSARTAN REDUCED KIDNEY ANGIOTENSIN II LEVELS IN THE STREPTOZOTOCIN-DIABETIC RAT

(1) DR. ERMAN ARIE (1) DR. GENKIN INNA (1) DR. MILO GAI (1) DR. VAN DIJK DAVID (1) MRS. DAVID ISKRA (1) PROF. GAFTER UZI

(1) RABIN MEDICAL CENTER, DEPARTMENT OF NEPHROLOGY AND HYPERTENSION

Introduction: The renin-angiotensin system has been implicated in the pathogenesis of diabetic nephropathy. There is increasing clinical and experimental evidence that angiotensin converting enzyme (ACE) inhibitors or angiotensin II (Ang II) subtype AT1 receptor blockers diminish proteinuria and retard progressive glomerulosclerosis. The aim of the study was to investigate the effects of losartan and enalapril on the intrarenal Ang II and cortical ACE activity in the diabetic rat.

Patients / Methods: Eighty eight male Wistar rats were allocated to 5 groups:1- Control, nondiabetic, rats (n=20), 2- Control rats receiving losartan (80mg/kg/day, n=10), 3- Diabetic rats (streptozotocin 55mg/kg, n=20), 4- Diabetic rats as above receiving losartan (80mg/kg/day, n=18), 5- Diabetic rats as above receiving enalapril (20mg/kg/day, n=20). Losartan and enalapril were dissolved in tap water. The study lasted 21 days. At the end, the kidneys were rapidly removed and weighted. The right kidney was homogenized in cold methanol and the left kidney was kept in cold saline. Serum and renal cortex ACE activity was determined by radioassay. Plasma and kidney angiotensin II were determined after a methanol-extraction procedure with a radioimmunoassay.

Results: Urinary protein excretion (UPE), kidney weight (KW) and plasma (PAII) and kidney angiotensin II (KAI) increased significantly in untreated diabetic rats compared to controls (UPE-25.2±8.6 vs. 14.2±4.3 mg/24h, KW- 2.39±0.33 vs. 2.03±0.17g, PAII-427±190 vs. 294±141 pmol/L, KAI-197±118 vs 139±57 fmol/g KW , P<0.05). Administration of losartan for 21 days prevented proteinuria and the increase in kidney weight (17.6±6.0 mg/24h, 2.16±0.2g, respectively), increased PAII (759±368 pmol/L) and reduced kidney ACE activity and KAI compared to untreated diabetic rats (KACE- 23±14 vs. 12±9 pmol/mg/min, KAI-197±118 vs. 26±10 fmol/g KW, P<0.001). Losartan administration to control rats reduced significantly kidney angiotensin II compared to controls (68±20 vs. 139±57 fmol/g KW, P<0.001, respectively). Enalapril inhibited renal cortex ACE activity and reduced to a lesser extent kidney angiotensin II.

Conclusions: Losartan profoundly reduced kidney Ang II in diabetic rats. This may be due to its inhibitory effect on renal cortex ACE activity and probable inhibition of uptake of Ang II from the circulation. Thus, the renoprotective effect of losartan is, in part, related to the reduction in kidney angiotensin II levels.
A COMBINATION OF 1,25-DIHYDROXYVITAMIN D3 AND SODIUM VALPROATE IS HIGHLY EFFECTIVE IN SUPPRESSION OF PROSTATE CANCER CELL LINE (PC3) GROWTH

(1) DR. GAVRILOV VLADIMIR (1) MR. STEINER MICHAEL (1) PROF. SHANY SHRAGA

(1) CLINICAL BIOCHEMISTRY, BEN GURION UNIVERSITY, BEER SHEVA

**Introduction:** 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) the active metabolite of vitamin D3, is possessing an anticancer activity. However, its use in medical practice is restricted because of hypercalcemic effect. Co-treatment of reduced doses of 1,25(OH)2D3 and other anticancer compound may result in an effective and well tolerable therapy. In the present in-vitro study we investigated the anti-prostate cancer effect of combined treatment by 1,25(OH)2D3 and sodium valproate.

**Patients / Methods:** Androgen resistant prostate cancer cell line (PC3) was grown in RPMI-1640 medium. The PC3 cells were treated for 4 days with 1,25(OH)2D3 (1x10-7M), or sodium valproate (1mM), or both. The effects of these treatments on PC3 cells proliferation, cell cycle and apoptosis, were evaluated by crystal violet test, propidium iodide method and APOPercentage Assay kit of Biocolor Assays firm, respectively. An unpaired t-test was used for statistical analysis.

**Results:** Treating PC3 cells with 1,25(OH)2D3 or sodium valproate alone, decreased cells growth by 27% (p<0.001) and 44% (p<0.001), respectively, while a simultaneous use of both drugs resulted in maximal suppression (65%) of PC3 cells growth (p<0.001, as compared to control and single drugs treatments). The cell cycle analysis showed that treatments with single drugs caused a decrease in PC3 cells transition from G1 to S phase. However, the combined treatment resulted in not only the decreased transition from G1 to S phase, but this phenomenon was accompanied by a pre-G1 peak, characteristic for apoptosis. Direct analysis of the pro-apoptotic effects (APOPercentage Assay), showed that the combined treatment with both drugs increased apoptosis by 2.6-fold, while sodium valproate alone caused only 1.6-fold increase, and 1,25(OH)2D3 alone did not change this parameter. Thus, these results showed that the simultaneous use of 1,25(OH)2D3 and sodium valproate suppresses PC3 cells proliferation by decreased cells transition from G1 to S phase and by notable increase of apoptosis.

**Conclusions:** The present results support our hypothesis that the co-treatment by 1,25(OH)2D3 and sodium valproate is superior in inhibiting prostate cancer cells growth, than a single drug treatment. Further elaboration of such combined treatments may lead to the development of new protocols for the treatment of prostate cancer patients.
SEX HORMONES AND VITAMIN D ANALOGS MODULATE THE EXPRESSION OF 1A-HYDROXYLASE 25-HYDROXY VITAMIN D IN HUMAN MALIGNANT CELLS

(1) DR. SOMJEN DALLA (1) DR. KATZBURG SARA (2) MRS. FRYDMAN VERONICA (3) PROF. POSNER GARY H. (1) DR. LIMOR RONA (1) MRS. SHARON ORLY (4) DR. KOHEN FORTUNE (1) PROF. STERN NAFTALI

(1) INSTITUTE OF ENDOCRINOLOGY, METABOLISM AND HYPERTENSION, TEL AVIV SOURASKY MEDICAL CENTER AND SACKLER FACULTY OF MEDICINE, TEL AVIV UNIVERSITY, TEL AVIV, ISRAEL (2) DEPARTMENT OF CHEMICAL SERVICES, THE WEIZMANN INSTITUTE OF SCIENCE, REHOVOT, ISRAEL (3) DEPARTMENT OF BIOLOGICAL REGULATION, THE WEIZMANN INSTITUTE OF SCIENCE, REHOVOT, ISRAEL (4) DEPARTMENT OF CHEMISTRY, THE JOHNS HOPKINS UNIVERSITY, BALTIMORE, MD. USA.

**Introduction:** 1a- hydroxylase 25- hydroxy vitamin D (1-OHD3) mRNA is expressed in various cancerous cell types including human colon, ovary and prostate. It was further shown that 1,25(OH)2D3 (1,25) inhibits cell proliferation in different cancer cells. To date, no studies on the hormonal modulation on the expression of 1-OHD3 mRNA in cancer cells derived from colon, ovarian, prostate and adrenal have been reported. This study addresses these questions.

**Patients / Methods:** Four cancer cell lines: adrenal (H295R), colon (320D), ovarian (A2870) and prostate (C4-24) cells were used throughout. The relative 1-OHD3 mRNA expression in these cells was determined by real time PCR. The effect of various hormones [E2, dihydrotestosterone (DHT)], phytoestrogen [biochanin A (BA)] and its carboxy derivative (cBA), and vitamin D derivative [JK1624 F2-2 (JKF)] on 1-OHD3 mRNAs expression in these cells was assessed.

**Results:** All four human cancer cell lines expressed 1-OHD3 mRNA, which was differentially modulated in a cell- and hormone- specific manner. E2 increased the expression of 1-OHD3 mRNA in colon cancer cells, inhibited its expression in prostate and adrenal cancer and had no effect in ovarian cancer. DHT stimulated 1-OHD3 mRNA expression in colon and ovarian cancer cells, inhibited its expression in prostate cancer and had no effect in adrenal cancer. cBA increased 1-OHD3 in all cancers except prostate, whereas BA, from which cBA is derived, decreased 1-OHD3 in all cancers. Finally, the vitamin D analog JFK increased 1-OHD3 expression in adrenal and ovarian cancer but reduced 1-OHD3 mRNA in colon and prostate cancer.

**Conclusions:** Several human cancer cell lines of different tissue sources express mRNA for 1-OHD3 , which is uniquely modulated in a cell- and hormone- specific manner by sex steroids, phytoestrogens and vitamin D analogs. This might form the basis for the use of ancillary cancer type-specific anti-tumor therapy via the induction of 1-OHD3, leading to the synthesis of 1,25, which is an established inhibitor of cell proliferation.
SYNERGISTIC ACTIVATION OF ANTIOXIDANT RESPONSE ELEMENT BY 1,25-DIHYDROXYVITAMIN D3 AND CARNOSIC ACID IN DIFFERENTIATING LEUKEMIC CELLS

(1) MRS. BOBILEV IRENE (1) MRS. ROSSOVA VICTORIA (1) PROF. LEVY JOSEPH (1) PROF. SHARONI YOAV (1) DR. DANILENKO MICHAEL

(1) CLINICAL BIOCHEMISTRY, FACULTY OF HEALTH SCIENCES, BEN-GURION UNIVERSITY AND SOROKA MEDICAL

Introduction: Physiologically active form of vitamin D(3), 1,25-dihydroxyvitamin D(3) (VD) has demonstrated anti-cancer activity, but its clinical use is precluded by hypercalcemia. We have previously shown that carnosic acid (CA), a polyphenolic diterpene derived from the plant Rosmarinus officinalis (rosemary) enhanced differentiation and antiproliferative action of low, nontoxic concentrations of VD in myeloid leukemia cells (HL60 and U937). Since CA is a powerful antioxidant we were interested in determining whether antioxidant response element (ARE) is involved in these synergistic effects. ARE is found in promoters of genes encoding phase II detoxifying enzymes, e.g., gamma-glutamylcysteine synthetase (GCS) and NAD(P)H:quinone oxidoreductase (NQO1), which play an important role in protection against a variety of carcinogens.

Patients / Methods: To assess ARE transactivation, U937 cells were transiently transfected with NQO1-ARE or GCS-ARE luciferase reporter plasmids followed by treatment with CA (up to 10 microM), VD (1 nM and 100 nM) or the combination of low concentrations of these agents. Induction of NQO1 protein was revealed by Western blotting. The involvement of ARE in the induction of HL-60 cell differentiation was investigated by the measurement of the levels of CD11b and CD14 myeloid differentiation markers following treatment with NQO1-ARE decoy oligonucleotide.

Results: CA alone induced a strong concentration-dependent ARE transactivation in both types of the reporter constructs. The effect of CA was comparable with that induced by the classic ARE inducer, tert-butylhydroquinon. VD alone even at a high concentration (100 nM) had almost no effect. However, it synergistically enhanced the activity of CA in this system. Furthermore, a substantial induction of NQO1 protein observed in non-transfected U937 cells treated with increasing concentrations of CA. As determined by the expression of CD11b and CD14, ARE decoy treatment substantially reduced potentiation of VD-induced differentiation by CA, strongly suggesting the involvement of ARE in differentiation enhancement by CA.

Conclusions: Our results suggest that plant polyphenols potentiate VD-induced myeloid differentiation of leukemic cells via activation of the ARE system. These data may present a mechanistic basis for the development of combinatorial, differentiation-based approaches to chemotherapy and chemoprevention of myeloid leukemia using VD compounds and plant polyphenols.
Abstract Code: P90

CAROTENOID DERIVATIVES MEDIATES ACTIVATION OF THE ANTIOXIDANT RESPONSE ELEMENT TRANSCRIPTION SYSTEM

(1) MRS. SALMAN HAGAR (1) PROF. LEVY JOSEPH (1) PROF. SHARONI YOAV

(1) FACULTY OF HEALTH SCIENCES, BEN-GURION UNIVERSITY OF THE NEGEV AND SOROKA MEDICAL CENTER OF KUPAT HOLIM, BEER-SHEVA, ISRAEL

Introduction: Activation of the transcription factor Nrf2 regulates expression of phase II enzymes which encode for proteins that protect against damage of electrophiles and reactive oxygen intermediates. This is a major cellular strategy for reducing the risk of cancer, inflammation and chronic degenerative diseases. Many phase II genes are regulated by the antioxidant response elements (ARE) which are targets of the transcription factor Nrf2. Under un-stimulated conditions, Nrf2 is bound to its partner, Keap1, which represses Nrf2 activity. Inducers of this system are diversified in their chemical structure, however, they share some common properties: all are chemically reactive and nearly all are electrophiles which react with Keap1 to disturb its inhibitory activity on Nrf2. Hydrophobic carotenoids such as lycopene, which lack any electrophilic group, were recently found by us to be potent activators of the ARE transcription system. Our aim is to demonstrate that activation of the Nrf2/ARE transcription system by carotenoids is mediated by their oxidation and cleaving products.

Methods/ Patients: We fractionated partially oxidized lycopene into unidentified hydrophilic derivatives which are extracted by ethanol and the intact carotenoid which is not soluble in ethanol, and tested ARE activation in T47D mammary cancer cells. Then carotenoid cleaving enzymes were overexpressed in order to examine if the products of these enzymes activate ARE. To validate this approach, we transiently overexpressed beta-carotene dioxygenase I, which cleaves at the center of beta-carotene, producing retinal that is oxidized to retinoic acid, thus transactivates retinoic-acid response element (RARE).

Results: The ethanolic extract activated ARE with similar potency to the unfractionated lycopene whereas the intact carotenoid had no effect. As expected, transactivation of RARE was higher in dioxygenase I overexpressing cells whereas no change was found in ARE transactivation. Transient overexpression of the carotenoid cleaving enzyme, beta-carotene dioxygenase II which cleaves carotenoids at eccentric positions producing apo-carotenals, increased ARE transactivation by lycopene and beta-carotene.

Conclusions: Eccentric cleavage products of lycopene and beta-carotene are the active mediators in the activation of the Nrf2/ARE transcription system by these carotenoids. This activation most likely occurs by the electrophilic groups in these products which might form adducts with key enzymes such as Keap1.
Abstract Code: P91

COOPERATIVE ANTICANCER EFFECTS OF VITAMIN D3 DERIVATIVES AND PLANT POLYPHENOLIC ANTIOXIDANTS IN A SYNGENEIC MOUSE MODEL OF MYELOID LEUKEMIA

(1) MRS. SHARABANI HAGAR (1) MRS. SHABTAY AYELET (1) MR. BARVISH ZE’EV (1) MR. STEINER MICHAEL (1) PROF. LEVY JOSEPH (1) PROF. SHARONI YOAV (1) DR. DANILENKO MICHAEL

(1) DEPARTMENT OF CLINICAL BIOCHEMISTRY, BEN-GURION UNIVERSITY OF THE NEGEV, BEER-SHEVA, ISRAEL.

Introduction: 1alpha,25-dihydroxyvitamin D3 (1,25D3) is a potential anticancer agent. However, it has a marked toxicity at pharmacologically active doses. Using a syngeneic mouse model of myeloid leukemia, we found here that the antiproliferative effects of low, non-toxic doses of 1,25D3 or its low-calcemic analog can be substantially enhanced by plant polyphenolic antioxidants in both cell culture and animal studies. Furthermore, we show that this enhancing effect is associated with cell cycle arrest and reduction in the intracellular levels of reactive oxygen species (ROS).

Patients / Methods: Proliferation of murine myelomonocytic leukemia cells (WEHI-3B D-) was determined by Coulter counting. Cell viability was detected by trypan blue exclusion assay. Cell cycle progression and apoptosis (annexin V binding) were measured by FACS analysis. Protein levels were determined by Western blotting. In vivo tumorigenicity assay was performed in Balb/c mice inoculated intraperitoneally with WEHI-3B D- cells.

Results: When added alone, 1,25D3, its analog, 1,25-dihydroxy-20,16-ene-5,6-trans-cholecalciferol (Ro25-4020) and polyphenols (carnosic acid, crude rosemary extract, curcumin, and silibinin) inhibited cell proliferation in vitro. When combined at low concentrations, polyphenols and vitamin D derivatives markedly synergized in growth inhibition. This was accompanied by G1 cell cycle arrest and elevation of the cell cycle inhibitory proteins (p21Cip1 and p27Kip1). No significant induction of apoptosis was observed, as there was no increase in either annexin V binding to cells or the sub-G1 cell population under these conditions. The antiproliferative effect of vitamin D derivative/polyphenol combinations was associated with the cooperative antioxidant action, i.e. reduction in the intracellular ROS levels. Intraperitoneal (i.p.) inoculation of WEHI-3B D- cells in mice resulted in the development of solid tumors on the abdominal wall. Treatment with Ro25-4020 or 1,25D3 (i.p., 3 times a week) combined with either carnosic acid-rich rosemary extract or purified silibinin (mixed with food) resulted in a delay in tumor formation and an enhanced (at least an additive) reduction in tumor size.

Conclusions: These results indicate that vitamin D3 derivatives and plant polyphenols cooperate in the anti-leukemic effect not only in cell culture but also in the animal model without toxicity. Thus, these combinations may be used as an alternative to conventional cytotoxic chemotherapy of myeloid leukemias.
Various hormones and growth factors are implicated in progression of prostate cancer (PCa), but their role and underlying molecular mechanism(s) remain poorly understood. We recently reported that the full length and truncated isoforms of human growth hormone (GH) receptor (GHR) mRNA are differentially expressed in androgen-sensitive LNCaP and androgen-insensitive PC3 and DU145 human PCa cell lines, as well as in benign prostatic hyperplasia and prostate adenocarcinoma patient tissues. We also reported that in LNCaP cells, GH activated the JAK2/STAT5, MAPK (ERK1/2) and PI3K/AKT/PKB pathways and induced a transient, rapid, striking increase in androgen receptor (AR) levels, followed by a slower reduction, with only modest parallel changes in serine-phosphorylated AR.

Methods/ Patients analysis), induced by starvation medium (intrinsic pathway) or by tumor necrosis factor α (extrinsic pathway) and also the signaling pathways activated by leptin.

Results: Surprisingly, GH (1-1000ng/ml; 24-72h) caused a clear pro-apoptotic effect, seen as a major, dose-dependent increase (up to 10-fold) in cleaved (inactivated) poly-(ADP-ribose)-polymerase (cPARP89), an enzyme normally responsible for DNA repair and downstream substrate of the apoptotic effector caspase-3. More recently we studied the effects of leptin, another cytokine, in the PCa cell lines. Leptin rapidly (5-10min) activated JAK2-STAT3 and MAPK pathways in PC3 and DU145 cells; overexpression of genes for full-length leptin receptor (LRb), JAK2 and kinase negative (KN) mutant of HER2 (epidermal growth factor receptor family member), increased the effects of Leptin on JAK2 phosphorylation in all cells, but more so in AR-positive LNCaP and PC3/AR cells. Interestingly, leptin induced rapid, JAK2-mediated transactivation of KN-HER2 in LRb/JAK2-overexpressing PCa cells, also greater in AR-positive cells. In addition, leptin induced a longer term biphasic, 3-6h activation of KN-HER2 and MAPK, returning to baseline by ~24h.

Conclusions: The GH- and leptin-induced activation of common signaling pathways, the GH effects on AR protein and on apoptosis and the regulation of GHR in PCa patient tissues, suggest that the possible interaction between GH and leptin, most likely in concert with other hormones and growth factors, may play an important role in progression of human prostate cancer.
SP-1 PARTICIPATES IN MEDIATION OF INSULIN-INDUCED TRANSCRIPTION OF PKC DELTA

(1) MRS. HOROVITZ-FRIED MIRIAM (1) MR. JACOB AVRAHAM (2) PROF. COOPER DENISE R. (1) PROF. SAMPSON SANFORD R.

(1) FACULTY OF LIFE SCIENCES, BAR-ILAN UNIVERSITY, ISRAEL (2) DEPARTMENT OF BIOCHEMISTRY, UNIV. OF SOUTH FLORIDA, USA

Introduction: We have shown that Protein Kinase C (PKC) delta is essential for insulin-induced glucose transport in skeletal muscle. Insulin rapidly induces tyrosine phosphorylation and physical association of PKC delta with insulin receptor and stimulates PKC activity. We recently reported in several in vivo and in vitro models of delta skeletal muscle that insulin stimulation increases PKC delta RNA expression and PKC delta protein levels within 5 min. These effects were blocked by inhibitors of either translation or transcription. The purpose of this study was to identify the transcription factor involved in rapid PKC delta gene transcription. One potential candidate transcription factors is SP-1, a ubiquitous transcription factor involved in regulation of target genes participating in specific signaling pathways, and is utilized by insulin for induction of gene transcription.

Patients / Methods: Studies were done on the L6 rat skeletal muscle cell line. PKC delta protein levels were determined by Western blotting techniques, and PKC delta RNA levels were determined by RT-PCR. Association between SP-1 and the PKC delta promoter was analyzed by the chromatin IP assay.

Results: Insulin rapidly stimulated SP-1 phosphorylation and increased SP-1 levels in the nuclear fraction of L6 myotubes. Inhibition of SP-1, either pharmacologically or by suppression of SP-1 by RNAi, nearly completely abrogated insulin-induced increase in PKC delta promoter activity. In addition, SP-1 inhibition blocked insulin-induced increases in both PKC delta RNA expression and PKC delta protein levels. We also found that insulin increases association of SP-1 with the promoter region of PKC delta. Insulin had no effect on nuclear NFκB levels, and inhibition of NFκB was without effect on insulin-induced increases in PKC delta RNA and protein levels.

Conclusions: We conclude that insulin rapidly stimulates SP-1, which mediates the ability of this hormone to induce the rapid transcription of a major target gene utilized in the insulin signaling cascade. This work was supported by the Russell Berrie Foundation and D-Cure, Diabetes Care in Israel.
INSULIN REGULATES PROTEIN KINASE C EPSILON ACTIVITY AND LEVELS IN L6 SKELETAL MUSCLE CELLS

(1) INBAR AYA (1) MRS. HOROVITZ-FRIED MIRIAM (1) MRS. CIPOK MICHAL (1) PROF. BRODIE CHAYA (1) PROF. SAMPSON SANFORD R.

(1) FACULTY OF LIFE SCIENCES, BAR-ILAN UNIVERSITY, ISRAEL

Introduction: Protein Kinase C (PKC) is a family of serine-threonine kinases involved in numerous cell processes. In skeletal muscle, the activity and levels of expression of certain PKC isoforms, such as alpha and delta, are regulated by insulin stimulation. While PKC epsilon is expressed in skeletal muscle, little is known about its regulation and role in insulin signaling in this tissue. It was suggested that PKC epsilon down regulates insulin signaling, but studies on mechanisms of activation and expression of this isoform in response to insulin have not been reported. We investigated the effects of insulin on PKC epsilon activity and its abundance in the L6 rat skeletal muscle cell line, a recognized model for the study of insulin effects.

Patients / Methods: Experiments were done on differentiated L6 skeletal muscle cells. PKC epsilon protein levels were determined by Western blotting, and RNA levels were determined by RT PCR. PKC activity was measured on PKC epsilon immunoprecipitated from lysates of control and insulin-stimulated cells. 35-S methionine was used for studies on PKC epsilon degradation and protein synthesis.

Results: Insulin stimulation caused a rapid increase in tyrosine phosphorylation and activity of PKC epsilon, and resulted in an increase of total PKC epsilon protein and RNA levels. Furthermore, insulin decreased the degradation rate of PKC epsilon and increased the de novo synthesis of the protein. In order to examine the potential role of PKC epsilon in insulin signaling, we immunoprecipitated PKC epsilon and immunoblotted for various proteins in the signaling pathway. We found that PKC epsilon did not bind to upstream components in insulin signaling, such as IR and IRS, but it did to the downstream components Akt/PKB and GSK3. Insulin increased the association between PKC epsilon and Akt/PKB but not between PKC epsilon and GSK3. Overexpression of kinase dead PKC epsilon decreased insulin-induced phosphorylation of phospho-Akt/PKB(ser473) but not phospho-Akt/PKB(thr308).

Conclusions: The findings suggest that insulin regulates PKC epsilon levels by decreasing its degradation and increasing its synthesis, and that PKC epsilon might play a role in insulin-induced regulation of Akt/PKB. This work was supported by the Russell Berrie Foundation and D-Cure, Diabetes Care in Israel.
INDUCTION OF EGF-LIKE GROWTH FACTORS AMPHIREGULIN AND EPIREGULIN BIOSYNTHESIS BY PROSTAGLANDIN E2 IN HUMAN GRANULOSA CELLS

(1) DR. BEN-AMI IDO (1) DR. FREIMANN SARIT (1) MRS. ARMON LEAH (1) MRS. DANTES ADA (2) PROF. RON-EL RAPHAEL (3) PROF. SEGER RONY (1) PROF. AMSTERDAM ABRAHAM

(1) DEPARTMENT OF MOLECULAR CELL BIOLOGY, WEIZMANN INSTITUTE OF SCIENCE, REHOVOT (2) IVF UNIT, DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, ASSAF HAROFEH MEDICAL CENTER, ZERIFIN (3) DEPARTMENT OF BIOLOGICAL REGULATION, WEIZMANN INSTITUTE OF SCIENCE, REHOVOT

**Introduction:** Local prostaglandin production in the preovulatory follicle has a critical role in cumulus expansion and oocytes maturation. Furthermore, COX-2, a key enzyme of prostaglandin biosynthesis, was found to be over-expressed in pre-neoplastic changes of the ovarian surface epithelium, as well as borderline and benign tumors. Recently, EGF-like growth factors amphiregulin (Ar) and epiregulin (Ep) were found by us to be synthesized in human granulosa cells under LH stimulation. They play a crucial role in cumulus expansion and resumption of oocyte meiosis, as demonstrated in rat. They were also demonstrated to be involved in various cancer development, such as ovarian, breast, endometrial as well as other malignancies. We aimed to evaluate whether PGE2 may affect ovulation by induction of Ar and Ep synthesis in human granulosa cells obtained from women undergoing IVF treatment.

**Patients / Methods:** The effect of PGE2 on mRNA expression of Ar and Ep was studied using quantitative real-time RT-PCR. The effect of COX-2 inhibitor on LH induced Ar and Ep biosynthesis, was achieved by preincubation of the cells with 50 µM of specific COX-2 inhibitor, nimesulide. The signal transduction pathway of Ar and Ep biosynthesis by PGE2 was examined by preincubation with specific PKA inhibitor (H89), and specific MEK inhibitor (UO126) that attenuate the MAPK cascade.

**Results:** PGE2 demonstrated dose dependent increase of Ar and Ep mRNA level, reaching a peak level at 10-6M. Time dependent experiments of PGE2 between 2 and 24 hours demonstrated maximum mRNA expression at 2 hours and decreased thereafter. The expression level of Ar and Ep was significantly lower in the presence of H89 and UO126 compared to control cells. Interestingly, co-stimulation of the cells with nimesulide and hLH resulted in significant reduction of Ar and Ep biosynthesis.

**Conclusions:** Since PGE2 elevates the expression of Ar and Ep in a dose and time dependent manner in human granulosa cells, it is suggested that PGE2 may be involved in ovulation, at least in part, by their induction. This elevation involves cAMP –PKA and MAPK pathways. Furthermore, the negative effect of COX-2 inhibitors on the ovulatory process may be accounted for by the reduction of Ar and Ep synthesis.
AMPRIEGULIN AND TACE/ADAM17 ARE INVERSELY REGULATED IN HUMAN GRANULOSA CELLS

(1) DR. FREIMANN SARIT (1) DR. BEN AMI IDO (1) MRS. ARMON LEAH (1) MRS. DANTES ADA (2) PROF. RON-EL RAPHAEL (1) PROF. AMSTERDAM ABRAHAM
(1) DEPARTMENT OF MOLECULAR CELL BIOLOGY, THE WEIZMANN INSTITUTE OF SCIENCE, REHOVOT (2) IVF UNIT, DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, ASSAF HAROFEH MEDICAL CENTER, TEL-AVIV UNIVERSITY, ZERIFIN

Introduction: Amphiregulin is an EGF-like factor that binds the epidermal growth factor receptor. Recently, amphiregulin was found to be involved in oocytes maturation and cumulus expansion in a murine model. In order to mediate its effects, amphiregulin must first undergo a specific cleavage by disintegrin and metalloproteinases (ADAMs). It has been hypothesized that this growth factor is synthesized in granulosa cells and functions via paracrine effect on cumulus cells. Indeed we have demonstrated that amphiregulin is synthesized in primary cultures of human granulosa cells upon LH stimulation. It was demonstrated that tumor necrosis factor alpha-converting enzyme (TACE/ADAM17) cleaves amphiregulin in human keratinocytes, fibroblasts and colon cancer cells. We aimed to compare the expression of ADAM17 and amphiregulin in human granulosa cells following LH stimulation, and to compare the basal level of ADAM17 expression in granulosa versus isolated cumulus cells shortly after oocytes retrieval.

Patients / Methods: Human granulosa were collected from women undergoing IVF treatment due to male factor following ovum pickup and cultured for 7 days in gonadotropin free medium. Following LH stimulation, mRNA and protein levels of amphiregulin and ADAM17 were measured. In another set of experiments granulosa and cumulus cells were cultured separately. Total protein was extracted one day following culturing and ADAM17 level was measured.

Results: LH stimulation of granulosa cells, increased amphiregulin expression in the mRNA and protein levels and was also detected in the condition medium. In contrast, ADAM17 protein gradually decreased following LH treatment. Interestingly, ADAM17 protein level was 2-fold higher in cumulus versus granulosa cells following one day culturing.

Conclusions: We demonstrate for the first time that LH stimulation attenuates ADAM17 expression in human granulosa cells concomitantly with elevation in amphiregulin biosynthesis. Taken together with the higher ADAM17 protein level in cumulus versus granulosa cells one day following culturing suggests a preferential paracrine action of amphiregulin on cumulus cells rather than autocrine effect on granulosa cells.
SYNCHRONIZATION OF SEASONAL FLUCTUATION OF BIRTHS OF TWO POPULATIONS

(1) DR. GOLDSTEIN ABRAHAM (2) PROF. SACK JOSEPH

(1) GONDA BRAIN RESEARCH CENTER, BAR-ILAN UNIVERSITY (2) SACKLER SCHOOL OF MEDICINE, TEL-AVIV UNIVERSITY

Introduction: Human populations show seasonal patterns of birth rates which are quite stable in any particular population, but vary widely across different geographical locations. These differences have been attributed to climatic factors, yet additional factors have been found to play a role. We report how two populations sharing the same geographical location and environmental conditions synchronize their seasonal patterns of birth rates over a relatively short period of time. Over the last 30 years the annual birth pattern of the Jewish and Muslim populations in Israel changed from qualitatively different patterns to similar ones.

Patients / Methods: Data were extracted from the Israel Central Bureau of Statistics on monthly birth rates during 1970-2003. Birth rates were corrected for month length and were detrended using a moving average of 12 months. Synchronization of the annual patterns of the two populations was examined in a time-series cross-correlation analysis.

Results: The peak birth rate of the Muslim population moved from winter to late summer, resembling the Jewish pattern, whereas the Jewish population increased its secondary winter peak. A spring trough, reflecting summer conceptions, remained fairly stable in both populations, and is probably related to the effect of extreme hot temperatures or long photoperiod during July-August on fertility or conception. Before 1985 monthly birth rates of both populations had a maximum cross-correlation of 0.61 at a 3-month lag, but after 1985, the maximum correlation was 0.811 at a 0-month lag. During the first decade in the study, the maximal cross-correlation was at 3-month lag (0.631) and at 0-lag the correlation was virtually zero. This pattern changed already during the following decade and turned into a 0-lag maximum of 0.553. These changes were not related to climate, religious holidays, or marriage seasonality, and did not occur in neighboring countries.

Conclusions: Findings indicate that environmental factors underlie the pattern of a spring trough and the late-summer and winter peaks. Social factors appear to affect the relative amplitude of the peaks and overall magnitude of fluctuations, favoring the notion that social factors only alter the impact of environmental factors which are the main drive of the annual conception rhythm.
Abstract Code: P98

INCREASED CELL CYTOPLASMIC PROCESSES AND EXPRESSION OF CONNEXIN 43 ARE ASSOCIATED WITH A REDUCTION IN PROGESTERONE SECRETION IN HUMAN CUMULUS CELLS

(1) DR. BAR-AMI SHALOM (2) PROF. VLODAVSKY ISRAEL (3) DR. KHOURY CAMELLIA (4) PROF. SEIBEL MACHELLE (5) DR. ELLENBOGEN ADRIAN (6) PROF. MAYERHOFER ARTUR
(1) DR. JAFFE ANAT

(1) ENDOCRINOLOGY AND DIABETES UNIT, HILLEL YAFFE MEDICAL CENTER, HADERA & INSTITUTE OF EVOLUTION, HAIFA UNIVERSITY, ISRAEL (2) DEPARTMENT OF CANCER AND VASCULAR BIOLOGY RESEARCH CENTER, TECHNION, HAIFA, ISRAEL (3) ENDOCRINOLOGY LABORATORY, SAINT VINCENT DE PAUL HOSPITAL, NAZARETH, ISRAEL (4) OBSTETRICS AND GYNECOLOGY, UNIVERSITY OF MASSACHUSETTS, BOSTON, USA (5) OBSTETRICS AND GYNECOLOGY, HILLEL YAFFE MEDICAL CENTER, HADERA, ISRAEL (6) ANATOMICAL INSTITUTE, TECHNISCHEL UNIVERSITNT MUNCHEN, GERMANY

Introduction: Following the gonadotropic surge the compact cumulus mass is turned into an expanded structure associated with: massive accumulation of hyaluronic acid, a reduction in both cell cytoplasmic processes and gap junctions between the cumulus cells and increased progesterone (P4) secretion. The association between cumulus cell to cell contact and P4 secretion was studied.

Patients / Methods: Cumulus cells isolated form mature human cumulus oocyte complexes were cultured for 7 days on non-coated plastic alone (plastic) or on native extracellular matrix (nECM), derived from bovine corneal endothelial cells, in absence or presence of the human follicle-stimulating hormone (hFSH) or human chorionic gonadotropin (hCG). Morphological changes, connexin 43 expression and P4 and estradiol secretion were studied.

Results: A morphological examination revealed a vast increase in the density of cytoplasmic processes of human cumulus cells (hCC) when cultured on nECM in the presence or absence of gonadotropins. Furthermore, culture on nECM, increased the connexin C43, the main protein of gap junctions in the ovary, as detected by immunocytochmistry. P4 secretion was significantly decreased (twofold, P<0.005) in hCC cultured on nECM. Addition of hFSH or hCG significantly increased P4 secretion in hCC cultured on plastic. On the contrary, in hCC cultured on nECM, addition of hFSH or hCG was virtually ineffective in P4 secretion. Furthermore, in the presence of either gonadotropin, P4 secretion was significantly lower in hCC cultured on nECM compared to plastic. The P4:E2 ratio was increased with increasing cell-plating densities regardless of whether hCC were cultured on nECM or plastic. However, the P4:E2 ratio was significantly reduced in hCC cultured on nECM, both in the presence or absence of gonadotropins.

Conclusions: This study indicates that hCC - P4 secretion is reduced when cultured on nECM and this effect is associated with an increase in cumulus cell-to-cell contacts and increased density of the gap junction specific connexin protein. Minimal steroidogenic activity in conjunction with a high incidence of communication between the hCC is the normal status of the cumulus mass before its maturation. The present model system may in part represent the specific situation of immature cumulus mass.
Abstract Code: P99

PROTEOLYSIS OF MITOCHONDRIAL STEROIDOGENIC ACUTE REGULATORY (STAR) PROTEIN BY LON: UNEXPECTED EFFECT OF PROTEASOME INHIBITORS

(1) MR. GRANOT ZVI (2) MR. KOBILER OREN (1) DR. MELAMED-BOOK NAOMI (1) DR. EIMERL SARAH (2) PROF. OPPENHEIM AMOS B (1) PROF. ORLY JOSEPH


Introduction: StAR is a vital mitochondrial protein promoting transfer of cholesterol into steroid making mitochondria. StAR synthesis and activity are acutely upregulated by trophic hormones, while termination of StAR activity commences upon import of the protein into the mitochondrial matrix. We have shown that inside the organelle, StAR is rapidly degraded in the matrix, probably to prevent functional damage due to excessive accumulation of ‘useless’ protein of no physiological role. Unexpectedly, the mitochondrial degradation of StAR was inhibited by two threonine protease inhibitors, the proteasome inhibitors MG132 and clasto-lactacystin beta-lactone (cLbL). Last year, we reported the identification of Lon protease as the prime participant in mitochondrial StAR degradation. Such insight was made possible by expressing murine StAR in E.coli cells endowed with normal and mutated mitochondrial protease homologs. However, due to the gram-negative outer envelope of the E.coli cell, we could not provide essential confirmatory L tested in the bacterial evidence for the inhibitory effect of MG132 and cL model. Therefore, in the present study we designed an in vitro degradation assay L can inhibit a serine protease such βproviding direct evidence that MG132 and cL as Lon protease.

Patients / Methods: Over-expressed His-tagged StAR and human Lon protease were purified from bacterial lysates on Ni-NTA beads and in vitro degradation assay was examined by use of Western-blot analyses. Lon activity was also assessed using an on-line fluorogenic assay of FITC-casein degradation.

Results: Cell free degradation assays using StAR and FITC-casein as substrates showed that Lon-mediated degradation was ATP-dependent and readily blocked by MG132 (IC50 = 20 microM) and cLbL (IC50 = 3 microM); epoxomicin, representing a different class of proteasome inhibitors, had no effect. Lon assays performed with FITC-casein showed a sigmoid curve of substrate-dependent activity rates, indicating a positive cooperativity. Both MG132 and cLbL acted as non competitive inhibitors of Lon.

Conclusions: We show that Lon can mediate the rapid turnover of StAR in steroidogenic cells and revealed the potential use of MG132 and cLbL as selective inhibitors of mitochondrial proteolysis. These findings have promoted current studies exploring the unorthodox association of lactacystin with the unique serine-lysine dyad active site of Lon.