

Presentation Abstracts

INSULIN AND IGF-1 RECEPTORS AND CANCER

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Recent studies have demonstrated a role for both the insulin receptor (IR) and the IGF-1 receptor (IGF-1R) in cancer development, growth and cancer-related mortality. Epidemiological studies showed a relationship between total IGF-1 circulating levels and the relative risk of developing most of the common epithelial cancers including prostate, colon, and breast cancer. In vitro studies have shown an increased expression of the IGF-1R in cancer cells and furthermore, blocking the activation of the IGF-1R inhibits cancer growth both in vitro and in vivo. This has led to the development of numerous humanized IGF-1R blocking antibodies and a number of small tyrosine kinase inhibitors (TKI). Many of these compounds are in preclinical, phase 1 and phase 2 clinical trials. In some studies, complete or partial responses have been seen, though the proportion of patients responding is limited and often the severity of the side-effects have resulted in cessation of the trials. Interestingly, there appears to be some resistance to the blocking effects of the antibodies and studies have demonstrated a compensation by other tyrosine kinase receptors such as the IR, EGFR or PDGFR. Studies are also ongoing using a combination of anti-IGF-1R antibodies and other inhibitors of other signaling molecules such as mTOR or PI3'kinase.

On the hand, interest has also focused on the role of insulin in promoting cancer in obesity and Type 2 diabetes (T2D). Again, interest has arisen from epidemiological studies that find an association between c-peptide and serum insulin levels and cancer risk and cancer-related mortality in obesity and/or T2D. In addition, it has been demonstrated that cancer risk in T2D was greater in patients on sulfonylureas or insulin compared to metformin therapy. In the case of breast cancer, studies have convincingly shown that prognosis is worse when the breast cancer samples express higher levels of IR that is activated. In these case the expression of IR-A, a mitogenic sub-type of the IR is also expressed at high levels. To demonstrate the direct causality between endogenous hyperinsulinemia and cancer growth and metastases, we have utilized such a mouse model. Our results show that reducing the hyperinsulinemia or blocking the IR/IGF-1R tyrosine kinases blocks the extra growth of the tumors considered secondary to the hyperinsulinemia. Furthermore, we show an increase that increased metastases is also correlated with hyperinsulinemia.

Thus the increased cancer seen in obese individuals and type2 diabetic patients maybe due in part to endogenous hyperinsulinemia.

FATE DECISIONS DURING NEURAL CREST ONTOGENY

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The dorsal neural tube first generates neural crest cells that exit the neural primordium following an epithelial-to-mesenchymal conversion to become sympathetic ganglia, Schwann cells, dorsal root sensory ganglia and melanocytes of the skin. Following the end of crest emigration, the dorsal midline of the neural tube becomes the roof plate, a signaling center for the organization of dorsal neuronal cell types. Recent lineage analysis performed before the onset of crest delamination revealed that the dorsal tube is a highly dynamic region sequentially traversed by fate-restricted crest progenitors. Furthermore, prospective roof plate cells were shown to originate ventral to presumptive crest and to progressively relocate dorsalward to occupy their definitive midline position following crest delamination. These data raise important questions regarding the mechanisms of cell emigration in relation to fate acquisition, and suggest the possibility that spatial and/or temporal information in the dorsal neural tube determines initial segregation of neural crest cells into their derivatives. In addition, they emphasize the need to address what controls the end of neural crest production and consequent roof plate formation, a fundamental issue for understanding the separation between central and peripheral lineages during development of the nervous system.

A GENETIC APPROACH FOR INTRA-CEREBRO-VENTRICULAR DELIVERY

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Administration of synthetic or purified peptides directly into the brain ventricles is a method commonly used by neuroscientists for exploring physiological and behavioral functions of gene products. Intravenous administration is controlled by the blood-brain barrier, which limits its effectiveness, and current approaches for acute or chronic intra-cerebro-ventricular delivery have significant technical drawbacks resulting from both the chemical properties of the delivered substance, and the experimental procedures.

This lecture will describe a genetic approach for the delivery of secreted peptides or proteins into the cerebrospinal fluid (CSF) using a choroid plexus-specific and lentiviral-based genetic system. Using a choroid plexus-specific promoter, we established a lentiviral-based system, which offers inducible and reversible delivery of a gene product into the CSF. The system is composed of two complementary lentiviral vectors. The 'Effector' construct consists of a choroid plexus-specific promoter that drives the expression of reverse tetracycline trans activator (rtTA) protein and the reporter green fluorescent protein (GFP). The 'Target' construct includes the tetracycline-responsive element (TRE) DNA sequence, upstream to the nucleotide coding sequence of the requested gene of interest, followed by the reporter red fluorescent protein (RFP). Transcription initiation of the gene of interest and the RFP is mediated only in the presence of the inducer, doxycycline (Dox). A mixture of the two lentiviruses is injected ICV and the delivered genes are incorporated into the DNA of the choroid plexus cells. Initiation of transcription, limited to the choroid plexus cells by the choroid plexus-specific promoter, is induced by administering Dox-containing drinking water, and results in secretion of the final processed gene product into the CSF. The functionality of this system was demonstrated using the over-expression of the two established neuropeptides, corticotropin-releasing factor and gonadotropin-releasing hormone, modulating anxiety-like behavior and estrus cycle, respectively.

NEURODEGENERATION AND NEUROPROTECTION IN AN ANIMAL MODEL OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an inflammatory, neurodegenerative and demyelinating disease of the central nervous system. Despite consensus about the importance of axonal damage and neuronal death in MS, the underlying molecular mechanisms have not been well defined.

To elucidate the molecular events leading to neurodegeneration, we used experimental autoimmune encephalomyelitis (EAE), an animal model mimicking some features of MS. We investigated the contribution of plasma membrane calcium ATPase 2 (PMCA2) to neuronal pathology and death in the inflamed spinal cord. As PMCA2 is an essential calcium pump and PMCA2 expression is reduced in spinal cord neurons during EAE, we hypothesized that perturbations in PMCA2 levels could be a cause of neuronal damage. In agreement with this concept, silencing of PMCA2 expression induced death of spinal cord neurons. Knockdown of PMCA2 was followed by a decrease in collapsing response mediator protein 1 (CRMP1). CRMPs have been implicated in microtubule assembly and dendritic integrity and therefore, such changes could cause cytoskeletal and synaptic abnormalities leading to neuronal dysfunction and loss. Consistent with this notion, silencing of CRMP1 expression was followed by neuronal death, *in vitro*. CRMP1 expression was also decreased in EAE and administration of an AMPA/kainate receptor antagonist at onset or peak of the disease restored both PMCA2 and CRMP1 levels to control values and ameliorated clinical symptoms. Thus, perturbations in PMCA2 and CRMP1 expression could be additional mechanisms associated with AMPA/kainate receptor-mediated glutamate excitotoxicity in EAE.

Highlighting further the importance of PMCA2 in EAE, neurological deficits were more severe in PMCA2-heterozygous mice than in wild-type littermates. Accordingly, axonal loss was more pronounced in the spinal cord of PMCA2-heterozygous mice than wild type controls whereas the inflammatory reaction and glial activation did not show major differences. These findings support the notion that PMCA2 plays a critical role in neuronal injury during EAE.

INTERACTIONS BETWEEN CANCER GENES AND THE IGF-I SIGNALING PATHWAY

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The involvement of the insulin-like growth factors (IGF) in cancer biology has been the focus of extensive research. The IGF-I receptor (IGF-IR) is overexpressed in most malignant cells, where it displays potent antiapoptotic, pro-survival activities. Transcriptional regulation of IGF-IR gene expression constitutes a key control mechanism with important roles in normal growth control as well as in cancer development. Using a DNA affinity chromatography protocol linked to mass spectroscopic proteomic analyses we identified a number of nuclear proteins with oncogenic or antioncogenic properties that regulate IGF-IR transcription. Transcription factors with tumor suppressor activity, including p53, BRCA1, VHL, WT1, and others, were shown to negatively regulate IGF-IR expression. The etiology of neoplasias associated with *loss-of-function* mutation of tumor suppressors is, in many cases, linked to the inability of mutant forms to suppress IGF-IR gene transcription. In addition, we have recently identified a novel mechanism for IGF-IR autoregulation. Specifically, we showed that IGF-IR is localized in the nucleus of breast cancer cells. Furthermore, the IGF-IR (or fragments of the protein) binds to the IGF-IR gene promoter in an estrogen receptor-dependent fashion and controls IGF-IR gene expression. These data is consistent with the notion that the IGF-IR, in addition to its classical role at the cell membrane level, can also function as a transcriptional enhancer in breast cancer. Understanding the molecular basis of these complex interactions will be of significant value both in basic as well as translational terms.

MULTI-CELLULAR, MULTI-AGENT ANGIOGENIC CONTROL MECHANISMS: THE CORPUS LUTEUM AS A MODEL

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The corpus luteum (CL) lifespan is characterized by a rapid growth, differentiation and controlled regression, accompanied by an intense angiogenesis and angioregression, respectively. The short period of angiogenesis (until day 5 of cycle) is followed either by maintenance and stabilization of the vasculature during pregnancy, or controlled regression (induced by prostaglandin F2a ;PG) of the microvascular tree in a non-fertile cycle. Uncontrolled angiogenesis will cause reproductive defects and pathologies such as cancer, atherosclerosis and infectious diseases.

Using functional genomics we compared PG induced gene expression profiles in PG refractory (d 4 of estrous cycle) versus responsive (d 11) bovine CL. Interestingly, quite a few of the novel PG-regulated genes identified were related to angiogenesis (being either pro or anti-angiogenic): FGF2, pentraxin 3 (PTX3), thrombospondins (THBSs) and their cell-adhesion receptor (CD36). However, pro and anti-angiogenic factors were distinctly regulated by PG; there was a pronounced PG-induced upregulation of the FGF2 in d 4 CL while THBSs, CD36 and PTX3 were dramatically induced after PG in d 11 CL, associated with luteolysis. FGF2 is a well-known angiogenesis inducer while THBSs and PTX3 bind and inhibit FGF2 actions. We next examined if genes differentially expressed in d 4 vs d 11 CL were confined to a specific cell type. RNA was isolated from steroidogenic and endothelial cells (EC) enriched from the CL using magnetic beads. While some PG- regulated genes: NRG-1, *SELE* and *SELP* mRNAs showed cell-specific localization, *FGF2*, *PTX3*, *THBSs* were present in both steroidogenic and EC compartments of the CL. These results suggest a functional relationship between FGF2 activity and the luteolytic response. Moreover, administration of PTX3 and THBS-mimetic peptides into early stage CL may counteract FGF2 action and restore sensitivity to luteolytic actions of PG. The CL therefore provides a relevant model for studying the balance between angiogenic inducers and inhibitors.

CHILD WITH EXTENDED DELETION OF MONOCARBOXYLATE TRANSPORTER 8 (MCT8): EIGHT-YEAR FOLLOW-UP AND A TRIAL OF HIGH-DOSE LIOTHYRONIN

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Objective: The monocarboxylate transporter 8 (MCT8) has a pivotal role in neuronal T3 uptake. Mutations of this transporter determine a distinct X-linked psychomotor retardation syndrome (Allan-Herndon-Dudley Syndrome, AHDS) that is attributed to low intracellular levels of T3. We describe the cytogenetics analysis of the MCT8 gene in a patient with the syndrome. We also evaluate the clinical and endocrine effects of long-term elthroxine treatment and a trial of high-dose liothyronine. In that trial we attempted to overcome the T3 uptake resistance through alternative transporters.

Methods: The six exons of MCT8 gene were individually amplified by PCR. The length of the deleted region was determined by SNP array, followed by PCR-based mapping to define the exact borders of the deleted segment. The clinical and endocrine data of the patient during 6.5 years of elthroxine treatment and two periods (3 month each) of low- and high-dose of liothyronine were evaluated.

Results: An extended deletion of the MCT8 gene (comprised of 5 out of 6 exons) was detected. MCT8 dysfunction was associated with partial resistance to T3 at the hypothalamus and pituitary level, with normal responsiveness at the peripheral organs (liver and cardiovascular system). Liothyronine administration had no beneficial effect on the neurological status of the patient.

Conclusion: Liothyronine administration had no therapeutic effect in our patient with severe MCT8 dysfunction due to extended deletion of its gene. Yet, this treatment might be considered in early life, especially in patients with residual function of MCT8.

NEWBORN SCREENING FOR CONGENITAL ADRENAL HYPERPLASIA USING CUTOFF BASED ON GESTATIONAL AGE AND BIRTH WEIGHT

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Objective: To evaluate whether congenital adrenal hyperplasia (CAH) patients can be detected by newborn screening before the occurrence of life threatening salt wasting while maintaining overall adequate levels of prevalence, sensitivity and specificity.

DESIGN: In a two year pilot (2008 and 2009), neonatal screening was performed measuring 17 α -hydroxy-progesterone (17OHP) using Auto DELFIA Neonatal 17OHP B024 kits (PerkinElmer). Cutoff levels were based on both gestational age and birth weight.

Results: Data obtained from patient archives revealed that nationwide the incidence of 21-hydroxylase deficiency (21OHD) was 1:19,000 live births (1:30,000 for Jews and 1:8,000 for Arabs). The M:F ratio was 1:2.5 suggesting that 21OHD male patients in the general population might have been missed or died early due to a salt-losing crisis.

In the 2008-2009 period 319,394 newborns were screened and 15 CAH patients were detected, 8 male and 7 female. The 17OHP levels were between 202 and 609 nmol/l. Overall prevalence was 1:21,300; among them 8 were Jews (1:28,000) and 7 were Arabs (1:10,000). Therapy was started at the median age of less than 6 days. Total recall rate was 0.02% (60 cases), there were 4 suspected cases referred to Endocrinology but were false positive. No false negative were reported. Sensitivity was 100%, specificity 99.98% and positive predicted value was 20%.

Conclusions: Severe salt wasting can be prevented by neonatal screening. Our screening, based on gestational age in combination with birth weight, reduces false positive results thus reducing the psychological distress of parents whose infants might have a potentially life threatening chronic disease as well as unnecessary load on the medical system.

FINAL HEIGHT OF SUBJECTS WITH NON CLASSICAL 21 HYDROXYLASE DEFICIENCY BY AGE AT INITIATION OF GLUCOCORTICIDS THERAPY AND GENOTYPE

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Background: Non-classical 21-hydroxylase deficiency (NC21OHD) is a mild form of congenital adrenal hyperplasia (CAH) associated with different degrees of postnatal virilization developing from infancy to adulthood. The genotype might be either homozygous or compound heterozygous for mild mutations or compound heterozygous for one mild and one severe mutation of the gene encoding 21-hydroxylase (*CYP21*). It has shown that subjects with classical form of CAH tend to be shorter than expected from their midparental height. The loss of height in CAH is partially due to effect of sex steroids on epiphyseal closure and partially due to glucocorticoid-induced suppression of growth.

Aims: 1. to determine whether NC21OHD compromises final height. 2. to look for clinical parameters affecting final height in this population.

Methods: Retrospective review of medical records of subjects with NC21OHD who have reached final height for age at diagnosis, age at initiation of therapy, midparental height and *CYP21* genotype. The SD score (SDS) for final height and corrected height SDS (defined as final height – midparental height SDS) were estimated for each subject.

Results: Final height was available for 104 patients (81 females) diagnosed at mean age of 10.5 (median 8.5, range 0.1-32.1 years). Genotype was available for 86 patients of whom 60 (58%) were homozygous for V281L or compound heterozygous for mild mutations, 17 (16%) were compound heterozygous for one mild and one severe mutation, and 9 (9%) were heterozygous for V281L. The mean final height SDS achieved by NCCAH patients was -0.55 ± 1 , and the mean corrected height SDS was -0.16 ± 0.7 . No significant correlation or association was found between final height SDS and genotype, gender, or age at diagnosis, although those with one severe mutation tend to be shorter.

Conclusions: Unlike classical CAH NC21OHD does not seem to affect final height significantly irrespective of clinical parameters such as gender, age of diagnosis, genotype or treatment. This result may have application on the management approach of these patients.

CONSTRUCTION OF GENE THERAPY VIRAL VECTORS TARGETING THYROID CARCINOMA CELLS

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Introduction: The successful use of tissue-specific promoters in targeted gene therapy for cancer depends on a high level of cell type specificity. In this study, we defined and characterized a minimal thyroid cancer-specific enhancer which provides maximal, thyroid-specific, transcriptional activity from the Tg promoter in human thyroid papillary (NPA), follicular (MRO,WRO), anaplastic (ARO) carcinoma and primary human goiter cell cultures. This minimal Tg enhancer is intended for use in "safe", gutless recombinant adeno-associated viral (rAAV) vectors.

Methods: CAT was used to measure the Tg enhancer/promoter transcriptional activity. Five gutless rAAV serotypes [2,4,5, 8-9(DJ) and 12] expressing eGFP were prepared. Infection efficiency was measured by calculating the percentage of green fluorescent cells following different time periods.

Results: The full length Tg enhancer (1.4kbp) gave 13 and 2% CAT conversion in follicular and papillary thyroid carcinoma cells, respectively. The minimal Tg enhancer/promoter (-2.8 and -2.2 kbp) construct achieved a level of transcriptional activity: follicular 38%, papillary 28% and anaplastic <1% CAT conversion. On gel shift and supershift analysis, the minimal Tg enhancer fragment was found to bind TTF1 using NPA and MRO nuclear extracts.

rAAV12nlsGFP infected 82.7% of NPA and 92% of WRO cells, peaking at 3 days, rAAV2nlsGFP infected 82.1% of ARO cells, peaking at 6 days after infection. Furthermore, for primary human papillary thyroid cells, rAAV12nlsGFP infected the tumor cells more efficiently than normal thyroid cells from the same patient (n=3). rAAV8-9(DJ)nlsGFP infected 100% of WRO cells, peaking at 3 days. Upon replacement of the CMV enhancer/promoter with the minimal Tg enhancer element into this virus, rAAVDJ-Tg-nlsGFP gave specific infection in WRO cells (26.3%) compared to pre-adipocyte cells (3%).

Conclusions: The minimal Tg enhancer element can serve to drive a Tg promoter-driven suicide gene within an AAV12/2/8-9(DJ) viral coat as a future tool in thyroid cancer gene therapy.

RET PROTO-ONCOGENE MUTATIONS IN ISRAEL-20 YEARS EXPERIENCE

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Introduction: Germ-line mutations in the RET proto-oncogene cause Multiple Endocrine Neoplasia (MEN)-2 syndrome, Familial Medullary Thyroid Carcinoma (MTC) and Hirschsprung's disease. Little is known about the incidence of RET mutations in Israel, the ethnic characteristics of its carriers, and their clinical presentation.

Patients and Methods: Analysis of RET-mutations was conducted at the biochemistry laboratory, Tel-Aviv Sourasky Medical Center. All results from 1990-2010, including demographic, clinical and genetic data were reviewed.

Results: A total of 208 RET gene mutations examinations were conducted. Indications for conducting the test included: MTC (33.6%), first degree relatives of mutation carrier (46.6%), combined endocrinopathies (9.6%), pheochromocytoma (3.4%), familial MTC (1.4%), and Primary Hyperparathyroidism (PHT) (14%). Only 2.85% of patients with sporadic MTC were positive. No mutations were found in patients tested due to PHT or pheochromocytoma alone. 36% of family members were carriers. In 7 out of 20 (35%) patients presenting with combined endocrinopathy, no mutation was found. In three of these patients partial sequencing was done. Only 2.88% from the patients examined were from Arabic origin, and no mutations were found in this group. Out of 54 positive samples 8 were sporadic mutations, and 46 belonged to patients from 8 families. In the sporadic cases 6 different mutations in exons 10, 11, 14, 15, 16 were found. The ethnic origin was diverse. Six families carried 4 different mutations compatible with MEN-2A. Two other families carried a mutation compatible with MEN-2B.

Conclusions: A variety of sporadic and familial mutations in the RET proto-oncogene in the Jewish population in Israel was found among different ethnicities. No Arab carriers were found in this cohort. A low incidence of positive tests was found in patients with sporadic MTC. The genotype-phenotype correlation found is similar to that described in the literature. The diversity of the mutations found in different exons underscores the importance of complete sequencing of the gene.

HEMITHYROIDECTOMY FOR PAPILLARY THYROID CARCINOMA –WHEN LESS IS SOMETIMES MORE

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Introduction: Although total thyroidectomy is standard treatment for papillary thyroid carcinoma (PTC), hemithyroidectomy may be sufficient for small, low-risk, intrathyroidal tumors. There are no clear guidelines for the long term follow-up of hemithyroidectomy.

Objective: To evaluate follow-up regimen and outcome in patients with suspected intrathyroidal PTC treated with hemithyroidectomy or total thyroidectomy at a single institute.

Patients and methods: The study sample consisted of 161 patients with PTC treated at a tertiary medical center in 2001-2010: 60 consecutive patients after hemithyroidectomy, and 101 patients after total thyroidectomy. Only patients without visible neck metastases preoperatively were included. Clinical data were collected from the medical files. Number of patient visits to the endocrine clinic, laboratory thyroid tests, neck ultrasound, and fine needle aspirations (FNAs) during follow-up were documented as well. Independent t-test was used to evaluate between-group differences, and Pearson correlation was used to evaluate the relationship between characteristics in the hemithyroidectomy group.

Results: Tumor size was significantly greater in the total-thyroidectomy group (16.9mms) than the hemithyroidectomy group (7.25mms). There was no significant difference between the groups in the rate of permanent surgical complications. In the hemithyroidectomy group, 37 patients (61.6%) had known bilateral thyroid nodules preoperatively; this finding was positively correlated with the performance of repeated FNAs during follow-up. The hemithyroidectomy patients visited the endocrine clinic less frequently than the total thyroidectomy patients, but they were referred more often to neck ultrasound and FNAs. Significantly more patients in the hemithyroidectomy group were re-operated for suspicious recurrent/persistent disease.

Conclusions: Hemithyroidectomy for PTC is associated with a significant laboratory, imaging and cytological test burden, frequently even more than total thyroidectomy for more advanced disease. It provides no clear benefit to the patient. Clinicians should consider these factors when planning initial treatment for PTC, especially in patients with known bilateral thyroid nodules preoperatively.

PARATHYROID HORMONE SELECTIVE VENOUS SAMPLING FOR PREOPERATIVE EVALUATION OF PRIMARY HYPERPARATHYROIDISM

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Background: Minimally Invasive parathyroidectomy (MIP) is preferably used for the treatment of primary hyperparathyroidism (PHPTH) caused by a single adenoma. When the sestamibi scan and cervical ultrasound localization are negative, the classical 4-gland exploration is usually recommended. In the present investigation we evaluated the use of preoperative PTH selective venous sampling (PTH-SVS) for localization of an adenoma in patients with negative or questionable sestamibi scintigraphy.

Methods: PTH-SVS was performed in patients with proven HPTH and a negative preoperative sestamibi scan, and in patients with persistent or recurrent HPTH. When a significant PTH gradient was detected preoperatively MIP was chosen.

Results: PTH-SVS was performed in 115 patients. In 33 patients with persistent or recurrent PHPT a significant gradient was detected in 24 patients, and 13 patients were cured by reoperation. In 82 patients with a negative sestamibi scan before the first operation, a significant gradient was detected in 66 patients, and successful MIP performed in 41 patients. In 6 of the patients with a significant preoperative PTH gradient, but negative bilateral neck exploration, the side of thyroid lobectomy was chosen according to the PTH gradient, and resulted in cure of all 6 patients.

Conclusions: The preoperative use of PTH-SVS in PHPTH with a negative or questionable sestamibi scan resulted in successful MIP in 50.0% of the patients. We recommend the use of PTH-SVS for preoperative localization in patients with PHPT and a negative or questionable sestamibi scan.

ATYPICAL FEMORAL FRACTURES - SINGLE CENTER DATA

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Background: Atypical femoral fractures (ATF) have drawn much attention during the last years, especially in light of their possible connection to bisphosphonate use. Reported cases have been reviewed and position statements of ASBMR and the Endocrine Society have been published with sets of criteria for more precise case definition. Our aim was to review the prevalence, clinical and radiological parameters of patients with ATF at our institution.

Methods: Computerized database of discharge diagnoses (2009-2010) was reviewed. ICD-9 diagnoses compatible with the location of the fracture below femoral neck were chosen (e.g.: Shaft, Supracondylar, Subtrochanteric, etc). Patients younger than 50 years old and those with major trauma were excluded. Admission femoral X-rays were examined by a senior radiologist. The fractures were classified as ATF or not-atypical according to the published criteria. Hospital files of patients with ATF were reviewed.

Results: Our hospital cares for 300 patients with femoral fractures annually. Forty-two patients answered the search criteria. Of those, only 16 (2.5%) were classified by the radiologist as having an ATF.

The diagnostic codes used in patients with ATF were: "shaft fractures" (62.5%), "supracondylar" (18.7%) and "subtrochanteric" (18.7%). Most fractures coded as "subtrochanteric" were intratrochanteric, and thus, were excluded. Fourteen were women, age 72 ± 12 , 52-94.

The average hospital stay was 7.9 ± 3.2 days. Five patients were functionally intact prior to fracture, the vast majority were frail–bedridden, psychiatric ward inpatients, or dependent in ADL. Two patients received PPIs, two patients received alendronate and one received both. None were on current steroid treatment.

Conclusions: ATF are not uncommon. In our analysis, 2.5% of all femoral fractures were atypical. Lack of uniform code designation makes the case identification difficult. Most patients with ATF are frail. Less than third (5/16) of our patients were exposed to medications linked to increased risk for ATF. Uniform code for ATF is needed to allow data collection.

INHIBITION OF OSTEOCLAST DIFFERENTIATION BY CAROTENOID DERIVATIVES

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Several epidemiological studies indicate that consumption of fruit and vegetables has a beneficial role in bone health. Bone remodelling, an essential process for bone health is mediated by osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells). The goal of this study is to determine if various phytonutrients inhibit osteoclast differentiation and by what mechanism. Our experimental system is based on induction of differentiation of the monocyte/macrophage murine cell line- RAW264.7 to osteoclasts by Receptor Activator of NF- κ B Ligand (RANKL). ROS stimulate osteoclast differentiation by increasing RANKL production in cells of the stromal/osteoblastic lineage as well as augmenting the NF- κ B mediated differentiation mechanism in osteoclast precursors. Osteoclast differentiation was measured by a quantitative assay for Tartrate Resistant Acid Phosphatase (TRAP) and by counting osteoclasts as TRAP positive multinucleated cells.

Differentiation was inhibited by various phytonutrients such as the carotenoid lycopene and its derivatives, the polyphenols carnolic acid, resveratrol and curcumin and the isothiocyanate sulforaphane. Our previous studies have shown that oxidized derivatives of carotenoids are mediating some of their biological effects. For example, activation of the transcription factor Nrf2, in cancer and osteoblast cells, was caused by aldehyde derivatives of carotenoids, probably through interaction with thiol groups in the inhibitory protein Keap1. Thus, we analyzed the structure-activity relationship of a series of dialdehyde carotenoid derivatives in inhibition of osteoclast differentiation. We found that the degree of inhibition by these derivatives depends on the distance of the methyl group from the terminal aldehyde, which determines the reactivity of the conjugated double bond in reactions such as Michael addition to thiol groups in proteins. Moreover, the carotenoid derivatives attenuated the NF κ B signal through inhibition of I κ B phosphorylation (western blot).

In conclusion, various phytonutrients inhibit osteoclast differentiation. The effect of the carotenoid derivatives on this system is mediated, at least partially by inhibition of the NF- κ B pathway.

POST FRACTURE OSTEOPOROSIS TREATMENT PROGRAM, IS IT EFFICIENT?

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Objectives: To assess effectiveness of hospital based Fractures Prevention Program (FPP) in the fracture prevention treatment in patients with previous fragility fractures.

Patients and methods: FPP was initiated in Rambam Health Care Campus in March 2009. All patients with fragility fractures were referred from the Department of Orthopedic Surgery to the Bone and Mineral Metabolism Unit for fractures prevention treatment.

Results: 900 patients, aged 46-107 (75.18±11.7), 247 (27.4%) men, 653 (72.6%) women were hospitalized with fractures since March 2009: 593 (66%) for hip fractures, 60 (7%) for vertebral fractures, 247 (27%) for other fractures. 155 (17%) had previous fragility fractures. Prior to hospitalization 152 (23.2%) women have received a fracture prevention treatment: 134 (88.2%) with oral bisphosphonates (114 – alendronate, 20 - risedronate), 10 (6.5%) with raloxifen, 5 (3.3%) with teriparatide. Four (1.6%) men were treated before hospital admission with alendronate. 25OHD serum levels prior to hospitalization were available for 239 (26.5%) patients. Mean 25OHD was 26.5±14.7 (4-118) ng/ml; 85 (35.6%) patients had vitamin D deficiency (25OHD ≤20 ng/ml), 25 (10.5%) - severe vitamin D deficiency (25OHD ≤10 ng/ml).

154 (17.1%) patients, 23 (9.3%) men, 131 (20.1%) women, adhered to the FPP clinic visits: 98 (63.6%) had femoral neck fractures, 56 (36.4%) – other fractures. 746 (82.9%) patients stayed out of the FPP: 52 (10.6%) women and 1 (0.3%) men are treated in the community, 601 (80.4%) remain untreated, 18 (2.4%) died, 74 (10%) lost to follow-up. 165 (18.3%) patients are currently treated for osteoporosis in the FPP: 80 (48.5%) receive alendronate, 46 (27.9%) – risedronate, 5 (3%) – raloxifen, 32 (19.3%) – zoledronate, 28 (16.9%) - teriparatide, 16 (9.6%) – calcium and vitamin D prior to starting bisphosphonates. 53 patients are treated in the community.

Conclusion: Majority of the elderly patients remain untreated after fragility fractures. Men and hip fractures patients are more likely to remain untreated. Hospital based FPP increased by 10% the rate of FPT.

VITAMIN D INCREASES THE EXPRESSION OF FAK IN KERATINOCYTES LEADING TO ACCELERATED MIGRATION

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Introduction: The migration of epidermal keratinocytes is a critical element in the re-epithelialization phase of wound healing. Epidermal keratinocytes contain an autonomous vitamin D endocrine system, capable of synthesizing the hormonal form, calcitriol, degrading it and responding to it. We have previously shown that calcitriol accelerated the closure of the gap in an in-vitro scratch model.

Objective: to explore the effect of the hormone on the migration process and the mechanism of its motogenic action.

Methods: We used the immortalized non-tumorigenic HaCaT keratinocytes, which are thought to represent the population of basal keratinocytes in the absence of exogenous growth factors or active ingredients. We developed a "Scatter Assay" in which the dispersion of keratinocyte aggregates was monitored by phase and time-lapse microscopy. We developed a "multiple scratch assay" method to compare protein and mRNA levels in migrating and resting cells. The levels of proteins (activated or total) were determined by western blotting and GST pull down assay and the levels of mRNA were determined by real-time PCR.

Results: 24 hour pretreatment of HaCaT cells with calcitriol, significantly and consistently accelerated the migration of HaCaT cells. The effect of the hormone was dose dependent, already apparent at a concentration of 10 nM. The average speed of migration and the straightness of movement were significantly higher in calcitriol treated cultures. The effect on the migratory apparatus is manifested by the activation of the small G protein Rac1. By using pharmacological inhibitors we excluded the involvement TGF β HGF and cathelicidin as mediators of calcitriol action. We found that pretreatment with calcitriol increased the protein and mRNA levels of Focal Adhesion Kinase, FAK, that is known to play a pivotal role in cell migration. This leads to increased levels of activated FAK in migrating cells.

Conclusions: We conclude that treatment with calcitriol as a single agent prepares the keratinocyte for accelerated migration that may contribute to re-epithelialization during cutaneous wound healing.

BONE GLA PROTEIN INDUCES CARTILAGE AND VASCULAR CALCIFICATION VIA HIF1 α -DEPENDENT GLUCOSE METABOLISM

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Vascular calcification arises from a phenotypic transformation of vascular smooth muscle cells (VSMCs) into osteochondrocytic-like ones. Bone Gla Protein (BGP, osteocalcin) is commonly present in the calcified vasculature. Although the role of BGP in calcification is unclear, it was recently reported to act as an energy metabolism-regulating hormone. This study investigates the role of BGP in glucose metabolism and in cartilage and vasculature mineralization.

We established an *in-vitro* BGP-overexpression model in chondrocytes (ATDC5) and VSMCs (MOVAS). BGP overexpression stimulated chondrogenic differentiation and mineralization, increasing the expression of Sox9, Runx2, collagen type X, and staining for alkaline phosphatase, proteoglycans and mineral deposits in both ATDC5 and MOVAS cells. In addition, BGP overexpression enhanced glucose uptake and cell proliferation. Over the course of differentiation, BGP overexpression increased the expression of glucose transporters and key glycolysis enzymes, while downregulating gluconeogenesis enzymes. Both BGP overexpression and treatment with purified BGP resulted in stabilization of hypoxia-inducible factor 1 α (HIF-1 α) in both cell types, shown by silencing using HIF-1 α siRNA to be essential in mediating the direct metabolic effect of BGP. The *in-vivo* model of 1,25(OH)₂D₃-induced vascular calcification in young rats supported the *in-vitro* observations, showing a correlation between calcification, elevated BGP levels and increased HIF-1 α expression in aortae and bone growth plates. This study demonstrates a novel mechanism by which BGP locally shifts cells toward glycolytic breakdown of glucose, in a HIF-1 α -dependent manner, and stimulates calcification of cartilage and vasculature.

VITAMIN D LEVELS IN PEDIATRIC PATIENTS WITH MALIGNANCY

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Background: Multiple studies demonstrated an inverse association between vitamin D and its metabolites and cancer morbidity and mortality. Despite this impressive body of evidence, only a handful of studies estimated vitamin D status in pediatric patients with malignancy. We aimed to assess vitamin D status in a large cohort of pediatric cancer patients and survivors, and to define risk factors to vitamin D deficiency.

Methods: 25OHD levels were obtained in 116 consecutive patients (aged 12.1±6y, M=56) during their routine visits to the hemato-oncology department (mean time from diagnosis 4.13±3.8y). Patients or their parents were asked to answer a questionnaire regarding calcium intake and sun exposure habits.

Results: Average daily calcium intake was 783.6±476mg/day. Mean 25OHD levels were 21.9±9.1ng/ml. Eighteen patients (15.5%) were vitamin D deficient (<11ng/ml), and another 87 (75%) were vitamin D insufficient (11-32ng/ml). Only 11 patients (9.5%) were vitamin D sufficient. Younger age and the amount of sun exposure were associated with higher serum 25OHD levels (r=-0.25, p=0.007; r=0.29, p=0.02, respectively). No association was found with sun protection habits, calcium intake, disease type, gender, years since diagnosis, or undergoing SCT.

Conclusions: The prevalence of vitamin D deficiency and insufficiency in pediatric hemato-oncology patients is high, while daily calcium intake is significantly lower than the RDA. While these values may be similar to those of the general pediatric population in Israel, they are of particular concern in this patient population, which is at high risk for osteoporosis. Furthermore, given the current knowledge regarding the importance of vitamin D in the context of malignancy, maintaining an adequate vitamin D status may be important for recovery and prevention of recurrence of pediatric malignancy.

NUTRITION-INDUCED SERUM FACTOR AFFECTS MIRNAS LEVELS IN THE GROWTH PLATE

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Postnatal skeletal growth takes place in the cartilaginous growth center of the long bones, the epiphyseal growth plate (EGP) and is a genetically determined biological process that is modulated by environmental factors such as nutrition. The local and systemic mechanisms by which nutrition affects growth at the EGP are still not entirely elucidated. MicroRNAs (miRNAs) are small endogenous RNAs that regulate target mRNAs by binding to their 3'UTRs and were shown to be involved in a variety of functions, including skeletal development.

To study the nutrition-growth bond, pre-pubertal rats were subjected to 10 days of 40% food restriction (FR), followed by a renewal of the regular food supply (catch up; CU). A dramatic reduction in EGP height was observed in the FR group, followed by an instantaneous increase after restriction removal. Gene expression pattern was affected as well as several miRNAs.

To identify the mediator between the nutritional status and growth, serum derived from the three groups (control (AL), FR or CU), was added to the culture medium of the chondrocyte cell line, ATDC5, instead of the fetal calf serum. Proliferation was significantly reduced (by 15%; $p < 0.05$) in the presence of FR serum compared to AL. One day of refeeding was enough to correct this effect (AL vs. CU; NS). A significant reduction in the miRNAs observed in vivo was also noted in vitro (AL vs. FR; $p < 0.05$), followed by an increase with serum of the CU group.

These results are the first to show that miRNA respond to nutritional cues. It also implies the presence of a systemic mediator. Understanding the pathways involved in the transition from quiescence to proliferation in the EGP may lead to a better understanding of the children's growth process, and enable improved manipulation of growth in normal children as well as in those with special nutritional needs.

THE GLOBAL mTOR INHIBITOR TORIN1 IS MORE EFFECTIVE THEN THE mTORC1 INHIBITOR, EVEROLIMUS, ALONE OR IN COMBINATION WITH HDACi, IN SUPPRESSING NEUROENDOCRINE TUMORS CELL PROLIFERATION

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Background: RAD001 (everolimus) blocks proliferation and interacts with the PI3K/Akt/mTOR pathway in different neuroendocrine tumour (NETs) cell lines; however, its effects are limited by non-targeting mTORC2, which is responsible for the compensatory activation of Akt and development of resistance to mTORC1 inhibitors. Torin1, a globally inhibitor of both mTORC1 and mTORC2, seems to impair cell proliferation to a greater degree than rapamycin; however, its effects in NET cells are largely unknown. Histone deacetylases inhibitors (HDACi) represent a new class of anti-cancer agents, based on potency and specific HDAC target of inhibition.

Aims: To examine the effects of Torin1 vs. everolimus, alone or in combination with HDACi, on cultured NET cells.

Methods: Two NETs cell lines (BON1 and RIN) as well as cells extracted from human NETs after surgical excision were treated with everolimus, Torin1 and HDACi. Proliferation assays were used to determine the effects of the drugs on cell proliferation. Western blotting was used to analyze the expression of p-Akt, cyclin D1, cyclin D3, p27 and cleaved PARP, HIF 1- α , VEGF.

Results: Treatment of NETs cells with HDACi (AN-7 and LBH589) inhibited cell viability with a greater effect observed with LBH589. Incubation of NETs cells with everolimus resulted in a significant dose-dependent decrease in viable cell number. Incubation of cells with everolimus 50nM in combination with AN-7 (80mM) or LBH589 (10nM) exerted a greater decrease in viable cell number (up to 50% decrease; $P < 0.0001$) than either of the drugs individually. This effect was also observed in cultured cells derived from two patients with NETs. Torin1 significantly inhibited cell viability to a greater degree than everolimus in combination with HDACi (up to 65%; $p < 0.0001$).

Conclusions: In NETs cells, Torin1 shows a greater inhibitory effect on tumor cell proliferation than did the combination of everolimus with an HDACi.

ACTIVATION AND ROLE OF PI3K AND PI4K IN MAPK ACTIVATION DURING GnRH ACTIONS

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Gonadotropin releasing hormone (GnRH) is a hypothalamic decapeptide that serves as a key regulator of the reproductive system. Interaction of GnRH with its receptor (GnRHR), which is a G-protein coupled receptor (GPCR), leads to intracellular mechanisms that include activation of Mitogen-activated protein kinase (MAPK) cascades to mediate the expression of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Here we describe the role of PI3K and PI4K in GnRH-induced ERK1/2 phosphorylation in the α T3-1 and L β T2 gonadotrope cell lines. Therefore, we incubated α T3-1 and L β T2 cells for 1h with Wortmannin (WT) at 10nM and 10 μ M or LY294002 (LY) at 10 μ M and 100 μ M, doses known to inhibit PI3K and PI4K respectively, before stimulation with GnRH (100nM) or PMA (100nM) for 10min. Wortmannin gave a significant inhibition of ERK1/2 activation by GnRH or PMA at the two doses examined, with a more pronounced inhibition observed in the more mature L β T2 cells. LY294002 also gave a significant inhibition of ERK1/2 activation by GnRH at the two doses examined in L β T2 cells. For further examination we co-transfected α T3-1 cells with a dominant negative (DN) form or wild-type (wt) of PI4K110 (PI4KIII β) with ERK-GFP. Indeed, while the wt-PI4K had no significant effect, the DN-PI4K markedly reduced the effect of GnRH on ERK1/2 phosphorylation. Finally, we examined GnRH-induced Akt activity which is a downstream effector of PI3K target. Therefore, α T3-1 cells were treated with GnRH (100nM) for increasing period until 240min. The basal phosphorylation of Akt in both sites was markedly high, reduced rapidly within 5min of stimulation and remained low for 60min (Ser473) and for 15min (Thr308). After 240min of stimulation the level of phosphorylation returned almost to its basal level at both sites. Hence, we conclude that PI3K and PI4K seem to play a role in GnRH-induction of ERK1/2 activation in pituitary gonadotrope cells.

mTOR INHIBITOR TORIN1 INDUCES ANTIPROLIFERATIVE EFFECTS IN MtT/E CELL LINE AND HUMAN PITUITARY TUMORS

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As for many tumor types, it has been shown that the Akt pathway is overexpressed and activated in human pituitary tumors. Thus, pituitary tumors may be sensitive to the anti-proliferative effects of mTOR inhibitors. However, non-functioning pituitary tumors are rapamycin-resistant. Torin1, second-generation ATP-competitive mTOR kinase inhibitor (TKI), suppresses both mTORC1 and mTORC2 complexes. To evaluate the *in vitro* effects of mTOR inhibitor Torin1 on pituitary cells, a rat non-secreting pituitary tumor cell line, MtT/E, and human non-functioning pituitary adenoma (NFGPA) cells were used.

Treatment of MtT/E cells with Torin1 induced a significant dose- and time-dependent decrease of cell viability and cell number. Incubation of cells from four NFGPAs with Torin1 significantly reduced the number of viable cells by 25-45%. The anti-proliferative effects of Torin1 on pituitary tumor cells were found to be mediated by G0/G1 cell cycle arrest associated with cyclin D1 and cyclin D3 suppression, apoptosis reflected by increased fraction of cleaved caspase and subG1 events and autophagy tested with an autophagy marker, LC3. Expression of phosphorylated-p70S6K and phosphorylated-Akt was significantly reduced by Torin1. Interestingly, the protein expression of the negative regulator of PI3K, the PTEN phosphatase, was significantly decreased by Torin1 in MtT/E cells.

Our results show that Torin1 potently inhibits pituitary cell proliferation suggesting that TKIs may be a promising antiproliferative therapy for pituitary adenomas. This therapeutic manipulation may have beneficial effects particularly for patients harboring invasive pituitary tumors unresponsive to current treatments.

CRF RECEPTOR TYPE 2 ACTIVATION IN THE VMH IS REQUIRED FOR ENERGY BALANCE REGULATION FOLLOWING METABOLIC CHALLENGES

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Background: Corticotropin-releasing factor (CRF) and its related Urocortins are key regulators of energy balance. Stressful stimuli, food deprivation and leptin administration remarkably alter hypothalamic CRF type 2 receptor (CRFR2) expression, suggesting an important role for this receptor in regulating energy homeostasis during challenge.

Methods: To examine the role of CRFR2, expressed by the ventromedial hypothalamus (VMH), in modulating energy balance, a lentiviral-based system for site-specific knockdown (KD) of CRFR2 was established and VMH-specific CRFR2 KD mice were generated. Mice were tested both on basal conditions and following exposure to physiological perturbations to homeostasis.

Results: Reduced expression of VMH-CRFR2 did not affect basal metabolic parameters suggesting that under basal state VMH-CRFR2 does not play a crucial role in maintaining energy homeostasis. In the 24h period following food deprivation challenge, in order to regain energy homeostasis, control mice increased their food intake and reduced their physical activity. In contrast, CRFR2 KD mice increased their food intake only up to 75% of the control mice and maintained similar activity levels. Meal structure analysis showed that CRFR2 KD mice failed to increase their meals number and to prolong their meal duration. Moreover, CRFR2 KD mice showed reduced respiratory exchange ratio during the light phase compared to control group. This maladaptive recovery suggests that hypothalamic CRFR2 signaling is essential for re-establishing homeostasis following metabolic challenge. In addition, insulin tolerance test revealed reduced insulin sensitivity in CRFR2-KD mice which could be due to reduced suppression of the counterregulatory responses.

Conclusions: Our results support an important role for VMH-CRFR2 neurons in the control of food intake and energy expenditure in response to homeostatic challenge and suggest a role for these neurons in glucose sensing.

MIRNA ABLATION IN POMC NEURONS LEADS TO SEVERE OBESITY AND GLUCOCORTICOID DEFICIENCY

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MicroRNAs have important roles in neuronal differentiation and survival, as well as in neuronal plasticity in the mature nervous system. To investigate the role of miRNA in the central hub connecting stress response and appetite regulation, we generated a transgenic mouse model in which Dicer was specifically deleted from POMC expressing neurons, using a cre-lox system. On PND1, POMC-mRNA expression is similar in wild type and Dicer-KO mice but at six weeks of age no POMC neurons could be detected in the arcuate nucleus by ICH, indicating that miRNAs are essential for survival of these neurons. Similarly, POMC-mRNA expression in the hypothalamus of Dicer-KO mice was reduced by 96% from the levels in WT animals ($p=0.0009$). AgRP, Leptin receptor and NPY mRNA levels were reduced by 87% ($p=0.003$), 32% ($p = 0.02$), and 26% ($p=0.09$) respectively. POMC and CRFR1 mRNA levels were undetectable in the anterior pituitary gland of Dicer-KO mice. Consequently, basal and stress-induced corticosterone levels were undetectable in these mice. Weight gain and fat mass were significantly more prominent in Dicer-KO mice: 15-20 week- old Dicer $-/-$ and $+/-$ males weighted 42 ± 2.9 and 32 ± 0.5 g respectively ($p=0.003$) and had 21.1 ± 3.4 % and 9.3 ± 0.9 % fat mass ($p=0.0029$). Although free thyroxine levels were similar between the groups, total tri-iodothyronine levels were significantly higher in the Dicer-KO (45.4 ± 3.7 ng/ml) in comparison to wild type mice (32.7 ± 4.4 ng/ml; $p= 0.017$). Dicer-KO were glucose intolerant, responding with higher glucose levels after a glucose load (357 ± 34 vs. 247 ± 13 mg% at 30 min ($p=0.007$). Nevertheless, total ($p =0.009$), HDL ($p=0.003$) and LDL ($p=0.001$) cholesterol levels were significant lower in Dicer-KO, whereas there were no differences in triglyceride values. In conclusion, postnatal POMC neuronal death due to microRNA ablation leads to development of obesity and increased fat mass despite glucocorticoid deficiency.

GnRH-STIMULATION OF GONADOTROPIN A SUBUNIT (GSU) GENE EXPRESSION VIA CALCINEURIN INVOLVES ACTIVATION OF A NFAT TRANSCRIPTION FACTOR AND THE TORC COACTIVATOR

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GnRH induces gonadotropin gene transcription through activating various protein kinases and increasing levels of intracellular calcium which activates calmodulin. We previously reported that GnRH also activates the calcium/calmodulin-dependent phosphatase, calcineurin, and in this study we investigated its role in transcription of the gonadotropin α subunit (GSU) gene. Calcineurin over-expression is sufficient to induce promoter activity, and is required for both basal activity and the GnRH effect. We mapped the calcineurin responsive region to within 80 bp, which includes a region, comprising TTTCCTGTT, previously reported to be important for basal and GnRH-induced promoter activity, although the binding factor was never identified. To clarify which calcineurin target is responsible for transducing these effects, we examined Nuclear Factor of Activated T-cells (NFAT) transcription factors which bind a TTTC sequence, and saw that GnRH induces nuclear import of NFAT3 in a calcineurin-dependent manner. NFAT3 was detected at the GSU promoter only after GnRH treatment, and its knock-down substantially reduced GnRH-stimulated GSU promoter activity. In some cells, calcineurin also targets the CREB coactivator, Transducer of Regulated CREB activity (TORC), and we found that TORC1 over-expression induced GSU promoter activity, while its knock-down abolished the GnRH response. However, activation of the GSU by GnRH was not affected by over-expression of dominant negative CREB, suggesting that a different factor is responsible for TORC recruitment to this promoter. Although we expected GnRH to induce nuclear accumulation of TORC, this did not occur, and TORC appeared to shuttle between the cytoplasm and nucleus constantly. However GnRH did induce changes in the TORC protein, inducing an initial degradation of TORC, much of which is already N-terminal truncated, and facilitating a transcription-independent increase in levels of intact TORC. As the N-terminus of TORC was previously shown to interact with various transcription factors, this likely represents a means of TORC activation, through a mechanism that has yet to be elucidated but appears to involve calpain cleavage and the proteasome.

ACTH SECRETING PITUITARY MACROADENOMAS: OUTCOME OF MULTIMODAL THERAPY

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Introduction: Cushing's disease is rare, with an incidence of 1/1,000,000. It is commonly associated with a pituitary microadenoma, however, 4-10% of patients have a macroadenoma. Patients with macroadenomas have higher plasma ACTH levels, reduced suppression after dexamethasone and lower surgical cure rates, necessitating radiation and medical therapies.

Aim of the study: To evaluate the biochemical and anatomical presentation of pts with ACTH secreting pituitary macroadenomas, the need of different therapeutic options and the long- term remission rates.

Methods: Multicenter retrospective study. Clinical, biochemical, radiological and therapeutic data were retrieved from charts.

Results: Twelve patients (5 males and 7 females) with ACTH secreting pituitary macroadenomas (mean size 27.7 ± 10.3 mm) were included. Mean age at diagnosis was 41.4 ± 11.7 y and mean follow up was 6.5 ± 4.2 y (range 2-15 y). At diagnosis, mean plasma ACTH and mean urinary free cortisol were 21.6 pmol/L ± 15.6 (nl:2-10.1) and 1185 ± 1616 nmol/day (nl:20-208 nmol/day), respectively. Three patients had evidence of visual field defects, and 5/12 patients had partial hypopituitarism. Transphenoidal surgery was the primary therapy in 11/12 pts and medical treatment with pasireotide in one. Postoperative remission rate was 36% (4/11 pts). Reoperation was performed in one patient. Six patients were referred to pituitary radiotherapy, two had conventional radiotherapy and 4 had stereotactic radiotherapy.

Conclusions: Transsphenoidal surgery alone frequently fails to cure Cushing's disease caused by ACTH secreting pituitary macroadenomas, therefore, postoperative pituitary irradiation and/or medical therapy is often necessary and effective.

INTEGRATION OF HOMEOSTATIC AND HEDONIC SIGNALS IN CONTROL OF ENERGY HOMEOSTASIS

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The central nervous system coordinates both the control of energy homeostasis, i.e. the balance of food intake and energy expenditure as well as peripheral fuel metabolism. Here, the hypothalamus receives information from the periphery of the body about the fuel sources available either via hormonal signals such as leptin and insulin as well as directly by nutrient components such as amino acids, glucose and fatty acids and processes this information to coordinate a reflex-like response to adapt feeding and energy expenditure. However, this regulation can be overruled by higher brain functions assessing numerous aspects, such as the rewarding aspect of food and social interactions. The presentation will focus on the coordinate regulation of these different aspects of energy homeostasis as well as the neuroanatomical basis for their integration.

MANAGEMENT OF PATIENTS WITH ADRENOCORTICAL CARCINOMA

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The prognosis of patients with adrenocortical carcinoma (ACC) is still poor. Although most diagnostic and therapeutic strategies are not evaluated according to criteria of evidence-based medicine, most experts usually agree in general on most “standard” diagnostic work-up and treatment options. However, even these recommendations of expert groups are often not yet translated into clinical practice. The main obstacle for optimal practice is the rarity of the disease with an incidence of only 1-2/million/year. Furthermore, ACC represents a heterogeneous group of neoplasia which makes it difficult to predict outcome and response to treatment. In general, patients with ACC should be considered for surgical resection which is feasible in all patients with stage I-II and most patients with stage III disease. In the majority of cases, adjuvant treatment with mitotane is considered necessary and in some cases, radiotherapy can be helpful to decrease the rate of local recurrence. In those patients who are diagnosed at a time point when metastatic spread has already taken place systemic treatment options include chemotherapy in combination with mitotane. Currently, drug regimens including etoposide, doxorubicin and cisplatin versus streptozotocin have been tested in the first randomized trial on ACC patients. Due to improved international networking (www.ensat.org), further clinical trials have become reality including testing of an IGF1 receptor antagonist in stage IV ACC patients and evaluation of adjuvant mitotane in low risk patients after complete resection of ACC.

THE IMPORTANCE OF microRNA BIOLOGY TO YOUR RESEARCH: WHAT, WHY AND HOW

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miRNAs are a subset of endogenous small RNA molecules that regulate gene expression post-transcriptionally by affecting mRNA stability or translational repression. The study of this recently discovered family of genes is an exponentially growing field in life sciences and medical research. Diverse methods have been developed for the study of miRNA involvement both under normal and pathological conditions. Bioinformatics tools enables prediction of miR- target interaction for studying the regulation of any gene of interest based on conservation data, and these predictions can be verified by *In vitro* assays. High throughput methods for the profiling of miRNA expression patterns as microarrays or deep sequencing are available, suggesting potential miRNAs as biomarkers for specific physiological conditions. In the whole animal context, modified protocols for *in Situ* hybridization and real time PCR are often used to explore miR expression pattern in specific tissues. Transgenic mouse models for miRNA reporter, knockout or over-expression are generated as mouse models for miRNA-based diseases. Finally human studies include SNP analysis and the measurement of circulating miRNAs and their correlation with pathological conditions. Implementation of miRNA technologies in research opens new possibilities for thrilling discoveries.

INTRODUCTION TO OPTOGENETICS: THE FUTURE IS ALREADY HERE

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Halorhodopsin (NpHR) and Channelrhodopsin-2 (ChR2) are recombinant light-gated ion channels that transport chloride ions or cations, respectively. Each channel is selectively activated by different wavelength of light. These channels can be used for the precise control over neuronal activation or inhibition in genetically altered neuronal cells. These (and similar) channels have been studied intensively in recent years in various model systems such as: neuronal cell cultures, brain slices, in-vivo in transgenic animals such as c.elegans and mice and in mammals that were virally infected to express these opsins.

The electrophysiological properties of these transporters are well elucidated and they were found to be non-toxic, wavelength specific and very precisely controlled by the light stimulus as set by the experimenter (up to single-spike resolution).

This genetic based technique offers an exciting opportunity for the study of the neural substrates of behavior. By genetically manipulating certain cell types or brain structures to express these channels and together with an optic setup enabling the delivery of wave-length specific light onto desired brain locations, one can switch on or off, reversibly and in a very precisely controlled manner, certain neural circuits in the mammalian brain while it is engaged in a task.

GUTLESS ADENO ASSOCIATED VIRUS FAMILY: A NEW TOOL AND POTENTIAL TREATMENT PARADIGM FOR THREE ENDOCRINE SYSTEMS

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Background: Traditionally genetic manipulation *in vitro* has involved the transfection, electroporation or lipofection of plasmids into cell lines appropriate for the particular question. Unfortunately the ability to use these techniques in primary cultures and *in vivo* is usually not transferable. To deal with this issue different viral based systems have been developed that are able to infect primary systems *in vitro* or *in vivo*. The search for the optimal viral vector system has been the challenge for many investigators. We present here an introduction to the Gutless recombinant Adeno-Associated Virus (rAAV) as we have applied it to some endocrine related projects. Gutless rAAV vectors are considered safe for *in vivo* use for both animals and humans. Over the last decade more than 17 human clinical have been initiated using different rAAV serotypes. Each serotype has a different range of tissue specificities.

Aim: In the current presentation our aim was to screen 5 different rAAV serotypes in primary adipose, smooth muscle and thyroid tissue in culture, in order to determine which would be the most efficient and effective for further genetic manipulation. Furthermore, we wish to demonstrate an application of the most optimal rAAV serotype in cultured vascular smooth muscle cells (VSMC).

Methods: Primary human preadipocytes (HPA), VSMC and primary human thyroid cells were prepared from tissue taken during operations under local Helsinki supervision. Cells were transduced with 5 rAAV serotypes all with the ability to express nuclear directed eGFP upon cell infection. Infection efficiency was determined by the % of green fluorescent cells counted at different time periods. Furthermore, VSMC were infected with the optimal rAAV serotype able to express a 12-lipoxygenase (12-Lo) knockdown sequence under the CMV promoter. In this case VSMC cell death was measured by trypan blue exclusion and visual estimation.

Results: HPA were optimally infected by AAVDJ, which showed strong maximal eGFP expression from 6-30 days post infection. VSMC were optimally infected by both AAV2 and AAVDJ, with maximal eGFP expression over 6-10 days and 10-70 days respectively. Human primary thyroid cells in culture were optimally infected by AAV12 with a maximal eGFP expression 3 days after infection. 12-Lo knockdown AAV2 particles induced massive cell death with a similar time course as eGFP expression. This VSMC death was preventable by the addition of the 12-Lo product, 12HETE with the virus. Control vectors did not kill VSMC.

Conclusions: Once optimized, these vectors may prove useful new tools for the future treatment of human vascular disease. The gutless rAAV family of viruses will be useful tools for future endocrine research.

המוגלובין מסוכרר כמדד אבחנתי לסוכרת: האם לאמץ בישראל כעת: בעד ונגד

מיכה רפפורט

מחלקה פנימית ג' והשרות לסוכרת, מרכז רפואי אסף הרופא צריפין

המוגלובין מסוכרר (Hba1c) מקובל מזה שנים רבות כמדד מנחה טיפול בסוכרת אך לא כמדד אבחנתי. נייר העמדה העדכני לשנת 2010 של האגודה האמריקאית לסוכרת ממליץ כי רמת המוגלובין מסוכרר שווה או גדולה מ 6.5% היא אבחנתית לסוכרת.

המוגלובין מסוכרר יש יתרונות רבים בכל הנוגע לאבחנת סוכרת. יתרונות אלו כוללים דיוק, יציבות מעבדתית וביולוגית וקשר מוכח לסיבוכי סוכרת. יחד עם זאת מדד זה אינו נעדר חסרונות ולכן אינו מייתר את המדדים האבחנתיים האחרים לסוכרת שנשארים תקפים.

הסיבה העיקרית להמלצת האגודה האמריקאית לסוכרת לכלול את רמת המוגלובין מסוכרר במדדי הסוכרת האבחנתיים היא האחידות בערכי ושיטות המעבדה שהושגה בשנים האחרונות בארצות הברית. בישראל עדיין לא הושגה האחידות הנדרשת המאפשרת שימוש במדד זה לאבחנת סוכרת. בימים אלו נעשה בישראל בשיתוף משרד הבריאות והמועצה הלאומית לסוכרת ניסיון לאמץ שיטה מעבדתית אחידה לביצוע בדיקת המוגלובין מסוכרר.

STEROID METABOLOMICS

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Individual steroid metabolites have been used as disease biomarkers in monogenic diseases. Beyond their specific effects, sex steroids mineralo- and glucocorticoids affect general body habitus and body composition and determine the individual's position in the continuum of body phenotypes: man and woman, short and tall, thin and obese, muscular and flaccid, young and aged. Analyses of steroids in plasma, urine and other body fluids by gas chromatography mass spectrometry (GCMS) provide a high-throughput profile of steroids. Traditional interpretation of GCMS output involved the semi-quantitative estimation of specific metabolites, as represented by the area under the curve of specific peaks, or the ratio between peaks representing metabolites of substrate and product of given enzymes. We utilize a fully quantitative GCMS output by introducing reference curves for each of the most telling 39 metabolites. Using an all-inclusive analysis of a steroidal array in the form of a subject steroidal fingerprinting, patients are stratified by their unique steroidal fingerprinting, and fingerprints are clustered according to specific clinical conditions. Thus, a subject's fingerprint is his unique profile of all 39 (and potentially 60) metabolites we currently quantify, and is a mark of his/her unique phenotype. To identify signatures of complex diseases, we use the steroid metabolome, which is fingerprinting-based. Bioinformatic methods and tools for data mining have been borrowed from microarray analyses, evaluating and generating disease signatures from subjects' steroidal fingerprints.

ASSESSMENT OF CORTISOL SECRETION IN VARIOUS BODY FLUIDS: "PITFALLS AND TECHNICAL ASPECTS"

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Despite a wealth of apparently well-validated means of testing, the detection of diminished function of the hypothalamic pituitary adrenal axis (HPA) continues to challenge practicing clinicians. Inevitable variation exists not only in the outcome of different types of testing, but also in the ever changing methodology, antibodies and equipment used in commercial assays, leading to further variation in the proposed cutoff cortisol levels predicting normalcy. Despite these limitations and the growing understanding that normal levels of cortisol during dynamic testing should be applied in an assay and laboratory-dependent fashion, the cutoff levels for cortisol in the ACTH tests have remained unchanged over the years in clinical practice.

On the average, more than 90% of serum cortisol is protein-bound, and changes in binding proteins can alter measured serum total cortisol without influencing free concentrations of this hormone. Although total cortisol generally correlates well with the free fraction, there are clinical conditions such as major surgery, severe illness, acute phase of septic shock and stress in which large changes in cortisol binding globulin (CBG) and albumin concentration take place, thus raising a real need for measurement of free cortisol concentration. In our laboratory, we are measured bound and free cortisol in various body fluids.

Salivary cortisol is unaffected by cortisol binding globulin (CBG) and hence, allows to bypass CBG- related variations in serum total cortisol. The measurement of salivary cortisol offers a simple, stress-free and convenient, though indirect, method to assess circulating cortisol and particularly serum free cortisol levels. The expression of 11beta dehydrogenase in the parotid gland is a source of potential serum unrelated regulation of salivary cortisol. In this context, the direct measurement of serum free cortisol by equilibrium dialysis may refine the interpretation of the 1 \mp g ACTH test and allow further insight not afforded by testing based on serum total cortisol alone. The best index of increase adrenal glucocorticoid secretion is urinary free cortisol (UFC) measurements performed using a 24-h urine collection.

The measurement of free urinary cortisol is also one of the most useful screening tests for Cushing's syndrome. It well known that immunoassays currently employed by most clinical laboratories have significant limitations, especially concerning specificity and steroid / steroid interference. The immunoassays for UFC are using liquid-liquid extraction to eliminate interfering compounds, but are still susceptible to interferences from cortisone and/or other endogenous steroid metabolites and synthetic glucocorticoids, such as prednisolone. Another limitation of immunoassays is the lack of an internal standard to monitor variable recovery of cortisol in the extraction process. These limitations of immunoassays for UFC have led to the development of more specific methods based on liquid chromatography with ultraviolet detection (LC-UV), liquid chromatography- mass spectrometry (LC-MS), and gas chromatography- mass spectrometry (GC-MS) The chromatographic methods not only have reduced interference for cortisol quantification, but also allow quantification of cortisone, an endogenous metabolite of UFC.

HORMONE ASSAYS: ARE THEY RELIABLE?

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Hormone assays nowadays are mostly performed by immunoassay methods on automatic analyzers. These have the advantage of high specificity, sensitivity and precision, high throughput and low cost.

Sometimes, albeit rarely, a hormone result might be inaccurate due to analytic, pre-analytic or post-analytic factors. Some common analytical factors are:

1. Assay interference: immunoassays are susceptible to four classes of assay interference: (a) crossreactivity problems, (b) auto-antibodies to the analyte, (c) heterophilic or animal antibody interference with assay reagents and (d) in vivo or in vitro drug interactions
2. Hormone molecular heterogeneity: different variants of hormone molecules (HCG for example) might be detected differently by various assays and yield confounding results.
3. Assay standardization: most hormones lack international reference standard (IRP) preparations and therefore various immunoassays may yield dissimilar results.
4. Free thyroid hormone immunoassays appear sensitive to alterations in serum albumin, binding proteins and free fatty acid (FFA).

It is most difficult for the laboratory to proactively detect an inaccurate result from a single measurement. The physician should suspect an analytical problem when a reported value is inconsistent with the clinical status of the patient.

Once an erroneous test result is suspected, some simple means can be applied to address the issue e.g., neutralization of heterophilic Abs with blocking agent, dilution of the sample or use a different assay. In cases where analyte-Ab interference are suspected, precautions such as measuring Ab presence can be taken, as is routinely done in the case of Thyroglobulin.

None of the above, however, would identify all cases and guarantee a "true" result.

INFLAMMATORY AND ANTI-INFLAMMATORY PATHWAYS IN INSULIN RESISTANCE INDUCTION IN OBESITY

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The marked increase in obesity during the last decades and its strong association with insulin resistance and type 2 diabetes, have elicited interest in the underlying mechanisms of obesity-induced insulin resistance. Obesity is currently viewed as a low-grade, chronic inflammatory state, termed metaflammation, orchestrated by metabolic cells in response to excess nutrients and energy. It is associated with higher secretion of proinflammatory cytokines like tumor necrosis factor α (TNF) from adipose tissue, and with reduced secretion of the anti-inflammatory, anti-diabetic hormone adiponectin. The balance between the various cytokines and adipokines, secreted from adipose tissue, plays an important role in modulating insulin action.

We have previously shown that TNF induces insulin resistance by promoting serine phosphorylation of insulin receptor substrate (IRS)-1, and thereby compromises insulin signal propagation. We have now identified p38MAPK as an important mediator of hepatic insulin resistance both *in vivo* in animal models of obesity and *in vitro* in liver cells exposed to TNF. Whereas stress kinases like JNK phosphorylate IRS-1 directly, p38MAPK activation by TNF and additional stress stimuli, initiates ErbB receptors signaling and activation of a PI3K signaling cascade, culminating in serine phosphorylation of IRS-1 and impaired cellular response to insulin. The relevance of this novel mechanism has been confirmed also in muscle cells. Furthermore, we found that AMPK activation in muscle cells by adiponectin or by AICAR, an AMP analog, attenuates IRS-1 serine phosphorylation under stress conditions. Adiponectin receptor-1 (AdipoR1) expression in skeletal muscle plays an important role in insulin resistance and diabetes. Recent work in our lab indicates that AdipoR1 expression in human skeletal muscle is subjected to posttranscriptional regulation, including alternative splicing and translational control. These mechanisms play an important role during myogenesis and are important for whole-body insulin sensitivity.

Collectively, our studies provide new insights into the mechanisms underlying the development of insulin resistance in obesity.

ADIPOSE TISSUE FOAM CELLS IN OBESITY: ROLES IN TISSUE REMODELING AND INSULIN RESISTANCE

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Adipose tissue macrophages (ATMs) have been suggested to participate in the pathogenesis of insulin resistance and type 2 diabetes. In AT of obese but not lean mice and humans we have identified lipid-laden macrophages (foam cells) in crown-like structures of macrophages surrounding the adipocytes. We have developed a novel flow cytometric method for purifying AT foam cells and compared their genomic profiles to non-foam ATMs from lean mice. Results were confirmed by real-time PCR, immunohistochemistry and western blotting. The foam cells showed enhanced expression of lipid uptake/metabolism proteins such as Lpl, Fabp4, Fabp5, Fabp7, Ldlrap. Also upregulated in foam cells were extracellular matrix (ECM) proteins, including collagens (Col1, Col3, Col4, Col6), integrins (ItgaV, Itga6) and ECM modifying factors such as MMPs (MMP3, MMP12, MMP14) and cathepsins (CtsK, CtsL). AT foam cells were not clearly conformed to M1 or M2 polarities, as their corresponding mRNA levels were mixed. We have tested the functional significance of AT foam cells by selectively disrupting highly up-regulated genes in vivo; as an example Lpl (30 fold increase in foam cells) was deleted in macrophages using the *LysM-Cre* Loxp technology. HFD *lpl*^{Δmye} mice showed reduced expression of Lpl not only in ATMs but also in whole epididymal adipose tissue (but not other tissues). These mice showed significant reduction in total cholesterol (22% reduction) and LDL (41%) without change in FFA, TG and FFA levels in both fasting and fed state. Both mice showed similar weight gain but HFD *lpl*^{Δmye} had a significant decrease in glucose tolerance test (33% reduction), insulin (29%) and HOMA-IR (32%). At the adipose tissues levels HFD *lpl*^{Δmye} showed robust fibrosis and abnormal foam cells formation. Taken together, this study identified foam cells in adipose tissue in response to adiposity in both mice and humans. The results of the genomic analysis of the foam cells and the *lpl*^{Δmye} mice suggest that AT foam cells function in lipid uptake, AT remodeling and may contribute to the pathogenesis of insulin resistance and hypercholesterolemia.

HYPOTHALAMIC NEURONAL TOLL-LIKE RECEPTOR 2 PROTECTS AGAINST OBESITY

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Toll-like receptors (TLRs) are a class of pattern recognition receptors (PRRs) that plays a fundamental role in the activation of the immune response against invading pathogens, as well as under sterile pathological conditions. As such, these receptors were also found to be involved in the development of obesity. This linkage was previously attributed to the destructive peripheral inflammatory processes that are often associated with obesity. Here, we introduce a novel non-immunological role of TLR2, known to recognize lipid components, as a central negative regulator of food intake in the hypothalamus. We discovered that TLR2 deficient mice (TLR2D) developed mature-onset obesity and showed decreased glucose tolerance and insulin sensitivity compared with their WT controls. Using chimeras in which the immune system of the host mice was replaced at adulthood with wild-type cells, we demonstrated that TLR2 has a non immunological role in preventing age-related obesity. The increased appetite of the TLR2D mice was associated with reduced levels of the hypothalamic anorectic peptide, α -MSH, within their arcuate nucleus, suggesting a role for this receptor in the central regulation of obesity. We further found that TLR2 expression was induced by metabolic POMC+ neurons in the hypothalamus of middle aged mice. *In vitro* cultures of a hypothalamic neuronal cell line with the well known pharmacological activators of TLR2 further substantiated the direct role of this receptor in regulating metabolic signals within hypothalamic neurons, possibly by modifying the levels of key adipokines. In summary, our study attributes a novel protective physiological role to TLR2 in the regulation of body weight and food intake, which extends beyond its defined pathological inflammatory-related functions.

CNS SYSTEM INFLAMMATION AND THE CONTROL OF GLUCOSE METABOLISM

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Recently, it has been recognized that defined neuronal populations in the central nervous system (CNS) not only regulate energy homeostasis, i.e. food intake and energy expenditure, but also control peripheral glucose metabolism. We could demonstrate that insulin not only acts directly on hepatocytes to suppress hepatic glucose production but that simultaneous insulin-dependent regulation of hypothalamic neurons is required for efficient suppression of hepatic glucose production. Through the generation and characterization of cell type-specific insulin receptor knockout mice we could demonstrate that agouti-related peptide (AgRP) -expressing neurons in the arcuate nucleus of the hypothalamus confer insulin's ability to suppress hepatic glucose production. More recently, we could demonstrate that under conditions of diet-induced obesity neuronal insulin resistance occurs. Interestingly, saturated fatty acids such as palmitate can induce neuronal insulin resistance in a toll-like receptor (TLR) -dependent manner. To further delineate the intracellular signaling mediators of inflammation-activated insulin resistance in neurons, we have generated neuron-specific JNK-1-deficient mice. Strikingly, these animals are protected from diet-induced neuronal insulin resistance despite an unaltered obesity development, thus leading to improved peripheral glucose metabolism. Taken together, these experiments reveal a critical role for neuronal insulin signaling in control of peripheral glucose metabolism and characterize TLR-dependent activation of JNK-1 as a crucial mediator of neuronal insulin resistance, in turn affecting peripheral glucose metabolism upon obesity development.

CONTROVERSY IN CLINICAL ENDOCRINOLOGY: GRAVES' OPHTHALMOPATHY

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Graves' ophthalmopathy (GO) is present in about 50% of patients with Graves' hyperthyroidism. It may range from mild to moderately severe to (rarely) sight-threatening. It is commonly believed that an autoimmune response against antigen(s) shared by the thyroid and the orbit is responsible for GO, the TSH receptor being the most likely culprit.

From a clinical point of view two aspects are still debated: 1) how to manage Graves' hyperthyroidism in patients with associated GO; 2) which is the more appropriate medical management of active GO.

Prompt restoration and stable maintenance of euthyroidism should be obtained in all patients. Antithyroid drugs and thyroidectomy *per se* do not influence the natural course of GO. On the other hand radioiodine therapy can be associated with progression of *de novo* appearance of GO, particularly in smokers. This can be prevented by oral glucocorticoids administration. In patients with mild GO, the choice of thyroid treatment is largely independent of GO. Moderate-to-severe and active GO should be treated without delay. In these patients the choice between conservative (antithyroid drugs) or ablative (radioiodine, thyroidectomy or both) treatment is presently expert-opinion rather than evidence-based. The potential use of biological agents, such as rituximab, which counteracts pathogenetic mechanism of both hyperthyroidism and GO, requires further evaluation in randomized clinical trials.

Glucocorticoids (GC) are used in the management of GO in view of their anti-inflammatory and immunosuppressive effects. GC have been employed either locally or systemically, the latter route being less effective, but may be considered in patients with absolute contraindications to systemic use of GC. Oral GC have an overall favourable response rate in about 60% of GO patients, but recurrence of eye disease is not uncommon and side effects are frequent, especially iatrogenic Cushing's. These considerations led to the use of intravenous (iv) GC pulse therapy. ivGC pulse therapy is widely employed with many differences in regimen of administration, total dose, interpulse interval and duration of the treatment and there is no evidence for the superiority of any of these schedules. Recent randomized clinical trials have shown that the iv route is more effective and better tolerated than the oral route. A favourable response is observed in ~80% of cases, with low prevalence of Cushingoid features. However particular attention should be paid to possible liver toxicity of ivGC. GC can be used either alone or associated with orbital radiotherapy. The combined regimen has been shown to be more effective than either treatment used alone. GC are effective only when GO is active, i.e. of short duration, progressive and with significant phlogistic manifestations.

In patients in whom GC therapy fails the medical armamentarium is rather limited. Orbital radiotherapy can be considered in those patients who did not receive it in association with GC. Indeed, recent randomized clinical trials have confirmed that orbital radiotherapy is an effective and safe therapeutic procedure. Somatostatin analogs have been extensively investigated, based on the observation that somatostatin receptors are expressed in orbital tissues of GO patients. Four well-designed randomized clinical trials have shown that either octreotide LAR or slow-release lanreotide have no role (apart from marginal and questionable eye changes) in the management of GO. Novel somatostatin analogs, such as SOM230, with different receptor specificity, should be evaluated. Finally, interesting results have been recently reported using rituximab, a chimeric-murine monoclonal antibody targeting the CD-20 antigen. A randomized clinical trial using rituximab will be started shortly.

INSULIN AND GLUCAGON SHARE THE SAME MECHANISM OF NEUROPROTECTION IN DIABETES: ROLE OF GLUTAMATE

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In patients with acute ischemic stroke, diabetes and hyperglycemia are associated with increased infarct size, more profound neurologic deficits and higher mortality. Notwithstanding extensive clinical and experimental data, treatment of stroke-associated hyperglycemia with insulin is controversial. Diabetes and even early pre-diabetic insulin resistance are not only characterized by hyperglycemia, but are also associated with increased levels of amino acids in the circulation, including the neurotoxic glutamate. The pleiotropic metabolic effects of insulin include a reduction in the concentration of amino acids in the circulation. Here we show that the deleterious effect of stroke-associated diabetes is mediated by increased blood and CNS glutamate and that the reduction of glutamate levels by insulin or other agents within a very brief therapeutic window, significantly improves the neurological outcome after brain injury. Decreasing plasma concentrations of glutamate in diabetic rats with insulin or glucagon after transient middle cerebral artery occlusion (tMCAO) or traumatic brain injury (TBI) lowers blood and CSF glutamate, improves brain histology and preserves neurologic function. The neuroprotective effect of insulin and glucagon was similar, notwithstanding their opposite effects on blood glucose. The therapeutic window of both hormones overlapped with the short duration (~30 min) of elevated brain glutamate post injury in rodents. Similar neuroprotective effects were found after administration of the glutamate scavenger oxaloacetate in TBI model, which has no effect on glucose metabolism. These data indicate that insulin exerts a neuroprotective effect within a very brief therapeutic window that correlates with its capacity to reduce glutamate, rather than with its effect on glucose levels. Other, safer approaches to a reduction of glutamate without affecting glucose levels may be of use in the immediate aftermath of acute ischemic stroke or TBI.

AHNAK IS A NOVEL REGULATOR OF GLUT4 GENE EXPRESSION IN RAT AND HUMAN ADIPOCYTES: ROLE IN OBESITY AND INSULIN RESISTANCE

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High levels of free fatty acids like arachidonic acid (AA) have a major role in the pathogenesis of insulin resistance, obesity and type 2 diabetes (DM2). While elevated levels of AA repressed GLUT4 expression via a specific GLUT4 promoter region (-222/-197 bp), the mediator remained elusive. Using this region as bait for mediators in AA-treated cardiomyocytes, followed by mass spectrometry analysis, we detected the AHNAK/desmoyokin giant protein in association with the GLUT4-promoter (GLUT4-P). This association was confirmed by ChIP assay. In subcutaneous adipose tissue obtained from obese patients undergoing bariatric surgery, AHNAK mRNA levels correlated with the degree of weight loss ($R^2=0.943$; $p=0.005$). Similarly, AHNAK mRNA levels were ~2 fold increased in adipocytes of aged/obese rats, compared to lean controls. Transient expression of AHNAK in primary rat adipocytes repressed transcription from both GLUT4-P and a synthetic 3xIRS-LUC promoter reporter. This repression was partially curtailed by insulin, acting via nuclear exclusion of AHNAK, as observed by immunofluorescent staining. AHNAK gene silencing by siRNA enhanced Glut4 protein levels by 2-fold and protected GLUT4 expression from AA-induced repression. Thus, AHNAK emerges as a novel regulator of GLUT4 gene expression and as a potential therapeutic target for insulin resistant states like obesity and DM2.

AHNAK GENE EXPRESSION IS INCREASED IN HUMAN OBESITY AND DECREASED AFTER BARIATRIC SURGERY

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Background: AHNAK is a giant phospho-protein that is increased in adipose and muscle tissues in animal models of obesity. Previous results from our group suggest that AHNAK represses the transcription activity of GLUT4 gene leading to insulin resistance. In order to study the relevance of AHNAK to human obesity we studied the expression of AHNAK in obese patients.

Methods: We isolated adipocytes from subcutaneous (SCad) and visceral abdominal (Vad) adipose tissue biopsies obtained from 38 patients undergoing elective surgery (15/23 M/F; age 39.7 ± 13.6 (Mean±SD); BMI 39.4 ± 9.6 kg/m²; and HOMA 3.11 ± 2.52). AHNAK mRNA was quantified using real-time PCR. Adipocyte diameter, as measured by microscopy, was 502 ± 307 μm for Vad and 701 ± 423 μm for SCad. AHNAK mRNA expression was also determined in visceral fat biopsies obtained from 6 patients before and after weight loss after bariatric surgery.

Results: SCad AHNAK mRNA correlated with BMI ($R^2=0.38$; $p=0.029$), weight ($R^2=0.372$; $p=0.033$), and fasting plasma glucose levels ($R^2=0.414$; $p=0.018$). Further, Vad AHNAK mRNA levels positively correlated with adipocyte volume ($R^2= 0.493$; $p=0.004$) and AST levels ($R^2=0.354$; $p=0.034$). The correlation between AHNAK gene expression and obesity is supported by a good correlation between the extent of weight loss and the reduction of AHNAK mRNA in the 6 patients where biopsies were taken before and after weight loss ($R^2=0.943$; $p=0.005$). Further, we found a significant correlation between AHNAK mRNA expression in Vad and SCad from the same patient (correlation coefficient 0.555; $p=0.001$).

AHNAK mRNA level in VAd and SCad was higher in patients with hyperlipidemia ($p=0.016$ and $p=0.047$ respectively). However, there was no correlation between AHNAK mRNA expression in adipocytes and basal insulin levels, HOMA, HbA1c, or a diagnosis of diabetes.

Conclusions: The correlation between the AHNAK levels in human adipocytes, and the degree of obesity and derangement in metabolic parameters, suggests a potential role for AHNAK in the pathogenesis of obesity and the metabolic syndrome.

MORE MUSCLE, LESS FAT, BETTER THAN DIET: THE POSITIVE EFFECTS OF ANG 1-7 TREATMENT IN THE METABOLIC SYNDROME

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The metabolic syndrome (MetSyn) affects over a billion people worldwide and is a leading health concern due to its link to cardiovascular disease. During MetSyn development, adipose tissue expands, but once its capacity to efficiently store energy is exceeded, accumulation of ectopic fat takes place. Skeletal muscles are a major site of insulin-stimulated glucose disposal, and a direct correlation was delineated between intramuscular adipocytes and insulin resistance. Moreover, skeletal muscle loss, sarcopenia, is characteristic of MetSyn and is further enhanced during diet, which is recommended to MetSyn sufferers. We previously showed that rats fed on high-fructose diet (HFrD) and treated with Angiotensin 1-7 (Ang1-7): (a) did not develop MetSyn; (b) had smaller adipocytes with less fat inflammation; and (c) had more myogenic cells without fully differentiated adipocytes in skeletal myofiber cultures. To shed light on the underlying mechanisms, we conducted high throughput real-time PCR analyses. The most significant results are presented. On the HFrD background, Ang1-7 downregulated NOX4 and PKC β and upregulated PPAR α expression in epididymal fat. These results suggest that Ang 1-7 exerted antioxidant effects and a triglyceride lowering effects that may involve fatty acid oxidation through PPAR α . In gastrocnemius muscles, HFrD elicited upregulation of leptin, the atrophy associated PAI-1 and the oxidative stress-associated Nox2 and induced downregulation of myosins specific to fast and slow-twitch myofibers. In HFrD-fed, Ang 1-7 treated rats, the expression of these myosins was upregulated and the level of PAI-1 returned to control levels. Hence, Ang 1-7 downregulated pathways involved in deleterious effects of HFrD in skeletal muscle. Further, Ang1-7 enhanced the expression of slow and fast myofibers, possibly indicating new mechanisms by which sarcopenia can be retarded during HFrD. Together, these results point to molecular pathways by which Ang1-7 provides multi-system protection from the metabolic sequels of exposure to high fructose.

**VITAMIN D METABOLITES AND VITAMIN D LESS-CALECMIC
SYNTHETIC ANALOGS INDUCE REACTIVE OXYGEN SPECIES (ROS)
FORMATION AS A SIGNAL TO INHIBIT HUMAN ARTERIAL VASCULAR
SMOOTH MUSCLE CELL PROLIFERATION**

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Although most vitamin D's actions are traditionally ascribed to 1, 25(OH)₂D₃ [1,25D] acting on classical vitamin D receptors, there is now evidence that 24, 25 (OH)₂D₃ [24,25D], formerly considered merely an inactivation product, has important independent biological effects as well. Synthetic less-calcemic vitamin D analogs are presumed to act through classical vitamin D receptors but induce lesser rise in serum calcium *in vivo*. Here we examined the effects of these various vitamin D receptor modulators on ROS in arterial vascular smooth muscle (VSMC) harvested from the human umbilical artery, in the context of their known modulatory effects on VSMC proliferation as reported by us in earlier communications (Am. J. Hypertens. 2000; 13:396; J. Steroid Biochem. Mol. Biol. 2004; 89-90:397; Circulation 2005; 111:1666). With the exception of very low concentrations, [1,25D], [24,25D], and 25 (OH)D₃ [25D] and the less calcemic synthetic analogs JKF and QW, all decreased VSMC proliferation by 30-60% along with parallel increments in cell metabolic activity as reflected by the ATP-generating system creatine kinase BB (CK). These vitamin-D related agents also increased ROS formation as examined by direct visualization in a fluorescent microscopy system. There were differences in the induction of ROS, which was minimal with [25D] and [1, 25D], potent with [24,25D] and extremely potent with JKF and QW. When the formation of ROS was blocked by diphenylene iodonium (DPI), an inhibitor of NADPH oxidase, the effect of all vitamin D-related compounds on ROS formation was entirely aborted. Likewise, in the presence of DPI, none of the vitamin D-related compounds was able to inhibit VSMC proliferation and CK induction was also attenuated. These results establish a link between the inhibitory effect of vitamin D metabolites and analogs on VSMC growth and ROS formation. ROS formation apparently serves to allow the transduction of vitamin-D induced signals aimed at slowing down VSMC proliferation. This is an energy requiring process which is also blocked when ROS generation is inhibited. These events in the vasculature must be further studied especially in times in which liberal use of mega-doses of vitamin D in clinical medicine is highly fashionable.

THE GLUCOKINASE MUTATION T206P IS COMMON AMONG MODY PATIENTS OF ASHKENAZI JEWISH DESCENT

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Maturity onset diabetes of the young (MODY) is characterized by an autosomal dominant mode of inheritance, a primary defect in insulin secretion with nonketotic hyperglycemia, the age of onset under 25 years and a lack of autoantibodies. Eleven genes were found to be involved in the etiology of the disease, while glucokinase (GCK), Hepatic Nuclear Factor 1 α (HNF1 α) and Hepatic Nuclear Factor 4 α (HNF4 α) are the most common cause.

The aim of the study was to characterize the genetic basis of MODY in the different ethnic groups of the Israeli population.

The cohort included 151 patients with clinically identified MODY and their first degree family members. The coding regions including the intron–exon boundaries of GCK, TCF1 and HNF4A were examined. Molecular analysis of the three genes was performed on genomic DNA. Exons of the three genes were amplified by PCR with specific primers. All PCR products that showed consistent abnormal migration on DGGE were subjected to sequence analysis and compared to the GeneBank sequence.

Mutations were identified in only 32 families with a distribution of 3% in HNF4 α , 78% in GCK and 19% in HNF1 α . All these mutations were family specific, except T206P. This mutation was identified in 6 unrelated families, all from a ethno-origin, thus indicating an ethno-genetic correlation. A simple, fast and relatively cheap restriction-digestion assay was developed to identify this mutation in Jewish- Ashkenazi patients.

We propose that clinically identified GCK-MODY patients of Jewish-Ashkenazi origin be first tested for this mutation.

THE INSULIN-LIKE GROWTH FACTOR I RECEPTOR (IGF-IR) TRANSLOCATES TO THE NUCLEUS AND AUTOREGULATES *IGF-IR* GENE EXPRESSION

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Introduction: The IGF system plays a crucial role in the biology of breast cancer. Most of the biological actions of IGF-I and IGF-II are mediated by the IGF-IR, a membrane-bound heterotetramer with antiapoptotic and cell survival activities. Recent studies have shown that the IGF-IR can be modified by the small ubiquitin-like modifier protein-1, 2, and/or 3 (SUMO), with ensuing translocation to the nucleus. The functional significance of IGF-IR SUMOylation in the specific context of breast cancer has yet to be elucidated.

Aim: To investigate the potential nuclear localization of IGF-IR in both cells and to address the putative autoregulation of *IGF-IR* gene expression.

Material and Methods: Human breast cancer-derived MCF7 cells (ER-positive) and C4.12.5 (ER-depleted) cells were derived by clonal selection of MCF7. A proteomic approach based on genomic *IGF-IR* DNA affinity chromatography followed by Western blot analysis was used to verify association of nuclear IGF-IR with the *IGF-IR* promoter. ChIP analysis was performed to confirm the results. IGF-IR and insulin receptor (IR) subcellular localizations were assessed by confocal microscopy.

Results: Among other proteins found to bind to the *IGF-IR* promoter we identified the IGF-IR in ER-depleted, but not ER positive, breast cancer cells. ChIP analysis confirmed the direct *in vivo* binding of IGF-IR to *IGF-IR* promoter DNA, suggesting that the IGF-IR (or a fragment) may act as transcriptional enhancer. The functional relevance of binding data was assessed by cotransfection experiments with IGF-IR expression vectors along with an *IGF-IR* promoter luciferase reporter. Furthermore, confocal imaging experiments detected IGF-IR and IR staining in the cytoplasm, nuclear and perinuclear areas in both cells.

Conclusions: Our studies demonstrate that IGF-IR is localized in the nuclear areas and that nuclear IGF-IR may act as a modulator of its own promoter. Taken together, we provide evidence that the IGF-IR may act as a transcriptional enhancer.

* Elected as best basic abstract

IMPACT OF INFANCY LENGTH ON CHILD GROWTH IN 22 SUBSISTENCE-BASED SOCIETIES

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Context: Humans evolved to withstand energy crises by a predictive adaptive response, decreasing their body size. Thus, short-term adaptations to energy crises defer the infancy-childhood transition age (ICT), culminating in short stature. In natural-fertility human societies, this transition is associated with weaning from breastfeeding and the mother's new pregnancy. We therefore used the inter-birth interval (IBI) as a surrogate for the ICT. **SAMPLE:** The sample used is 22 natural-fertility societies of foragers, horticulturalists and pastorals from Africa, South America, Australia and Southeast Asia.

Hypothesis: We hypothesized that late ICT would be associated with smaller adult size and predicted that the IBI will negatively correlate with body size.

Results: The IBI (range 28.6-45.1 mo) correlated negatively with average adult bodyweight for females $r=-.537$, $p=.012$, and males, $r=-.475$, $p=.025$ and with adult BMI for females ($r=-.467$, $p=.033$) but not for males ($r=-.387$, $p=.075$). IBI correlated negatively with 1-year old bodyweight and with gestation to one year weight gain for males ($r=-.678$, $p=.015$; $r=-.694$, $p=.018$, respectively) but not for females ($r=-.473$, $p=.088$; $r=-.473$, $p=.088$, respectively), and positively with the age 3 mass as % of adult mass for both females ($r=.695$, $p=.008$) and males ($r=.662$, $p=.014$, fig 2). Juvenility and adolescent growth variables did not correlate with the IBI. *IBI correlated negatively with population density ($r=-.526$, $p=.044$). For males, less so for females, BMI correlated negatively with population density ($r=-0.636$, $p=.011$; $r=-.512$, $p=.051$, respectively3). When categorizing societies by economy-type, the IBI was longest in foragers, with a mean 38 mo, and shortest in mixed peasant economies with a mean IBI of 28 mo, $p < 0.001$.*

Conclusions: This inter-population study of natural-fertility human societies shows population density to correlate negatively with BMI, and adult size to be adaptively smaller when BMI is low. The mechanism for this adaptation utilizes the trade-off of infancy length against adult size, supporting the ICT theory, of negative relation between the ICT age and adult height.

EMPIRICAL TESTING OF THE 'INFANCY – CHILHOOD TRANSITION (ICT) THEORY' ON GROWTH REGULATION

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Introduction: The ICT theory on child growth claims a central role for the age at ICT in determination of growth pattern and ultimate adult height, such that delayed ICT (DICT) results in short stature, and early ICT – tall stature. The theory has been proven by multiple human observations. To empirically test the theory in experimental animals the ICT was assumed to be controlled by weaning from lactation.

Hypothesis: Early weaned animals will be longer than late weaned.

Methods: Sprague-Dawley pups, which usually are weaned at age 21 days, were weaned by transfer to foster non-lactating mothers at age 16, 21 or 26 days, and separated from these mothers at age 30 day. Growth was followed at weekly intervals until age 90 days.

Results: Early weaning animals (day 16) grew faster as of day 30 to reach a 60 day length of 41.90 ± 0.49 cm and 90 days length of 44.19 ± 0.75 , as compared to late weaning rats (26 days) with lengths of 35.68 ± 1.54 ($p < 0.001$) and 38.65 ± 1.9 ($p < 0.001$), respectively.

Weights of the early weaning rats were 362.5 ± 38.28 and 428.56 ± 31.56 at 60 and 90 days, as compared to 281.5 ± 21.53 ($p < 0.001$) and 365.13 ± 19.61 ($p < 0.001$), respectively, in the late weaning animals.

On the other hand the BMI grew smaller by early weaning. BMIs of the early weaning rats were 0.206 ± 0.02 and 0.219 ± 0.01 at 60 and 90 days, as compared to 0.222 ± 0.02 ($p < 0.05$) and 0.245 ± 0.01 ($p < 0.05$), respectively, in the late weaning animals.

Conclusions: Delayed (DICT) as compared to early ICT results in shorter and heavier animals. The ICT theory, as developed by human observations, is supported to be valid in experimental rats. Weaning may control the ICT.

THE LENGTH OF INFANCY CONTROLS INFANTILE AND JUVENILE DEVELOPMENT, AND THIS ADAPTIVE RESPONSE IS TRANSMITTED TRANS-GENERATIONS

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The preadult life history of rats is divided into infancy, as defined by lactation, and juvenility to reach in the female vaginal opening at a mean age of 35 days, whereas in the male, testes reach adult size of 2.3 ml mean age 37 day (day 21 F1).

Hypothesis: The length of infancy controls infantile and juvenile development, and this adaptive response is transmitted trans-generations.

Methods: Sprague-Dawley pups (generation F1), which usually are weaned at age 21 days, were weaned by transfer to foster non-lactating mothers at age 16, 21 or 26 days, and separated from these mothers at age 30 day. At age 60 females and males were mated within the weaning groups and generation F2 pups were followed for their infantile and juvenile developmental milestones.

Results: Generation F2 pups shifted their infantile developmental milestones such that males pups of early weaning parents (day 16) had earlier fur development at age 8.7 ± 0.67 as compared to 10.38 ± 1.6 in pups of late weaning parents (26 days) ($p < 0.05$). pinnae detachment was also earlier in d16 F2 (10.7 ± 0.82) as compared to 13.13 ± 0.64 in d26 F2 ($p < 0.05$). Eye opening occurred at day 15.7 ± 0.48 in d16 F2, as compared to 16.38 ± 0.52 ($p < 0.05$) in d26 F2.

Generation F2 pups shifted their infantile developmental milestones such that females pups of early weaning parents (day 16) had earlier fur development at age 8 ± 0.53 as compared to 10.75 ± 0.71 in pups of late weaning parents (26 days) ($p < 0.05$). pinnae detachment was also earlier in d16 F2 (10.63 ± 0.74) as compared to 13.38 ± 0.52 in d26 F2 ($p < 0.05$). Eye opening occurred at day 15.5 ± 0.53 in d16 F2, as compared to 16.25 ± 0.46 ($p < 0.05$) in d26 F2.

Conclusions: The age at weaning programs life history adaptively, to be transmitted trans-generation. Shorter infancy results in a trans-generational shift to earlier infantile and juvenile development.

AUTOSOMAL RECESSIVE HYPONATREMIA DUE TO ISOLATED SALT WASTING IN SWEAT ASSOCIATED WITH A MUTATION IN THE ACTIVE SITE OF CARBONIC ANHYDRASE 12

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Introduction: Genetic disorders of excessive salt loss from sweat glands have been observed in pseudohypoaldosteronism type I (PHA) and cystic fibrosis that result from mutations in genes encoding epithelial Na⁺ channel (ENaC) subunits and the transmembrane conductance regulator (CFTR), respectively. We identified a novel autosomal recessive form of isolated salt wasting in sweat which leads to severe infantile hyponatremic dehydration.

Results: Three affected individuals from a small Bedouin clan presented with failure to thrive, hyponatremic dehydration, and hyperkalaemia with isolated sweat salt wasting. Linkage analysis and genes sequencing ruled out an association to the CFTR and ENaC genes. Using positional cloning we identified an association of a Glu143Lys gene mutation in *carbonic anhydrase 12* (CA12) with the disease.

Conclusion: Carbonic anhydrase is a zinc metalloenzyme that catalyzes the reversible hydration of carbon dioxide to form a bicarbonate anion and a proton. Glu143 in CA12 is essential for zinc coordination in this metalloenzyme and lowering of the protein-metal affinity reduces its catalytic activity. This is the first presentation of an isolated loss of salt from sweat gland mimicking PHA, associated with a mutation in the CA12 gene not previously implicated in human disorders. Our data demonstrate the importance of bicarbonate anion and proton production on salt concentration in sweat and its significance for sodium homeostasis.

* Elected as best clinical abstract

A NOVEL C-TERMINAL FSH β MUTATION CAUSE PRIMARY AMENORRHEA IN THREE SIBLINGS

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Context: Inactivating mutations of the FSH β subunit gene, causing isolated FSH deficiency and hypogonadism are very rare autosomal recessive disorder. To date, only six women with delayed puberty and isolated FSH deficiency have been reported worldwide.

Objective: Clinical and molecular studies in three Palestinian sisters with impaired pubertal development and primary amenorrhea.

Patients and Methods: A 15y old female presented with delayed puberty (breast tanner II, pubic hair tanner IV), primary amenorrhea, and undetected basal serum FSH. LH peak during LHRH test was extremely high at 135mIU/ml while FSH remained low at <0.1mIU/ml. Abdominal sonography showed an infantile uterus. Two additional sisters presented later with a similar phenotype. Full pubertal development and menarche were easily achieved by estrogen and later progesterone replacement therapy. DNA was extracted from peripheral leukocytes and sequenced for FSH beta gene.

Results: A homozygous 1base pair frameshift deletion mutation in exon3 (**354delGfs9**) of the FSH β gene was found in all three affected patients predicting an alteration of the 9 amino acids following codon 118 and a premature stop codon at position 127. The parents and two healthy sisters were heterozygous for this deletion mutation.

Conclusions: A novel FSH β mutation has been detected in three hypogonadal sisters. This is only the fifth FSH β mutation reported world wide and the first mutation not involving the cystine knot and the three β hairpins of the protein that are essential to hormone binding. Further studies are required to determine the crucial role of the c-terminal 11 amino acids of FSH β on the function of FSH given the severe clinical phenotype observed in our patients.

SEXUAL INTERESTS AND HYPOGONADISM IN PRADER-WILLI SYNDROME (PWS)

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Background: Hypogonadism is a major feature of Prader-Willi syndrome, but clinical manifestations are variable. Sexual interests and behavior in this population have not been previously described.

Objectives: We studied PWS adolescents and young adults to assess (1) satisfaction with physical and sexual development (2) frequency of romantic and sexual experiences, (3) aspirations and expectations regarding marriage, (4) investigate the relation between sexual interests and hormone levels, and (5) assess the desire for hormonal replacement therapy.

Methods: The study population consisted of 27 individuals (13 males) ages 17 to 32 (mean 23.5) years with genetically confirmed PWS. Mean IQ was 75 (range 50 – 100). We conducted structured interviews using questionnaires specifically designed for this study.

Results: There was a significant negative correlation between IQ and body image in both males and females. IQ showed a positive correlation with interest in dating and romantic activities. Approximately half of PWS males and females reported having gone on a date and kissing romantically. All males and 64% of the females wished to be married. Seventy-seven percent of PWS males wanted hormonal treatment to increase phallic size. We found no correlation between hormone levels and sexual interests. Only 43% of PWS females wanted hormonal medication to achieve regular menstruation.

Conclusions: Despite documented hypogonadism, PWS young adults are interested in sexual and romantic issues. The range of sexual activities and expectations is variable. Understanding specific sexual characteristics of each individual is important in order to offer proper anticipatory sexual guidance counseling and for appropriate recommendations for hormone replacement.

LACK OF ASSOCIATION BETWEEN SEROCONVERSION AND CATCH-UP GROWTH IN CHILDREN WITH CELIAC DISEASE

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Objective: To assess the association between seroconversion and catch-up growth during the first year of gluten-free diet (GFD) in children with celiac disease (CD).

Study design: Medical records of all biopsy-proven CD children diagnosed between January 1999 and August 2009 were reviewed, and only prepubertal patients were included. Growth parameters and celiac antibodies were documented before initiation of GFD, after 6 months (period 1) in 55 patients (21 males, age 0.9-12.2 y), and after 12 months (period 2) in 37 patients of the original cohort. All growth data were transferred to standard deviation scores (SDS).

Results: Mean height velocity SDS was significantly higher in period 1 compared with period 2 (2.90 ± 3.20 vs. 0.20 ± 2.08 , $p < 0.001$) irrespective of the serological status of the patients, while the difference in mean weight-SDS gain approached a statistical significance (0.47 ± 0.82 vs. 0.15 ± 0.38 , $p = 0.074$). Mean height-SDS and mean weight SDS levels after 6 months of gluten withdrawal were significantly higher than corresponding baseline levels both in seropositive patients (-0.47 ± 0.91 vs. -0.82 ± 0.82 , $p < 0.001$ and -0.59 ± 1.17 vs. -1.11 ± 1.33 , $p < 0.001$, respectively) and seronegative patients (-1.02 ± 1.14 vs. -1.50 ± 1.12 , $p < 0.001$ and -1.19 ± 1.27 vs. -1.45 ± 1.40 , $p = 0.048$, respectively). Similarly, these growth parameters were significantly higher at the end of period 2 compared with the beginning of that period, but only in seropositive patients: -0.43 ± 0.97 vs. -0.53 ± 0.91 , $p = 0.029$ and -0.53 ± 0.86 vs. -0.75 ± 0.88 , $p = 0.009$ for height-SDS and weight-SDS, respectively. Mean levels of height velocity SDS, and the gain of weight-SDS, BMI and BMI-SDS were similar in the seropositive and seronegative groups, in both periods of the study.

Conclusions: The most remarkable catch-up growth in children with CD is expected during the first 6 months of GFD, irrespective of the serology status.

INVESTIGATING OOCYTES AND INTESTINE LINEAGES BY THE RECONSTRUCTION OF CELL LINEAGE TREES

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The embryonic development and post-natal maintenance of the female germline has recently become a subject of active scientific debate, with doubts as to the clonal relation between adult stem cells and primordial germ cells as well as conflicting evidence of post-natal oocyte renewal. Here we analyze acquired somatic mutations to reconstruct lineage trees of hundreds of oocytes as well as of other cell types like the intestine that serves as a validation for the reliability of this method. These cells were sampled from mismatch-repair deficient mice at various ages. In the reconstructed lineage trees we validated the reliability of this method by the examination of different topological aspects of the intestinal reconstructed tree which were validated by other methods that were employed in the past in this tissue. In the oocytes lineage we have shown that oocyte cluster distinctly from cells of bone marrow origin, show no lineage barrier between ovaries and increase in depth (number of cell divisions since the zygote) with mouse age, an increase accelerated after unilateral ovariectomy. The deeper oocytes in older mice may be pre-natal, entailing depth-guided oocyte maturation or post-natal, entailing oocyte renewal in the adult mouse. Our results have important implications to the understanding of the lineage origins of adult stem cells and to oocyte aging.

THE REGULATION AND TOPOLOGY OF UBIQUITINATION IN MAMMALIAN OOCYTES DURING MEIOSIS

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The cyclin-dependent kinase 1 (CDK1) is a master regulator meiosis in oocytes. The accumulation of Cyclin B1, upon reinitiation of meiosis, brings about CDK1 activation. Prior to anaphase I, the ubiquitination and subsequent proteasomal degradation of Cyclin B1, results in CDK1 inactivation, a prerequisite for the extrusion of the first polar body (PBI), representing the completion of the first meiotic division. Proteasomal degradation in mammalian cells is mediated by the formation of polyubiquitin chains, which are known to be linked through lysine 48 of the ubiquitin protein. However, ubiquitin has 6 other lysine residues capable of chain formation, the functions of most of which are unknown. We aimed at investigating the control of ubiquitin-mediated degradation on CDK1 activity during meiosis, in mouse oocytes. In particular, we explored the ubiquitin chain topology needed for PBI emission. We demonstrate herein that while the extrusion of polar body is completely inhibited by a global blockage of proteasomal degradation, the addition of a pharmacological inhibitor of CDK1 can induce both cytokinesis and anaphase. This effect is abrogated upon the introduction of a PLK1 inhibitor. Astonishingly, the extrusion of PBI in the presence of Proteasome and CDK1 inhibitors can be reversed when the CDK1 inhibitor is washed. In addition, by micro-injecting single-lysine ubiquitin mutants, we found that the K11R, but not the K48R mutated ubiquitin, blocked PBI extrusion, indicating that the lysine 11 topology, rather than the classic lysine 48 topology, is involved this process. Furthermore, we show that the K11R-induced inhibition of PBI emission can be rescued using CDK1 inhibitor. Taken together, our data sheds light on the nature of *in vivo* ubiquitination, and its control of CDK1 during mammalian meiosis.

* Elected as best basic abstract

TRANSCRIPTIONAL REGULATION OF MITOCHONDRIAL PROTEASES PROTECTING MITOCHONDRIAL HOMEOSTASIS DURING STEROID HORMONE SYNTHESIS

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Rational: High efficiency vital steroid hormone biosynthesis requires intense expression of StAR (Steroidogenic Acute Regulatory) protein known to facilitate transfer of cholesterol substrate to the inner mitochondrial membranes, where steroidogenesis ensues. StAR activity is terminated by its import into the organelle matrix, where its rapid accumulation can create a cytotoxic 'protein-overload stress'. To prevent that, the mitochondrial quality control machinery degrades StAR by concerted action of nuclear-encoded ATP-dependent protease/chaperone complexes, LON and AFG3L2 (L2). We sought the mechanism by which 'StAR-overload stress' generates mitochondria-to-nucleus communication culminating in upregulation of LON and L2 transcription to better protect the mitochondria during such stress.

Results: **(a)** *Up to a 3-fold increase of StAR degrading mito-protease gene products (mRNA/RT-qPCR and Western/protein) were observed in rat ovarian follicles induced to ovulate in vivo (eCG/hCG administrations) when Lon, Afg3l2, and other mitochondrial proteases (Spg7, Clpp and Yme111) were studied; selective upregulation was observed at the level of Lon and Afg3l2 expression in ovarian granulosa cells treated with FSH and testosterone.* **(b)** Functional mapping of human LON promoter revealed proximal regions necessary for activity and consensus binding sites (EMSA) for NRF-2 necessary for its activity. In addition, we have identified an upstream strong inhibitory element assessed to bind a key repressor factor E2F-4.

Discussion: Using powerful endocrine cell models, our findings suggest a regulated mechanism that can modify the expression of normally regarded house-keeping genes encoding mitochondrial proteases. To do so, the mitochondria somehow generate as yet to be defined signal that activates in the nucleus at least two pivotal of transcription factors, i.e., NRF-2 that is required for diverse mitochondrial functions such as respiration, biogenesis, mtDNA transcription and replication, and E2F-4 known to be a critical repressor in G₁-arrested cells. Thus, hormone producing cells of the adrenal and the gonads provide a unique model to study transcriptional regulation of mitochondrial proteases engaged in maintenance of the organelle homeostasis under crisis conditions such as heat-shock, hypoxia, aging (LON), and neuronal disorders (SPG7, AFG3L2).

MENOPAUSAL TRANSDERMAL ESTROGEN AND INTRAUTERINE LEVONORGESTREL DOES NOT INCREASE CRP LEVELS WHEREAS ORAL HORMONE TREATMENT MAY

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Background: The controversy regarding the pros and cons of menopausal hormone treatment [HT] is still ongoing since the intriguing results of the WHI study. Laboratory and experimental evidence have shown that inflammatory processes play a central role in the development, progression and outcomes of atherosclerosis. Several studies suggest that patients at high risk of developing atherothrombotic disease suffer from chronic systemic inflammation. More specifically, increased levels of C-Reactive Protein (CRP), a marker of systemic inflammation, have been associated with a higher risk of cardiovascular morbidity and mortality. CRP levels are associated not only with the presence of atherosclerosis but also with its clinical severity.

Objective: To assess CRP levels in menopausal patients treated with oral HT vs menopausal transdermal estrogen [TDE] and intrauterine levonorgestrel [Mirena IUD].

Methods: Menopausal women [n=148] under various forms of HT, or controls, have undergone CRP measurement.

Results: Patients receiving oral conjugated equine estrogen + gestagens [n=25] had mean± SD CRP level of 5.36±2.9, significantly higher than the CRP levels in all the other treatment groups or control [**P<0.001**]. The combination of TDE and Mirena had normal mean CRP concentration, not significantly different from control, or TDE and progesterone [Evorel], oral estrogen + drospirenon [Angelique], or TDE and vaginal progesterone.

Conclusion: The significantly increased CRP levels associated with oral conjugated equine estrogen + gestagens menopausal treatment may possibly represent an increased risk of CV morbidity and mortality as suggested by the WHI study. Other forms of HT, especially the TDE and intrauterine or vaginal gestagens are not associated with high CRP levels.

THE SPECTRUM OF GONADAL AND HYPOTHALAMIC FUNCTION IN ADOLESCENTS AND ADULTS WITH PRADER-WILLI SYNDROME (PWS)

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Background: Hypogonadism, a cardinal feature of Prader-Willi syndrome (PWS) is characterized by variable clinical manifestations. The etiology is heterogeneous and there is no consensus regarding treatment.

Objective: To characterize the spectrum and causes of hypogonadism in a cohort of PWS adolescents and adults.

Methods: We measured reproductive hormonal profiles of 19 males (m) and 16 females (f) ages 15 to 32 years with genetically confirmed PWS. Puberty was assessed by Tanner staging; blood was sampled for gonadotropins, sex-steroids and the gonadal-specific peptides, inhibin B (INB) and anti-Mullerian hormone (AMH).

Results: We found four distinct hormonal profiles based on INB and FSH levels: Group A (m:f; 8:1): hypergonadotrophic (primary gonadal) hypogonadism with elevated FSH levels (> 15 IU/l) and undetectable inhibin B. Group B (m:f; 4:4): hypogonadotrophic hypogonadism with FSH < 0.5 IU/l and inhibin B < 7 pg/ml. Group C (m:f; 3:5): partial gonadal and hypothalamic function with inhibin B > 20 pg/ml and FSH 2-10 IU/l. Group D (m:f; 4:6): mild hypothalamic and severe gonadal dysfunction (FSH 0.5-10 IU/L and INB < 20 pg/ml). There were significantly more males in group A vs C or D (P<0.05). Mean breast Tanner stage and testosterone levels were highest in group C (p<0.03), mean LH was highest in group A (p<0.001), and mean AMH was highest in group B (p<0.005). No differences were found in genetic subtype, age and BMI among the four groups.

Conclusion: We characterized four distinct phenotypes of hypogonadism in PWS adolescents and adults ranging from primary gonadal to hypothalamic hypogonadism; the minority had gonadotropin deficiency. Determining individual reproductive hormone patterns, including INB, may be important for assessing fertility feasibility in women and for recommending contraception to females or hormonal replacement therapy for both genders.

THE ROLE OF SPERM LIGANDS IN FORWARD AND HYPERACTIVATED MOTILITY, CAPACITATION AND ACROSOME REACTION, IN HUMAN SPERM

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Introduction: Mammalian sperm are activated by sperm ligands but the nature of the stimulating ligands is still unknown. MAPKs are key regulatory enzymes in signal transduction. We have recently characterized human sperm MAPKs, and implicated ERK in forward and hyperactivated motility and acrosome reaction (AR). Here we examine the effect of EGF and TGF- β 3 upon ERK activation and the role of ERK in forward and hyperactivated motility, capacitation and acrosome reaction.

Patient/Methods: Sperm samples from healthy donors and patients were obtained from Sheba Medical Centre Sperm Bank, Tel-Hashomer Hospital.

Results: EGF and TGF- β 3 activated ERK within 5 minutes. The effect was persistent and still detectable in sperm after capacitation. Incubation of normal spermatozoa with EGF, increased forward motility within the first 5 minutes, and hyperactivation after capacitation. Incubation with TGF- β 3 increased both forward and hyperactivated motility within the first 5 min. Later, we examined whether the ligand-induced motility is mediated *via* ERK-dependent mechanism, by adding, U0126 a selective inhibitor of MEK. Indeed, pre-incubation with the inhibitor reduced the percentage of motile sperm and abolished the effect of the ligand on forward motility. Both EGF and TGF- β 3 stimulated also sperm AR and the effect was mediated by ERK. We have also identified several proteins that were phosphorylated on tyrosine during capacitation and the effect was markedly reduced in the presence of a MEK inhibitor.

Conclusions: EGF and TGF- β 3 stimulate sperm motility and AR *via* an ERK-mediated cascade. Protein tyrosine phosphorylation, a hallmark of capacitation is also mediated by ERK.

LONG ACTING HORMONES DESIGNED BY GENE FUSION AND GENE TRANSFER ARE SAFE FOR USE IN CLINICS

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Glycoprotein hormones are used clinically in the treatment of many diseases. One major issue regarding the clinical use of many peptides is their short half-life span in the body, due to the rapid clearance from the circulation. The low stability of peptides has thus often posed a difficulty to researchers and hindered their adoption in potential medical applications. Thus, at the clinical level, there is a need for a regime of frequent injections of the peptides into the patients to overcome this low stability factor. The major strategies for overcoming this problem by pharmaceutical companies are based on chemical techniques and using specific peptidase inhibitors or cocktails. To overcome this problem, we used genetic engineering techniques that have been found successful for designing long acting hormones. Using overlapping PCR and gene transfer techniques, we succeeded to add the signal sequence of O-linked oligosaccharides to the coding sequence of the hormones. The cassette gene that have been used contains the sequence of the carboxyl-terminal peptide (CTP) of human chorionic gonadotropin β (hCG β) subunit. The CTP contains 28 amino acids with four O-linked oligosaccharide recognition sites. It was postulated that the O-linked oligosaccharides add flexibility, hydrophilicity and stability to the protein. On the other hand it was suggested that the four O-linked oligosaccharides play an important role in preventing plasma clearance and thus increasing the half-life of the protein in the circulation. Using this strategy we succeeded to ligate the CTP to the coding sequence of follitropin (FSH), thyrotropin (TSH), erythropoietin (EPO) growth hormone (GH) and thus to increase the longevity and bioactivity of these proteins in-vivo. Interestingly, the new analog of FSH was found not immunogenic in humans and it is already passed successfully clinical trials phase III. Moreover, FSH long acting was approved by the European Commission (EC) for treatment of fertility. All designed variants were successfully expressed in Chinese Hamster Ovary Cells (CHO). Designing long acting peptides will diminish the cost of these drugs and perhaps reduce the number of injections in the clinical protocols.

PRIMARY HYPERPARATHYROIDISM: UNSOLVED ISSUES AND TREATMENT

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Primary hyperparathyroidism (PHPT) is a common endocrine disorder characterized by elevated calcium and inappropriately high levels of serum PTH. Depending on severity, it may be accompanied by hypercalcemic symptoms, nephrolithiasis, hyperparathyroid bone disease, bone loss and neuromuscular weakness. Over the past 20 years a milder form of PHPT, characterized by the absence of the above clinical manifestations (asymptomatic PHPT) has increasingly been recognised as a clinical problem.

The primary aim of management is to normalize serum calcium and reduce PTH levels, leading to improvement in any associated symptoms. Parathyroidectomy (PTx) is the only curative treatment and in experienced hands it is successful in up to 95% of patients.

Surgery is an effective choice also in PHPT patients with asymptomatic PHPT who meet surgical criteria. In these patients, parathyroidectomy (PTx) has been shown to normalize parathyroid hormone [PTH] and serum calcium, and to increase bone mineral density (BMD). Parathyroidectomy has also been shown to normalize serum calcium and PTH and increase BMD in asymptomatic PHPT patients who do not meet the surgical criteria. Further studies are needed to confirm whether surgery benefits neurocognitive and cardiovascular symptoms. Studies of the natural history of asymptomatic PHPT indicate that in the absence of surgery some patients show stability in biochemical measures (serum calcium and PTH levels) and BMD, however this is only temporary. Current guidelines therefore recommend that patients are regularly monitored and eventually appropriately managed by medical therapy. The guidelines define regular monitoring as annual monitoring of serum calcium and PTH, and bi-annual 3-site BMD.

However there are few alternative treatment options in patients who are ineligible for, or unwilling to undergo, surgery and those in whom PTx has failed. Medical therapy targets the compromised organ(s). Current options include the off-label use of bisphosphonates, selective estrogen receptor modulators and hormone replacement therapy, and the recently approved calcimimetics cinacalcet.

Bisphosphonates and hormone replacement therapy effectively increase BMD and decrease bone turnover, but have no significant impact on serum calcium or PTH levels. The calcimimetic cinacalcet reduces serum calcium and PTH and raises serum phosphorus in these patients, but has no effect on BMD. Medical management should be offered to patients with contraindication to surgery or unwilling to have PTx. It could also be considered in selected asymptomatic PHPT patients who meet the surgical criteria for PTx.

DISSECTING THE CENTRAL STRESS RESPONSE USING SITE-SPECIFIC GENETIC MANIPULATION IN ADULT MICE

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The biological response to stress is concerned with the maintenance of homeostasis in the presence of real or perceived challenges. This process requires numerous adaptive responses involving changes in the central nervous and neuroendocrine systems. When a situation is perceived as stressful, the brain activates many neuronal circuits linking centers involved in sensory, motor, autonomic, neuroendocrine, cognitive, and emotional functions in order to adapt to the demand. However, the details of the pathways by which the brain translates stressful stimuli into the final, integrated biological response are presently incompletely understood. Nevertheless, it is clear that dysregulation of these physiological responses to stress can have severe psychological and physiological consequences, and there is much evidence to suggest that inappropriate regulation, disproportional intensity, or chronic and/or irreversible activation of the stress response is linked to the etiology and pathophysiology of anxiety disorders and depression.

Understanding the neurobiology of stress by focusing on the brain circuits and genes, which are associated with, or altered by, the stress response will provide important insights into the brain mechanisms by which stress affects psychological and physiological disorders. The CRF/Urocortin system is fundamental in orchestrating the organisms stress response. In addition to its hypophysiotropic action, CRF integrates the behavioral responses to stress within the central nervous system. This lecture will present an integrated multidisciplinary approach from gene to behavior using mouse genetics and animal models aim in elucidating the contribution of different members of the CRF/Urocortin family of peptides and receptors to the central stress response. Defining the contributions of known and novel gene products to the maintenance of stress-linked homeostasis may improve our ability to design therapeutic interventions for, and thus manage, stress-related disorders.

* Winner of Lindner Award

DIFFERENTIAL EXPRESSION OF NOVEL ADIPONECTIN RECEPTOR-1 TRANSCRIPTS IN SKELETAL MUSCLE OF SUBJECTS WITH NORMAL GLUCOSE TOLERANCE AND TYPE 2 DIABETES

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Objective: Adiponectin receptor-1 (AdipoR1) expression in skeletal muscle has been suggested to play an important role in insulin resistance and diabetes. We aimed at evaluating the presence of novel AdipoR1 splice variants in human muscle and their regulation under physiological and pathophysiological states.

Research Design and Methods: AdipoR1 5'UTR mRNA transcripts, predicted from bioinformatics data, were evaluated in fetal and adult human tissues. Expression and function of the identified transcripts were assessed in cultured human skeletal muscle cells and in muscle biopsies obtained from individuals with normal glucose tolerance (NGT) and type 2 diabetes (T2D) (n=49).

Results: Screening of potential AdipoR1 5'UTR splice variants revealed a novel highly abundant muscle transcript (R1T3), in addition to the previously described transcript (R1T1). Unlike R1T1, R1T3 expression was significantly increased during fetal development and myogenesis, paralleled with increased AdipoR1 protein expression. The 5'UTR of R1T3 was found to contain uORFs that repress translation of downstream coding sequences. Conversely, AdipoR1 3'UTR was associated with enhanced translation efficiency during myoblast-myotube differentiation. A marked reduction in muscle expression of R1T3, R1T1 and R1T3/R1T1 ratio was observed in individuals with T2D, as compared with NGT subjects, paralleled with decreased expression of the differentiation marker myogenin. Among NGT subjects, R1T3 expression was positively correlated with insulin sensitivity.

Conclusions: These results indicate that AdipoR1 receptor expression in human skeletal muscle is subjected to posttranscriptional regulation, including alternative splicing and translational control. These mechanisms play an important role during myogenesis and may be important for whole body insulin sensitivity.

* Winner of Chowers Award

PRECLINICAL TESTING OF NOVEL THERAPEUTIC APPROACHES IN ENDOCRINE TUMOR MODELS

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Endocrine tumors represent a heterogeneous group of neoplasia. Dependent on their cellular origin these tumor cells often retain specific functional properties including hormonal secretion and responsiveness to endocrine regulatory pathways. While some tumors are causing clinical symptoms primarily due to autonomous hormone production others are defined by effects related to their proliferative capacity and ability for metastatic spread. Only a few cell lines are available for endocrine tumors which furthermore do not reflect these heterogeneous functional properties and specific therapeutic response rates of individual tumors. Preclinical tumor models can aid in investigation of a number of aspects including elucidation of functional mechanisms as well as development of therapeutic approaches. Following this approach we have utilized a number of different *in vitro* and *in vivo* models for endocrine tumors. In the proposed presentation some examples including development and testing of novel liposomal agents with targeted properties, preclinical testing of vascular disrupting agents will be highlighted. To facilitate patient individual treatments and thereby to optimize therapeutic efficacy, we are currently aiming at the development and characterization of patient-individual tumor models. To investigate whether morphological and functional characteristics between tumor samples after mouse engraftment in comparison to the original tumor would be comparable, we started examination of implanted material and original patient tumor by histology and immunohistochemistry. First comparisons indicate that the implanted tumors keep the characteristics of the original tumor material in the murine host. These findings need to be further substantiated and additional endpoints such as vascularization and endocrine potential need to be examined. Nevertheless, these tumor models have the potential to evaluate individualized treatment modalities in the future.

The Epidemic of Primary Hyperaldosteronism: Where is the Beef?

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At the onset of this century, the medical literature has been densely seeded by reports that primary hyperaldosteronism is not as rare as previously believed and accounts for ~10% of the cases of hypertension, rather than 0.5-2% as then cited by leading textbooks of the time. Driven by powerful academicians in the hypertension and adrenal field and reinforced by data coming from all over the world, albeit nearly exclusively from referral centers, this new information incited much enthusiasm and was the actual driving force in the formation of the Endocrine Society's sponsored guidelines for the diagnosis and treatment of primary hyperaldosteronism. By the time these guidelines were released, some 2-3 years ago, it became apparent, though not explicitly admitted, that the distinguished members of the guidelines writing committee were somewhat skeptical of the already widespread expectations that a tide of hyperaldosteronism might over flood endocrine practice. Indeed, the claim that one tenth of the hypertensive population harbor primary hyperaldosteronism had little chance of substantiation even at its glorious days. First, hypertension itself is far more common than ever appreciated and its prevalence rises steadily with age at a time in which longevity itself continues to increase. Second, PRA, the basis for the hailed Aldosterone/PRA ratio declines with age, making it difficult to use particularly in the population in which hypertension is now especially prevalent. Third, none of the reports on the epidemic of primary hyperaldosteronism is based on a population study, making the true prevalence of this disease among hypertensive subjects difficult to determine. Fifth, recent reports do not support the initially claimed widespread presence of this disease. Nevertheless, the hyperaldosteronism epidemic legend had a distinct positive impact, as it revived not only the pursuit of this diagnosis under proper clinical circumstances (e.g., young hypertensive subjects, resistant hypertension even with normal K+, adrenal incidentaloma), but invigorated interest in aldosterone and its impact on the cardiovascular system, thus leading to new insights on its role in cardiac, renal and cerebrovascular disease. The revelation of novel interactions between aldosterone and adipose tissue and aldosterone and the brain may well be the most important inadvertent sequels of the short lived epidemic of primary hyperaldosteronism.

PHEOCHROMOCYTOMA: UPDATE

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Pheochromocytoma is a rare and fascinating catecholamine-secreting tumor derived from neural crest cells, which refers to both medullar adrenal tumors and paragangliomas. It was the subject – according to a PubMed search - of 2341 medical articles in the last 5 years. Selected papers with the highest impact on the global perception of the disease were chosen to present the most recent interesting updates. First, a systematic review of the publications reveals a large variety of non-classical clinical presentations of pheochromocytomas ranging from silent tumors to tako-tsubo syndrome; as well, new publications concerned diagnostic biochemical markers, functional imaging protocols and operative therapeutic approaches of pheochromocytoma. Then, it appears that the field which undoubtedly benefitted the more striking advances is the genetics of pheochromocytomas and paraganglioma: to the known mutations in the REarranged in Transformation (RET) proto-oncogene, in the Von-Hippel Lindau (VHL)-tumor suppressor gene, and in the neurofibromatosis type 1 (NF-1) tumor suppressor gene, new mutations in 5 genes coding the mitochondrial Succinate Dehydrogenase (SDH) Complex (SDHA, SDHB, SDHC, SDHD, SDHAF2) were associated with high predisposition to the disease. All these mutations share a neuronal apoptotic pathway, but in the very last months, an absolutely novel mutation in the gene of a putative transmembrane protein TMEM127 was associated with adrenal pheochromocytoma, opening the way to exploration of totally new pathophysiologic pathway of the disease and to probably other candidate genes. As a result, the proportion of hereditary pheochromocytoma raised from classically admitted 10% to near 32 %. The question of performing a genetic screening in cases of pheochromocytoma is thus critical, and some publications describe relationship between genotype and phenotype (localization of the tumor, catecholamine profile...) which can orientate toward specific gene screening. Finally, new data concerning identification of predictive markers of malignancy as well as new therapeutic possibilities for malignant pheochromocytoma will be reviewed.

**CONGENITAL ADRENAL HYPERPLASIA DUE TO STEROID 21-
HYDROXYLASE DEFICIENCY**
Review of the Endocrine Society Clinical Practice guideline

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Clinical practice guideline for congenital adrenal hyperplasia (CAH) was recently developed by a Task Force which included clinicians experienced in treating CAH. Additional experts were also consulted.

Consensus Process: Consensus was guided by systematic reviews of evidence and discussions. The evidence-based guidelines were developed using the Grading of Recommendations, Assessment and Evaluation system to describe the strength of recommendations and the quality of evidence.

Screening and Diagnosis: A universal newborn screening for severe steroid 21-hydroxylase deficiency followed by confirmatory tests is recommended. The diagnosis rests on clinical and hormonal data; genotyping is reserved for equivocal cases and genetic counseling.

Treatment: Glucocorticoid dosage should be minimized to avoid iatrogenic Cushing's syndrome. Mineralocorticoids and, in infants, supplemental sodium are recommended in classic CAH patients. Prenatal treatment of CAH continues to be regarded as experimental. The Task Force recommends against the routine use of experimental therapies to promote growth and delay puberty. Surgical guidelines emphasize early single-stage genital repair for severely virilized girls, performed by experienced surgeons. Bilateral adrenalectomy should be avoided. Clinicians should consider patients' quality of life, consulting mental health professionals as appropriate. At the transition to adulthood, monitoring for potential complications of CAH is recommended. Finally, judicious use of medication during pregnancy and in symptomatic patients with nonclassic CAH is recommended.

BONE PHYSIOLOGY—UPDATE AND IMPLICATIONS FOR NEW THERAPIES

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Bone remodeling is a continuous process that is essential for the biomechanical integrity, function and response to various stimuli of the skeleton. Bone remodeling occurs unsynchronized in many microscopic sites and is composed of a short phase (2–3 weeks) of bone resorption by specific cells, the osteoclasts, followed by a long phase (3–4 months) of bone formation by the osteoblast. Secondary mineralization that will finalize the cycle, may take another 12–18 months. Imbalance between bone formation and bone resorption will lead to changes in bone quantity and quality, that may be expressed as clinical signs and symptoms. A negative balance in bone remodeling is the pathophysiological mechanism that will lead to post-menopausal, senile and glucocorticoid-induced osteoporosis, to mention some examples. Major advances in the understanding of the origin, differentiation, function and the various control mechanisms of the osteoclast, the osteoblasts and its derivative cells, as the osteocyte, have been achieved during the last few years. A major common, though not exclusive pathway that controls the various stages in osteoclasts' development, function and life span, the receptor activator of Nf kappa B (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG), had been elucidated. The discovery of the Wnt, low density lipoprotein-like protein 5 (LRP5), beta-catenin pathway as a major regulator of osteoblasts' recruitment, differentiation and function as well as its negative and positive regulators and the cross-talk between osteoblasts, osteocytes and osteoclasts, are major breakthroughs in understanding bone physiology and pathophysiology.

There are new therapies already in use, in clinical trials and being developed that affect bone resorption and/or bone formation and are based on this newly-gained knowledge. These drugs may reduce the rate of bone fractures and its devastating clinical effect in osteoporosis, prevent disuse bone loss and enhance bone repair and fracture healing.

A short review of the above-mentioned mechanisms will be discussed.

HIF-1 ALPHA REGULATES ECM SECRETION IN THE HYPOXIC GROWTH PLATE

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Growth plate chondrocytes are professional secretory cells, engaged in the secretion of extracellular matrix (ECM) to form the cartilaginous template of developing bones during endochondral ossification. Unlike most other tissues, the growth plate is avascular and therefore hypoxic. The ability of chondrocytes to survive and secrete vast amounts of ECM under hypoxic conditions raises questions concerning the mechanism underlying this adaptation. Hypoxia inducible factor-1 (HIF-1), a key regulator of cellular hypoxic response, is necessary for chondrocyte survival under hypoxia, limiting the ability to study its effects by a loss-of-function approach. In order to bypass this limitation and investigate the possible role of HIF-1 in ECM secretion by growth plate chondrocytes, we utilized mice with temporally activated cKO of Hif-1 α , the oxygen sensitive subunit of HIF-1, in these cells (Hif-1 α /fCol2ERCre).

We show here for the first time that Hif-1 α inactivation in the growth plate results in the intracellular accumulation of major ECM components in the hypoxic central region of the growth plate. This was accompanied by ER stress and activation of the unfolded protein response. In addition, the content of the ECM surrounding the cells was reduced and resulted in cell-matrix detachment. Taken together, these results indicate that Hif-1 α inactivation in the growth plate inhibits proper folding and secretion of cartilage ECM under hypoxia. To further understand the molecular mechanisms by which HIF-1 α regulates cartilage ECM folding, we examined the involvement of HIF-1 α in posttranslational modifications of cartilage ECM. We show that upon Hif-1 α inactivation, there was a reduced expression of collagen prolyl-4 hydroxylase subunits, which are required for collagen hydroxylation. Moreover, the growth plate was significantly more hypoxic and protein glycosylation was impaired. In this work, we establish a new role for HIF-1 α in the regulation of cartilage ECM folding and secretion under hypoxia. In addition, we provide evidence for the importance of HIF-1 α in post-translational modifications and biosynthesis of cartilage ECM.

A SERM-LIKE ACTIVITY OF DIETARY COMPOUNDS IN BONE AND CANCER CELLS

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Various phyto-nutrients, carotenoids, polyphenols and isothiocyanates were found by us to inhibit estrogen signaling, in breast and endometrial cancer cells. In addition, we and others have shown that these phyto-nutrients induce the antioxidant response element (ARE) and the Nrf2 transcription factor. Using overexpression of Nrf2 and siRNA for this gene, we demonstrated that Nrf2 is involved in the phytonutrient-induced inhibition of the estrogenic activity. Although the effect of estrogens in breast and endometrial cancer is harmful, it is beneficial for bone formation. Thus, we investigated the effect of the phyto-nutrients on estrogenic activity in osteoblasts. We found that the dietary compounds, which inhibit estrogenic activity in cancer cells, did not inhibit and even stimulated the expression of estrogen-induced genes in osteoblast-like cells. The effect of glucocorticoids in bone is opposite to that of estrogens and glucocorticoid treatment leads to bone resorption and osteoporosis. Thus, we determined whether phytonutrients inhibits glucocorticoid activity in bone. We found that the expression of glucocorticoid-dependent bone-destroying gene (RANKL) and the glucocorticoid inhibition of bone-supporting genes (osteocalcin, osteoprotegerin) were both reversed by the phytonutrients. The phytonutrients increased estrogen receptor- α level in bone cells nuclei but reduced its level in nuclei of breast cancer cells and did not affect the level of glucocorticoid receptors. As discussed above, Nrf2 was found to be involved in the inhibition of estrogenic activity in breast cancer cells. In contrast, in bone cells, over-expression of Nrf2 enhanced estrogen-induced transcription but reduced glucocorticoid-induced transcription, similar to the effect of the phyto-nutrient. In addition, reduction of Nrf2 level, by siRNA, leads to a decrease in phytonutrient supported activity of estradiol in bone cells. In addition to their positive effect on osteoblasts which can lead to increased bone formation, the dietary compounds were found to interfere with RANK-Ligand dependent osteoclastic differentiation, which can lead to reduction in bone resorption. In conclusions, dietary phyto-nutrients, which inhibit estrogenic activity in cancer cells, do not inhibit and even stimulate estrogen signaling in osteoblastic bone cells but inhibit the deleterious effects of glucocorticoids in these cells. The results suggest that Nrf2 is partially involved in these activities.

BONE LOSS IS FAT GAIN – THE ROLE OF SIRTUIN 1 IN BONE

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Bone loss is an inevitable consequence of aging. While for most organs, aging pathologic processes accumulate with advancing age and different diseases occur in different individuals, bone loss occurs virtually in everyone if they live long enough, resulting in most in osteoporosis. Age-related bone loss is characterized by reduction in the osteoprogenitor pool in the marrow accompanied by increased marrow adipogenesis leading to decreased bone formation.

Sirtuin 1 (*Sirt1*), the mammalian homologue of yeast Sir2, is a member of the sirtuin family of highly conserved NAD⁺-dependent deacetylases that was found to regulate life span in lower organisms and affect metabolic processes in mammals. First identified for its role in chromatin remodeling associated with gene silencing, *Sirt1* was then discovered to be a mediator of the life-extending effect of calorie restriction in lower organisms. *Sirt1* deacetylates histones and a host of key regulatory proteins affecting transcription and function. Although there is no definite evidence that *Sirt1* regulates lifespan in mammals, over-expression of *Sirt1* in mice confers protection against obesity, impaired glucose tolerance, and Alzheimer's disease. Its role in bone and in osteoporosis has not been studied yet.

We have recently uncovered that *Sirt1* is a major regulator of bone mass by influencing osteoblast differentiation from its mesenchymal marrow stem cell (BM-MSC) progenitor. Using mice with a germ line mutation in *Sirt1*, we show that *Sirt1* haplo-insufficient mice have a dramatic reduction in bone mass, accompanied by increased marrow adipogenesis. Importantly, we identified a novel bone-specific target of *Sirt1*, a critical inhibitor of bone formation, which is negatively regulated by *Sirt1*. These findings have potential CLINICAL implications suggesting that *Sirt1* is a target for promoting bone formation as an anabolic approach for treatment of osteoporosis.

**Poster
Abstracts
Group A**

MALIGNANCY PREDICTORS OF ADRENAL INCIDENTALOMAS

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Background: Adrenal incidentalomas are common on imaging studies and should be assessed for hormone secretion and risk for malignancy.

Objective: The aim of this study was to identify the adrenal incidentaloma characteristics which predict malignancy.

Methods: We performed a retrospective study of all adrenalectomies performed in three academic hospitals in Jerusalem between 1999-2008. Hormone secretion and imaging characteristics were analyzed. The prevalence of each variable and its correlation with the final diagnosis were studied.

Results: Two-hundred thirty five patients were studied. Sixty percent of the patients were women and the mean age was 52 years. In 28.9% of the patients, the lesion was an incidental finding; and mean tumor diameter was 4.8 cm. Hormonal hypersecretion was found in 67.8%.

12.8% of all adrenal lesions were malignant: 43% of which were adrenocortical carcinomas and 57% were metastasis. Sixty-nine percent of all adrenal lesions were benign and 16.6% were pheochromocytomas.

A multiple logistic regression analysis showed that tumor size correlated with the risk for malignancy ($p=0.001$), and that hormone secretion was associated with a lower risk for malignancy ($p=0.05$). A ROC analysis showed that tumor diameter of 4.6 cm was the optimal cut-off size for differentiating between benign and malignant tumors with a sensitivity of 77% and specificity of 69%. The incidence of malignancy in patients that were operated due to imaging findings suggestive of malignancy was 17.6%.

Conclusions: In our cohort, lesion size on CT imaging was the most powerful predictor of malignancy, while hormonal hypersecretion was associated with a final diagnosis of a benign tumor. Most lesions suspected to be malignant based on imaging studies were benign. Despite of the infrequency of malignancy under these circumstances, adrenalectomy is probably recommended to allow an early diagnosis of rare adrenocortical carcinomas.

CREATININE CORRECTED 24-HOUR URINARY CATECHOLAMINE METABOLITES FOR THE DIAGNOSIS OF PHEOCHROMOCYTOMA

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Background: The assessment of 24-hour urinary excretion rates of catecholamine metabolites (24UMET) remains the first-line biochemical investigation for pheochromocytoma. The diagnostic accuracy of this test is based on proper collection and reporting of urine volume, which, in daily life, poses an obstacle. Alternative test of creatinine-corrected urinary catecholamine metabolite level ratio (CCR) was examined by Heron et al. (1996), but measurement of CCR is still not in routine use. The diagnostic validity of CCR for the diagnosis of pheochromocytoma was investigated.

Methods: Retrospective analysis of our patients' files, evaluated due to suspicion of pheochromocytoma during the years 2007-2010, was conducted. Medical history, 24UMET, CCR results, imaging and pathological reports were examined. The presence of pheochromocytoma was confirmed at post-surgery pathology. In patients with abnormal urine results, the absence of pheochromocytoma was documented by further laboratory and imaging procedures. Sensitivity, specificity, positive predictive value and negative predictive value of 24UMET and CCR for the diagnosis of pheochromocytoma were evaluated.

Results: Out of 111 patients tested, 16 (14%) had inadequate urinary collection and were thus not included in the analysis. The results of 95 patients; 41 male and 54 female, aged 42-80 years are presented in the table.

Table : Comparative evaluation of CCR [Metanephrine<<220ug/gr.creat] and 24UMET [Metanephrine 30-180ug/24h*]

| Patient/test | CCR(+) | CCR(-) | 24UMET(+) | 24UMET(-) |
|---------------------------|--------|--------|-----------|-----------|
| PH-positive (n=6) | 6 | 0 | 5 | 1 |
| PH-negative (n=89) | 5 | 84 | 11 | 78 |
| Positive predictive value | 54% | | 31% | |
| Negative predictive value | 100% | | 98.6% | |
| Sensitivity | 100% | | 83% | |
| Specificity | 94% | | 87.6% | |

* Mayo criteria

Lately, we have started to evaluate overnight-CCR [ON-CCR] as a modification of the CCR from 24 hours. Two patients with pheochromocytoma had elevated CCR in both 24hour and ON test. Two other patients without pheochromocytoma had normal ON-CCR, 24UMET and CCR

Conclusions: CCR is a sensitive test with a negative predictive value of 100%. Therefore, it can be a good screening test for pheochromocytoma. Overnight CCR should be further studied to determine its possible role as a simplified diagnostic test.

PREVALENCE OF METABOLIC SYNDROME IN PATIENTS WITH ADRENAL INCIDENTALOMAS

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Background: Adrenal incidentalomas are found on abdominal CT scans in about 4.4% of cases. Many of them are attributed to Cushing syndrome and adrenal myelolipomas.

Aim: The aim of our study was to evaluate the presence of metabolic syndrome in patients with adrenal incidentalomas.

Patients and methods: Metabolic syndrome was documented when three or more of the following parameters were present: waist circumference >88cm/102 cm [Female/Male], serum triglycerides >150 mg/dl, glucose >100 mg%, HDL-cholesterol <50/40 mg/d [Female/Male], blood pressure >130/85 mm/Hg, in patients with adrenal incidentaloma.

A total of 105 patients [69 females and 38 males; aged 67.3± 10.8; range 42 - 84 yr] with CT features of cortical adenoma participated in our study. We evaluated 24- hour urine catecholamines excretion, 24- hour urinary free cortisol, dexamethasone suppression test, and blood potassium level.

Adrenal androgens status included dehydroepiandrosterone sulfate, 17-hydroxyprogesterone, and total testosterone. The presence of metabolic syndrome was documented.

Results: Myelolipomas on abdominal CT scans were found in 29 patients [27%]. Hypertension was found in 53 patients [49%], hyperlipidemia in 56 patients [52%], and diabetes mellitus in 36 participants [33.6%]. Metabolic syndrome was present in 45 patients [43%.]

Conclusion: We conclude that metabolic syndrome is common in patients with adrenal incidentalomas.

MULTIDISCIPLINARY APPROACH TO A PATIENT WITH HURTHLE CELL CARCINOMA

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We present a subject with complicated Hürthle cell carcinoma [HCC] treated by multidisciplinary coordinated approach

Case report: A 67 years old man, was referred to our center because of a local recurrence of left [LT] central neck [CN] lesion with a cytological HC features. On 2005, he had a total thyroidectomy with bilateral CN dissection [BL-CND] due to intra-operative interpretation of frozen section as Medullary cell carcinoma. The final pathologic diagnosis was left 3.5cm benign HC adenoma. 5 years later he was sent to a LT-CND of the 2.5 cm lesion. The pathologic examination confirmed HCC with local invasion into muscle fibers. Revision of the former pathology was again HC adenoma. He was treated with 150MCi ¹³¹I. In whole body scan few ¹³¹I uptakes were seen in the CN. Follow-up ultrasound [US] demonstrated 2 lesions: a 7 mm on the LT-side which was positive for HCC by FNA, and a 3mm on the right paratracheal region, not accessible to FNA. In order to study the viability of the lesions, PET-FDG scan was performed. Both lesions had clear uptakes. After integrating these results we realized that the 7mm lesion absorbed ¹³¹I in the post-surgical treatment and is not a new metastasis and the other 3mm lesion is metabolically highly active. So, a third operation was planned. Since the ability to localize a 3mm lesion in a heavily changed field was low, we used a new technique of US-guided tattooing of the lesions. This procedure enabled operative localization and successful surgical excision of both HCC lesions.

Conclusion: 1-Surgically treated patients with HC adenoma must be further followed. 2-Uptake of treatment dose ¹³¹I does not always lead to cell death. 3-Even a small lesion of HCC can be seen on a PET-FDG scan. 4-Preoperative US-guided tattooing is uncomplicated and has a role in operative localization of very small lesions. This case exemplifies the advantages of treatment by a multi-disciplinary coordinated approach.

N-t-BOC-HEXYLENIAMINE DERIVATIVE OF 7-(O)-CARBOXY-METHYL DAIDZEIN INHIBITS THE IN VITRO GROWTH OF HUMAN THYROID CANCER THROUGH ESTROGEN RECEPTOR β –DEPENDENT PATHWAYS INVOLVING THE FORMATION OF REACTIVE OXYGEN SPECIES

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Thyroid cancer incidence is up to three fold higher in women than in men, suggesting the possible involvement of estrogens in its pathogenesis. The present study investigated the effect of a novel isoflavone-derived anti-estrogenic compound developed in our laboratory, the N-t-boc-hexylenediamine derivative of 7-(O)-carboxymethyl daidzein [cD-tboc] in human thyroid cancer cells. **First:** the mRNA expression of estrogen receptor α and β (ER α and ER β) was confirmed in several human thyroid cancer cell lines, in human non-malignant, in goiterous cells and in papillary thyroid cancer cells harvested during thyroidectomy. All cell types expressed both ER α and ER β with a variably higher abundance of ER β over ER α . **Second:** DNA synthesis and creatine kinase (a marker of estrogenic genomic response) were increased in response to estradiol-17 β (E2), the ER α agonist PPT as well as the ER β agonist DPN. **Third:** as determined by DNA synthesis, the XTT assay and direct microscopic visualization, cD-tboc markedly inhibited cell growth in all types of human thyroid cancer by-60-90%, be it of cell line- or patient-derived origin and also slowed down, albeit to a lesser extent, the growth of non-cancerous human thyroid cells (0-50%). Very significantly, cD-tboc abolished E2-induced cell growth in cancer cells, but only partially in goiter and normal cells (70 vs. 45%). **Fourth:** functionally critical for the growth-inhibitory effect of cD-tboc was its ability to increase (ROS) formation, since inhibition of NADPH-oxidase activity by DPI not only abolished ROS formation, but also partially inhibited the cytotoxic effects of cD-tboc. **Fifth:** cD-tboc could not induce cancer cell death when ER β was inactivated either by co-incubation with its antagonist PTHPP (10 vs 70%) or human anaplastic thyroid cancer cell line transfected with ER β SiRNA (5 vs 70%) but not ER α SiRNA. In the latter cells, the expression of ER β was markedly suppressed (0 vs 70%). This is the first evidence that cD-tboc acts as an anti-human thyroid cancer agent *in vitro* in a variety of cell types (including cancer cells removed from human thyroid cancer patients) via ER β -dependent mechanism(s) involving ROS formation.

BILATERAL CORTISOL SECRETING ADENOMAS TREATED BY PARTIAL ADRENALECTOMY

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Cushing's syndrome due to bilateral cortisol-secreting adenomas (BiCA) is very rare. The preferred surgical approach of partial adrenalectomy is not well known in Israel. We present a case of Cushing's syndrome due to BiCA and describe the treatment course.

Introduction: BiCA is classified among the macronodular adrenal hyperplasias. It is characterized by distinct adenomas (usually two or three), with internodular atrophy. Previous literature reported only several dozens of cases of BiCA, most of them in middle aged females of East Asian origin. Currently, the first line surgical approach in BiCA is partial adrenalectomy (also called adrenal sparing surgery), at least unilaterally. Having undergone this procedure, the patient may have a chance to remain glucocorticoid independent.

We found three case-reports of successful steroid withdrawal 10 to 16 months after laparoscopic bilateral parietal adrenalectomy due to BiCA. In these cases the zona glomerulosa functioning was preserved.

Case report: A 47-year-old female from the Philippines presented to our department with full blown Cushing's syndrome. Diagnosed with ACTH independent Cushing's syndrome, she was referred to adrenal imaging. CT scan demonstrated two adrenal macronodules, one in each gland, surrounded by atrophied glands. The patient underwent right total adrenalectomy but, remained hypercortisolemic. In order to try and preserve normal cortical functioning we decided to perform partial adrenalectomy on the other adrenal. The patient underwent the operation without any complications and consequently attained biochemical remission.

The pathology report was in accordance with the diagnosis of BiCA: 1cm and 3cm adenomas surrounded by atrophied cortical adrenal tissue.

After the operation, the patient did not need mineralocorticoid replacement to control potassium level, but is still glucocorticoid-dependent 6 months later.

Conclusion: In our single experience, partial adrenalectomy was an uncomplicated surgical approach. The surgeons and endocrinologists should be aware of this adrenal sparing procedure in treating bilateral adrenal disease.

CONGENITAL ADRENAL HYPERPLASIA IN DUE TO HSD3B2 MUTATION

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Background: 3 β -Hydroxysteroid dehydrogenase (3 β -HSD) deficiency is a rare cause of congenital adrenal hyperplasia (CAH). It results from mutations in the structure of type II 3 β -HSD gene (*HSD3B2*) and is classified as classical and nonclassical forms. Classical 3 β -HSD deficiency is characterized by salt wasting. In males it is associated with incomplete virilization of the external genitalia, whereas females exhibit normal external genitalia or mild virilization.

Subjects and methods: The patient is a full term female infant. Her parents are 2nd degree cousins of Jewish ethnicity from the Caucasus. Physical examination was unremarkable with normal external genitalia. Newborn screening for 17-hydroxyprogesterone showed elevated level (153 nmol/l). Repeated venous sample revealed a 17- hydroxyprogesterone level of 181 nmol/l, testosterone >55 nmol/l, androstendione >34.5 nmol/l, cortisol 292 nmol/l and aldosterone 1000 pmol/l. On the 6th day of life she developed salt wasting (serum K-7.3 meq/l Na-132 meq/l) and a combined therapy with hydrocortisone, fludrocortisone and saline was initiated. Karyotype was 46XX; Abdominal and pelvic US revealed normal uterus and adrenal hyperplasia.

Genetic analysis: Evaluation of the P450c21 and P450c11 genes for the common mutations in the Jewish population was negative. Sequencing of *HSD3B2* was performed.

Results: A homozygote missense mutation in exon 4 of the *HSD3B2* gene was found. This C>A mutation results in the substitution of proline for threonine in codon 222 (P222T) and has been reported previously. The P222T protein was found unstable, with absent enzyme activity both in vivo and in vitro.

Conclusions: The P222T mutation causes classic 3 β -HSD deficiency CAH. This case emphasizes the importance of neonatal newborn screening for CAH, particularly in the absence of ambiguous genitalia.

THE EXPERIENCE OF RECEIVING RADIOACTIVE IODINE TREATMENT DURING ISOLATION AT HOME - FROM KNOWLEDGE TO INTERVENTION

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Background: The purpose of the study was to examine the emotional experience of receiving radioactive iodine treatment in isolation at home in patients with thyroid cancer. The primary mission was to explore ways to improve isolation care outside the hospital in order to ensure effective handling of the treatment and the patient's well-being.

Method: The research tool was a semi-structured in-depth interview, recorded and transcribed in accordance with the guidelines of Smith and Osborne for qualitative research. The study group included 11 patients with thyroid cancer attending Rabin Medical Center who had received radioactive iodine therapy in isolation at home.

Findings: Patients raised several common emotional issues during the interview, which we divided into four major themes, each containing approximately 3-5 categories: dealing with a "friendly" cancer; coping within the family; body disclosure; and isolation.

Discussion and Conclusions: The findings were analyzed in light of current theories of "the uncanny body", loneliness, and aloneness, and a psycho-medical preparatory intervention was formulated. The main component of the intervention was the controlled and systematic transfer of information from medical staff to patient to enhance the mechanisms that can turn isolation into a positive experience and to curtail those with a negative impact. Specifically, we focused on the following measures: individual conversations with the patient before onset of treatment; the production of an informational booklet about iodine treatment; and the development of a database of patients who underwent this experience who can provide a real-time response and support to new patients and staff alike.

POSTTRANSCRIPTIONAL REGULATION OF HUMAN ADIPONECTIN RECEPTOR 2

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Objective: Adiponectin plays a central role in glucose and lipid metabolism. Adiponectin receptors (AdipoR1 and 2) expression has been suggested to play an important role in insulin resistance and diabetes. In this study we aimed at evaluating novel alternatively spliced AdipoR2 variants and receptor isoforms and their regulation under physiological and pathophysiological states.

Results: Using Bioinformatics analysis several novel human AdipoR2 splice variants were identified. Two distinct 5'UTR mRNA transcripts (T1, T2), which encode the wild-type receptor (WT-R2), are expressed in various human tissues, with highest abundance in liver, as demonstrated by real-time PCR. Similarly, WT-R2 expression was found by western blot analysis to be highest in liver. 5'-RACE analysis suggested that both transcripts share an identical promoter. Analysis of T1 and T2 mRNA stability revealed that both transcripts have a similar half-life time in actinomycin-treated human hepatoma HepG2 cells. The distinct AdipoR2-5'UTRs were cloned into pGL3-promoter vector. Analysis of their translation efficiency, by dual Luciferase assay in both HepG2 and HEK293 cells, demonstrated higher translation efficiency of T2 compared with T1. Treatment of HepG2 cells with the insulin-sensitizing drug rosiglitazone enhanced WT-R2 expression in a dose dependent manner, without a significant effect on T1 or T2 mRNA levels or their 5'-UTR-dependent translation efficiency, pointing to additional mechanisms in the posttranscriptional regulation of AdipoR2 receptor. Another identified splice variant T7, encodes a truncated receptor isoform (Tr-R2). T7 and Tr-R2 were found to be expressed predominantly in liver, however, Tr-R2 expression is significantly lower compared with the WT receptor. WT-R2 and Tr-R2 expression levels were lower in liver of obese diabetic ob/ob mice when compared to lean non-diabetic mice.

Conclusions: Human AdipoR2 is encoded by two distinct splice variants which differ in their 5'-UTR-dependent translation efficiency. Further studies are necessary in order to understand the regulation of these transcripts and their role in insulin resistance and diabetes.

CELLULAR MECHANISM OF INSULIN MIMETIC MATERIAL DERIVED FROM YEAST

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Glucose Tolerance Factor (GTF) is a dietary agent extracted from brewer's yeast. GTF reversed glucose intolerance in diabetic animals and humans. We found that oral treatment with GTF decreased blood glucose and lipids and potentiated insulin action in type 1&2 diabetic animals. We also found that addition of GTF to diabetic rats inhibited the nephropathy and retinopathy in these animals. In vitro studies done in our laboratory showed that GTF increased glucose transport into adipocytes and myocytes in insulin-like mode. When a combination of GTF and insulin was supplemented to the cells, a synergy between GTF and insulin was detected.

The aim of our study was to investigate the effects of GTF on the cellular level and to follow its involvement with insulin pathway.

GTF was extracted and partially purified from yeast. Differentiated 3T3-L1 or L-6 cells were treated with either insulin or GTF. Cells were lysed, and western blot analysis was performed with antibodies for phosphorylated key proteins in insulin pathway. Treatment of 3T3-L1 and L-6 cells with GTF increased phosphorylation of key proteins along insulin signaling pathway, in a time and dose-dependent manner. Whereas GTF increased tyrosine phosphorylation of IRS-1 and stimulated the activation of Akt and MAPK, it did not affect tyrosine phosphorylation of insulin receptor (IR). To further investigate this finding, we treated CHO cells over expressing insulin receptor (CHO-IR) with either insulin or GTF. Whereas a remarkable elevation in phosphorylation of IR was detected when these cells were treated with insulin, we could not find any phosphorylation above control values when CHO-IR cells were treated with GTF.

Our data demonstrates that GTF acts through insulin-signaling pathway, not via insulin receptor. Our findings present GTF as a novel oral "insulin-like" material, for future treatment of diabetes.

CONSTRUCTION OF ADENO-ASSOCIATED-VIRAL (AAV) VECTORS FOR THE TREATMENT OF OBESITY

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Background and Aim: The eradication of adipocytes apoptosis induction may be an appropriate therapeutic approach for the long term treatment of human clinical obesity. We have recently observed that fat cells express platelet-type 12-lipoxygenase (12-LOX), which is important for cell survival. To bridge the gap between *in vitro* and *in vivo* systems, we chose to screen gutless adeno-associated virus (AAV) serotypes for their ability to infect cultured fat cells. Currently we compared four rAAV serotypes expressing green fluorescent protein (GFP). Additionally we studied the effect of optimized AAV infection on fat cell death, using 12-LOX knockdown sequences.

Methods: 3T3-L1 and Human fat cells were infected with four gutless rAAV serotypes (2, 4, 12, D-J), expressing eGFP or nlsGFP protein. Infection efficiency was measured by calculating the percentage of green fluorescent cells after different time period. Furthermore, the cells were infected with the optimal rAAV serotype able to express a 12-LOX knockdown sequence under the CMV promoter. Cell death was measured by visual estimation and trypan blue exclusion.

Results: AAVDJ and AAV12 were the most efficient rAAV serotype of those tested for infecting cultured fat cells. The peak response was after 6-30 days of viral exposure for AAVDJ and 3 days for AAV12. 12-LOX knockdown AAV12 particles induced cell death with a similar time course as eGFP expression. Most significantly, this cell death was preventable by the addition of the 12-LOX product, 12hydroxyeicosatetraenoic acid (12HETE), but not 5- or 15HETE with the virus.

Conclusions: Using the rAAV12 or rAAVDJ vehicle we should be able to infect into fat cells 12-LOX antisense knockout vectors that should be able to reduce fat tissue mass *in vitro* and *in vivo*. This new tool will allow us to develop gene therapy protocols for the future treatment of obesity and its consequent other human pathologies.

IDIOPATHIC REACTIVE HYPOGLYCEMIA (IRH): A POTENTIAL ROLE OF ALTERED GLP-1

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IRH pathogenesis, is still far from being clarified. We hypothesized that excessive GLP-1 levels could account for hypoglycemia seen in IRH subjects. Previous studies have reported either excessive or normal insulin levels in IRH. Non have assessed gut hormones- versus corresponding glucose-dynamics.

Insulin- compared to corresponding glucose-dynamics revealed that in IRH, insulin secretory dynamics are inadequate. Insulin responsiveness to maximal glucose level revealed a subnormal response compared with controls. Neither hyperinsulinism nor an increased insulin sensitivity was found.

We studied insulin, glucagon and GLP-1 responses to oral glucose load, over a 5-hr time course. Glucose, insulin, glucagon and GLP-1 responses and responsiveness were expressed (absolute levels and ratio of hormone change toward corresponding glucose change, respectively).

Symptomatic hypoglycemia was observed 3-4 hours following ingestion of glucose in IRH subjects, with spontaneous recovery. Neither hypoglycemia symptoms, nor a chemical hypoglycemia was documented after an overnight fasting. There was an initial hyperglycemia indicating the presence of impaired glucose tolerance (IGT) in 4 out of the 11 subjects with IRH we studied. 3-4 years prior to the study these subjects did not demonstrate IGT. Glucagon was not significantly different between the two groups. However, glucagon responsiveness in the after-peak glucose curve, failed to fully compensate for the decline of glucose toward hypoglycemic levels, and was inferior to that in controls.

Basal as well as post load levels of GLP-1 in IRH was significantly elevated, with a temporal relationship between peak plasma concentrations of GLP-1 and glucose, but not between GLP-1 and insulin.

Diminished insulin response, is incompatible with a causative role of GLP-1 via the glucose-dependent insulin secretion.

Separating the responses to glucose into before-peak and after-peak, reveals a failure of insulin to match up with glucose in before-peak, with failure of glucagon in the after-peak to fully compensate for hypoglycemia.

THE FREQUENCY OF KETOACIDOSIS AT DIABETES ONSET HAS DECLINED OVER 20 YEARS IN A PEDIATRIC TERTIARY CENTER

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Background: Diabetic ketoacidosis (DKA) is the leading cause of acute morbidity and mortality in children with type 1 diabetes (T1D). Some studies have shown a decrease in the frequency of DKA over the past decades while others have shown no change or an increase.

Objective: To determine whether the frequency of DKA and the clinical characteristics of children at diagnosis of T1D have changed over the past two decades.

Methods: In three time periods, 76 (1986-1987), 86 (1996-1997) and 245 (2006-2007) patients aged <20 years were newly diagnosed with T1D in one tertiary care center. Retrieved from the patients' files were data for clinical characteristics and laboratory evaluation at diagnosis. Comparative analysis was performed in the 3 time periods.

Results: Frequency of DKA at diagnosis was 40% in 1986-1987, 42% in 1996-1997 and 29% in 2006-2007, the latter decrease was significant ($p=0.04$). No significant differences in the proportions of patients with severe or moderate DKA were found over time. Age at diagnosis, percent of patients aged <6 years and proportions of pre-pubertal patients at onset did not change significantly over time. Mean weight-SDS significantly increased (from -0.72 ± 1.8 in 1986-1987 to -0.27 ± 1.2 in 2006-2007, $p<0.05$), while percentage of weight loss (~6.5%) before diagnosis remained unchanged. For the entire cohort, children aged <2 years presented more often with DKA (85%) compared to older children (32%), $p<0.0001$. Ethiopian patients had higher rate of DKA at diagnosis (57.8%) compared to the rest of the cohort (33%), $p=0.04$.

Conclusions: The overall frequency of DKA in children with newly diagnosed T1D decreased in the past decade, though the degree of metabolic decompensation remained unchanged. However, children aged <2 years and Ethiopian children are still at high risk for DKA at diagnosis.

WORLD DIABETES DAY: SURVEY OF HEALTH CARE PROFESSIONALS IN CENTRAL REGION OF ISRAEL, IN CLALIT HEALTH SERVICES

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Since Health Care professionals (HCP) are key promoters in diabetes care the diabetes team decided to address HCP for the World Diabetes Day (WDD) for two consecutive years.

In 2009, we invited HCP to answer a questionnaire about Diabetes risk (6 questions with one point each). Waist circumference, body mass index (BMI) were measured. In 2010, HCP were sent via e-mail a questionnaire about their beliefs about their ability to treat diabetes.

298 HCP (46% of HCP), age above 50 (43%) answered the questionnaire (56% female, 21% male, 23% missing data). Risk score for diabetes was 1.792(SD 1.08). BMI < 25kg/m², between 25 BMI 30 kg/m², between 31 and 40kg/m², above 40kg/m² was found in 37.4%, 36.6%, 23.6% and 2.4% of the HCP respectively. The waist circumference was normal in 47.6% of HCP and 39.6% performed physical activity. In 2010, 106 of the HCP (11% response rate) rate 7.8 on a scale of 1 to 10, their satisfaction from their work in the field of diabetes. Sixty eight percent of HCP consider that work load impede diabetes care, 92% that individual treatment is the best way to diabetes care, 88% that group treatment should be part of diabetes care , 68% that group treatment could decrease work load, 31% already participated in group therapy and 59% would like to start group treatment for diabetic patients.

In conclusion majority of HCP are women and have a greater rate of participation in WDD. The medium risk for diabetes, high prevalence of obesity and low rate of pursuing physical activity in HCP should encourage intervention directed to the HCP toward healthy lifestyle. HCP have a relatively high satisfaction of their work in the field of diabetes and consider one to one treatment as the best way to deliver diabetes care.

ESTRADIOL-17 β INHIBITS HUMAN VASCULAR SMOOTH MUSCLE CELL PROLIFERATION – POTENTIAL ROLE OF REACTIVE OXYGEN SPECIES (ROS)

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Background: The lower incidence of cardiovascular disease (CVD) in premenopausal women than in men of similar age has led to the speculation that estradiol-17 β (E2) plays a role in protection from vascular pathologies. Protective effects have been demonstrated on the arterial wall, lipid metabolism and fibrinolytic system. However, diabetic women seem to lose these E2 derived protective effects. Estrogens have been shown to function as redox active substances exerting both pro-oxidative and anti-oxidative actions in the cytoplasmic and mitochondrial compartments.

Objective: We hypothesize that E2 affects VSMC proliferation in part through modulation of ROS and that this effect may differ under normal and high glucose conditions due to the increased oxidative state induced by hyperglycemia.

Methods and Results: E2 induced ROS production in VSMC was assessed in high and low glucose concentrations by fluorescent microscopy using the 2', 7'-DCF method. Treatment of VSMC with 30nM E2 for 1 hour resulted in increased ROS under normal and high glucose conditions. Pre-treatment with DPI or rotenone, inhibitors of NADPH oxidases and the mitochondrial respiratory chain complex1 respectively, prevented E2 induced ROS formation. Expression of NADPH oxidases in VSMC was assessed by western blot analysis at 1h, 24h and 48hrs post treatment with 30nM E2 for 1h, demonstrating an early transient reduction in NOX4. VSMC proliferation under normal and high glucose was assessed by thymidine incorporation after 24h exposure to 30nM E2, demonstrating inhibition of proliferation under normoglycemia but not hyperglycemia, with loss of the inhibitory effect with DPI pretreatment under conditions of serum deprivation.

Conclusions: Our results suggest that E2 inhibition of VSMC proliferation is mediated at least partially through E2 induced ROS formation under normoglycemia but not hyperglycemia. Our results may further suggest that E2 induced ROS formation occurs in both cytoplasmic and mitochondrial compartments with possible cross talk between them.

ANALYSIS OF THE PROLIFERATIVE AND DIFFERENTIATIVE EFFECTS OF INSULIN ANALOGUES IN KERATINOCYTES

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Background: Exogenous insulin is the only treatment available for type 1 diabetes patients and for part of the patients with type 2 diabetes. The long-acting insulin analogues (glargine, detemir) mimic the basal insulin secretion during a 24 hour-period and the short-acting analogues (lispro, aspart) mimic the bolus insulin secretion after a meal. Dermatological ailments are among the most serious complications associated with diabetes. The insulin receptor and insulin-like growth factor-1 receptor are expressed in skin keratinocytes. The receptors can be stimulated by insulin and IGF-1, resulting in the activation of an intracellular signaling pathway. It was shown in our lab that both insulin and IGF-1 lead to increased proliferation of keratinocytes. However, whereas insulin supported keratinocytes differentiation, IGF-1 was shown to inhibit this process. The biological effects of the newly developed insulin analogues in the skin have not yet been investigated.

Aim: Examine the proliferative and differentiative effects of short- and long-acting insulin analogues in keratinocytes, as well as the signaling pathways involved, in comparison to regular human insulin and IGF-1.

Materials and methods: Primary cultures of keratinocytes were produced from newborn BalB/C mice skin using methods established in our lab. Glucose uptake was examined using 2-deoxyglucose uptake, proliferation rate was assessed by means of thymidine incorporation, and differentiation was evaluated by western blot analysis with specific antibodies against markers of skin differentiation.

Results: Treatment of keratinocytes with insulin, IGF-1, humulin, glargine, detemir, lispro or aspart led to a significant elevation in glucose uptake compared to control. In addition, all of these treatments resulted in significant elevations in proliferation rates. We are currently determining the differential actions of the various types of insulin on IR and IGF-1R phosphorylation. In addition, we are analyzing the signaling pathways activated by insulin analogues in skin keratinocytes.

EVIDENCE OF DIRECT MITOGENIC ACTIVITY OF INSULIN AND THE INSULIN RECEPTOR IN PROSTATE CANCER DERIVED CELL LINES

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Background: Beside its normal spectrum of metabolic effects, insulin also acts as a growth factor and has the ability to promote mitogenic activity. Thus, hyperinsulinemia, a consequence of insulin resistance, is regarded as a potential risk factor for the development of cancer in patients with diabetes. However, the mechanism of action of insulin in prostatic cancer has not yet been completely elucidated. The aim of this study was to investigate whether insulin can directly induce mitogenic activity in prostate cancer-derived cell lines and to reveal the role of insulin receptor (IR) in mediating this activity.

Methods: we employed a number of prostate cancer cell lines (LNCaP, P69, C4-2 and PC3) representing early and advanced stages of the disease. Insulin doses ranged between 0-500 ng/ml. Insulin-stimulated proliferation rates were measured by hemocytometer cell counting. Cell-cycle dynamics were evaluated by propidium iodide staining and FACS analysis. Activation of the insulin receptor was assessed by immunoprecipitation assays. Expression levels of the receptor were measured by western immunoblotting.

Results: Insulin induced cell proliferation in a dose-dependent fashion in the LNCaP and C4-2 lines, but not in P69 or PC3 lines. Cell cycle analyses showed that insulin can positively influence LNCaP and C4-2 lines to progress towards the G2/M phase. Immunoprecipitation assays show that in all of the cell lines expressing the IR, insulin activates IR but not IGF-IR.

Conclusion: In the model studied, insulin exhibited direct mitogenic activities mediated exclusively through the IR. Further research is needed to fully dissect the molecular mechanism underlying the biological actions of insulin in prostate cancer.

METABOLIC SYNDROME WITH FASTING HYPERGLYCEMIA IS MORE COMMON IN ETHIOPIAN ISRAELIS THAN WEST AFRICANS OR AFRICAN AMERICANS

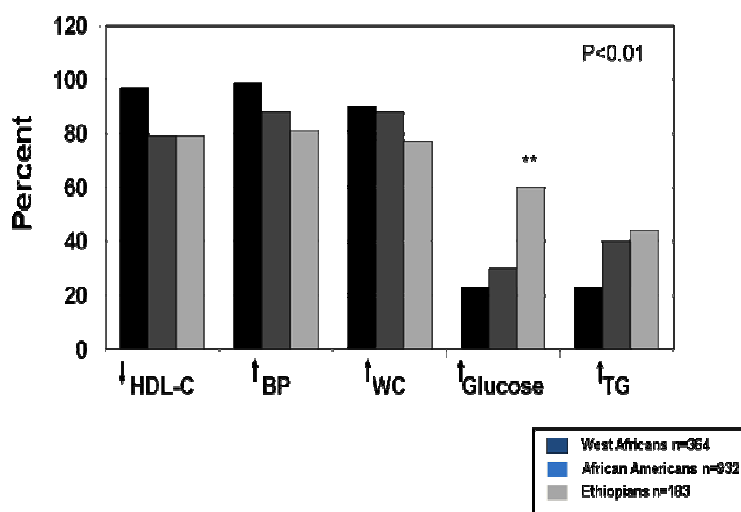
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Jewish Ethiopians lived for centuries as an isolated, rural community. Mass migration from sub-Saharan Africa to Israel occurred in the latter decades of the 20th century. On arrival in Israel, undernourishment was common, and type 2 diabetes mellitus (T2DM) and Metabolic Syndrome (MetS) occurred in <1% of the population. Within 10 years of residence in Israel, the medical profile of the population changed. We had access to data from 183 Ethiopian Israelis living in Hadera, an Israeli city with a high concentration of Ethiopian immigrants (age 53±20 (mean±SD), range 15-90, BMI 24.3±4.0, range 15.2-39.4), years of residence in Israel 9±2y, range 2-19y). The prevalence of Ethiopians with normal fasting glucose, fasting hyperglycemia, and T2DM was 54%, 30%, 16% respectively. The prevalence of the MetS in each of these 3 groups was 15%, 44% and 85%, respectively (P<0.01). As observed in West Africans and African Americans, the three variables that most often led to the diagnosis of the MetS in Ethiopians were: low HDL-cholesterol, hypertension and central obesity (Figure). However, in Ethiopians with MetS, the prevalence of fasting hyperglycemia was significantly higher than in either West Africans or African Americans (both P<0.01) (Figure). For the development of optimal screening programs for early identification of risk for T2DM in Ethiopians, it is important to know if fasting hyperglycemia or MetS is a better predictor of progression to T2DM. Furthermore, prospective studies are needed to determine whether the high rate of fasting hyperglycemia in Ethiopians with MetS indicates that progression to T2DM will occur more rapidly than in West Africans or African Americans.

Variable Distribution in Metabolic Syndrome



SAFETY AND EFFICACY OF BIPHASIC INSULIN ASPART (NOVOMIX®30, NOVOMIX®50, NOVOMIX®70 OR COMBINATIONS) IN ISRAELI PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Most type 2 diabetes (T2DM) patients need insulin therapy during the time course of their disease. Biphasic insulin aspart (BIAsp) provides treatment choices targeting both fasting (FPG) and postprandial (PPPG) plasma glucose.

In a 13-week, multi-centre, open-label, non-randomized, uncontrolled, observational study including 338 Israeli patients with T2DM already treated with insulin, we evaluated the safety and efficacy of BIAsp (NovoMix®30, NovoMix®50, NovoMix®70 or combinations) in routine clinical practice. The dose and choice of BIAsp was by the discretion of physician. The patients (50.3% male) had an average age of 62.1±10.8 years (Mean±SD) with a BMI of 32.4±6.3 kg/m² and diabetes duration of 15.0±8.3 years. Overall, the rate of hypoglycaemia (episodes per patient year) was significantly reduced from baseline to end-of-treatment in total cohort for both symptomatic and major hypoglycaemia (Table). A total of 13 serious adverse events (SAEs) and one non-serious adverse drug reaction (ADR) were reported. SAEs were not related to the trial drug. HbA_{1c}, FPG and PPPG were lowered after 13-week treatment with different BIAsp regimens (p<0.0001). No clinically relevant weight gain was observed. To conclude, in routine clinical practice in Israel, BIAsp treatment in patients with T2DM is safe and well tolerated, and is associated with a significant improvement in glycaemic control.

Change from Baseline to week 13 (hypos, rate per patient year, other parameters, mean±SD)

| | NovoMix30 (N=106) | NovoMix50 (N=91) | NovoMix70 (N=14) | Combination (N=127) | Total (N=338) |
|-----------------------|----------------------|---------------------|---------------------|------------------------|------------------|
| Symptomatic hypos | -0.38 | 0.04 | -0.06 | -0.55 | -0.32 |
| Major hypos | -0.05 | 0.04 | 0.00 | -0.41 | -0.16 |
| Minor hypos | 0.00 | -0.03 | 0.00 | -0.07 | -0.03 |
| HbA _{1c} (%) | -0.7±1.3 | -0.6±1.5 | -0.6±0.9 | -0.9±1.4 | -0.7±1.4 |
| FPG(mg/dL) | -17.5±52.3 | -17.6±77.0 | -50.3±57.6 | -23.5±74.2 | -21.5±67.1 |
| Weight (kg) | 0.8±4.5 | 0.4±2.9 | -0.8±1.8 | 0.7±3.6 | 0.6±3.7 |

**Poster
Abstracts
Group B**

INTRAMUSCULAR GLUCAGON STIMULATION TEST FOR ASSESSING ADRENAL FUNCTION IN SHORT CHILDREN

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

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

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

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

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

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

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

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

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

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

ETHNIC AND GENDER INEQUITIES IN THE EVALUATION OF REFERRED SHORT CHILDREN

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

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

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

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

PATIENTS WITH LARON SYNDROME SECRETE INCREASED AMOUNTS OF PROLACTIN

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

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

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

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

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

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

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

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

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

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

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

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

Results: A mean reduction of $32 \pm 15\%$ in SSK was observed during the first $1\frac{1}{2}$ years of hGH treatment. Continuation of treatment resulted in a progressive mean increase in SSK over the previous value of 192 ± 158 (SD) % in boys and of $224 \pm 164\%$ in girls. Stopping hGH resulted in a further increase in SSK of 46 to 61% from the treatment value. Cholesterol increased progressively to above normal values in most patients, so did insulin.

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

HEAD SIZE AND GROWTH RESPONSE TO hGH IN CHILDREN WITH IGHD

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

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

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

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

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

Conclusions:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Methods: HaCaT keratinocytes were exposed to TNF α or IFN γ for 24 hours and then for 5 hours to vitamin D₃, 25-hydroxyvitamin D₃, 1 α -hydroxyvitamin D₃ or calcitriol. mRNA levels of CYP24A1, VDR and 25-hydroxyvitamin D 1 α -hydroxylase (1 α (OH)ase) were assayed by real-time PCR.

Results: TNF α but not IFN γ upregulated the expression of VDR. However, both cytokines upregulated the expression and activity of 1 α (OH)ase, while not affecting vitamin D 25-hydroxylase activity. These effects of the cytokines on the various components of the keratinocyte vitamin D endocrine system are manifested as increased expression of CYP24A1 following exposure of the cells to vitamin D₃. By using pharmacological inhibitors of ERK, JNK and NF- κ B pathways we demonstrated that activation of each of these pathways is necessary for the induction of 1 α (OH)ase by both cytokines, while only the JNK pathway was obligatory for the induction of VDR by TNF α .

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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The type I GnRH receptor (GnRHR) mediates the pituitary functions of GnRH, as well as its anti-proliferative effects in sex hormone-dependent cancer cells. Here we examine and compare the signaling of GnRHR in pituitary gonadotrope vs. prostate cancer (CaP) cells. We first noticed that the expression level of PKC α , PKC β II and PKC ϵ is much higher in α T3-1 and L β T2 gonadotrope vs. LNCaP and DU-145 cells, while the opposite is seen for PKC δ . Activation of PKC α , PKC β II and PKC ϵ by GnRH is transient in α T3-1 and L β T2 gonadotrope cells and prolonged in LNCaP and DU-145 cells. On the other hand, the activation and redistribution of the above PKCs by PMA was similar for both gonadotrope and CaP cells. Activation of ERK1/2 by GnRH and PMA was robust in pituitary cells, with a smaller effect observed in the CaP cells. The Ca²⁺ ionophore A23187 stimulated ERK1/2 in gonadotrope but not in CaP cells. GnRH, PMA and A23187 stimulated JNK activity in gonadotropes, with a more sustained effect in CaP cells. Sustained activation of p38 was observed for PMA and A23187 in Du-145 cells, while p38 activation by GnRH, PMA and A23187 in L β T2 cells was transient. Thus, differential expression and redistribution of PKCs by GnRH and the transient vs. the more sustained nature of the activation of the PKC-MAPK cascade by GnRH in gonadotrope vs. CaP cells respectively, may provide the mechanistic basis for the cell context-dependent differential biological responses observed in GnRH interaction with pituitary gonadotropes vs. prostate cancer cells.

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

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

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

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

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

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

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

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

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