Alternative splicing of Lysyl Oxidase-like 4 in ovarian carcinoma

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Introduction: Lysyl oxidase (LOX) is an amine oxidase that is usually synthesized and secreted by fibrogenic cells. Four LOX-like (LOXL) genes have been identified so far in mammalian genomes, encoding four different LOX-like proteins: LOXL1, LOXL2, LOXL3 and LOXL4. All members of the LOX family show a highly conserved C-terminus region that contains the catalytic domain. The N-terminus of the LOX isoforms is less conserved among the different members and is thought to determine the individual role and tissue distribution of each isoenzyme. LOXL4, the least studied member of the LOX-like family enzymes, undergoes a process of alternative splicing in cancer, in a site- and stage-specific manner that we have previously shown. The purpose of the current study was to uncover the splicing mechanism that is responsible for this process.

Patients/ Methods: I. ShRNAs for four splicing factors: SF2/ASF, SRp55, hnRNP-A1 and hnRNP-A2, were transfected in two cell lines: U-87 MG cell line (human glioblastoma) and NCI-H460 (human large-cell lung carcinoma). II. Over-expression of SF2 was performed in MST0-211H cell line (human malignant mesothelioma), HeLa cell line (human epithelial cervical cancer) and MCF10A cell line (human mammary epithelial line). III. Western blotting for SF2/ASF and tubulin. IV. RT-PCR for LOXL4 full length, splice-variant1 (splv1) and splice-variant2 (splv2) mRNA expression.

Results: We examined LOXL4 expression in U-87 MG cells. When untreated, these cells express the full length and splv2, almost equally. The silencing of two factors, SF2/ASF and hnRNP-A1, resulted in a dramatic change in the expression pattern of LOXL4. For both silenced factors, LOXL4 full-length mRNA expression was much stronger, while the shortest variant, splv2, completely vanished. The silencing of hnRNP-A2 led to a smaller decrease in splv2, while SRp55 silencing did not seem to change LOXL4 splicing. In NCI-H460 cells, which normally express small amounts of all variants, no significant changes were found following silencing. In an attempt to further establish the splicing factor responsible for LOXL4 splicing, we over-expressed SF2/ASF in MST0-211H cells, which normally express only the full length LOXL4. Expression of SF2/ASF resulted in the appearance of splv2, while dramatically reducing the expression of the full length. Similar results were seen in HeLa cells. Over-expression of SF2/ASF in MCF10A cells, which untreated, have the unique quality of expressing splv2 alone, caused only a slight increase in the expression of splv2.

Conclusions: These results demonstrate for the first time, that LOXL4 is a direct target of the splicing factor SF2 SF2/ASF. Furthermore, in concordance with our previous in-vivo findings, it can be concluded that LOXL4 splicing occurs similarly in other epithelial cancer types, such as breast cancer and mesothelioma.

Preclinical analysis of IGF-IR antibody MK-0646 in endometrial cancer

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Introduction: Endometrial cancer is one of the most frequent gynecological cancers in Western countries. The involvement of the insulin-like growth factors (IGFs) in the initiation and progression of endometrial cancer has been well established. The IGF-I receptor (IGF-IR), which mediates the proliferative and antiapoptotic activities of IGF-I and IGF-II, emerged in recent years as a promising molecular target in cancer therapy. The aim of the present study was to evaluate the hypothesis that interfering with the IGF-IR signaling pathway in endometrial cancer could revert the transformed phenotype, decrease proliferation, induce apoptosis, and render the cells more sensitive to chemotherapy. To this end we used a recently developed humanized monoclonal antibody (MK-0646, Merck Oncology), directed against IGF-IR. MK-0646 was shown to block IGF-I binding to the receptor, leading to IGF-IR degradation.

Patients/ Methods: To evaluate the effect of IGF-IR inhibition on IGF-I-mediated signaling, human endometroid (ECC-1) and serous papillary (USPC-1) endometrial cancer cell lines were treated for various periods of time (40 min, 1 h, 3 h, 5 h) with antibody MK- 0646, in the presence of IGF-I during the last 10 min of the incubation period.

Results: Results of Western blots using antibodies against total and phospho-IGF-IR, AKT, and ERK showed that MK-0646 decreased the IGF-I-stimulated phosphorylation of IGF-IR, AKT and ERK in both ECC-1 and USPC-1 cells. In addition, MK-0646 induced a significant decrease in total IGF-IR levels. To evaluate the potential effect of IGF-IR inhibition on apoptosis, cells were treated with IGF-I for 24-48 h, in the absence or presence of MK-0646, after which apoptosis was assessed by Caspase-3 and cleaved PARP measurements. Results obtained showed that MK-0646 abrogated the antiapoptotic activity of IGF-I

Conclusions: Taken together, our results suggests that specific IGF-IR blockade could be a useful therapeutic approach in endometrial cancer.

Transcriptional regulation of gonadotropin genes by GnRH through its effects on histone H3 methylation and phosphorylation

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Introduction: Luteinizing hormone and follicle stimulating hormone control reproductive development and function. Both hormones comprise a common α- and a hormone specific β-subunit, which are regulated by the gonadotropin releasing hormone (GnRH). We have demonstrated that GnRH can regulate gonadotropin subunit gene transcription at the level of chromatin, through the displacement of histone deacetylases. This allows 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 lysine 36 (H3K36me3) and/or phosphorylation of H3 at serine 10 (H3S10p), modifications previously shown to be implicated in yeast and mammalian transcriptional regulation.

Results: Our data shows that GnRH increases nuclear protein levels of H3S10p and phosphorylated MSK1 in gonadotrope cells. As MSK1, which is activated by ERK or p38MAPK, targets H3S10, this suggests that GnRH may activate subunit gene transcription through regulating H3S10p. H3S10p is reported to be required for histone acetylation by GCN5, which we have found is associated with the LHβ promoter. ChIP studies revealed the presence of H3K4me3 on all three subunit gene promoters. While nuclear protein levels of H3K4me3 are unaltered by GnRH, ChIP studies normalized against levels of total H3 present at the promoters, demonstrate an increase in H3K4me3 at the subunit gene promoters after GnRH treatment. GnRH also increases nuclear protein levels of H3K36me3 and H2BK120ub, modifications implicated in transcription elongation, suggesting that GnRH may regulate elongation through controlling these modifications.

Conclusions: These results therefore suggest that GnRH regulates gonadotropin subunit gene transcription through the induction of various histone modifications. The function of, and mechanism behind, each of these modifications are currently being studied

Sustained activity of the EGF receptor is an absolute requisite for LH-Induced oocyte maturation and cumulus expansion

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Introduction: Maturation of the oocyte and expansion of its surrounding cumulus cells constitute part of the responses of the ovarian follicle to the preovulatory surge of LH. It was previously shown that activation/phosphorylation of the epidermal growth factor receptor (EGFR) mediates the ovulatory response to LH. Unlike other systems in which the EGFR is rapidly dephosphorylated, in the ovary, it stays phosphorylated for few hours following LH stimulation. We hypothesized that this sustained activity of the EGFR is required to mediate LH action in the ovary through the continuous activation of its downstream ERK1/2.

Patients/ Methods: Intact preovulatory follicles were isolated from 25-day-old PMSG-primed Wistar female rats. They were exposed to either LH or forskolin in the presence or absence of specific inhibitors of their downstream effectors. At the end of incubation, the follicles were incised, and the cumulus oocyte complexes were monitored for the meiotic status of the oocyte as well as for the extent of cumulus expansion. Activation of ERK1/2, as indicated by its state of phosphorylation, was examined by Western Blot analysis. The expression of prostaglandin-endoperoxide synthase 2 (Ptgs2, also known as Cox2), as well as that of MAPK phosphatases (MKPs), was determined by RT-PCR.

Results: We demonstrated that a short-term exposure of ovarian follicles to LH, as well as a transient activation of the adenylyl cyclase by forskolin, is sufficient to trigger oocyte maturation and cumulus expansion. By contrast, termination of the activity of the EGFR, at time points that are earlier than 3 h of exposure to LH, severely impaired these responses. In addition, the sustained activity of the EGFR was essential for an extended phosphorylation of the ERK1/2 downstream signaling molecules, which by itself is required for oocyte maturation and cumulus expansion. Interestingly, the continuous activity of the EGFR was also necessary to maintain the up-regulation of Ptgs2, an indispensable gene for cumulus expansion. In search for a mechanism that may be responsible for the prolonged ERK1/2 activity in the ovary, we screened the inducible MKPs, which specifically dephosphorylate the active ERK1/2. This experiment revealed, for the first time, that MKP-3 up-regulation is temporally correlated with ERK1/2 dephosphorylation, pointing toward MKP-3 as the potential enzyme responsible for ERK1/2 shutdown in this system.

Conclusions: Our data demonstrate that a prolonged activity of the EGFR is absolutely required to mediate the LH-induced responses in rat preovulatory follicles. We propose that this mechanism allows translation of the short systemic surge of LH into deferred, multiple responses that bring about the spatiotemporal coordination of a wide range of events, collectively defined as ovulation. This study highlights the fact that the sustained, nonclassical activity of the EGFR-ERK1/2 pathway is necessary
for the gonadotropins-induced ovulatory response.

The Indispensible role of ROS in Ovulation

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Introduction: Ovulation, that is an essential prelude for successful reproduction is stimulated by the preovulatory surge of the pituitary luteinizing hormone (LH). The biochemical and molecular effects of this gonadotropin in the preovulatory follicle culminate in the release of a mature ovum surrounded by the cumulus cells. Since ovulation has biophysical and biochemical features that are characteristic of an inflammatory reaction, reactive oxygen species (ROS) may play a role during this process. ROS in the ovary may originate from inflammatory cells, such as macrophages and neutrophils, which are present during ovulation and produce large amounts of free radicals. Ovarian ROS may also be generated by the LH-induced cyclooxegenase (Ptgs2) expression, an enzyme that catalyzes the initial oxidation step in the conversion of arachidonate to prostanoids associated with inflammatory conditions. ROS are also byproducts of monooxegenase reactions mediated by P450 systems in steroidogenic cells. The NADPH dependent generation of superoxide was shown to increase during the early preovulatory phase in the ovary of cycling female mice. Taking this information into consideration, we hypothesized that ROS may be involved in the signaling cascades leading to ovulation.

Patients/ Methods: The role of ROS in ovulation was examined in-vivo in PMSG/hCG-primed mice injected into their ovarian bursa by broad-range anti-oxidants, such as NAC and BHA. An ex-vivo approach of intact ovarian follicles culture was employed to further elucidate the molecular mechanism of ROS involvement in the LH-induced preovulatory responses such as cumulus expansion and progesterone secretion. Western blot analysis was employed to analyze the state of EGFR and MAPK42/44 phosphorylation/activation. Real-time PCR analysis was performed to assess the expression profiles of ovulation essential genes. For this purpose large antral follicles obtained from PMSG-primed mice were incubated with LH in the presence or the absence of antioxidants.

Results: Administration of broad range scavengers of oxidative species such as BHA and NAC into the ovarian bursa of PMSG/hCG treated mice significantly reduced the rate of ovulation. This observation gained further support by ex-vivo experiments performed on isolated intact ovarian follicles. In this system, LH-induced cumulus mucification/expansion, that is a necessary prerequisite for ovulation was prevented by the antioxidants mentioned above. Along this line, H2O2 fully mimicked the effect of LH, bringing about an extensive mucification/expansion of the follicle-enclosed cumulus-oocyte complexes. Progesterone production that is another parameter tightly associated with ovulation was also impaired in isolated follicles incubated with LH in the presence of the antioxidant agents. The inhibitory effect of scavengers of ROS on LH-induced ovulatory responses in the ovarian follicles was also manifested at the molecular level. The LH-stimulated up-regulation of genes such as Ptgs2, Has2, TNFAIP6, Pgr and Cebpb, the expression of which is a prerequisite of normal ovulation, was substantially attenuated upon the addition of the antioxidants to the culture. These antioxidants also inhibited the LH-induced phosphoryilation and activation of the EGFR as well as that of its downstream effector, p42/44 MAPK.

Conclusions: In the present work, we employed in-vivo studies in combination with ex-vivo and molecular analyses to demonstrate the indispensible role of ROS in ovulation as well as in other ovulatory responses such as cumulus expansion, progesterone secretion and specific genes up-regulation and activation. These observations raise the novel idea that reactive oxidants present in the preovulatory ovarian follicles are essential for the ovulatory response.

Designing long acting analogs of glycoprotein hormones using site-directed mutagenesis and gene transfer

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Introduction: 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.

Patients/ Methods: To overcome this problem, we used genetic engineering techniques that have been found successful for designing long acting hormones. Using site-directed mutagenesis and overlapping PCR techniques, we succeeded to add the signal sequence of O-linked oligosaccharides to the coding sequence of the hormones. The cassette gene that has been used contains the sequence of the carboxyl-terminal peptide (CTP) of human chorionic gonadotropin □ (hCG□) subunit. The CTP contains 28 amino acids with several proline and serine residues and four O-linked oligosaccgaride 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 circulation.

Results: 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. GH-CTP was found to be safe in monkeys and it passed successfully clinical trials phase I in humans.

Conclusions: Ligation of CTO to the coding sequence of the hormone has no effect on expression, secretion and in vitro bioactivity of the hormone. On the other hand, it increases the half-life and bioactivity in vivo. Furthermore, it seems that the chimerical proteins contain the CTP are not immunogenic. Designing long acting peptides will diminish the cost of these drugs and perhaps reduce the number of injections in the clinical protocols.

A novel mutation in GPR54/Kiss-1R leads to GnRH resistants hypogonadotrophic hypogonadism in a highly consanguineous family

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Introduction: GPR54, the kiss1 receptor is essential for normal gonadotropin-released hormone physiology and for puberty, and defects in it cause isolate hypogonadotropic hypogonadism (IHH) or precocious puberty.

Patients/ Methods: To identify the genetic cause of IHH in a highly consanguineous family of Israeli-Arab origin

Results: All patients that were available for endocrine analysis showed IHH that was resistant to GnRH stimulation and were treated with partial success. A novel homozygous p.F272S mutation in GPR54 was identified in the affected patients, the unaffected parents or siblings carried the mutation in a heterozygote state. This mutation resides in the 6th transmembrane domain of the receptor in a highly conserved amino acid and was suspected to lead to inactivation of the receptor. Functional analysis measuring activation of inositol specific phospholipase C (PLC) confirmed complete inactivity of the receptor.

Conclusions: These results enlarge the clinical spectrum associated with mutation in the GPR54

Early metabolic effects of bariatric surgery in type II diabetic patients

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Introduction: The effect of bariatric surgery on glycemic control of diabetic patients has been extensively studied. Diabetes resolution after bariatric procedures is reported to be as high as 85%. The degree of hyperglycemia improved in most cases shortly after surgery. Yet, there is a relative paucity of data regarding the metabolic outcomes of bariatric operations in Israel. The aim of this study was to examine the early effects of surgery on weight loss as well as glycemic control parameters in one medical center in Israel..

Patients/ Methods: One hundred and seventy seven patients underwent bariatric procedures in our hospital during the last two years (2008-9). Of those, 37 (20.9%) were diabetic. Pre- and postoperative data was studied retrospectively. The parameters chosen were: weight, fasting glucose, HBA1C, dosage of anti diabetic drugs and lipid profile.

Results: Twenty eight patient's records were available for analysis. One patient died few days after surgery from massive Pulmonary emboli and another 8 patients were lost to follow up. The mean follow-up period was 9 months. Twenty two patients underwent sleeve gastrectomy (21 laparoscopic and one open), 4 underwent laparoscopic Roux-en-Y gastric bypass and 2 underwent gastric banding. Mean body mass index (BMI) before surgery was 41.9 (±4.9) and the mean weight was 116.6Kg (±15.6). Significant weight reduction was observed at 1, 3 and 6 months after surgery with mean weight reductions of 11.3, 19.3 and 27.1 kilograms respectively ($p<0.05$). Three months after surgery, subjects lost 42.2% (±13.5%) of their excess weight and after 6 months the excess weight loss reached 58.1% (±22.3%). Improvement in diabetic control was observed in the vast majority of patients (27 of 28) and diabetes remission in 9 patients (32.1%). Thirteen patients were treated with insulin before surgery. Of those, 5 stopped insulin treatment after surgery and the other 8 decreased their daily dose by more than half. After the operation, triglyceride level decreased from 207±129 mg/dl to 148±66 mg/dl, ($p=0.026$) with no influence on HDL or LDL levels. The subgroup of patients who experienced complete normalization of carbohydrate metabolic parameters (9 patients) had lower HbA1C before surgery (7.23 versus 8.79 $p=0.016$) and were less likely to have received insulin preoperatively (2 of 9 versus 11 of 18, $p=0.077$), compared to the non-cure group. The following parameters did not influence the chances of diabetes resolution: age, gender, pre–intervention BMI, rate or magnitude of weight loss or the type of surgery.

Conclusions: Our data suggest a lower rate of diabetes resolution than the rates previously reported. Improvement of diabetes control after bariatric surgery was observed in most of our patients (96.4%), which is consistent with previous data. Patients who were better controlled and possibly patients who were treated by oral anti diabetic agents had a higher rate of diabetes resolution. Surprisingly, our results did not support the correlation between the magnitude of weight loss and the chance of diabetes remission.

Cortisol, but not ACTH/CRH, increases circulating ghrelin in man

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Introduction: We have recently explored the involvement of ghrelin in the eating response to stress in humans and found that ghrelin levels increased in parallel to cortisol after a standardized psychological stress. To further elucidate this interaction, we examined the ghrelin response to pharmacological manipulation of the HPA axis.

Patients/ Methods: Following approval from the local Ethical Committee, six lean, healthy male volunteers were examined on two occasions. Blood samples were collected every 30 minutes for two sequential periods of two hours. Initially, a baseline period was followed by intravenous injection of ACTH 250 μg. Subsequently, metyrapone (2-3 g) was administered at midnight and in the following morning the initial 2-hour sampling was followed by intravenous injection of hydrocortisone 100 mg.

Results: Mean total ghrelin levels during the 2-hour period after metyrapone administration was significantly lower than during the period following ACTH administration ($p=0.033$). After ACTH stimulation, there was a positive correlation between total ghrelin and cortisol AUC ($r=0.876$, $p = 0.021$). Mean acylated ghrelin levels were lower during the post metyrapone sampling than in the baseline period ($p=0.058$). Furthermore, acylated ghrelin levels significantly increased after acute hydrocortisone administration ($p = 0.032$) and was positively correlated with the decrease in ACTH ($R = 0.825$, $p = 0.043$) and the increase in cortisol ($r = 0.86$, $p=0.06$). There was a highly positive correlation between total and acylated ghrelin levels during all phases of the study ($r=0.96$, $p=0.002$).

Conclusions: In conclusion, increased cortisol levels secondary to ACTH stimulation or hydrocortisone administration is associated with increments in plasma ghrelin levels, whereas central stimulation of the HPA axis by blocking cortisol synthesis with metyrapone is associated with decreased plasma ghrelin levels. Collectively, this suggests that stress-induced elevations in ghrelin levels may be secondary to the rise in peripheral cortisol, independent of central elevation of ACTH and possibly CRH levels.

The coexistance of TSH receptor and thyroperoxidase mutations in the same kindred

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Introduction: Inherited hypothyroidism occurs in approximately 1 of 20,000 live births. We recently reported a high prevalence of mutations of the thyroperoxidase (TPO) and TSH receptor (TSHR) genes in consanguineous communities of Northern Israel. The aim of the present study was to determine the genetic background of an extended family with familial occurrence of congenital hypothyroidism (CH) and high rate of hyperthyrotropinemia.

Patients/ Methods: Thirty members belonging to extended kindred of an Arab-Muslim origin, two of whom with CH were enrolled. TSHR and TPO gene mutations were detected by sequencing.

Results: Three novel nucleotide substitutions in the extracellular region of the TSHR were identified in the same allele, two of which produced amino acid substitutions (Q90P and P264S). As one of the 2 subjects with CH had no TSHR gene mutation, other gene defects were sought. Two TPO gene mutations (G493S and R540X), which we previously described in the same population, were identified in different alleles. Thirteen individuals were heretozygous for the TSHR mutation, 9 of which were also heterozygous for one of the two TPO gene mutations. Of the 17 individuals heterozygous for one of the two TPO gene mutations, 4 had normal TSHR alleles. Of the 2 individuals with CH, one was homozygous for the TSHR gene mutation and one was compound heterozygous for the TPO mutations. Another homozygote for the TSHR mutation was born prior to the institution of neonatal screening. Genotype-phenotype correlation based on TSH concentration revealed no differences between heterozygotes for either TPO mutations (N=8) and WT (N=6). In contrast, heterozygotes for the TSHR mutation had significantly higher TSH compared to WT family members (7.1±0.3 vs. 2.3±0.5). TSH values in the two homozygotes for the TSHR mutation were 31 and 58 mU/L, and both had low FT4 levels.

Conclusions: Mutations of TPO and TSHR genes were found to coexist in the same consanguineous kindred. Patients homozygous for the TSHR gene mutation and compound heterozygous for the TPO gene mutation presented with hypothyroidism. The mild hyperthyrotropinemia of heterozygotes with the TSHR gene mutation was not aggravated by the coexistence of a TPO defect in only one allele.

Impact of pregnancy on outcome and prognosis of survivors of differentiated thyroid cancer

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Introduction: Differentiated thyroid cancer usually affects women of child-bearing age. During normal pregnancy, several factors may have a stimulatory effect on normal and nodular thyroid growth. The present study aimed to determine if pregnancy in thyroid-cancer survivors poses a risk of progression or recurrence of the disease

Patients/ Methods: A retrospective evaluation of consecutive women who were followed at a single Endocrine Institute for differentiated epithelial thyroid cancer and who had at least one pregnancy and delivery after receiving treatment for thyroid cancer between 1992 and 2009. The patients' medical records were reviewed for data including age at diagnosis, pathological cancer staging, number of operations, number and doses of radioactive iodine treatments the patient underwent, interval from diagnosis to pregnancy, thyroglobulin (Tg) levels and neck ultrasound findings before and after pregnancy, and thyroid stimulating hormone (TSH) levels during pregnancy. Tg levels and neck ultrasound findings were compared before and after pregnancy. Disease progression in pregnancy was defined as a significant increase in Tg level, a new imaging finding or enlargement of a known pre-pregnancy mass suggestive for thyroid cancer metastasis within a year after delivery. The demographic and disease-related characteristics and levels of TSH measured in pregnancy were correlated with disease progression during pregnancy using Pearson correlation analysis.

Results: Sixty-three women met the study criteria. The mean time to the first delivery after completion of initial thyroid-cancer treatment was 5.16±3.76 years, the mean duration of follow-up after delivery was 4.66±3.75 years. Forty women had one and 23 women had more than one delivery, for a total of 90 births. Biochemical and/or imaging evidence of thyroid cancer progression during the first pregnancy after treatment was documented in 9 patients (14.2%). Three of them also showed disease progression during a second pregnancy. Another 3 patients showed no disease progression during the first pregnancy but did so during the second. TSH was measured 4.87±2.24 times during pregnancy and mean TSH level was 2.53±4.03 mIU/ml. There was no correlation of most of the indices evaluated with disease progression during pregnancy. A strong positive correlation with cancer progression during pregnancy was noted for persistence of thyroid cancer before pregnancy and total I-131 dose administered.

Conclusions: Pregnancy does not cause thyroid cancer progression or recurrence in thyroid-cancer survivors who have no structural or biochemical evidence of disease persistence before pregnancy. However, in the presence of such evidence, disease progression may occur during pregnancy, yet not necessarily as a consequence of pregnancy. A non-suppressed TSH level during pregnancy does not stimulate disease progression in thyroid-cancer survivors and may be an acceptable therapeutic goal, especially in those with a history of miscarriages or preterm deliveries.

Radioiodine therapy for graves' disease is associated with increased rate of the metabolic syndrome

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Introduction: Although the treatment of Graves' thyrotoxicosis with radioactive iodine is generally perceived as clinically efficient, cost effective and safe, most radioiodine-treated patients become hypothyroid rapidly after radioiodine treatment and need life-long thyroxine replacement. Further, observational studies show that radioiodine therapy is linked to increased mortality due to cardio- and cerebrovascular disease. Here we evaluated the cardiometabolic outcome of radioiodine-treated (RI) vs. medically treated (Med) subjects with Grave's disease as assessed after ≥ 3 years of follow up.

Patients/ Methods: All subjects were actively recruited to undergo complete physical examination and blood testing which included glucose, HBA1C, CRP, liver, kidney and thyroid function. Non-diabetic patients also underwent 75 gram oral glucose tolerance test. Arterial stiffness was evaluated using applanation tonometry and pulse wave analysis by different standard devices which assess distinct measures of arterial stiffness: pulse wave velocity (PWV), augmentation index, and large/small artery compliance (C1 and C2). Additionally, all subjects were referred for ambulatory blood pressure monitoring.

Results: Sixty five RI-treated and 33 Med-treated patients with Graves' disease were included in the study. Mean age (53±12 vs. 48±13.6, p=NS), gender (M-28% vs. 21%, p=NS), duration of the disease (5.2 vs. 5.3 yrs, p=NS), and thyroid function tests (TSH: 2±1.4 vs. 1.6±1.1, p=NS) were similar in the RI and Med groups, respectively. Post-RI-therapy patients had higher BMI, waist circumference, systolic blood pressure, PWV, hs-CRP levels, higher prevalence of the metabolic syndrome and lower C2 than antithyroid drug-treated patients. After adjustment to age and gender RI- treated patients had higher BMI (27.4±5.7 vs. 24.3±3.6, p=0.03), hs-CRP levels (4.14±5.3 vs. 1.62±1.44, p=0.047), and higher prevalence of the metabolic syndrome (52.3 vs.15.6%, p=0.003).

Conclusions: Radioiodine treated patients with Grave's disease gain more weight and have higher rate of the metabolic syndrome compared with medically treated subjects with this condition. The difference is seen despite normal and indistinguishable thyroid hormone levels with thyroxin treatment. We suggest that in the choice between these two major therapeutic modalities, physicians and patients should be aware of this emerging difference in outcome. Additionally, RI-treated patients should be carefully followed in an attempt to reduce the risk of weight gain and the metabolic syndrome.

Prognostic value of post thyroidectomy thyroglobulin levels in patients with differentiated thyroid cancer

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Introduction: Thyroglobulin (Tg) is an excellent biological marker for recognition of persistent or recurrent thyroid cancer. Most studies have looked at the diagnostic value of Tg but not at the prognostic value over time. Furthermore, very few studies evaluated the prognostic value of Tg levels at the early point after total thyroidectomy and before iodine ablative treatment.

Patients/ Methods: Our center has a registry of patients with well differentiated thyroid carcinoma who were followed at our institute since 1973. In the present study, data on the clinical, laboratory and outcome characteristics of 420 patients with post operative and preablation Tg values (baseline thyroglobulin) after total thyroidectomy were collected from the registry.

Results: Patients were classified into 4 groups according to baseline Tg levels (0-2, 2-10, 10-100, >100 ng/ml). Higher Tg levels were associated with a shift to male gender, larger tumor size (P=0.01, P=0.02 respectively) and more extensive disease (P<0.0001). In addition, higher Tg levels were related to persistence of disease and disease presence at last follow up (P<0.0001). The 10 ng/ml cut-off level identified patients with persistent disease with a sensitivity and specificity of 73%, PPV 43% and NPV 89%. In multivariate analysis the following variables were predictive of persistent disease: baseline Tg levels, male gender, lymph node involvement, distant metastases, higher invasiveness and larger tumor size. Yet, the predictive power of baseline Tg levels was relatively weak (OR 1.002, 95% CI 1.00-1.04).

Conclusions: Post-operative Tg level is a weak prognostic marker, however it can be used with other disease characteristics in decision making regarding treatment and follow-up of well differentiated thyroid carcinoma patients.

Papillary Microcarcinoma of the Thyroid

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Introduction: No increased mortality has been reported in patients with thyroid microcarcinoma (PMC), however neck recurrences and distant metastases have been described. The study aim was to compare patients' outcomes after total thyroidectomy versus hemithyroidectomy for treatment of PMC.

Patients/ Methods: Two hundred ninety-three patients from two major medical centers in Israel were included. The mean follow-up period was 7.2±6.8 years.

Results: Total thyroidectomy (TT) was performed in 214 patients and hemithyroidectomy (HT) in 79 patients. Mean tumor size was 6.3±3 mm. Lymph node metastases and extraglandular extension were more frequent in the TT group than in the HT group, 24.8% vs 1.3 ($p<0.001$) and 11.7 vs 3.8 ($p=0.042$), respectively. Permanent complications were also more frequent in the TT group than the HT group (14.0% vs 5.1%) ($P=0.034$). The cumulative incidence of recurrence at the end of follow-up was 11.6% in the TT group and 14.3% in the HT group (p=NS). Considering low risk patients only (monofocal tumors, no lymph-node involvement, n=74 in the TT group vs n=66 in the HT group) neck recurrence was found in 9% of patients in the HT group but none in the TT group. In the HT group all locoregional recurrences were diagnosed using ultrasonography, compared to 50% in the TT group. The incidence of recurrence was higher in patients with multifocal tumors and lymph-node involvement in both groups.

Conclusions: Hemithyroidectomy is associated with a lower rate of complications compared with total thyroidectomy, however higher rate of locoregional recurrences should be expected.

A new form of autosomal recessive hypophosphatemic rickets is associated with an inactivation mutation in the ENPP1 gene

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Introduction: Human disorders of phosphate (Pi) handling and hypophosphatemic rickets have been shown to result from mutations in PHEX, FGF23 and DMP1, presenting as X-linked recessive, autosomal dominant and autosomal recessive patterns, respectively.

Patients/ Methods: We have characterized an enlarged consanguineous Bedouin family displaying autosomal recessive Hypophosphatemic rickets. Two patients presented with short stature and bowing legs. A third patient has normal stature and was apparently healthy except for delayed healing of post traumatic fracture of his left tibia. The structure and size of the family were suitable for positional cloning of the causing gene through linkage study. After excluding linkage to the three above mentioned known genes, we carried out a genome wide search for the chromosomal region containing the mutated gene.

Results: We identified linkage to the chromosomal locus 6q23. The linkage interval of 7.39Mb contains 70 genes including the ecto-nucleotide pyrophosphatase/ phosphodiesterase 1(ENPP1) gene, a known player in phosphate metabolism. The cDNA coding region for ENPP1 was directly sequenced and a novel mutation Y901S that presented homozygously in all patients, and segregated as expected in the healthy family members was found. The possibility that this variation represents polymorphism, was excluded by testing 236 control Bedouins of the same geographic region. Moreover, The tyrosine in position 901 resides in the nuclease-like domain, and is strictly conserved in ENPP1. ENPP1 generates inorganic pyrophosphate that is an essential physiologic inhibitor of calcification. The function of the mutation was tested by transfection of a pSVT7 expression vector harboring the mutated full-length ENPP1 coding sequence into cells and measuring the NPP activity in comparison to the activity in cells transfected with the normal sequence vector. We found that the mutation reduced NPP activity by 96% to 4% of residual activity. ENPP1 is a glycosylated ecto-enzyme, with its amino terminus in the cytoplasm and extracellular catalytic domains. We found that the cell membrane localization of the mutated protein is comparable to the wild type enzyme

Conclusions: ENPP1 generates inorganic pyrophosphate (PPi), an essential physiologic inhibitor of calcification, and previously described inactivating mutations in this gene were shown to cause aberrant ectopic calcification disorders, whereas no aberrant calcifications were present in our patients. Our surprising result suggests a different pathway involved in the generation of ARHR and possible additional functions for ENPP1.

The magic of replacement therapy – treating severe hypoparathyroidism with continuous infusion of PTH 1-34

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Introduction: Hypoparathyroidism is usually treated with low-phosphate diet, calcium supplements, and vitamin D analogs. However, metabolic control is not always achieved, and treatment may lead to nephrocalcinosis and renal insufficiency. Twice-daily PTH 1-34 injection was suggested as an alternative treatment. We describe an 18 year old female treated successfully with PTH 1-34 administered continuously via a pump.

Patients/ Methods: The patient has glycogen storage disease type III, severe hypoparathyroidism, and celiac disease. Extensive molecular studies were performed in order to achieve a specific diagnosis.

Results: The patient was diagnosed with glycogen storage disease type III at age 1 year. She presented at age 5 years with hypoparathyroidism, and was treated with calcium and magnesium supplements and alfacalcidol. She was reportedly clinically stable with that regimen until 2007, albeit severe hyperphosphatemia persisted. She was first admitted to our hospital on 3/2007 and had two prolonged hospitalizations with severe hypocalcemia, severe hyperphosphatemia, and hypomagnesemia and was dependent on intravenous calcium and magnesium to prevent tetany. CT scan revealed multiple brain calcifications. EEG showed general epileptic activity. At the first hospitalization treatment with PTH 1-34 at 20micgX2/day was started. Significant improvement was initially observed, but after one year treatment was no longer effective and the patient became again dependent on intravenous calcium and magnesium. We initiated treatment with continuous infusion of PTH 1-34 via an insulin pump. Within 24 hours we observed a dramatic increase in serum Ca and Mg and a decrease in serum PO4. Urinary calcium excretion decreased. EEG normalized. After one year of therapy, the patient continues to have normal Ca, PO4 and Mg levels, and is very happy with the treatment, reporting better sleep, better academic performance, and an improvement in quality of life. The current PTH 1-34 dose is 18micg/24h. Recently she was found to have celiac disease. Molecular diagnostic studies: Sequencing for the paracellin gene (PCLN1=CLDN 16) revealed normal coding exons. Sequencing for the CaSR revealed compound heterozygosity A986S / R990G. The patient's mother and brother both carry the A986S polymorphism, while the patient's father carries the R990G mutation. All 3 are normocalcemic. HLA typing found the patient to be positive for DQB1*03 – compatible with celiac disease. A genome-wide homozygosity screen is pending.

Conclusions: This patient presented a complex diagnostic and therapeutic challenge. We do not know if her three diseases are related and how they affect each other's clinical course. This case demonstrates the biochemical advantages of PTH replacement therapy, including the avoidance of side effects such as nephrocalcinosis and renal failure.

The impact of increase in effective osteoporosis drugs availability on the incidence of osteoporotic fractures in the Negev population

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Introduction: Osteoporosis is characterized by decreased bone mass and distortion of the skeletal microarchitecture, resulting in increased vulnerability for fractures following mild trauma. Osteoporosis is more common among postmenopausal women, however, men are also affected. Previous surveys carried-out in our area in the pre-effective drug era (late '1970th) and on the verge of the widespread introduction of effective anti-osteoporosis drugs (about 2000), indicated a marked increase in the incidence of hip fractures in the population at risk of women and men age 50 and older, which was mainly attributed to an increase in the fracture incidence in the very old (75 years and older) and to aging of the population. The present study was aimed to evaluate the impact of widespread availability of anti-osteoporosis drugs on the incidence of hip fractures in the same population.

Patients/ Methods: The study included women and men 50 years and older with radiographic evidence of a new fracture caused by low impact trauma. Only residents of the Negev were included. Incidence rates of hip fractures were calculated based on population data obtained from the official Central Bureau of Statistics. Data on osteoporosis drug use in the period of 6 month prior and 6 month following the fracture event were restricted to 75% of the patients who belonged to the Clalit health Services and were extracted from the hospital charts and the Ofek computerized database.

Results: Compared with previous surveys conducted close to the inclusion of effective anti-osteoporosis drugs in the health basket of the State of Israel and immediately after, we observed a persistent increase in the rate of use of effective anti-osteoporosis drugs (bisphosphonates, raloxifene, calcitonin, teriparatide) as well as of all anti-osteoporosis drugs (including calcium and vitamin D preparations) during the 6 months period preceding the fracture event. Anti-osteoporosis drug use was consistently higher in the period following fracture event, but the rate did not increase from that observed immediately after the introduction of effective anti-osteoporosis drugs into the health basket . The absolute rate of use of anti-osteoporosis drugs remained relatively low: use of all treatments before the fracture event was 31% in women and 14% in men, which changed after the fracture event to 53% and 19%, respectively. Use of effective anti-osteoporosis drugs treatments before the fracture was 18% in women and 9% in men, which changed after the fracture event to 27% and 4.5%, respectively. A statistically signifucant 45% decrease in the incidence of proximal-hip fractures in wome 75 years and older was observed in the current survey compared to the year 2000 survey [from 1,900/100,000 (95% CI 1,537–2,356) to 1,044/100,000 (95% CI 1,253-870)]. In men 75 years and older, a 37% decrease in the incidence of hip fractures was observed [from 1,053/100,000 (95% CI 710–1,505) to 649/100000 (95% CI 482-857)], which did not reach statistical significance.

Conclusions: These results draw a direct line between an increase in the use of anti-osteoporosis preparations and reduction in the incidence of proximal hip fractures in the Negev population in the 7-9 years period since the inclusion of effective anti-osteoporosis drugs in the health basket . This is underlined by the more pronounced decline in hip fractures observed in women, among whom the use of anti-osteoporosis drugs was far more widespread than in men, implicating causality between use of anti-osteoporosis drugs and the decrease in fracture incidence. Despite the increase in the anti-osteoporosis drugs availability, the majority of the population at risk, including et extra-risk, who experienced fracture events, still does not receive them, suggesting that osteoporotic fracture incidence could be decreased even further by filling of the treatment gaps through encouragement of the use of osteoporosis drugs by patients at risk, both women and men.

Post fracture osteoporosis treatment program, is it efficient?

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Introduction: Fragility fractures resulting from low trauma events such as a fall from standing height are common in the elderly. Up to 50% of women and 30% of men will experience an osteoporotic fracture during their lifetime. Patients with fragility fractures have a fivefold increase in risk for further osteoporotic fractures. Orthopedic care usually does not include initiation of fracture prevention treatment.

Patients/ Methods: Fractures Prevention Program (FPP) was initiated in Rambam Health Care Campus in March 2009.All patients (pts) with fragility fractures were referred from the Department of Orthopedic Surgery to the Bone and Mineral Metabolism Unit for fracture prevention treatment.

Results: 347 pts, aged 46-107 (74.5±12.7), 93 (27%) men and 254 (73%) women were hospitalized with fractures in 2009. During ten months period, 228 hip fractures, 23 vertebral fractures and 98 fractures at other sites (proximal and distal humerus, olecranone, wrist, distal femur and femoral shaft, tibia, fibula, and ankle) were diagnosed, 65 (18.7%) patient had previous fragility fractures. Prior to hospitalization only 56 (22%) women have received a fracture prevention treatment: 51 (19.7%) were treated with oral bisphosphonates (48 – Alendronate, 3 - Risedronate), 4 (1.6%) – with SERM (Raloxifen), 1 (0.4%) – with Recombinant PTH (Forteo). None of the men was treated before hospital admission. 25OHD serum level prior to hospitalization was available for 83 (24%) patients. 25OHD level was 60.2±23.7 (14.3-113) nmol/L, 26 (31%) patients had vitamin D deficiency (25OHD ≤50 nmol/L), 7 (8.4%) patients had severe vitamin D deficiency (25OHD ≤25 nmol/L). 82 (23.6%) pts, 8 (8.6%) men and 74 (29%) women, adhered to the FPP clinic visits, including 30 (53.6%) women previously treated in the community. 47 (20.6%) program participants had femoral neck fractures and 35 (29.4%) – fractures at other locations. 265 (76.4%) pts stayed out of the FPP: 26 (7.5%) women continued their community initiated treatment, 1 (0.3%) man was started on therapy by his family physician, 44 (12.7%) patients refused to join the FPP and remain untreated, 27 (7.8%) – were lost for follow-up, 157 (45.2%) – were unable to reach the clinic and remained untreated in the community, 10 (2.9%) patients died. 109 (32.2%) pts are currently treated for osteoporosis, including 27 in the community: 55 (16.3%) pts receive alendronate, 19 (5.6%) – risedronate, 2 (0.6%) – raloxifen, 10 (3%) – zoledronate, 10 (3%) - teriparatide, 13 (3.8%) – calcium and vitamin D only to ensure vitamin D replenishment prior to bisphosphonate treatment. 229 (67.8%) patients remain untreated.

Conclusions: Despite high prevalence of osteoporosis in the elderly, most patients remain untreated even after fragility fractures. Hospital based FPP increased by 10% the rate of post fracture treatment. Male patients and patients with hip fractures are more likely to stay untreated after hospitalization. Taking into account post-fracture disability and low compliance, a structured post fracture hospital initiated program with intravenous zoledronate might lead to better results and is worth to be tested.

Osteoprotegerin as an independent marker of subclinical atherosclerosis in osteoporotic postmenopausal women

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Introduction: Osteoprotegerin (OPG) appears to represent the molecular link between bone resorption and vascular calcification, and may help to explain the high prevalence of atherosclerosis and osteoporosis in postmenopausal women. We investigated a possible association between serum OPG levels and arterial stiffness in postmenopausal women with osteoporosis.

Patients/ Methods: 70 postmenopausal women with osteoporosis and cardiovascular risk factors but without coronary artery disease were evaluated for metabolic, inflammatory parameters and serum OPG levels. Pulse wave velocity (PWV) and augmentation index (AIx) were performed as a simple noninvasive recording of the two artery sites pressure waveform using SphygmoCor (version 7.1, AtCor Medical, Sydney, Australia).

Results: Serum OPG levels were significantly, positively associated with AIx ($r=0.39$, $p=0.003$) and with PWV ($r=0.81$, $p<0.0001$). No association between OPG levels and hemodynamic variables or measures of glucose metabolism was observed. Among inflammatory markers, OPG was significantly, positively associated with fibrinogen ($r=0.323$, $p=0.015$). In a multiple linear regression analysis, OPG was independent predictor of PWV (standardized beta=0.75, $p<0.0001$) and AIx (standardized beta=0.41, $p=0.01$).

Conclusions: Serum OPG is potentially an independent predictor of early vascular adverse changes in osteoporotic postmenopausal women.

Bone turnover markers in the follow-up of bisphosphonate treated osteoporotic patients

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Introduction: Tools for assessment of long term effects of bisphosphonates (BP) in osteoporotic patients are needed, especially, due to the occasional clinical reports of severely suppressed bone turnover and atypical skeletal fragility, as well as presumably increased risk of osteonecrosis of jaw in patients with carboxy terminal telopeptide (CTX) plasma level <0.2 ng/ml. Drug holiday is a common practice after long term BP use. Data about the rate of bone turnover recovery after long term BP treatment is scarce.

Patients/ Methods: 300 patients(pts) aged 68.56±8.14 (mean±SD) years were evaluated after 4-10 years of alendronate therapy.Two bone turnover markers (BTM): aminoterminal propeptide of type I collagen (PINP) and carboxy terminal telopeptide (CTX) were assessed in pts' fasting morning plasma samples during long term BP treatment.

Results: Plasma levels (mean±SD, ng/ml) after 4 years of treatment were 7.7±25.32 and 0.2± 0.09, after 5 years 11.23±26.02 and 0.13±0.23, after 6 years 10.08±22.32 and 0.12±0.19 after 7 years8.09±23.67 and 0.11±0.21, after 8 years 6.94±22.49 and 0.006±0.18, after 9 years 6.94±22.49 and 0.006±0.18, after 10 years 12.17±27.17 and 0.1±0.21 for P1NP and CTX respectively. There was no significant change in the levels of both bone turnover markers over the duration of therapy. Normal premenopausal range, a target range for BP treated pts, for CTX was 0.025-0.573 ng/ml and for P1NP - 15.13-58.59 ng/ml, as reported by the manufacturer (Roche). One (0.3 %) pt had CTX values below premenopausal range, 156 51.9) %) had CTX levels <0.2 ng/ml, 26 (8.75%) pts had P1NP levels below premenopausal in all treatment duration groups. None of the pts reported abnormal healing after oral cavity procedures. Forty five pts that were assessed during BP treatment, stopped treatment for drug holidays. From these 30 were evaluated 6 months after treatment discontinuation: 9 (20 %) demonstrated a significant rise in P1NP (increase by ≥ 10 ng/ml) and in CTX(> 0.2 ng/ml), in 2 pts (4.4%) only P1NP value increased and in 5 (11.1%) only CTX. 31 pts were evaluated 1 year after treatment discontinuation: in 5 (11.1%) increase in both markers was observed, in 1 (2.2%) a rise in P1NP and in another (2.2%) a rise in CTX only was observed.

Conclusions: Most pts on long term bisphosphonate treatment had bone turnover markers levels within normal premenopausal range irrespective of treatment duration. Suppressed bone turnover was observed in a small subset of patients. After treatment discontinuation only one fifth of the pts demonstrated increase in bone turnover.

Sirtuin 1 (sirt1) is a regulator of marrow Adipogenesis

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Introduction: Bone loss is an inevitable consequence of aging. Age-associated bone loss results from insufficient osteoblasts attributed to a shift of multipotential mesencymal stem cell progenitors towards the adipocyte lineage at the expense of the osteoblast lineage. Sirtuin 1 (SIRT1) a NAD+-dependent deacetylase was found to be a key regulator of life span in lower species and in multiple metabolic pathways and age-associated diseases in mammals. First identified for its role in chromatin remodeling and gene silencing, it was then discovered to be the mediator of the life extending effect of calorie restriction. Importantly, SIRT1 was found to represse the transcription of the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ), a master regulator of adipocyte differentiation and its transcriptional activity (Picard et al. Nature 2004). By recruitment to the PPARγ promoter together with the corepressors NCoR and SMRT SIRT1 represses PPARγ gene expression. Since osteoblasts and adipocytes originate from a common progenitor, we sought to test the hypothesis that SIRT1 is involved in the marrow mesenchymal stem cell fate to differentiate into an osteoblast or adipocyte.

Patients/ Methods: Studies were performed in the murine embryonic mesenchymal stem cell line C3H10T1/2 and in primary bone marrow mesenchymal cell cultures obtained from 12 week-old female SIRT1+/- mice and their WT counterparts (general SIRT1 ablation in inbred 129 sv mice is lethal). SIRT1 over-expression in C3HT101/2 cells was modified through retroviral infection with pBABE-SIRT1 with puromycin selection. Adipogenesis was induced with insulin, dexamethasone, indomethacin IBMX and rosiglitazone. Adipogenesis was assessed 14 days post induction by Oil-Red-O staining. RNA was extracted and gene expression was determined by Real Time PCR. Protein expression was evaluated by western blotting.

Results: Following induction of adipogenic differentiation Sirt1+/- –derived bone marrow mesenchymal stem cell cultures exhibited increased adipocyte formation, corresponding with decreased osteoblast formation, as determined by Oil-Red-O staining. There was a five fold increase in PPARγ gene expression and a 3 fold increase in CEBPα gene expression 48 hours post adipogenesis induction in SIRT1-derivd cultures as compared to WT-derived cultures. A reciprocal finding was demonstrated in SIRT1-overexpressing C3HT101/2 cells with reduced adipogenesis compared to control cells.

Conclusions: These results indicate that SIRT1 plays a role in the bone marrow mesenchymal progenitor cell fate to differentiate into an osteoblast or an adipocyte possibly via its influence on PPARγ. Our findings suggest that SIRT1 is involved in the pathogenesis of osteoporosis.

11ß-hydroxysteroid dehydrogenase type 1 as a cause of and target for metabolic syndrome and age-related cognitive impairment: hope or hype?

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In Cushing's syndrome, chronic glucocorticoid excess exerts a host of adverse effects on body (visceral obesity, insulin resistance/type 2 diabetes, dyslipidaemia, atherosclerosis) and brain (depression, cognitive impairment). However, in most cases of these prevalent disorders plasma cortisol levels are not elevated and thus any relevance of glucocorticoids to pathogenesis has been obscure. Glucocorticoid action on target tissues is determined by the density of nuclear corticosteroid receptors and by intracellular metabolism by 11ß-hydroxysteroid dehydrogenases (11β-HSDs) which catalyse the interconversion of active cortisol (corticosterone in rodents) and inert cortisone (11-dehydrocorticosterone). In metabolic tissues (liver, adipose) and CNS the 11ß-HSD type 1 isozyme predominates which catalyses the regeneration of active steroids, thus amplifying glucocorticoid action.

Obese humans and rodents show ~2-fold increased 11ß-HSD1 selectively in adipose tissue. Transgenic modelling of this recapitulates metabolic syndrome whereas 11ß-HSD1 knock-out (11β-HSD1$^{-/-}$) mice have improved glucose tolerance, a 'cardioprotective' lipid profile, insulin sensitization, reduced visceral fat accumulation with high fat diet and lower weight gain despite hyperphagia. 11β-HSD1$^{-/-}$ mice also have increased angiogenesis in response to experimental myocardial infarction and resist atherosclerosis. In obese diabetic humans, selective 11ß-HSD1 inhibitors have shown beneficial effects in early phase clinical trials.

11ß-HSD1 is also highly expressed in the adult CNS and its inhibition protects hippocampal cells from neurotoxic challenge in vitro. 11ß-HSD1 is elevated in aged rat hippocampus and correlates with the degree of cognitive decline suggesting an aetiological role. Importantly, 11ß-HSD1$^{-/-}$ mice resist glucocorticoid-associated impairments of cognitive function with ageing and indeed the 11ß-HSD inhibitor carbenoxolone improves cognitive performance in elderly humans. Thus 11β-HSD1 appears a promising target for therapy of metabolic syndrome/obesity spectrum disorders and age-related cognitive impairment and is a prototype for tissue-specific manipulation of the effects of glucocorticoids.

The effect of ERα and ERβ specific agonists on cell proliferation and energy metabolism in human vascular smooth muscle cells

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Introduction: In cultured human vascular smooth muscle cells (VSMC) estradiol-17β (E2) induced a biphasic effect on DNA synthesis, i.e., stimulation at low and inhibition at high concentrations, whereas the specific activity of the brain isozyme of creatine kinase (CK) was dose- dependently stimulated. We now investigate the effects of ERα and ERβ specific agonists compared to E2 on different parameters in vascular cells.

Patients/ Methods: VSMC were treated with 0.3 or 30nM E2, 42 or 420nM 2,3-bis (4-hydroxyphenyl)-propionitrile (DPN ;ERαβ specific agonist) and 39 or 390nM 4,4',4''-[4-Propyl-(1H)-pyrazol-1,3,5-triyl]tris-phenol (PPT;ERα specific agonist) and the effects on DNA synthesis, CK, the expression of mRNA for ERs, 12 lipooxygenase (12LO), 15 lipooxygenase (15LO), 1α vitamin D hydroxylase and ROS production were analysed.

Results: Treatment with PPT at both concentrations increased DNA synthesis, while DPN at both doses inhibited DNA synthesis, and the effect of E2 on cell proliferation was biphasic. PPT and DPN similar to E2 stimulated dose-dependently CK. Raloxifene (Ral), a specific ERα antagonist, inhibited the stimulation of DNA synthesis by either PPT or by low dose of E2, but did not affect the decreased cell proliferation by either DPN or by high dose of E2. LO inhibitor baicalein inhibited E2 and DPN effects but not those of PPT. Real-time PCR revealed that PPT had no effect on ERα but DPN stimulated it. Both PPT and DPN inhibited ERβ, while E2 did not affect any ER. E2 stimulated the expression of both 12 and 15LO, whereas PPT inhibited 12LO with no effect on 15LO and DPN inhibited 12LO and stimulated 15LO. E2 increased mRNA for 1α vitamin D hydroxylase whereas PPT had no effect and DPN inhibited its expression. ROS production was induced by all hormones as well as by 12 and 15HETE and was inhibited by DPI which also abolished E2 and DPN induced inhibition of proliferation.

Conclusions: we provide herein evidence for the separation of mediation via ERα and ERβ pathways in the different effects of E2 on VSMC. The exact mechanism has still to be analysed in future experiments.

The role of PERK in the regulation of beta-cell function and survival in type 2 diabetes

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Introduction: In type 2 diabetes, hyperglycemia and elevated free-fatty acids (FFA) induce metabolic stress leading to beta-cell dysfunction and apoptosis. In the short-term, glucose augments proinsulin biosynthesis and insulin secretion, however, chronic exposure to high glucose increases beta-cell apoptosis. It is not clear whether sustained stimulation of proinsulin biosynthesis adversely affects beta-cell survival in type 2 diabetes. PERK, an endoplasmic reticulum (ER) membrane associated kinase, phosphorylates eIF2alfa leading to transient attenuation of translation. Robust activation of PERK-eIF2alfa by FFA causes apoptosis through activation of ATF4 and CHOP. However, ATF4 may have a pro-survival effect by amelioration of the cellular antioxidant capacity. We hypothesized that PERK-eIF2alfa coordinate proinsulin biosynthesis and the beta-cell response to metabolic stress and that dysregulation of PERK activity under conditions of hyperglycemia results in beta-cell apoptosis.

Patients/ Methods: Human and Psammomys obesus (P. obesus) islets and INS-1E beta-cells were incubated overnight at 3.3, 16.7 or 22.2 mmol/l glucose with and without 0.5 mmol/l palmitate. PERK and eIF2alfa expression and phosphorylation and ATF4 and CHOP expression were analyzed by Western blot. The role of PERK in the regulation of proinsulin biosynthesis and beta-cell apoptosis was studied by RNAi knockdown of Perk in INS-1E cells. Proinsulin biosynthesis was analyzed by incubating islets or INS-1E cells with 3.3 or 16.7 mmol/l glucose for 1 h followed by metabolic labeling with L-[2, 3, 4, 5-3H]leucine and immunoprecipitation using anti-insulin serum. beta-cell apoptosis was assessed using the Cell Death ELISA PLUS assay (Roche Diagnostics, Manheim Germany).

Results: Incubation of human and P. obesus islets at 16.7 or 22.2 mmol/l glucose decreased basal PERK and eIF2alfa phosphorylation. Moreover, prolonged exposure to hyperglycemia attenuated FFA-stimulated PERK and eIF2alfa phosphorylation indicating that glucose inhibited PERK activity under conditions of metabolic stress. To study the impact of PERK inhibition on beta-cell function and survival, we knocked down Perk in INS-1E cells. This resulted in a 3-fold increase of proinsulin biosynthesis and beta-cell apoptosis at all glucose concentrations. Reducing proinsulin synthesis to basal levels by cycloheximide reduced beta-cell apoptosis, suggesting that the induction of beta-cell apoptosis by PERK inhibition may be related to augmented proinsulin synthesis. Glucagon-like peptide 1 (GLP-1) was shown to alleviate ER stress induced by pharmacological agents and FFA. Perk knockdown INS-1E cells were treated with the GLP-1 analogue exendin 4. This increased the expression of ATF4, a downstream target of eIF2alfa expression and decreased beta-cell apoptosis. Finally, treatment of Akita mice, an animal model of beta-cell ER stress with exendin 4 improved hyperglycemia, increased serum insulin levels and preserved the islet architecture and insulin content

Conclusions: Chronic hyperglycemia inhibits PERK in islets resulting in increased proinsulin biosynthesis, which in turn renders the beta-cell susceptible to stress and apoptosis. Thus, PERK might be an important link between hyperglycemia and beta-cell stress in type 2 diabetes. Reducing ER protein load under conditions in which PERK is down-regulated, such as hyperglycemia may ameliorate beta-cell survival. Moreover, GLP-1 signaling increases the expression of ATF4 despite of decreased eIF2alfa activity, thereby protecting the beta-cells from apoptosis. GLP-1 based therapy prevents beta-cell ER stress in vivo leading to improvement of diabetes.

AHNAK gene silencing increases GLUT4 gene expression and translocation in skeletal muscle-derived L6 cells

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Introduction: AHANK is a giant phosphor-protein that participates in hyperlipidemia -mediated cellular signaling in cardiac muscle. Enhanced expression of AHNAK has been associated with poor muscle fitness in human patients.

Patients/ Methods: Therefore, we investigated in skeletal muscle-derived L6 cells (wild type and GLUT4myc) how AHNAK gene silencing affects a. GLUT4 gene expression, b. basal and insulin stimulated GLUT4 translocation.

Results: Compared to empty vector, transient overexpression of AHNAK C-terminal, middle- and N-terminal in L6-WT repressed basal activity of GLUT4-P to 56±15%, 44±14% and 35±19% (mean±SEM), respectively. Applying siRNA to L6 myotubes, we found that AHNAK gene silencing by 40% increased endogenous levels of Glut4 protein by 3.8-fold. More so, AHNAK gene silencing in L6 Glut4myc cells increased basal and insulin-stimulated cell surface levels of GLUT4 to 152±19% and 230±39%, respectively.

Conclusions: our data introduce AHNAK as a negative regulator of insulin sensitivity that reduces cellular GLUT4 and impairs its function, thus contributing to the pathogenesis of insulin resistance. Hence, AHNAK may serve as potential molecular target for obesity and type 2 diabetes therapies.

Development of therapeutic cellular products for the treatment of diabetes-related complications

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Introduction: Degenerative diseases are by far the most common cause of human morbidity and mortality. A prime example is diabetes, being an incurable chronic disease currently affecting about 170 million people, a number which the WHO is expecting to double by 2025. Complications of diabetes mostly occur through vasculopathies, involve gradual deterioration of body function in all diagnosed patients. Current treatments are of limited effect, and thus most patients succumb to complications for which new therapies are clearly required. The objective of regenerative medicine is the reversal of the disease process thereby inducing recovery and improving patients' functionality and quality of life. One of the most promising innovative treatments for diabetes is autologous stem cells-based treatment modality that can repair damaged tissues.

Patients/ Methods: Currently, most studies and treatments are using the bone marrow (BM)-derived cells. Procedures for obtaining BM cells entails pain and discomfort and require the use of anesthesia. The alternative is mobilizing cells from the BM to the peripheral circulation by pre-treating the patient with granulocyte colony-stimulating factor (G-CSF). However, this was reported, to result in increased blood viscosity, metabolic demands, and platelet counts To circumvent risks and discomfort associated with existing methods, we are constantly developing new approaches enabling to obtain cells from un-mobilized peripheral blood collected from the arm vain.

Results: We describe here a cell population named BC1. BC1 produced from blood contains a significant number of highly viable cells composing a mixture of endothelial progenitor cells (EPCs) and multipotent adult stem/progenitor cells (MASPCs) involved in blood vessels' regeneration. Morphologically, BC1 shows elongated and spindle large activated stem/progenitor like cells. Specific cellular markers and activity tests prove the production of functional EPCs. Cells implanted into irradiated NOD/SCID mice successfully migrated and engrafted in the mice BM as detected 7weeks after transplantation. In addition, purified blood cells stored for various durations successfully completed the production process upon termination of banking period, yielding cell population composed of EPCs and MASPCs.

Conclusions: Experiments employing human blood resulted in production of a therapeutic cellular product named BC1 indicate of a very promising cells that should be further tested in relevant animal, such as hind limb ischemia, before subjected to clinical studies.

Preparation and characterization of recombinant N82K human leptin, a naturally occurring obese-phenotype-inducing mutation in human leptin gene

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Introduction: A novel homozygous mutation of the leptin gene was recently reported in an Egyptian child with severe early onset obesity. This mutation results from the substitution of asparagine (AAC) by lysine (AAA) at codon 103 of a non-mature (signal peptide-containing) leptin and corresponds to the N82K mutation in the mature protein. The patient had very low serum leptin levels, raising the question of whether the obese phenotype resulted from low leptin levels or from its lower intrinsic activity. To answer this question, we characterized the functional consequences of the N82K mutation.

Patients/ Methods: Wild-type (WT) human leptin was mutated accordingly (N82K), expressed in Escherichia coli at high yield, purified to homogeneity as a monomer and its receptor binding capacity and biological activity was compared to WT human leptin prepared by the same methodology.

Results: Circular dichroism analysis of the mutated leptin indicated proper refolding and a secondary structure identical to that of the WT human leptin. In contrast to WT human leptin, the N82K mutant did not form a detectable complex with human leptin binding domain (hLBD) and its binding capacity to hLBD assessed in a nonradioactive receptor-binding assay was at least 500-fold lower than that of WT human leptin. The biological activity of the N82K mutant, tested in two cell bioassays, was reduced by more than three orders of magnitude relative to WT human leptin.

Conclusions: In view of our results we conclude that the reported obese phenotype in Egyptian child originates not only from low serum leptin levels but also in not mainly from the N82K mutant's almost total lack of intrinsic leptin activity.

The incidence of Type 1 diabetes (T1D) in Israeli children following the second Lebanon war: A population-based study

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Introduction: The contribution of psychological stress to the emergence of T1D is still controversial. Several studies have demonstrated a higher prevalence of stressful life events in T1D children compared with healthy children. However, other studies failed to show a change in diabetes incidence in Croatia and Bosnia-Herzegovina during armed conflicts. During the Second Lebanon War (July 7th-August 14th, 2006), the civilian population in the northern regions of Israel was under rocket attacks that claimed the life of 52 civilians. Over 4300 were wounded, among them 2774 were diagnosed with acute stress disorder. We aimed to evaluate trends in the incidence of T1D before and after the war in the northern regions compared with the other regions of Israel.

Patients/ Methods: Data on T1D was obtained from the Israel juvenile diabetes register, that contains new diabetes patients aged 0 to 17 years since 1997. We included in the study new T1D cases diagnosed between 2002 (when ascertainment rate was much improved) and 2007. The annual and seasonal incidence of T1D (expressed as rate per 100,000) was based on data obtained between 9/2006 to 8/2008 (two post-war years) and between 9/2002 to 8/2006 (four pre-war years). The northern and southern regions were defined and population numbers were obtained from the Israel Central Bureau of Statistics.

Results: The completeness of the data was estimated to be 97.4%. In the six study years, 1822 new T1D children were reported (53% males), 668 of them (37%) after the war. During pre-war years, T1D incidence was lower in the northern regions compared with the other regions [odds ratio 0.8 ,95% CI 0.7-1.0] and increased after the war [OR 1.1, 95% CI 0.9-1.3] (Fig). Interestingly, the difference in T1D incidence between north and other regions was higher in the second year than in the first year after war. T1D incidence was higher in post-war years than in pre-war years in the northern regions [OR 1.3, 95% CI 1.1-1.6) but not in the other regions in Israel [OR 1.0, 95% CI 0.9-1.2]. This post-war elevation in the north was higher in males [OR 1.5, 95% CI 1.2-2.0] than in females [OR 1.1, 95% CI 0.8-1.5]. Both before and after the war, T1D incidence was higher in winter time than in summer time in all regions

Conclusions: The observed elevation in T1D incidence in the northern regions following the Second Lebanon War suggests that psychologically stressful situations may contribute to this elevation in T1D. Yet, other unknown factors may also play a role in the change in incidence of this multifactorial disease.

Clinical effects of moderately intensive glycemic control after cardiac surgery

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Introduction: The impact of intensive insulin treatment on the clinical outcomes of patients hospitalized in intensive care units (ICU) is highly controversial. The objective of the present study was to test the efficacy and safety of a protocol based on intensive insulin therapy in a surgical ICU and ward and to assess its impact on clinical outcomes.

Patients/ Methods: Patients undergoing cardiac surgery (n=203) over 8 months with diabetes or random blood glucose >150 mg/dl were treated in the ICU with intravenous insulin, followed by multi-injection protocol consisting of 4 glargine/aspart insulin injections in the ward, with a glycemic target of 110-150 mg/dl. The control group consisted of all patients (n=207) operated during a similar period immediately prior to protocol implementation. Data were prospectively collected and entered into a computerized database.

Results: During the intervention, mean blood glucose±SD was 151±19 mg/dl and 157±32 mg/dl in the ICU and ward, respectively vs 166±27 mg/dl and 184±46 mg/dl in the controls ($p<0.0001$). Intensive insulin treatment decreased the risk for infection from 11% to 5% (56% risk reduction, $p=0.018$), mainly by reducing the incidence of graft harvest site infection (6.9% vs. 2.5%, $p=0.034$). In patients with acute hyperglycemia after surgery, moderately intensive insulin treatment decreased the incidence of multi-organ failure from 3.2% to 0% ($p=0.038$) and the need for prolonged mechanical ventilation from 7.3% to 1.5% ($p=0.024$). The incidence of atrial fibrillation following coronary artery bypass graft decreased from 30% to 18% (39% risk reduction, $p=0.042$). The incidence of hypoglycemia (blood glucose <60 mg/dl) was low and similar between the groups (control - 2.5% vs 3% - protocol, p NS).

Conclusions: Moderate-intensity dynamic blood glucose control after cardiac surgery is effective, safe and associated with improved clinical outcomes.

Stress hyperglacemia and Mody 2

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Introduction: Introduction Stress hyperglycemia in children is usually benign, and does not mandate further investigations. However, in some clinical settings it may be the first sign of blood glucose abnormalities or even frank diabetes.

Patients/ Methods: Due to a febrile illness, a 2.7 years old boy with a viral infection and a 5 weeks old infant with a urinary tract infection were admitted. Routine urine and blood works revealed abnormally elevated glucose levels. Further evaluation demonstrated a noticeable glucose abnormality and elevated HbA1C levels.

Results: A history suggestive of familial diabetes in both patients led to genetic analysis, finding two known heterozygote MODY mutations in the Glucokinase gene: C233R and T206P respectively.

Conclusions: Stress hyperglycemia in children can be the first sign of monogenic diabetes. In cases in which a family history of diabetes mellitus is noted, it is reasonable to consider genetic evaluation.

Permanent neonatal diabetes due to an INS gene mutation

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Introduction: Neonatal diabetes (ND) is a rare disorder, defined as diabetes mellitus occurring in the first 6 months of life. It arises from mutations in the genes that play critical roles in the development of the pancreas, the insulin processing as well as the regulation of insulin release. We describe a case of ND due to a mutation in the insulin (INS) gene.

Patients/ Methods: The patient presented with fever and sustained hyperglycemia at the age of 3 months. Physical examination was unremarkable besides a horizontal crease on her right ear lobe. HbA1C was 4.1%, anti-GAD antibodies were negative, abdominal US was normal. Insulin treatment was started. Genetic analysis: DNA was extracted from peripheral lymphocytes and direct sequence of the KCNJ11, ABCC8 and INS genes had been undertaken.

Results: Genetic analyses of the Kir6.2 and SUR1 were normal. A heterozygote mutation for a missense mutation, G32S, in exon 2 of the INS gene was found. This G>A mutation at nucleotide 94 results in the substitution of serine (uncharged polar) for glycine (non polar) at codon 32 (p.Gly32Ser) and has been reported previously. Neither parents were shown to carry the mutation.

Conclusions: The G32S mutation disrupts the folding of the proinsulin molecule and results in misfolded protein and retention of the protein in the endoplasmatic reticulum, leading to β-cell apoptosis. We aimed to decrease endogenic insulin formation and minimize further apoptosis by intensive insulin treatment (basel-bolus regiment). However, adding short acting insulin therapy induced hypoglycemia, and therefore the patient is treated only by once-a-day injection of long acting insulin analog (0.3 u/kg/day). For the past year, the patient is doing well and her HbA1C is stable on 7%.

Does prolonged aldosterone blockade affect adversely glucose control and arterial properties in type 2 diabetic patients?

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Introduction: Aldosterone blockade is potential therapeutic target for the prevention and treatment of cardiovascular disease in diabetic patients. However, several short-term studies suggest that aldosterone blockers may worsen glycaemic control and endothelial function in this population. The present study investigated the long-term (12 months) effect of spironolactone treatment on glucose homeostasis and vascular properties in patients with type 2 diabetes.

Patients/ Methods: In randomized, placebo controlled study, 52 patients with type 2 diabetes mellitus were assigned into two groups: Group 1 received spironolactone, Group 2 received placebo. Study patients were evaluated for electrolytes, glucose, HbA1C, insulin, c-peptide, lipid profile, hs-CRP, 24-hour urinary albumin excretion, aldosterone, plasma renin activity and endothelin. Insulin resistance was assessed by homeostasis model assessment (HOMA-IR) and adiponectin levels. Arterial elasticity was evaluated using pulse wave contour analysis (HDI CR 2000, Eagan, Minnesota).

Results: The two groups were similar at baseline in terms of hemodynamic and arterial elasticity parameters. After 12month, small artery elasticity index (SAEI) as we as large artery elasticity index (LAEI) improved significantly in patients received spironolactone compared to the placebo group ($p=0.001$ and $p<0.0001$, respectively). In univariate GLM analysis, endpoint SAEI and LAEI remain significantly associated with treatment assignment after adjustment for mean arterial pressure. Baseline potassium and aldosterone did not differ by treatment group but were significantly greater in group1 compared to placebo group at the end of the study. Glucose control and insulin resistance didn't worse by spironolactone treatment. Endothelin decreased in spironolactone and did not change in placebo group.

Conclusions: Prolonged spironolactone therapy improved arterial compliance in diabetic patients without deterioration in glucose tolerance. The findings of the present study suggest that beneficial vascular effects of aldosterone blockade may lead to decrease in future cardiovascular events in this population.

The timing of nephrology referral and long-term survival of new dialysis patients with diabetes

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Introduction: Diabetes is the leading cause of end stage renal disease in the western world. Nearly half of the incident dialysis patients have diabetes. The presence of diabetes is associated with high morbidity and mortality. Among the modifiable risk factors of mortality among dialysis patients is the timing of nephrology referral, a recognized significant predictor of short-term morbidity and mortality in patients who start dialysis treatment. We analyzed the long-term impact of the timing of referral on mortality of new dialysis patients.

Patients/ Methods: All consecutive patients who entered the hemodialysis program in our center between January 1, 2004 and December 31, 2007 were studied retrospectively. Patients with acute renal failure or advanced malignancy at presentation were excluded. Patients were classified as early referrals (ER) or late referrals (LR) depending upon whether they initiated hemodialysis <3 or >3 months after their first nephrology consultation. The survival analysis comparison between both groups was by the log-rank test. A Cox proportional hazards regression model identified factors that were independently associated with mortality risk.

Results: The ER group had 118 patients (59%) and the LR group had 82 patients (41%). They were similar in mean age (ER 66.7 years and LR 69.1 years, p=0.224), diabetes (ER 59% and LR 61%, p=0.77), mean hemoglobin level (ER 10.7 g/dl and LR 10.2 g/dl, p=0.086), and mean serum albumin (ER 3.4 g/dl and LR 3.2 g/dl, p=0.11). Over one-half (n=109, 54.5%) of all patients died during the follow-up period, 53 (44.5%) in the ER group and 56 (68%) in the LR group. The overall 4-year survival was 41.1% in the ER group compared to 18.7% in the LR group (p<0.0001). The 4-year survival was 24.4% in the diabetic group compared to 44.5% in the non diabetic group (p=0.029). The 4-year survival was 35.1% in the ER diabetic group and only 12.1% in the LR diabetic group (p= 0.001). The mortality rate (multivariate analysis) was associated with age (hazard ratio [HR]) 1.038 for each year, 95% Confidence Interval (CI)1.013-1.063), diabetes (HR 2.46, CI 1.383-4.376), late nephrology referral (HR 1.943, CI 1.161-3.252), and serum albumin level (HR 0.382 for an increase of each 1 g/dl, CI 0.256-0.570).

Conclusions: Long-term survival in dialysis patients was independently associated with diabetes and with time to referral for a nephrological consultation. Our results show a significantly higher mortality up to four years after the initiation of dialysis in late referred patients with chronic kidney disease compared to early referred patients. The impact of nephrology referral pattern is more pronounced in dialysis patients with diabetes. The referral pattern should be considered a modifiable risk factor for survival in the setting of end-stage renal disease and diabetes.

A novel mutation in the EIF2AK3 gene in a Palestinian family with Wolcott-Rallison Syndrome

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Introduction: Wolcott-Rallison syndrome (WRS) is a rare autosomal recessive disorder characterized by the association of permanent neonatal or early infancy type 1 diabetes mellitus, multiple epiphyseal dysplasia, growth retardation, and other variable multisystemic clinical manifestations.WRS results from mutations in the gene encoding the eukaryotic translation initiation factor 2 α-kinase 3 (EIF2AK3). This enzyme phosphorylates EIF2A to regulate the synthesis of unfolded proteins in the endoplasmic reticulum. Here we describe a novel mutation in the EIF2AK3 in a Palestinian family with Wolcott-Rallison Syndrome.

Patients/ Methods: A Palestinian infant, born to consanguineous parents, presented with early infancy type 1 diabetes mellitus, hypothyroidism, short stature, FTT, multiple skeletal epiphyseal dysplasia, elevated liver enzymes and hepatomegaly. Wollcot-Rallison syndrome was suspected and confirmed by molecular testing.

Results: DNA sequencing of the EIF2AK3 gene for the patient revealed a novel stop codon mutation, with replacement of Arginine (CGA) to stop codon (TGA) in codon 826.

Conclusions: To our knowledge, this is the first description of this disease in a Palestinian family with molecular confirmation, reinforcing the pathogenic significance of loss of the kinase domain in determining the extended phenotype of WRS, allowing accurate genetic counseling, early diagnosis of affected kindreds, early therapeutic interventions and avoiding complications.

Celiac in type 1 diabetes subjects - prevalence, metabolic control and growth parameters

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Introduction: Our objectives were to study the prevalence of biopsy-proven Celiac Disease (CD) among children and youth with type 1 diabetes mellitus (T1DM) and to study their metabolic control, nutritional status and growth parameters.

Patients/ Methods: In a retrospective chart review 11 patients were diagnosed with CD based on positive antibodies and positive jejunal biopsy. Control group compromised of two subjects matched by sex, age, and duration of diabetes for each CD patient (n=22). Patients with CD were further classified to those with good or poor compliance to gluten-free diet (GFD).

Results: 294 out of the 316 T1DM patients were screened for CD. We identified 11 patients with CD (3.74%). There was no difference in mean HbA1c levels between CD group and control group (p=0.94). However, CD patients with good compliance to GFD (n=7), had better metabolic control, throughout the entire follow-up, compared with those with poor compliance to GFD (n=4), without statistical significance due to small sample size (p=0.45). There was no difference in iron, ferritin, B12 and folic acid levels between CD group and control group. Patients with both T1DM and CD had growth impairment with significant difference between their target height and the height Z score compared with the control group (p=0.01). Among those with poor compliance to GFD growth impairment was severe.

Conclusions: Prevalence of CD among T1DM is 3.74%. There was no difference in metabolic control. However, patients with both T1DM and CD had significant growth impairment, with more pronounced impairment among children with poor compliance to GFD.

Efficacy and safety of DBCare, a food supplement, in patients with type 2 diabetes mellitus and inadequate glycemic control: A randomized, double-blind, placebo-controlled trial

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Introduction: Despite current oral hypoglycemic agents, most patients with type 2 diabetes mellitus (T2DM) do not maintain treatment goals, i.e. glycated hemoglobin level (A1C) <7%. Over time, uncontrolled diabetes may lead to microvascular and macrovascular complications. DBCARE is a traditional Indian herbal food supplement that contains 11 ingredients, some of which have been shown to have hypoglycemic properties in anecdotal animal and human studies. We report the first prospective, randomized, placebo-controlled study that evaluates the effect of DBCARE on patients with T2DM. Aim: To evaluate the efficacy and safety of DBCARE in patients with inadequately controlled T2DM despite oral hypoglycemic treatment.

Patients/ Methods: A prospective, 12-week, randomized, double-blind, placebo-controlled trial was conducted in a secondary referral center in Israel. Patients (above 18 yrs of age) with T2DM, on oral hypoglycemic agents, with A1C level >7.5%, were randomly allocated to receive DBCARE tablets (2 tablets three times daily) or placebo.

Results: 42 patients (26M/16F, mean age 60.8±9.4 years) were randomized to receive DBCARE (N=22) or placebo (N=20). Baseline clinical and biochemical characteristics of both groups were not statistically different (glucose 156.7±46.1 mg/dL, A1C 7.7±0.7%). From baseline to week 12, A1C levels declined 0.34±0.69% in the DBCARE group (p=0.039) and 0.23 ± 0.73% in the placebo group (p=0.224). In parallel, fasting plasma glucose (FPG) decreased 0.31±30.2 mg/dL in the DBCARE-treated group (p=0.96) and 9.6±44.7 mg/dL in the placebo group (p=0.41). Subgroup analyses of patients with baseline body mass index (BMI)>30 or <10 years duration of diabetes revealed A1C reduction of 0.50 ±0.53% (p=0.067) and 0.27±0.5%, (p=0.064), respectively at week 12 vs. baseline. Other parameters, including the homeostasis model assessment for insulin resistance and for insulin secretion, and C-reactive protein were not statistically different at 12 weeks vs. baseline. DBCARE was generally well tolerated. Three patients withdrew from the study, 2 from the treatment group and 1 from the placebo group.

Conclusions: DBCARE treatment was not effective in improving glycemic control in our cohort of patients with inadequately controlled T2DM, despite oral hypoglycemic treatment. Yet, patients with BMI>30 and with relatively newly diagnosed diabetes, benefited modestly from the treatment. Further studies are needed to evaluate the effect of DBCARE treatment on specific groups of patients, i.e. those with BMI>30, FPG>200mg/dL and recent onset diabetes at baseline. Trial Registration: Clinicaltrials.gov Identifier NCT0056004.

Relative expression of the mutant transcript determines clinical phenotype in family with dominant ABCC8 mutation and severe neonatal hyperinsulinism

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Introduction: Congenital Hyperinsulinism (CHI) is most frequently caused by ABCC8 gene mutations which can result in diffuse (recessive or dominant) or focal (paternal uniparental disomy) pancreatic disease. We present an unusual case of a maternally inherited ABCC8 mutation that causes disease in some but not all carriers due to a difference in the ratio between mutant and normal transcript expression.

Patients/ Methods: Clinical Data: Severe hyperinsulinemic hypoglycemia was diagnosed in a one day old girl of non consanguineous parents. A maternal cousin and sister had a similar phenotype.

Results: Molecular Data: DNA and RNA were extracted from leukocytes of the patient and family. A single heterozygous in-frame insertion mutation in exon 37 of the ABCC8 gene was found in the patient, her unaffected mother and the affected cousin. Expression of the mutation in COSm6 cells demonstrated normal protein membrane expression but no channel activity even in the heterozygous state. The finding of an apparently dominant ABCC8 mutation in a child with severe CHI and her unaffected mother suggested variable expression of the mutant allele. To test this, we reverse transcribed lymphocyte RNA and amplified the ABCC8 cDNA segment between exon 36 and exon 38. Purified PCR products were cloned into pGEM vectors and 20-4-different clones were sequenced for each RNA sample. Although genotypically identical, the relative expression of the mutant allele was much higher in the affected infant (19 mutated-m vs. 10 normal – n) than in the unaffected mother (9m: 30n) who carried mostly normal transcripts. Direct sequencing of PCR amplified cDNA labeled products also indicated that while the healthy mother expressed almost exclusively the normal transcript, her affected daughter produced primarily the mutant RNA.

Conclusions: We demonstrate for the first time that increased relative expression of a mutated ABCC8 transcript in lymphocytes (systemic) is associated and probably determines the phenotypic severity of CHI. The in-frame insertion mutation in exon 37 of the ABCC8 gene results in a protein that has no channel activity even in the heterozygous state. Further epigenetic studies may elucidate the mechanism involved in this variable expression of the mutated transcript.

Mitochondria-to-nucleus cross-talk: upregulation of mitochondrial protease transcription by 'protein stress' in steroid making cells

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Introduction: Being a highly metabolic organelle, mitochondria are subjected to various stresses such as ROS and unfolded protein accumulation. While undergoing functional differentiation, the mitochondria in steroidogenic cells are exposed to stress impacts exceedingly more than these organelles in any other cell type. That is due acquisition to the key proteins essential for steroid synthesis from cholesterol, i.e., the mitochondrial inner membrane monooxygenase CYP11A1, and STAR protein that translocates cholesterol substrate to CYP11A1. Upon completion, STAR is imported and floods the mitochondrial matrix with its inactive form. We suggest that the latter event imposes a 'protein stress' effect with potential organellar damage, to which the mitochondria operate a defense machinery of chaperone/proteases complexes acting to neutralize the 'ticking bomb' and rapidly degrade STAR. We addressed the question whether mitochondrial 'protein stress' can generate signaling to the nucleus and generate an upregulation of the organelle protease gene transcription (YES!).

Patients/ Methods: co-immunoprecipitations (coIP), in situ protein degradation assays by metabolic labeling, protein knock-down by siRNA, qRT-PCR and promoter analysis assays. The experimental models included hormone administered rat models and various cell lines.

Results: (a) STAR can physically interact with the membrane-bound m-AAA mitochondrial chaperone/protease complex (AFG3L2/SPG7), (b) Once reaching the mitochondrial matrix, a rapid STAR degradation is launched and proceeds consecutively by at least three proteases: the matrix LON protease, continuing with the inner membrane homo or hetero-oligomeric form of the AFG3L2/paraplegin protease/chaperone complexes, and a third yet unknown protease that concludes the elimination mission, (c) Simulation of mitochondrial 'protein-stress' caused by over-expression of STAR resulting in upregulation of Afgel2, Spg7 and Lon mRNAs levels and promoter activities. Consistent with this observation we show that hormonal induction of STAR in rats results in upregulation of the above proteases mRNAs levels in the ovary.

Conclusions: Our studies unravel the mechanism by which the mitochondria in steroidogenic cells respond to organelle stress. We named this effect as protein overload, or 'protein stress'. The findings suggest that in-house mitochondrial proteases serve as 'gate-keepers' of these organelles and prevent damage by a rapid removal of the threatening proteins. Proper activities of the proteases we describe herein are vital, as previously shown in the fatal neurodegenerative disorders hereditary spastic paraplegia and spinocerebellar ataxia type 28. We show that elevated mitochondrial 'protein stress' can signal upregulation of protease gene transcription in the nucleus.

Effect of sperm ligands on forward motility, hyperactivation and acrosome reaction, via ERK-mediated cascade

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Introduction: Mammalian sperm are activated by sperm ligands but the nature of the stimulating ligands is still not known. The signaling involved in ligand-stimulated spermatozoa is also not yet clarified and the main question in sperm biology is the identification of these sperm ligands and their mechanism of action. Mitogen activated protein kinases (MAPKs) are key regulatory enzymes in signal transduction. MAPKs are known to be involved in proliferation, differentiation, cell cycle control, apoptosis and transformation. We have recently characterized human sperm MAPKs, and implicated ERK and p38 in forward and hyperactivated motility and acrosome reaction (AR).However, the nature of the sperm ligands that activate ERK and p38 are not yet known. We have decided to concentrate our initial efforts on two potential sperm ligands, namely EGF and TGF-β3. We decided to compare their ability to activate ERK, and in parallel to stimulate forward and hyperactivated motility and acrosome reaction. Furthermore, we intend to find out if the activation of forward and hyperactivated motility and acrosome reaction by the above sperm ligands is mediated by ERK.

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

Results: EGF and TGF-β3 activated ERK within 5 minutes. The effect was persistent and still detectable in sperm after capacitation. Secondly, we explored the effect of these ligands on human spermatozoa forward and hyperactivated motility. Incubation of normal spermatozoa with EGF, increased forward motility within the first 5 minutes, and hyperactivation after capacitation. Incubation with TGF-β3 increased both forward and hyperactivated motility within the first 5 min. Later, we examined whether the EGF-induced forward motility is mediated via ERK-dependent mechanism, by adding a selective inhibitor. Indeed, pre-incubation with the inhibitor reduced the percentage of motile sperm and abolished the effect of EGF on forward motility. As a final point, we investigated the effect of both EGF and TGF-β3 on AR and whether this effect is mediated by ERK. The preliminary results have shown that both ligands induced AR.

Conclusions: EGF and TGF-β3 stimulate sperm motility and AR via an ERK-mediated cascade.

Molecular pathways leading to GnRH-induced cell proliferation and death in gonadotropes

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Introduction: 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 leads to proliferation of immature, partially differentiated gonadotropes. However, this response is no longer seen in mature, fully differentiated gonadotropes, in which GnRH decreases cell numbers instead. Several MAPK cascades are activated by GnRH in the gonadotropes. However, the mechanisms downstream to the MAPK cascades that are responsible for mediating the GnRH-induced cell proliferation and death are not yet understood. Moreover, it is not clear at which point GnRH stops inducing proliferation and which event is responsible for this switch. We hypothesize that the Bcl-2 family proteins, Bax and Hrk, as well as prohibitin are at least partially responsible for mediating the effects of GnRH on proliferation or cell death of the gonadotropes. Here, we show that GnRH increases the levels of cleaved PARP in mature gonadotropes, while leading to their slight decrease in immature gonadotropes. Additionally, our data shows that GnRH leads to an increase in the levels of Bax and Hrk in mature gonadotropes, while decreasing the levels of Bax in immature gonadotropes, and that GnRH does not change the levels of Bcl-2 in either cell type. In addition to Bax, Hrk and Bcl-2, we have also found an effect of GnRH on prohibitin, a protein that may also be involved in preventing cell proliferation. We have previously reported that the protein levels of prohibitin are higher in the nuclei of mature gonadotropes, when compared to immature gonadotropes. Here, we show that GnRH increase the prohibitin mRNA levels in mature gonadotropes. We have also found that the prohibitin levels in the nuclear fraction decrease following GnRH treatment, indicating export of the protein from the nucleus. Our data indicates that this export is ERK1/2-dependent.

Conclusions: Together, our findings suggest that the different effects of GnRH on mature and immature gonadotropes with regard to proliferation and apoptosis may be at least partially caused by the different effects of the hormone on Bax, Hrk and prohibitin.

Sphingosine kinase is not upregulated by GnRH or its analogues

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Introduction: The mechanisms whereby GnRH-a decreases ovarian failure in young women exposed to gonadotoxic chemotherapy are unknown. One of the suggested possible mechanisms is the gonadal up regulation of Sphingosine-1-Phosphate [S1P], an antiapoptotic molecule. To examine such a possible mechanism we have evaluated the activity of Sphingosine Kinase [SK] which is the physiological enzyme generating S1P.

Patients/ Methods: Human granulosa cells were retrieved by follicular aspiration for IVF, after informed consent. The granulosa cell cultures [GCC] were established after separation of the GC's on Percoll and cultured in M199 with FCS and antibiotics. After 2-3 days and preincubation in serum free medium, the GC's were incubated with native GnRH, GnRH-agonist, GnRH-antagonist, dimethyl-sphingosine (DMS, an SK inhibitor), and control medium. After 24 hours, the cells were trypsinized, lysed, and the SK activity was determined by conversion of added Sphingosine to SK followed by TLC separation with radioactive P32, by phosphoimaging. ATP- P32 was added for labeling SK, according to the method of Sara Spiegel [Richmond, VA, USA].

Results: There was no significant change in SK activity following incubation with either GnRH or its analogues. Neither the agonist, nor the antagonist or native GnRH affected SK activity. Our results are validated by the fact that DMS inhibited SK activity

Conclusions: Neither GnRH, nor its analogues increase the activity of SK in GCC, in vitro. However, S1P might be still possibly involved in the protective mechanism of GnRH-a against chemotherapy associated gonadotoxicity through an inhibitory effect on S1P-phosphatase.

The roles of MAPK in human sperm functions

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Introduction: Human spermatozoa are the final product of a complex differentiation process that takes place in the testes. These cells swim through the female reproductive tract to reach the fertilization site and transfer the male DNA into the egg. During their course they encounter various signals such as hormones, that make them capacitated, and only then they are capable to fertilize the egg. MAPKs are crucial signaling proteins that convey signals of multiple stimulations, such as proliferation, differentiation, motility and stress into cells. The signal is conveyed by protein phosphorylation. Spermatozoa are known as transcriptionally quiet cells, and therefore phosphorylations are considered to be major means of signaling. We investigated the PKC/MAPK pathway in human sperm flagellar motility, hyperactivated motility and acrosome reaction.

Patients/ Methods: Ejaculates were obtained by masturbation from healthy donors after 3 days of sexual abstinence. After liquefaction, semen was layered on top of a percoll gradient (40/80), centrifuged and spermatozoa were separated, washed and diluted into Ham's F-10 that was added with 4 mg/ml BSA. The cells were then incubated in 37C at 5% CO2. Stimulants were added for the indicated times.

Results: ERK1/2 and p38 are localized to the sperm mid-piece and tail. In response to PMA, a known stimulator of PKC, we observed a marked increase in ERK phosphorylation that could be abolished by specific PKC and MEK1/2 inhibitors. PMA also stimulated flagellar and hyperactivated motility, as well as the acrosome reaction. The PMA-induced increase in flagellar and hyperactivated motility could be abolished by the MEK1/2 inhibitors. However, p38 inhibitors could increase flagellar and hyperactivated motility. The PMA-induced acrosome reaction could be markedly decreased by both MEK1/2 inhibitors and p38 inhibitors.

Conclusions: We could demonstrate that ERK1/2 are activated downstream to PKC in human sperm, and stimulate sperm flagellar motility, hyperactivation and acrosome reaction. P38 has an inhibitory role in sperm hyperactivation and flagellar motility, and a stimulatory role in the PMA-induced acrosome reaction.

The role of FYN kinase in the release from metaphase in Mammalian oocytes

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Introduction: Meiosis in mammalian oocytes starts during embryonic life and arrests for the first time before birth, at prophase of the first meiotic division. The second meiotic arrest occurs after spindle formation at metaphase of the second meiotic division in selected oocytes designated for ovulation. The fertilizing spermatozoon induces the release from MII arrest only after deactivation of the oocyte's spindle assembly checkpoint (SAC). Src family kinases (SFKs) are nine non-receptor protein tyrosine kinases that regulate many key cellular functions. Fyn is an SFK expressed in many cell types, including oocytes, that regulates many cellula. Our aim was to study the involvement of Fyn in the organization of the meiotic spindle and in the exit from metaphase during meiosis in rat and mouse oocytes, as a model for mammalian oocytes.r functions.

Patients/ Methods: Endogenous Fyn kinase was inhibited either by exposing the oocytes to SFKs inhibitors (SU6656 or PP2) or by microinjecting them with dominant negative form of Fyn (DN-Fyn) complementary RNA (cRNA) together with Histone-H2-RFP and β-Tubulin-GFP cRNAs. Fyn localization, spindle structure and chromosomes segregation were assessed by live cell confocal microscopy or immunostaining.

Results: Exposure of oocytes at the metaphase of either first or second meiotic divisions to SFKs inhibitors resulted in disruption and condensation of the spindle structure, reduction of the spindle size, misalignment of the chromosomes and appearance of microtubule (MTs) filaments throughout the cytoplasm in a time and dose dependent manner. Fyn co-localized with the spindle MTs under the inhibitory effect of SU6656 and even after recovery from the drug. Microinjection of DN-Fyn cRNA into the oocytes or exposing them to SU6656 or PP2 inhibited the exit from meiotic and mitotic metaphases. After washing the inhibitor, the spindle recovered and the oocytes gained the ability to exit from metaphase.

Conclusions: Altogether, it is suggested that Fyn plays an essential role in signaling events that involve the SAC pathway and hence in regulating the exit from metaphase during meiosis.

Deciphering the luteal transcriptome: Insights into possible mechanisms regulating corpus luteum regression

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Introduction: Prostaglandin F2α (PGF2α) is the principal luteolytic hormone however, despite its widespread use, the mechanisms by which it induces luteolysis are not well defined. In the mature corpus luteum (CL) PGF2α initiates a series of events culminating in its demise. However, during early luteal phase the gland is refractory to exogenous PGF2α. This lack of responsiveness cannot be attributed to a deficiency of PGF2α receptors and in fact various cell responses were observed. We hypothesized that functional genomics and individual luteal cell isolation will be instrumental in elucidating these mechanisms

Patients/ Methods: Microarray and bioinformatics analyses were used to screen genes differentially expressed between day (D)4 and D11 CLs (PGF2α refractory and responsive, respectively) collected 4h and 24h after PGF2α administration. The mRNA and protein expression of selected genes were validate by qPCR and western blots, respectively. Cell localisation of selected genes was studied by: i) endothelial (EC) and steroidogenic cells enriched from CL, using BS-1 coated magnetic beads and ii) small and large–like luteal cells obtained after in vitro luteinization as well as cultured luteal EC

Results: Microarray studies revealed robust differences in luteal gene expression on d4 versus d11 of the luteal phase. Over 500 transcripts differentially expressed in D4 vs D11 were identified. Seventy six genes unique to the early luteal stage were identified as genes involved in cell cycle. There were 164 genes whose expression increased on d11 only, most of them are involved in apoptoic cell death and immune response. We then selected 11 genes affecting major cellular pathways that were differentially expressed in these two developmental stages. Quite a few of these genes are known to be involved in angiogenesis: FGF2, PTX3, thrombospondins (THBS 1/2) and their cell adhesion receptor –CD36 were expressed in the steroidogenic and EC compartments of the CL alike. However while FGF2, a proangiogenic factor, was markedly elevated by PGF2α on D4 CL, THBS1 and 2, which inhibit angiogenesis, were induced only in the mature gland undergoing luteolysis. Interestingly, PTX3 known to bind and inhibit FGF2 action was inversely expressed with this growth factor. Selectins, E and P adhesion molecules, were restricted to luteal EC. Surprisingly, a similar pattern was observed for galanin (GAL), a neuropeptide found in brain. Its localization to luteal EC or any EC type is revealed here for the first time. Neuregulin1 (NRG1) was more abundant in the steroidogenic luteal cells and specifically in the luteinized theca cells. As with GAL, NRG-1 expression was stimulated by PGF2α only on D11 (PGF2α-responsive) but not D4 CL (PGF2α-refractory)

Conclusions: These studies are beginning to decipher the long sought after mechanisms involved in PGF2α- induced luteolysis

Elucidation of mechanisms of the reciprocal cross-talk between Gonadotropin-Releasing Hormone (GnRH) and Prostaglandin receptors.

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Introduction: We have recently described a novel GnRH receptor signaling pathway mediated by the prostaglandins PGF2α and PGI2, which acts through an autocrine/paracrine modality to limit autoregulation of the GnRH receptor and inhibit LH, but not FSH release. Here we further explore the cross-talk between GnRH and the PGs receptors.

Results: GnRH stimulates arachidonic acid (AA) release from LβT2 gonadotrope cells via the Ca2+-independent phospholipase A2 (iPLA2) and not via the more common Ca2+-dependent cytosolic phospholipase A2α (cPLA2α). LβT2 cells express various PLA2 family members such as sPLA2IIE, sPLA2V, iPLA2-A, iPLA2-γ, cPLA2β and cPLA2γ, but surprisingly not the common cPLA2α. AA release was followed by a marked induction of COX-1 and COX-2 by GnRH, via the PKC/c-Src/PI3K/MAPK pathway. COX-2 transcription by GnRH is mediated by the two NFkB sites and the C/EBP site within its promoter. Indeed, GnRH stimulates p65/RelA phosphorylation (22-fold) in LβT2 cells and the two NFkB sites apparently act as a composite response element. Although GnRH stimulates cAMP formation in LβT2 cells, we found no role for cAMP acting via the CRE site in the COX-2 promoter. PGF2α, PGI2 or PGE2 had no effect on basal- or GnRH-stimulated ERK, JNK and p38MAPK activation and cellular Ca2+ elevation. Although, PGF2α, PGI2 and PGE2 reduced GnRH-stimulated cAMP formation, we could not correlate it to the inhibition of GnRH receptor expression, which is exerted only by PGF2α and PGI2.

Conclusions: The inhibition by PGF2α and PGI2 of the autoregulation of GnRH receptor expression is most likely mediated via inhibition of GnRH-stimulated phosphoinositide turnover and not by inhibition of Ca2+ elevation and MAPK activation.

Sibling affinity and environmental influences on the infancy-childhood transition age population-based sample of Israeli infants

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Introduction: The transition from infancy to childhood (ICT) at age 7-13 months is marked by a growth spurt, when GH-IGF-1 axis activity sets in. The ICT age correlates negatively with, and determines 50% of the final adult height. Delayed ICT results in adult short stature. Predictive adaptation to low or high energy availability modifies the timing of transition to adjust adult size to energy resources. Hypothesis: The ICT age is affected by the household environment and is governed by genetic factors.

Patients/ Methods: The study examined growth pattern in 239 boys and 261 girls from well-baby clinics, including 48 pairs of monozygotic (MZ), 58 dizygotic (DZ) twins and 288 siblings (SB), who were measured repeatedly for body weight and length over the first two years of life. Age at ICT was estimated using the Karlberg's ICP model without correcting for gestational age. The followings potential predictors of the ICT timing were evaluated: birth weight and length, maternal body weight and height at infants' birth, Apgar score, parental education and occupation, and season of year at infant's birth.

Results: Significant sex difference was found for the ICT age(11.0±1.9 mo, boys vs 10.3±2.0 mo, girls, $p<0.0001$), and for body length at 24m (mean F -1.0SDS, M -1.2 SDS, $p=0.02$). The ICT age showed significant sib-sib correlations, with highest within-pair correlation in MZ twins, $r=0.88$, vs. $r = 0.74$ in DZ and $r=0.439$ in SB (all at $p<0.0001$). SB correlation was significantly ($p<0.01$) lower than either DZ or MZ correlation. However, MZ and DZ correlations did not differ significantly ($p>0.05$).

Conclusions: 1.Significant intra-pair sibling correlations clearly suggest the existence of considerable familial effect in ICT age variation. It has a common environment component shared by siblings, and in particular twins, who share intrauterine and familial environment at a given time. 2. The contribution of genetic factors remains to be clarified.

Pelvic ultrasound in girls with precocious puberty is a useful adjunct in diagnosis and therapy monitoring

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Introduction: Gonadotropin-releasing hormone analogs (GnRHa) are known to be efficacious in the treatment of central precocious puberty (CPP). However, there are no clear-cut criteria for initiation of therapy or the means for monitoring suppression during treatment. We have previously shown that pelvic ultrasound can be used to differentiate CPP from premature thelarche (PT). The contribution of pelvic ultrasonogrphy to the decision on treatment initiation and to treatment monitoring has not been extensively studied. Aim: To prospectively assess the use of pelvic ultrasound in documenting progression of precocious puberty before GnRHa therapy and in monitoring suppression during therapy.

Patients/ Methods: Girls referred because of appearance of breast buds before age 8 years were recruited consecutively. All underwent general and endocrine evaluation. The diagnosis of CPP (n=25) was based on the clinical judgment of an experienced clinician after 6 months of follow up. Transabdominal pelvic ultrasound was performed with a 5-MHz real-time sector scanner on referral, 3 and 6 months later, and every 6 months thereafter.

Results: Before treatment a significant increase in height –SDS ($p=0.003$), uterine volume ($p<0.01$), fundus diameter ($p<0.05$) and endometrial thickness ($p<0.001$) was observed after 3 months of follow-up in girls with CPP but not in controls (girls with PT). Three months after beginning therapy there was a significant decrease in uterine length (mean 4.2±0.6 vs 3.8±0.7 cm , $p=0.01$), uterine volume (4.4±2.2 vs 3.0±1.0 ml , $p<0.003$) and ovarian volume (3.2±2.3 vs 1.9±1.0, $p<0.01$) but no significant change in height-SDS or bone age to chronological age ratio. No further changes in either height-SDS or ultrasound measurements were documented during 2 years of treatment.

Conclusions: The increase in uterine measurements indicating progression of puberty may be used as an adjunct when considering GnRHa treatment. The significant decrease in both uterine and ovarian measurements as soon as 3 months after therapy initiation suggest that ultrasound may be an early useful means for monitoring suppression.

The protective effect of GnRH-a against chemotherapy associated ovarian failure in stem cell transplantation [SCT]

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Introduction: GnRH-a has been shown to decrease ovarian failure in young women exposed to gonadotoxic chemotherapy. Recently, we have reported on a young woman, similarly treated, who has successfuly delivered twice after two bone marrow transplantations, against all odds.

Patients/ Methods: To examine whether GnRH-a may minimize the risk of premature ovarian failure [POF], in young women undergoing gonadotoxic chemotherapy conditioning for stem cell transplantation [SCT], the ovarian function of 98 young women, age 15-40, who have undergone SCT for various indications, were retrospectively evaluated. Of the 85 evaluable patients, 50 were treated with GnRH-a during the aggressive conditioning chemotherapy before SCT, whereas 35 did not. POF vs cyclic ovarian function[COF] was defined by regular cycles, hormonal profile, ultrasound, and/or pregnancy.

Results: Patients treated with GnRH-a resumed COF in 34% [17/50] compared to 11% [4/35] in women who did not receive GnRH-a, $P<0.05$. Lymphoma patients benefitted significantly from the GnRH-a cotreatment, $P=0.023$, whereas the others did not. 71.4% of the Hodgkin Llymphoma [HL] and 50% of the non-HL patients who received GnRH-a had COF after SCT vs 2/11 controls [no GnRH-a].

Conclusions: Administration of GnRH-a before and during gonadotoxic chemotherapy may minimize POF in lymphoma patients undergoing SCT, but not in leukemias and other indications. Due to the retrospective nature of this study, larger, prospective and RCT are awaited.

Correlations between pre- and postnatal measurements of penile and clitoral size

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Introduction: Ultrasound examination, usually at mid-gestation, is routinely used in standard clinical obstetric practice for the prenatal detection of various syndromes, fetal growth status, determination of fetal sex and detection of anatomic malformations, including external genitalia anomalies. A recently established reference range for prenatal penile length in relation to gestation age has led to frequent incorporation of micropenis into the prenatal diagnostic profile. Findings outside the normal range often cause parental anxiety, lead to further evaluation and sometimes to pregnancy termination. Comparisons of pre- and postnatal penile and clitoral size are lacking. Our objective was to correlate pre- and postnatal measurement of penile width and length and clitoral height and length.

Patients/ Methods: Fetal penile width and length and clitoral hieght and length in singleton pregnancies of randomly selected pregnant woman, were measured twice by high-resolution ultrasonography. Postnatal measurements were carried out twice during the first postnatal week. Correlation between pre- and postnatal measurements were calculated by the Pearson correlation test.

Results: Paired pre- and postnatal measurements were performed in 45 males and 47 females. The correlations between measurements of fetal penile and clitoral length and width/height and week of gestation were highly significant ($p \leq 0.001$). Correlations between fetal and postnatal penile length and width were not significant. There was significant correlation between fetal clitoral length and height and postnatal clitoral length ($r=0.34$, $p=0.019$, $r=0.36$, $p=0.012$, respectively).

Conclusions: The lack of correlations between pre- and postnatal penile measurements and the significant correlation between fetal and postnatal clitoral length suggests that while prenatal findings in females might be reliable indicators of postnatal measurements, this is not the case in males. This uncertainty in fetal penile measurements mandates exercising caution in parent counseling.

46, XX infant (SRY-Negative) with bilateral ovotestis, is there a causing gene?

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Introduction: Ovotesticular Disorder of Sexual Development (OT-DSD) (true hermaphroditism) is a rare disorder of sexual differentiation characterized by ambiguous external genitalia and gonads having both ovarian and testicular elements. Differentiation of testicular tissue in 46, XX individuals occurs either in XX males (mostly expressing the SRY gene), or in individuals with OT-DSD usually SRY negative. Although they are sporadic cases, there are some reports on familial recurrence. We report a rare case of scrotal hypospadias, and bilateral ovotesticular DSD with its unique clinical and molecular genetic analysis.

Patients/ Methods: Serum hormonal levels, HCG stimulation test, karyotype and gonadal biopsy were performed followed by PCR studies of DNA from peripheral leukocytes and gonadal tissues investigating the presence of the SRY gene. SNP microarray for homozygosity mapping is being performed.

Results: Karyotype was 46, XX. HCG test showed significant rise in testosterone levels. Pathology revealed the unique finding of bilateral similar ovotestis. PCR in peripheral leukocytes was SRY negative, while in gonadal tissues SRY expression was observed in the ovotesticular (testicular) tissue. SNP homozygosity mapping currently performed has early indication for a linkage which may direct to candidate genetic studies.

Conclusions: The bilateral existence of both ovarian and testicular tissues in the same gonad is rare. The presence of SRY in the ovotestis while negative in serum is unique even in OT-DSD. The far consanguinity in this case enables linkage (SNP) studies that may provide evidence that bilateral XX OT-DSD is caused by genes which are crucial in gender determining stages of sex development.

Gene regulation by microRNAs in cellular systems

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A central gene regulatory mechanism, recently identified, is regulation by small non-coding RNAs termed microRNAs. MicroRNAs control gene expression by binding to mRNA targets and leading them to facilitated mRNA degradation and translation inhibition. Currently there are hundreds of reported human miRNAs predicted to control at least half of the human transcriptome. MicroRNAs were observed to be important for a diverse range of biological processes such as differentiation and development, and to play a pivotal role during pathogenesis. I will present our computational and experimental efforts in identifying: (i) the complete microRNA repertoire; (ii) the characteristics of effective microRNA targeting; and (iii) novel functions microRNAs play in the cellular context.

Loss of miRNA activity in adult beta cells causes overt diabetes

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With the discovery of miRNAs, a large set of cellular regulators, we sought to explore their relevance to pathogenesis of diabetes. To this end we have obtained a conditional Dicer1 allele, the chief miRNA processing enzyme, and knockouted miRNA function specifically in mature beta cells through an inducible RIP-Cre recombination system . Our results show that miRNA loss in beta cells results in a striking diabetic phenotype, although histological studies did not reveal any change in tissue architecture. At the cellular level, Dicer^null beta cells show a dramatic decrease in insulin mRNA and protein levels. Strikingly, Dicer^null beta cells retain beta cell markers, such as MafA, Pdx1 and Nkx6.1, suggesting that regulation of insulin gene expression is affected in a relatively isolated fashion. Clues for the mechanism underlying reduction of insulin transcription emerged when we uncovered the increased expression of the insulin-associated transcriptional repressors, BETA3, Sox6 Insm1 and TLE4 in the mutant islets. We conclude that miRNAs are important in tuning the fine balance between transcriptional activators and repressors in the mature beta cell, proposing an intriguing, novel etiology for diabetes.

microRNA microRNAs and estrogen regulation

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The association between nutrition and growth is a common knowledge, however, in spite of the enormous effort of pediatric endocrinologists, dieticians and scientists, our understanding of the interaction of nutrition and linear growth in children is still lacking. The exact mechanism by which the body signals the EGP to grow or attenuate growth is still unclear. In the last couple of years our group has been extensively involved in trying to elucidate the mechanism governing the association between nutrition and growth. We have, together with others, identified the role of leptin in regulation of linear growth and we have shown that HIF1a, a transcription factor that is essential for EGP growth and development is responsive to nutritional status.

MicroRNAs (miRNAs) are small endogenous RNAs that regulate target mRNAs by binding to their 3'-UTRs. They have been reported to be involved in a variety of functions, including skeletal development and longitudinal growth.

Prompted by reports showing miRNA expression in cartilage, we investigated the potential role of miRNAs in regulating growth attenuation and Catch-up (CU) growth in the mature EGP. CU growth is a period of accelerated growth that occurs when growth inhibitory conditions resolve.

To study the mechanisms regulating nutritional induced CU growth, pre-pubertal rats were subjected to 10 days of 40% food restriction, followed by a renewal of the regular food supply. We found that under these mild food restriction conditions humerus and EGP lengths were significantly smaller compared to control. When food restriction was removed, there was an instantaneous increase in weight and EGP length, later accompanied by an increase in humerus length. Nutritional manipulation induced dramatic changes in the expression of numerous genes; however, no significant change was detected in known growth-related genes. Using miRNA microarrays, we found that numerous miRNAs were expressed in the postnatal EGP. Furthermore, nutritional manipulation led to significant changes in the expression of several miRNAs, including the cartilage-specific miR-140. We also noted a dramatic change in several potential targets of these miRNAs, which are expressed in the EGP. Some may have an anti-proliferative effect on the EGP or bone.

These results may have important implications for understanding the mechanism of the EGP growth. The present study is the first, to our knowledge, to show the involvement of miRNA in growth regulation in the post-natal EGP and the first to show the effect of nutrition on miRNA expression in vivo. Involvement of miRNA in the regulation of growth may open a new era of research and may enable the development of new treatment for children with growth abnormalities.

CV safety of anti diabetic drugs: review of the literature and ongoing studies

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The benefit of glucose lowering in preventing cardiovascular disease (CVD) in type 2 diabetes (DM) is supported mainly be data from long-term studies of patients with DM of short-duration and without pre-existing CVD. There are no good evidence to support CVD events reduction by intensive glucose control in patients with long-standing DM with existing CVD.

The potential risks or benefits of different glucose-lowering agents on CVD are currently a topic of great interest with many un-answered questions. The limitations of many of the studies published until now include: 1. Lack of pre-specified CVD outcome; 2. Short duration of follow-up; 3. Usage of combined anti-diabetic therapies hampering the ability to analyze separately the effect of each drug on CVD events; 4. Heterogeneity of baseline patients' characteristics. These limitations led the FDA in 2008 to require that all manufactures of new anti-diabetic drugs report CVD outcome before approval.

Both observational and interventional studies provide data supporting the safety and beneficial effect of metformin in reducing CVD both as mono-therapy and in combination with insulin. Two recent large observational studies have shown an increased risk of CVD and all-cause mortality in patients who were treated with sulphonylureas compared with metformin although the UKPDS, ADVANCE and BARI-2D trials which used sulphonylureas as part of a multi-drug treatment did not support these findings. Nateglinide, another insulin secretagogues was not shown to reduce CVD outcome in the recently published randomized control trial NAVIGATOR conducted in patients with impaired glucose tolerance.

Another widely-used glucose-lowering drug which has raised concern is rosiglitazone since the publication of a large meta-analysis which found an association between its usage and IHD. Further data did not support these findings including the recently published RECORD trial but all data show consistently that both rosiglitazone and pioglitazone (both PPAR agonists) increase the risk of congestive heart failure by about two-fold. These conflicting data led the FDA to publish an alert about the possible association between rosiglitazone and increased CVD risk and preclude the usage of these drugs in patients with heart failure.

Acarbose an α-glucosidase inhibitor have been found to reduce the risk of myocardial infarction and CVD events both in subjects with impaired glucose tolerance (STOP-NIDDM) and in a meta-analysis of patients with type 2 DM. However these findings are based on small number of events and need further confirmation by larger trials with pre-specified CVD outcome. Another promising group of medications are the GLP-1 agonists. Several published studies have shown their beneficial effects on CVD risk factors. In addition animal studies and small human studies suggest that GLP-1 agonists may have a protective effect on the myocardium during ischemia. However there are currently no results of long-term studies with GLP-1 agonists with pre-specified CVD end-points.

Glucokinase – a novel therapeutic target for type 2 diabetes Mellitus

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Type 2 diabetes (T2DM) is characterized by inadequate pancreatic beta-cell function, usually in the face of increased insulin requirements due to peripheral insulin resistance. Agnostic genome-wide association studies have shown that the majority of the genetic variants associated with increased risk of diabetes affect the function of genes that are important modulators of beta-cell function or mass. Thus, beta-cell dysfunction is of primary importance in the pathogenesis of T2DM, and modulators of beta-cell function or mass are rational, novel therapeutic targets. The beta-cell regulates insulin secretion in response to changes in circulating glucose levels. After entering the beta-cell through specific transporters, glucose is metabolized, causing an increase in the intracellular ATP/ADP ratio. This, in turn, causes closure of the ATP sensitive K channel, which results in membrane depolarization, followed by opening of the voltage-gated calcium channels that cause calcium to enter the cells, stimulating a cascade of events that ultimately results in insulin secretion.
Mutation analyses in a wide spectrum of monogenic diseases of glucose homeostasis have demonstrated that the primary regulator of glucose-mediated insulin secretion is glucokinase, the enzyme that catalyzes the first step in glucose metabolism. Heterozygocity for in-activating mutations in this gene cause one form of Maturity Onset Diabetes of the Young, while homozygocity for the same mutations cause severe neonatal diabetes. At the other end of the spectrum activating mutations in this gene cause hyperinsulinemic hypoglycemia of varying degrees of severity, depending on the activity of the mutation. More recently, common polymorphisms in the GCK gene have been associated with Type 2 Diabetes.
In addition to the beta-cell, glucokinase is expressed in the liver, where its expression is regulated by insulin and its activity is controlled by a peptide called the Glucokinase Regulator Protein. In the liver, activation of this enzyme results in glucose phoshorylation and glycogen synthesis, which is translated into decreased hepatic glucose production.
Recently, we identified a patient with a GK mutation that resulted in severe hypoglycemia that required partial pancreatectomy. Detailed examination of the pancreas revealed abnormally large pancreatic islets, some of which contained proliferating beta-cells. Based on this observation, we investigated the effect of glucokinase activation on beta-cell proliferation in a mouse model. Our findings show that glucokinase activation results in increased proliferation, and that this effect can be blocked by preventing beta-cell membrane depolarization, calcium entry, calcineurin signaling or insulin receptor-mediated signaling. Taken together, these studies demonstrate that increased intracellular glucose metabolism stimulates mouse beta-cell replication, which is regulated by a dual mechanism involving both calcineurin and the insulin receptor signaling, as both of these pathways are necessary for replication but neither is sufficient.

Small molecule glucokinase activators are currently under development by several pharmaceutical companies for the treatment of T2DM. While many challenges still remain, including potential issues with hypoglycemia, the possibility that these drugs could enhance beta-cell replication opens new prospects for the long-term effect of this class of drugs on the preservation of beta-cell mass in patients with T2DM.

SGLT Inhibitors in the treatment of diabetes: An overview

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Agents approved to treat T2DM act by increasing insulin levels, improving insulin sensitivity or altering intestinal glucose absorption. While efficacious, these agents can be associated with hypoglycemia, weight gain, fluid retention, congestive heart failure, osteoporosis and gastrointestinal adverse events. Sodium-glucose co-transporters (SGLT) are responsible for glucose uptake from the intestine (SGLT1) and from the lumen of the renal tubule (SGLT1 and SGLT2). Potent and selective inhibitors of SGLT 1 and SGLT2 are in clinical development for the treatment of diabetes. In preclinical models of insulin resistance and T2DM, SGLT2 inhibitors increase urinary glucose excretion, improve glycemic control while reducing insulin secretion, increase beta cell mass, reduce weight gain, improve insulin sensitivity and lipid profiles and reduce urinary albumin excretion. While SGLT1 inhibitors have advanced only to phase 1 clinical development, several oral SGLT2 inhibitors are being studied in phase 3 clinical trials. In subjects with T2DM, SGLT2 inhibitors increase urinary glucose excretion, lower fasting and postprandial glucose levels and HbA1c without increasing hypoglycemia, are associated with weight loss, and reduce systolic blood pressure. Despite increases in glucosuria, urinary output is only mildly increased and symptoms due to increased urinary output or dehydration have not generally been apparent. Except for mild increases in BUN and decreases in creatinine clearance, possibly due to an osmotic diuresis, no abnormalities in renal function have been noted. While increases in genital infections (balanitis and vulvovaginal mycotic infections) have been seen in subjects treated with SGLT2 inhibitors, increases in urinary tract infections have not been noted. In summary, although no agents are approved, in clinical trials, SGLT2 inhibitors have been shown to improve glycemic control without causing hypoglycemia and associated with weight loss. Given the novel insulin-independent mechanism, SGLT2 inhibitors are predicted to be efficacious when used in combination with other antihyperglycemic agent classes and to be efficacious across the spectrum of beta cell dysfunction from IFG/IGT to beta cell exhaustion. By virtue of weight loss and reductions in blood pressure, SGLT2 inhibitors may favorably affect cardiovascular risk. Reduced insulin demands associated with SGLT2 inhibitors could decrease beta cell stress and possibly slow the progression of T2DM. While long-term clinical data are needed to adequately profile safety and tolerability, SGLT2 inhibitors could represent a unique and valuable addition to the antihyperglycemic therapeutic armamentarium.

Sirt1 is a regulator of osteoblastogenesis and bone mass

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Introduction: The Silent Information Regulator (SIR) family of genes represents a highly conserved group of genes present in the genome of lower species up to mammalians. The encoded SIR proteins are involved in regulation of gene silencing, DNA repair and are key mediators of the beneficial effects of calorie restriction on lifespan. A well-characterized gene in this family is S. cerevisiae Sir2. To date seven mammalian homologues have been identified, where SIRT1 is the closest evolutionary to Sir2. SIRT1 has a NAD-dependent histone deacetylase activity and plays important roles in aging and in age-associated diseases. SIRT1 regulates epigenetic silencing and chromatin modification, partially by direct regulation of modifying enzymes. The sirtuins were connected to several age-related diseases such as neurodegenerative, vascular and metabolic diseases. Their role in osteoporosis hasn't been studied yet.

Patients/ Methods: To investigate the role of SIRT1 in bone we sought to characterize the bone phenotype in adult 3- month-old female haplo-insufficient mice (Sirt1+/-), and compared them to their Sirt1+/+ (WT) littermates. Complete Sirt1 KO is lethal or results in severe post natal malformations. Murine embryonic mesenchymal C3H10T1/2 cells over-expressing Sirt1 were compared to WT C3H10T1/2.We used microCT and histomorphometric analyses to study bone mass, architecture, formation and resorption. Marrow derived mesenchymal stem cells (MSCs) were used to evaluate osteoblastogenesis by ALP activity and mineralizd nodule formation. Osteoclastogenesis was evaluated in primary bone marrow cultures by TRAP staining. Serum 25-OHvitaminD3, E2, IGF-1 PINP and RANKL were determined.

Results: SIRT1 haplo-insufficient mice had a significant reduction in bone mass. There was a 30% decrease in trabecular BV/TV, as a result of a 23% reduction in trabecular number, no significant change in trabecular thickness and a 37% decrease in Conn.D. Decreased osteoblastogenesis was found in Sirt1+/- mice as indicated by reduced calcein labeling, decreased bone formation rate, a 50% reduction in alkaline phosphatase activity and a significantly reduced number of mineralized nodules. A reciprocal result was observed in C3H10T1/2 cells over-expressing Sirt1 in which there was a 50% increase in alkaline phosphatase activity compared to C3H10T1/2 cells. Osteoclast number was lower in Sirt1+/- mice and osteoclast generation upon exposure to RANKL and M-CSF was reduced in Sirt1+/- mice, resulting in smaller osteoclasts with fewer nuclei. Serum markers showed no difference between Sirt1+/- and WT mice.

Conclusions: Our results implicate a major role for SIRT1 in regulating osteoblastogenesis. SIRT1 activators may have a favorable effect inducing osteoblast formation and reducing bone loss for the treatment of osteoporosis.

Truncated beta epithelial sodium channel (ENaC) subunits responsible for multi-system pseudohypoaldosteronism support partial activity of ENaC

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Introduction: The major site of action of aldosterone in electrolyte regulation is epithelial cells where aldosterone induces expression of epithelial sodium channel (ENaC) subunits increasing ENaC activity at the apical cell surface. ENaC is constructed of three homologous subunits. The amino and carboxy terminal domains of the subunits are located in the cytoplasm, while the bulk of their structure is exposed outside of the cell, forming a funnel that directs ions from the lumen into the epithelial cell. Mutations in the alpha, beta and gamma ENaC genes (SCNN1A, SCNN1B and SCNN1G) are associated with multi-system pseudohypoaldosteronism (PHA), and mutations in the PY motif of beta and gamma subunits are associated with Liddle syndrome of hereditary hypertension.

Patients/ Methods: In this study we sequenced segments of the genomic DNA of a female infant diagnosed with multi-system PHA to identify the mutations responsible for her condition. She is the first case of PHA in an Ashkenazi family in Israel. After identifying two mutations in the SCNN1B alleles we generated mutant forms of beta-ENaC cDNA using a site-directed mutagenesis method we recently developed. The cDNAs were transcribed in vitro using T7-RNA polymerase to generate complementary RNAs (cRNAs) for expression studies in Xenopus oocytes. Amiloride-sensitive whole-cell inward Na+ current was measured 2-3 days after cRNA injection using the two-electrode voltage-clamp method while oocytes were clamped at –80 mV.

Results: We identified two frameshift mutations in the SCNN1B alleles of the patient. The p.Glu217fs (c.648dupA in exon 4) and p.Tyr306fs (c.915delC in exon 6) mutations produce shortened beta-ENaC subunits with 253 and 317 residues respectively instead of the 640 residues present in beta-ENaC subunit. Expression of normal alpha and gamma cRNAs together with mutant beta cRNA in Xenopus oocytes showed that the mutations drastically reduce but do not eliminate ENaC activity (3% of normal ENaC). Oocytes injected with both mutant cRNAs showed > 2 fold higher ENaC activity, indicating a synergism between mutant forms. Oocytes injected with alpha and gamma cRNAs without the beta cRNA showed no detectable ENaC activity.

Conclusions: The findings reveal that truncated beta-ENaC subunits are capable of partially supporting intracellular transport of the other two subunits to the membrane and the final assembly of a weakly active channel together with normal alpha- and gamma-ENaC subunits on the oocyte cell surface. Moreover, these results enhance our understanding of the long-term consequences of these types of mutations in PHA patients. The present findings should also be useful for prenatal diagnosis and early
treatment of multi-system PHA.

Expression of microRNA 21 in mammary stem cells is controlled by cytokine-STAT5 signaling

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Introduction: In the developing mammary gland, the transcription factors STAT5A/B initiate cascades of events that range from the specification of alveolar progenitors to the establishment of functional alveoli during pregnancy. Although many STAT5 target genes have been identified, the complexity of STAT5s function is still an enigma.

Results: MicroRNAs have emerged as another means of controlling the physiology of cells as each member can regulate multiple mRNAs. However, the transcriptional regulation of microRNA genes is poorly understood. Here we use ChIP-seq and expression analyses to define those microRNAs that are controlled by cytokines through STAT5A/B. ChIP-seq established cytokine-induced STAT5A binding to the miR21 gene promoter and expression analyses confirmed loss of miR21 expression in cells lacking STAT5. Notably, we determined that miR21 expression in mammary stem cells and alveolar progenitors was dependent on the presence of STAT5A.

Conclusions: Current experiments address the role of miR21 in mammary alveologenesis.

Calcitriol stabilizes cyclooxygenase-2 mRNA in epidermal keratinocytes

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Introduction: The biosynthesis of prostaglandins from arachidonic acid is mediated by the rate-limiting enzyme, cyclooxygenase (COX). Two isoforms of COX are known. COX-1 is expressed in many tissues under basal conditions, while COX-2 can be induced by various agents such as growth factors, cytokines, pro-inflammatory agents, and tumor promoters. We have previously shown that treatment with the hormonal derivative of vitamin D, calcitriol, increases PGE2 production by human epidermal keratinocytes and that this increase is due to up-regulation of COX-2 expression. The aim of this study was to explore the mechanism responsible for this effect of the hormone.

Patients/ Methods: The non-tumorigenic immortal HaCaT keratinocytes were employed as an experimental model. Cultures in the absence of serum, growth factors or active mediators were exposed to calcitriol for 2 and 16 hours. mRNA levels were quantified by real time PCR and protein levels by western blot analysis. The rate of mRNA degradation was followed after exposing the cells to the transcription inhibitor Actinomycin D for 30, 60, 90 and 120 minutes.

Results: Exposure of HaCaT cells to calcitriol for both 2 and 16 hours, brought about a similar marked and consistent increase of almost 4 fold in COX-2 mRNA levels. The maximal effect was attained already at 1nM of the hormone. While the half-time of the COX-2 transcript was around 20 minutes in control cultures there was not detectable decay of mRNA levels for 1 hour in calcitriol-treated cultures. The stabilizing effect of calcitriol was not associated with increased levels or changes of cellular localization of the mRNA stabilizing protein, HuR. Using specific pharmacological inhibitors to signaling pathways known to be affected by calcitriol in keratinocyte we found that activity of Src kinase family member(s) and PKC are obligatory for the stabilizing effect of calcitriol.

Conclusions: These findings demonstrate that treatment with calcitriol results in up regulation of COX-2 mRNA in keratinocytes, and that this effect is at least partially due to mRNA stabilization. This increase in COX-2 could provide an explanation to the dual effect of calcitriol on epidermal inflammation: pro-inflammatory in healthy skin and anti-inflammatory in inflamed skin and may contribute to the known anti-apoptotic effect of calcitriol on epidermal keratinocytes.

Differential role of PKC isoforms in GnRH and PMA activation of extracellular signal-regulated kinase (ERK) and Jun N-terminal kinase (JNK)

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Introduction: Gonadotropin releasing hormone (GnRH) is the first key hormone of reproduction. Signaling of GnRH in pituitary gonadotropes include sequential activation of phospholipase Cβ (PLCβ), PLD and PLA2, enhanced phosphoinositide turnover, Ca2+ mobilization and influx, activation of PKC and the MAPK cascades (ERK, JNK and p38) and formation of prostaglandins and leukotrienes, culminating in gonadotropin (LH and FSH) synthesis and release. Protein kinase C (PKC) is a serine/threonine lipid-activated kinase family. The many and sometimes opposing functions elicited by the PKC family suggest that different isoforms may mediate its diverse functions. Although PKC is implicated in MAPK activation by some GPCRs in general and by GnRH in particular, the nature of the PKCs mediating GnRH activation of the MAPKs is still unknown. Here we demonstrate for the first time the relative contribution of specific PKCs to ERK and JNK activation by GnRH and PMA in αT3-1 and LβT2 cells.

Results: The role of PKC isoforms (PKCs) in GnRH-stimulated MAPK (ERK and JNK) was examined in the αT3-1 and LβT2 gonadotrope cells. Incubation of the cells with GnRH resulted in a protracted activation of ERK1/2 and a slower and more transient activation of JNK1/2. Gonadotropes express conventional PKCs (cPKC) α and βII, novel PKCs (nPKC) δ, ε and θ, and atypical PKC (aPKC) ι/λ. The use of GFP-PKCs constructs and their translocation to membranes as a measure of activation, revealed that GnRH induced a rapid translocation of PKCα and PKCβII to the plasma membrane, followed by their re-distribution to the cytosol. PKCδ and PKCε localize to the cytoplasm and Golgi followed by rapid re-distribution by GnRH of PKCδ to the perinuclear zone, and PKCε to the plasma membrane. Interestingly, PKCα, PKCβII and PKCε translocation to the plasma membrane was more pronounced and more prolonged in PMA than in GnRH-treated cells. The use of selective inhibitors and dominant negative plasmids for the various PKCs has revealed that PKCβII, PKCδ and PKCε mediate ERK2 activation by GnRH, while, PKCα, PKCβII, PKCδ and PKCε mediate ERK2 activation by PMA. Also, PKCα, PKCβII, PKCδ and PKCε are involved in GnRH- and PMA-stimulation of JNK-1 in a cell-context dependent manner.

Conclusions: Thus, the contribution of selective PKCs to ERK and JNK activation is ligand- and cell-context dependent. We present preliminary evidence that persistent vs. transient redistribution of selected PKCs, or re-distribution of a given PKC to the perinuclear zone vs. the plasma membrane may dictate it's selective role in ERK, or JNK activation.

PPARα regulates systemic blood pressure by modulating the central Renin-Angiotensin sytem (RAS) predominantly through renin expression

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Introduction: We have previously shown that the absence of PPARα abolishes hypertension in the Tsukuba Hypertensive Mouse (THM), an animal doubly transgenic for human renin and angiotensinogen. Most of the protection appeared to stem from downregulation of the humoral renin-angiotensin system (RAS) as a result of a profound suppression of the human renin gene expression. However, in C57/Bl6 mice subjected to the aldo-salt model, the absence of PPARα seemed to affect thirst and salt appetite. As under this paradigm of mineralocorticoid-hypertension, the peripheral RAS is shut down yet the central RAS is typically activated, this suggested a potential role for PPARα a in the regulation of the central RAS. The goal of the present study was to specifically assess the contribution of PPARα to the expression of the central RAS system under various conditions of peripheral RAS activation.

Patients/ Methods: 21 C57/Bl6 mice, and 23 PPARalpha null (PPARKO) mice previously subjected to unilateral nephrectomy, underwent the aldo-salt protocol for 4 weeks (continuous SC infusion of a pressor dose of aldosterone via an osmotic minipump, and access to 1% NaCl drinking water), at the end of which they were studied in metabolic cages for 24 h. Similarly, 16 adult THM mice, and 20 THM lacking PPARalpha (THMKO) that had ad lib access to tap water were studied in metabolic cages. Blood pressure was recorded noninvasively. At the end of the experiments, animals were sacrificed, RNA was extracted from the hypothalami, and RAS component expression was assessed by real-time PCR. Immunohistochemistry of the RAS was performed on THM/THMKO brains.

Results: In both models absence of PPARα protected the animals from hypertension, and significantly affected drinking pattern. In the aldo-salt model, PPARKO mice drank significantly less salt water and ate less than the C57/Bl6 control mice (Table 1). In the THM model, absence of PPARα reduced drinking by 43% ($P=0.0001$), and urine output by 66% ($P<0.0001$). In both models, hypothalamic mouse renin expression was significantly lower in the absence of PPARα. In THMKO animals, human renin expression was 2 orders of magnitude lower than in THM. Additionally, human angiotensinogen was also significantly reduced. In contrast, in neither model did the absence of PPARα affect the level of the angiotensin II type 1 receptor or that of the angiotensin converting enzyme mRNA. Immunohistochemistry studies of THM/THMKO mice generally concurred with the mRNA results.

Conclusions: These studies highlight the role of PPARα in the expression of the central RAS, both when the peripheral RAS is overexpressed (THM model), or shut down (aldo-salt model), primarily by its effect on the expression of renin, the rate-limiting step of the system. The results underscore the importance of the central RAS in the generation and maintenance of systemic blood pressure, at least in part through its impact on drinking behavior and salt appetite.

A novel loss of function mutation in OTX2 is associated with phenotypically variable anophthalmia and isolated growth hormone deficiency

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Introduction: Heterozygote mutations of the gene encoding transcription factor OTX2 were recently shown to be responsible for ocular as well as pituitary abnormalities. Objective: To identify the genetic cause of anophthalmia and IGHD in a Sephardic Jewish family

Patients/ Methods: . Patients and Methods: The index case presented with unilateral anophthalmia and short stature underwent hypothalamic-pituitary axis evaluation as well as brain MRI. DNA was analyzed for mutations in the HESX1, SOX2 and OTX2 genes. Laser-scanning microscope was used to identify subcellular localization of the mutant protein. EMSA was performed to follow correct promoter binding and transactivation analysis was performed using the Luciferase assay

Results: Results: MRI in the proband revealed a small anterior pituitary gland, invisible stalk, ectopic posterior lobe and right anophthalmia. Endocrine evaluation showed IGHD. Molecular analysis yielded a novel heterozygous OTX2 mutation (c.270A>T, p.R90S) within the homeodomain. The paternal family has 4 other male cases of bilateral anophthalmia. Functional analysis revealed that the mutation inhibited the DNA binding activity of the protein and that the mutant OTX2 protein barely retained transactivation activities.

Conclusions: Conclusion: A novel loss-of-function mutation R90S in OTX2 is associated with familial anophthalmia and IGHD and is characterized by phenotypic variability

Familial Isolated Hypogonadotropic Hypogonadism caused by a novel homozygous mutation in a splice site of the GPR54 Gene

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Introduction: Isolated hypogonadotropic hypogonadism (IHH) is characterized by low gonadotropin levels and normal other pituitary hormones. Although familial IHH is mostly attributed to mutations in the gonadotropin-releasing hormone receptor (GnRHR) gene, the recently identified Kisspeptin/GPR54 signaling pathway gene mutations have become a significant etiology of IHH.

Methods: Clinical, endocrine, imaging, and molecular genetic characterization was performed in two IHH patients.

Results: A 16 y old Palestinian girl and her 20y old brother born to consanguineous parents presented with no pubertal development, infantile uterus on ultrasound and prepubertal descended testes, respectively. Both were normosmic and basal and stimulated gonadotrophins were prepubertal. Basal estradiol levels (for the girl) were low at 50 pmol/l and failed to rise in response to hCG. Her brain CT scan was normal. The brother had low basal testosterone (1.87 nmol/l). Combined therapy of hCG and HMG (LH and FSH) normalized testosterone levels but failed to increase testes size. Replacement with exogenous sex steroids achieved development of secondary sexual characteristics. DNA was extracted from 3 affected siblings (another affected sister) and other family members. Initial homozygosity studies using microsatellite markers (located in proximity to candidate genes: GnRHR, GPR54, GnRH, and Kiss1 did not yield a possible molecular etiology. SNP array studies thereafter revealed a relatively small area of homozygosity at the telomeric end of chromosome 19. Sequencing the GPR54 gene revealed a novel homozygous G>A mutation at the nt -1 canonical acceptor splice site of intron 1 in all 3 affected siblings. The mutation results in skipping of exon 2, leading to a frameshift which results in an altered protein from residue 82, with a premature stop codon at residue 151. (p. A82GfsX151). To assure the aberrant transcript formation we extracted RNA from transformed lymphocytes of the 3 affected siblings. Sequencing the reversed transcribed RNA at the exon 1 to 3 segment revealed that exon 2 was indeed missing with the expected frame shift resulting in a premature stop codon. The mother (menarche at 14y) was heterozygous, while a healthy sister and 5 normal Jerusalem Palestinians had the normal sequence.

Conclusions: A novel IVS1-1G>A mutation in GPR54 results in a severe IHH phenotype with failure to exhibit any pubertal maturation. Carriers of the heterozygous mutation may manifest a subtle phenotype. The subnormal gonadal response to hCG in patients may implicate a direct effect of kisspeptin/GPR54 on gonadal function. Further expression studies are currently performed.

Preimplantation genetic diagnosis (PGD) for inherited endocrine diseases: an ounce of prevention is worth a pound of cure.

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Introduction: Genetically inherited endocrine diseases may be severe, debilitating and sometimes fatal. Some of them carry a predisposition to cancer, and therefore in addition to the actual medical risk, they pose a significant mental burden on the known carriers. Preimplantation Genetic Diagnosis (PGD) enables the carriers of a known mutation to avoid a delivery of an affected child. Here we present our experience in PGD for some endocrine diseases.

Patients/ Methods: Five families were referred to the IVF-PGD Unit after being diagnosed as carriers of an autosomal recessive disease following the birth of an affected child: 3 couples were found to be heterozygotes for Persistent Hyperinsulinemic Hypoglycemia of infancy (SUR1), one couple was diagnosed as carriers for Congenital Adrenal Hyperplasia (CYP21B), and one couple diagnosed as heterozygotes for Hypoparathyroidism- Retardation- Dysmorphism syndrome (HRD). None of the patients was diagnosed by screening. One other patient was a female carrier of a RET mutation with MEN2 phenotype

Results: The patients were managed with standard IVF protocols, followed by polar body/ blastomere biopsy, single-cell PCR and transfer of the non-mutant embryo(s). Among the carriers of autosomal recessive diseases, average clinical pregnancy rate per patient per cycle was 53.2% (similar to the outcomes of first cycles in other IVF patients). All patients achieved an ongoing pregnancy/ live birth. The RET mutation carrier had 8 IVF cycles: in two cycles there were no healthy embryos for transfer, in another two cycles there was only one embryo available for transfer, and the desirable pregnancy was achieved only following the 8th cycle- an ongoing twin pregnancy

Conclusions: Some inherited endocrine diseases are preventable by PGD. Keeping in mind the possibility of referring patients for IVF with PGD may, in the long run, make the incidence of these diseases lower. For couples with an affected child, both the chance of having another affected child, and, on the other hand, termination of a pregnancy with an affected fetus, may be unacceptable. Fortunately for those, PGD is feasible, safe and effective, and therefore should be considered.

Multiple endocrine neoplasia type 1 (MEN-1), the hadassah-hebrew university medical center experience

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Introduction: MEN-1 is an autosomal dominant genetic disorder with a prevalence of 2-4 per 100,000. The main manifestations are parathyroid (PT), gastro-enteropancreatic (GEP) and pituitary tumors, but may affect other organ systems as well. MEN-1 is associated with significant morbidity and mortality with up to 50% dying before the age of 50. Treating MEN-1 affected subjects presents a unique diagnostic and therapeutic challenge. In the current study, we present our experience with MEN-1 affected patients, including clinical and genetic information.

Patients/ Methods: Clinical data was obtained for patients followed at Hadassah Medical Center between the years 2003-2009. Genetic analysis was carried out in the laboratory of Prof. A Calender, France, and the NIH, USA, and included direct sequencing and quantitative multiplex short fragment PCR of exons 2-10 of the menin gene. Clinical diagnosis of MEN-1 was defined as the presence of at least 2 out of the 3 main manifestations of MEN-1 (PT, GEP, pituitary).

Results: Our cohort included 25 subjects from 20 families. Clinical information was available on 22. Average age at presentation was 36 years (range 17-75). Initial presentation was hyperparathyroidism in 12 (55%), GEP tumor in 6 (27%) and pituitary tumor in 4 (18%). During evaluation and follow up, 19 (86%) developed hyperparathyroidism, 15 (68%) GEP tumors (7 non-secreting , 6 gastrinomas, 2 insulinomas) and 12 (55%) developed pituitary tumors (9 prolactin, 2 ACTH and 1 null). Two patients had metastatic carcinoid tumor, and 1 thymic carcinoid. Genetic testing was performed in 18 subjects. MEN-1 mutations were found in 12 (67%), and a genetic variant of unknown significance in one. Eleven (50%) patients underwent abdominal surgery for resection of tumor, 7 are treated with somatostatin analogs and 2 patients underwent peptide receptor radionuclide therapy (PRRT). Eight patients underwent parathyroid surgery, 6 treated with cabergoline for prolactinoma and 3 underwent pituitary surgery. Twenty two subjects are alive (age 45.4±10 years), whereas 3 died of metastatic GEP tumors at the ages 41, 45 and 56.

Conclusions: MEN-1 is a complex genetic disorder. Hyperparathyroidism is the most common and earliest manifestation. GEP tumors cause most of the morbidity and mortality associated with MEN-1. Menin mutations or variants were found in 72% of patients with clinically defined MEN-1.

Congenital IGF-1 deficiency protects from cancer development - additional support

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Introduction: In a previous study (1) we have found that homozygous isolated congenital IGF-I deficiency (such as in Laron Syndrome) protects patients from future development of malignancies. This was confirmed by another group (2). Aim: a) To find out whether congenital IGF-I deficiency combined with other pituitary hormone deficiencies also confers protection from cancer. b) To enlarge the population of patients with isolated congenital IGF-I deficiencies.

Patients/ Methods: By survey among endocrinologists in Israel and other countries, using a pre-structured questionnaire for patients and first and second degree family relatives.

Results: The main data obtained until Jan 15, 2010 are sunnerized in the following table. The data reinforce our previous findings (1) and those of Guevara-Aguirre in 75 LS pts. (2) that cong. IGF-1 deficiency protects from the development of malignancy. In the present study we found in addition that pts. with cong. IGHD, IGF-1 R mutation and IGF-1 gene deletion also fall into this category. Out of 6 pts. with a GHRH-R defect and out of 97 pts. with cong. MPHD including GH def. we found 1 pt. with cancer in each. Among the first degree family members (most heterozygotes) we found 27 cases of cancer. In addition, 30 out of 131 second degree relatives also reported malignancies.

Conclusions: a) The present survey underlines the important role cong. IGF-1 deficiency has on the development of malignancies. b) It is premature to define which pts. with GHRH-R defect or cong. MPHD are not protected from cancer.

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Peptide receptor radioligand therapy (PRRT) is an effective treatment for the long-term stabilization of malignant gastrinomas

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Introduction: Gastrinomas represent a rare group of neuroendocrine tumors usually located in the duodenum or pancreas. They secrete gastrin, being responsible for the clinical picture of severe acid-related peptic disease and diarrhea, known as the Zollinger-Ellison syndrome (ZES). While symptomatic control may be achieved with proton-pump inhibitors (PPIs) and somatostatin analogues (SSAs) treatment, little data is known regarding the possible anti-tumor effect of the peptide receptor radioligand therapy (PRRT) in gastrinomas patients. Aims: To assess the effect of PRRT on symptoms control, gastrin secretion and tumor load in patients with malignant gastrinomas, with progressive disease.

Patients/ Methods: We have retrospectively studied 11 consecutive patients with metastatic gastrinomas followed at two referral centers in Israel for a mean period of 5 years. The patients were symptomatically treated with PPIs (n=8), and/or with monthly injections of octreotide LAR (30 mg/month) (n=8) or lanreotide Autogel (120 mg/month) (n=1), all patients presented with an ECOG score of 0-1 (1), and received PRRT (90Yttrium- or 177Lutetium-DOTATOC) for progressive disease. Patients had serum gastrin measurements performed pre- and post-treatment, as well as radiological assessment before and every 3-6 months following PRRT, using the RECIST criteria for tumor response (2).

Results: The dosage of PRRT was 432.45±223.29 mCi (mean±SD), with a mean number of 2±1.26 courses/patient (range 1-4), depending on tumor uptake. PRRT was well tolerated in all patients, without serious side effects: 8/11 patients (73%) experienced a transient decrease in their blood counts, while in one patient (9%) a temporarily increase in creatinine levels appeared. Following PRRT, symptomatic improvement was observed in all patients, as well as significant suppression in gastrin levels, which decreased from 4831 mI/L to 932.6 mI/L ($p<0.001$) (normal 40-108 mI/L). Periodic radiological surveillance showed partial tumor response in 5/11 (45%). Tumor stabilization has been achieved in 6/11 (55%) patients, with no complete response. During the follow-up, 4/11 patients (36%) died due to tumor progression (mean time to progression of 8±2.8 months), in this group, the mean survival time following last PRRT reached 14±6.9 months. In 7/11 patients, the anti-tumor effect of PRRT persisted for a mean period of 11±3.16 months (ongoing).

Conclusions: In patients with malignant gastrinomas, PRRT has a valuable effect in reducing/stabilizing the tumor load, with a concomitant decrease in serum gastrin levels. Our data indicate an important anti-proliferative effect of PRRT on gastrinoma tumor cells, together with a significant clinical benefit with respect to disease symptoms, with minimal adverse effects.

Impaired arterial properties in active acromegaly are reversed by effective therapy

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Introduction: Active acromegaly is associated with increased cardiovascular and cerebrovascular morbidity and mortality. Normalization of GH and IFG-1 levels has been shown to improve some of the cardiac function abnormalities. The aim of the present study was to evaluate parameters of arterial function in active and controlled disease.

Patients/ Methods: The arterial properties of 18 subjects with acromegaly (7 males and 11 females) were studied by repeat non-invasive measurements of arterial properties using applanation tonometry and pulse wave analysis and assessing parameters of arterial stiffness: pulse wave velocity (PWV), central blood pressure, augmentation index (AIx), and large/small artery compliance (C1 and C2). By ultrasonography, common carotid artery far-wall intima-media thickness (IMT) and flow-mediated dilatation (FMD) of the brachial artery was measured. Nine subjects with active acromegaly (GH 7.76 ± 12mU/L, IGF-1 469.9 ± 246 ng/ml) and nine subjects with controlled or cured disease (GH 1.59 ± 0.1mU/L, IGF-1 160 ± 0.3ng/ml) were studied.

Results: Mean age was 48.3 ± 19 years in subjects with active disease and 56.4 ± 13years in those with controlled acromegaly, p=NS). Weight, waist circumference, body mass index, HDL- and LDL-cholesterol and triglycerides were similar in the two groups Glucose tolerance abnormalities were found in 5 (50%) patients with active disease and in two (27%) cured subjects, with significantly higher fasting plasma insulin level in the active group (21.9 ± 11.17 vs. 14.8 ± 3.8 MIU/mL, respectively, p=0.0075). Systolic blood pressure (SP) but not diastolic blood pressure (DP) was significantly higher in patients with active disease (128 ± 17 vs. 113.1 ± 12mmHg, p=0.005). Central (aortic) blood pressure was higher in the active disease group (SP: 118 ± 12 vs. 106 ± 12mmHg, p=0.04, DP: 74 ± 8 vs. 66 ± 6mmHg, p=0.02, respectively) as was systemic arterial resistance (1427 ± 323 vs. 1299 ± 215dynes/sec/cm(-5), p=0.006). Hypertension was found in three patients with active disease and two in cured subjects. Analysis of arterial function parameters were performed with adjustment for age, gender and SBP. Small artery elasticity index (C2) was decreased in the active disease group (6.71 ± 4.06 vs. 8.5 ± 4.2 ml/mmHg x 100, p= 0.03). There were no significant differences in other arterial stiffness parameters PWV, Alx, C1, IMT and measurement of endothelial function (FMD).

Conclusions: Patients with active acromegaly have decreased small artery elasticity index, increased systemic systolic BP, increased central SP and DP as well as increased systemic arterial resistance. These vascular derangements could contribute to the increased propensity for cardiovascular disease in acromegaly but are apparently reversed by effective treatment.

The Role of PPAR-delta in the adaptation of β-cells to high glucose levels

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Introduction: Pancreatic β-cells are programmed to adapt to elevated levels of glucose by increasing their insulin secretory function. The molecular mechanisms of this process are not fully understood. Peroxisome proliferator-activated receptor-delta (PPAR-delta), which has been shown to play essential roles in regulation of lipid and carbohydrate metabolism, has also been found to function as a lipid sensor in β-cells. Recently, we have found that high glucose levels activate PPAR-delta in vascular cells. Current reports have indicated that 4-hydroxyalkenals (the products of peroxidation of poly-unsaturated fatty acids), such as 4-hydroxynonenal (4-HNE), serve as endogenous ligands of PPAR-delta. Therefore, we asked whether the adaptation of β-cells to hyperglycemia is mediated by PPAR-delta and what could be the endogenous ligand of this nuclear receptor.

Patients/ Methods: The PPAR-delta agonist GW501515, its antagonist GSK0660 and 4-HNE were used in the INS-1E β-cell line and in isolated rat islets to study the role of PPAR-delta in modulating insulin secretion at varying glucose concentrations. Silencing of PPAR-delta in INS-1E cells and a luciferase reporter assay were employed to clarify the role of PPAR-delta activation in insulin secretion in β-cells.

Results: Increasing concentrations of glucose enhanced insulin secretory capacity of isolated rat islets and INS-1E cells. This effect was blocked in the presence of the selective PPAR-delta antagonist GSK0660. Both GW501516 and 4-HNE mimicked the effect of high glucose and increased insulin secretion, whereas GSK0660 blocked their effect. Using HPLC analysis we found that the capacity of INS-1E cells to generate 4-HNE was augmented significantly under high glucose conditions. The luciferase reporter assay in INS-1E cells revealed that high glucose levels, GW501516 and 4-HNE activated PPAR-delta, whereas the antagonist GSK0660 abolished this trans-activation effect. Silencing of PPAR-delta in INS-1E cells confirmed these results.

Conclusions: This study demonstrates that 4-HNE-depedent activation of PPAR-delta mediates the adaptive increase in insulin secretory capacity of β-cells exposed to high glucose levels.

Dicer is essential for mice islet cells survival

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Introduction: microRNAs (miRNAs) are a subset of endogenous small RNA molecules that repress gene expression post-transcriptionally, by mediating mRNA degradation or translational repression. Increasing evidence suggests aberrant miRNA function in human diseases, but despite rapid progress in the miRNA research field, little is known about their role in glucose metabolism and diabetes.

Patients/ Methods: We generated a transgenic mouse model in which Dicer, a key enzyme in the biogenesis of miRNA, is ablated from all the endocrine cells of the pancreas using the Cre-Lox system for site-specific recombination. Consequently, these cells lose all miRNA in the cells of the islets of Langerhans at developmental stages (E14.5 the latest).

Results: As a result from the Dicer deletion, the endocrine cells of the pancreas die progressively, resulting in virtually complete loss of all insulin and glucagon. As insulin was undetectable in the serum of adult mutant mice (EIA kit), we conducted whole pancreas insulin content assay. Strikingly, insulin content which was not significantly different at birth was reduced by four orders of magnitude at 12 weeks of age. These exceptional results were supported by immunohistochemical examination of pancreata showing no detectable insulin or glucagon positive cells in adult mice. Not surprisingly, the extreme hypo-insulinemia was accompanied by severe hyper-glycemia. Between birth and weaning the mutant mice experienced a marked deterioration in glycemic control. While at birth no differences in blood glucose levels were found between control and mutant mice, after weaning all the mutant pups displayed extreme hyperglycemia (>600mg/dl) which persisted through out their lives. Despite severe hypo-insulinemia, and in contrast to high-dose streptozotocin treated mice, the mutant mice did not display high blood ketone levels.

Conclusions: Challenging previously published reports, we demonstrated that deletion of Dicer in the endocrine pancreas at developmental stages (E14.5 the latest) does not prevent normal differentiation and function, but eventually results in programmed cell death, starting at the neonatal period. These findings are consistent with other developmental Dicer deletions models. Despite the complete loss of glycemic control, the mutant mice reach adult age. To the best of our knowledge, this is the first diabetes mouse model to survive untreated with constant severe hyperglycemia and virtually complete insulin depletion. This might be in part because mutant mice are spared from the lethal diabetic ketoacidosis (DKA), typical of other diabetes type 1 models. We suggest this might be due to the protective concomitant loss of glucagon, since it has been shown to play a critical role in the manifestation of DKA.

Expression and regulation of CRF receptor type 2 in the developing and mature mouse skeletal muscle

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Introduction: The corticotropin-releasing factor (CRF) and its family of ligands, including urocortin (Ucn)1, Ucn2 and Ucn3 and their cognate receptors, are involved in the maintenance and the adaptive responses necessary for energy homeostasis regulation. Studies focusing on the role of Ucn2 and Ucn3 signaling through their specific receptor, the CRF receptor type 2 (CRFR2), demonstrated diverse effects of CRFR2 signaling on energy homeostasis. Skeletal muscle (SM) tissue has been demonstrated to express high levels of the beta (β) alternative splice form of the CRFR2 gene (CRFR2β). CRFR2-null mice have enhanced glucose tolerance, increased insulin sensitivity and are protected from high-fat diet (HFD) induced insulin resistance. SM CRFR2β functions to inhibit interactions between insulin signaling pathway components by inhibiting insulin-induced Akt and ERK1/2 phosphorylation. However, little is known regarding SM CRFR2 regulation. To this end, we studied the regulation of CRFR2 expression during SM differentiation and examined the effect of different stressors on CRFR2 expression in the adult mouse

Patients/ Methods: We used RNase protection assays, RT-PCR and DNA sequencing in order to study CRFRs expression in SM tissue. The regulation of the CRFRs during the skeletal myogenic differentiation was determined using in vitro differentiation of the C2C12 cells. Luciferase reporter assays were used to study the activity of the CRFRs promoters during differentiation and to learn the involvement of putative muscle-specific transcription factors in CRFR2 promoter regulation. RNA extracted from SM of mice exposed to chronic variable stress (CVS) protocol or kept on HFD was used for determining CRFR2 and RBP4 expression

Results: We demonstrate a differential regulation of CRFR1 and CRFR2 mRNA expression, promoter activity and receptor functionality during the C2C12 myogenic differentiation. While C2C12 myoblasts exclusively expresses CRFR1, the C2C12 myotubes solely express CRFR2. In addition, using a site-specific mutagenesis we demonstrate the importance of the MEF2 consensus sequence, located at the 3' proximal region of CRFR2 promoter, to CRFR2 transcription. We further show that HFD and CVS challenges significantly increase the expression level of SM CRFR2 and RBP4, an adipokine whose serum levels are elevated in insulin resistant states

Conclusions: Our results are first to demonstrate the differential regulation of CRF receptors during SM differentiation and further contribute to our understanding of CRFR2 molecular and physiological regulatory mechanisms. Combining this new set of the data with our previously published findings we suggest that RBP4 and Ucn2 endogenously expressed by SM may synergistically act in an autocrine fashion to inhibit insulin signaling. A better understanding of SM CRFR2 pathways, its physiological roles and its regulation may pave the way for future management of type-2 diabetes and obesity

Differential expression of novel adiponectin receptor-1 transcripts in skeletal muscle of normoglycemic and type 2 diabetic patients

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Introduction: Adiponectin receptor 1 (AdipoR1) expression in human skeletal muscle has been suggested to play an important role in insulin resistance and diabetes. We aimed at evaluating the potential existence of novel AdiopR1 splice variants in human muscle and their regulation under physiological and pathophysiological states.

Patients/ Methods: The presence of 5'UTR mRNA transcripts of AdipoR1, predicted from bioinformatics data, was evaluated in fetal and adult human tissues. The expression and function of the identified transcripts was assessed in cultured human skeletal muscle cells and in muscle biopsies obtained from normoglycemic and type 2 diabetic patients (n=49).

Results: Screening of potential AdipoR1 5'UTR splice variants revealed a novel highly abundant skeletal muscle transcript (R1T3), in addition to the previously described transcript (R1T1). Unlike R1T1, R1T3 expression significantly increased during fetal development and during myoblast-myotube differentiation. R1T3 silencing was associated with a profound reduction in AdipoR1 receptor expression in human muscle cells. Type 2 diabetes resulted with a 4-fold and a 2.2-fold reduction in R1T3 and R1T1 expression, respectively, in human muscles as compared with normoglycemic subjects, paralleled with decreased expression of the differentiation marker myogenin. R1T1 and R1T3 levels, as well as R1T3/R1T1 ratio, were found to be directly correlated with the degree of whole body insulin sensitivity.

Conclusions: The finding of a novel muscle-specific 5'UTR splice variant suggests that AdipoR1 receptor expression in human skeletal muscle, as opposed to other tissues, may be subjected to posttranscriptional regulation during development and differentiation, a process that may be attenuated in insulin resistance and type 2 diabetes.

p38 mitogen-activated protein kinase dependent transactivation of ErbB receptor family – A novel common mechanism for stress-induced IRS-1 serine phosphorylation and insulin resistance

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Introduction: Stress stimuli such as TNF have been shown to induce IRS-1 serine phosphorylation and insulin resistance, by transactivation of the ErbB receptors. We aimed at elucidating the potential role of p38MAPK in mediating stress-induced ErbB receptors activation.

Patients/ Methods: Fao, HepG2 cells and ob/ob or C57/BL6 mice were used.

Results: High-fat diet fed mice and ob/ob mice exhibited elevated hepatic p38MAPK activation associated with glucose intolerance and hyperinsulinemia. Liver expression of dominant-negative p38MAPKα (DN-p38MAPKα) in ob/ob mice reduced fasting insulin levels and improved glucose tolerance, whereas overexpressing a constitutively-active p38MAPK activator, MKK3, in C57/BL6 mice induced glucose intolerance and hyperinsulinemia. Moreover, C57/BL6 mice overexpressing wild-type p38MAPKα exhibited reduced insulin-stimulated IRS-1 tyrosine phosphorylation. Fao or HepG2 cells exposed to TNF, anisomycin or sphingomyelinase demonstrated rapid transactivation of the ErbB receptors leading to PI3-kinase/Akt activation, and IRS-1 serine phosphorylation. p38MAPK inhibition either by SB203580, by siRNA or by DN-p38MAPKα decreased ErbB receptors transactivation and IRS-1 serine phosphorylation and restored insulin stimulated IRS-1 tyrosine phosphorylation. When incubating cells with specific ErbB receptors antagonists or utilizing cells lacking ErbB receptors, anisomycin- and TNF-induced IRS-1 serine phosphorylation was attenuated, despite intact p38MAPK activation. The stress-induced p38MAPK activation leading to ErbB receptors transactivation was associated with intracellular reactive-oxygen species (ROS) generation and was completely prevented by treatment with antioxidants.

Conclusions: Hepatic p38MAPK is activated following various stress stimuli in a ROS dependent manner. This event is upstream to ErbB receptors transactivation and essential for stress-induced IRS-1 serine phosphorylation and insulin resistance.

Nitric oxide synthase protects the pancreatic beta-cell from glucolipotoxicity-induced endoplasmic reticulum stress and apoptosis

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Introduction: Cytokines stimulate nitric oxide production leading to endoplasmic reticulum (ER) stress and apoptosis. Treatment of pancreatic beta-cells with glucose and free fatty acids induces nitric oxide synthase (NOS) and ER stress. However, the role of NO in glucolipotoxicity-induced ER stress in beta-cells is not clear.

Patients/ Methods: We studied the effect of high glucose and palmitate on the expression of NOS isoforms in INS-1E beta-cells, and rat and P. obesus islets. The effects of nNOS knockdown and NOS inhibition by NG-nitro-L-arginine methyl ester (L-NAME) on beta-cell function, ER stress and apoptosis under conditions of glucolipotoxicity were investigated.

Results: Overnight incubation of rat and P. obesus islets at 22.2 mmol/l glucose with 0.5 mmol/l palmitate induced the expression of nNOS, but not iNOS, contrasting the robust stimulation of iNOS by cytokines. NOS inhibition by L-NAME did not prevent the attenuation of glucose-stimulated insulin secretion and proinsulin biosynthesis or the depletion of islet insulin content observed under conditions of glucolipotoxicity. Moreover, treatment of beta-cells with palmitate and L-NAME together resulted in marked activation of the IRE1alfa and PERK pathways of the unfolded protein response (UPR), leading to apoptosis. Similarly, nNOS knockdown increased CHOP expression, JNK phosphorylation and caspase 3 cleavage in beta-cells exposed to high glucose and palmitate. Treatment of INS-1E cells with the JNK inhibitor SP600125 decreased beta-cell apoptosis induced by palmitate and L-NAME.

Conclusions: In beta-cells subjected to glucolipotoxic conditions, genetic and pharmacological inhibition of nNOS exacerbates ER stress and activates JNK. Therefore, induction of nNOS is an adaptive response to glucolipotoxicity, protecting beta-cells from ER stress and apoptosis.

The NFkB paradox: suppresion of GLUT4 gene expression with concomitant enhancement of GLUT4 translocation

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Introduction: The link between inflammation, insulin resistance and type 2 diabetes mellitus (DM2) is now well-established. While NFkB activity is enhanced in DM2, its role in regulating glucose transporter 4 (GLUT4) gene expression and function is yet unclear.

Results: GLUT4 regulation at transcriptional level was studied in vitro in differentiated L6 culture using co-transfection assays. Transient co-transfection GLUT4 promoter (GLUT4-P), with NFkB subunits (p65, p50 or both), dose-dependently suppressed GLUT4-P activity to 10%, 40% and 20% of basal levels, respectively. Glut4 function was assessed in Glut4-Myc L6 myoblasts. Compared to mock transfection in the basal state p65 transfection increased Glut4 translocation to the plasma membrane by 2 fold similar to insulin effect. Adding insulin to these cells further enhanced the translocation by 50%. While Glut4 translocation was unaffected by p50 transfection, equimolar amounts of both subunits increased translocation up to 300%, compared to basal, while insulin had no further effect. Over expression of NFkB subunits also increased 14-3-3 gene expression, while silencing significantly reduced 14-3-3 cellular levels. Further, p65 transfection enhanced 14-3-3 and AS160 protein – protein interaction a critical step for Glut4 translocation.

Conclusions: Thus, while NFkB represses GLUT4 promoter activity, it also upregulates Glut4 translocation machinary by enhancing 14-3-3 and AS160 interaction. These NFkB modes of action are potential targets for type 2 diabetes therapy.

Profile, target genes and regulation of microRNAs in ovarian carcinoma tumor progression

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Introduction: Ovarian cancer is the leading cause of death from gynecological cancers in western countries. The disease is asymptomatic in the early stages, and is usually diagnosed at an advance stage. Primary solid tumors, solid metastases, and effusions to the peritoneal and (ascites) pleural cavities characterize the tumor as it progresses. MicroRNAs (miRNAs) are small non-coding RNAs that exert a regulatory effect post-transcriptionally by binding target mRNAs and inhibiting gene translation. miRNA expression is deregulated in cancer. The aim of this study was to characterize the differences in miRNA expression pattern and the miRNA-regulating machinery between ovarian carcinoma cells in primary tumors vs. effusions.

Patients/ Methods: We analyzed snap-frozen primary ovarian carcinoma tumors and cells derived from peritoneal and pleural effusions. microRNA-array platforms were used to profile the expression of miRNAs at the two sites. The results of the array were validated on an independent set of samples by real-time PCR. Putative targets for miRNAs of interest were predicted using web available algorithms. Expression of target genes was assessed by Western blot, expression of the machinery molecules was analyzed by real-time PCR and Western blot.

Results: Using miRNA-array platforms, we identified three sets of miRNAs, one that is highly expressed in both primary carcinomas and effusions, one overexpressed in primary carcinomas, and one overexpressed in effusions. The most significant miRNAs were validated by real-time PCR on a validation set of samples. Our results show concordance between the training and the independent test cohorts for the reduced miR-145 and miR-214 and for the elevated let-7f, miR-182, miR-210, miR-200c, miR-222 and miR-23a in effusions. Using in-silico target prediction programs we identified potential target genes for the above miRNAs. We analyzed the expression of ZEB1 and c-Myc, targets of miR-200c. In addition, we analyzed PAK1 and PTEN, both predicted targets of miR-222. We found inverse correlations between the expression levels of the indicated miRNAs and of the predicted target genes. We further observed higher expression of the miRNA processing molecules Ago1, Ago2 and Dicer in effusions compared to primary carcinomas.

Conclusions: our data are the first to document different miRNA expression and regulation profiles in primary and metastatic ovarian carcinoma, suggesting a role in tumor progression.

Analysis of the interplay between the insulin receptor and IGF-I receptor signaling pathways in prostate cancer

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Introduction: Hyperinsulinemia is a consequence of insulin resistance. Beside its normal spectrum of metabolic effects, insulin also acts as a growth factor and has the ability to promote mitogenic activity. Thus, hyperinsulinemia is regarded 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 induces a mitogenic activity in prostate cancer-derived cell lines via its own receptor or the IGF1 receptor.

Patients/ Methods: we employed a number of prostate cancer cell lines (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 or with an MTT assay. Cell-cycle dynamics were evaluated by propidium iodide staining. Activation of the insulin receptor (IR) 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 PC3, C4-2 and P69 cell lines. Cell cycle analyses showed that insulin can positively influence C4-2 and P69 cells to progress towards the G2/M phase. With the insulin doses used immunoprecipitation assays showed significant activation of IR, but not IGF-IR.

Conclusions: 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.

The t-Boc derivative of 7-(O)-carboxymethyl daidzein is cytotoxic to the human follicular carcinoma cell line WRO and a negative growth modulator in non-malignant human goiter cells in vitro: Potential role of reactive oxygen species (ROS)

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Introduction: The incidence of thyroid cancer is up to 3 folds higher in women than in men, suggesting that estrogenic effects may be involved in the pathogenesis of this malignancy. We previously reported that a novel phytoestrogen derivative generated in our laboratory, the N-t-boc-hexylenediamine derivative of 7-(O)-carboxymethyl daidzein [cDtboc], possess potent anti-cancer effects several human cancer cell types expressing preferentially mRNA for estrogen receptor (ER)β relative to ERα. These earlier studies indicated that cDtboc exerted cytotoxic effects in thyroid cancer by the induction of apoptosis and not through cell necrosis. In the present study we compared the effect of cDtboc in the follicular thyroid carcinoma cell line WRO and cultured primary goiter cells originally harvested from 3 different patients.

Patients/ Methods: Cells were cultured and treated with the different compounds and DNA synthesis, CK specific activity, ROS formation and microscopical appearence were analysed.

Results: Both WRO and primary thyroid cells expressed ERα and ERβ with only slightly higher abundance of ERβ over ERα. In both WRO and goiter cells DNA synthesis and creatine kinase (CK, a marker of genomic response to estrogen agonists) increased in response to E2, the ERα agonist PPT and the ERβ agonist DPN. Very significantly, cDtboc abolished these effects completely in WRO but only partially in non-malignant goiter cells. Acting in the absence of E2, cDtboc alone was cytotoxic to WRO, as determined by DNA synthesis indices, the XTT assay and direct microscopic visualization, and much less so to human non-malignant goiter cells. A functionally critical effect of cDtboc was its ability to markedly increase reactive oxygen species (ROS) formation, which was particularly prominent in WRO, but was also detectable in goiter cells. The NADPH-oxidase inhibitor DPI not only abolished ROS formation but also partially inhibited the cytotoxic effects of cDtboc on WRO.

Conclusions: Hence, cDtboc possesses some antiestrogeic properties in thyroid cancer cells and is cytotoxic to thyroid cancer cells and, to a lesser extent, to non-malignant goiterous cells, acting in part via induction of ROS formation. Since WRO cells are clearly estrogen sensitive, thus resembling the estrogen-sensitivity of other thyroid cancer cell lines reported by us earlier (NPA, MRO and ARO), this property can be utilized to design highly effective estrogen-related, anti-thyroid cancer drugs. Further, the present study in goiter cells opens novel pathways through which the growth of non-malignant goiters can possibly be modulated.

Targeting of the IGF-IR as a potential therapeutic strategy in endometrial cancer

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Introduction: Endometrial cancer is the most frequent gynecologic cancer in Western countries. The majority of the cases can be divided into two categories: Type I cancers, which represent more than 80% of the cases, exhibit an endometroid histology, are generally estrogen dependent, and have a good prognosis. Type II cancers, also termed uterine serous papillary endometrial cancer (USPC), have serous papillary or clear cell histology and have a poor prognosis. The insulin-like growth factors (IGFs), have been implicated in the etiology of a number of malignancies, including endometrial cancer. However, no study has so far evaluated the expression of the IGF system in USPC. Moreover, no study has addressed the potential impact of IGF-IR targeting in endometrial cancer. The aim of our research was to evaluate the biological and molecular effects of treatment with a monoclonal antibody against IGF-IR in endometrial cancer cell lines.

Patients/ Methods: Type 1 (ECC-1 and Ishikawa) and Type II (USPC-1 and USPC-2) endometrial cancer cell lines were treated with the A12 monoclonal antibody (ImClone Systems Inc, New York) and assayed for proliferation, apoptosis, cell cycle progression, internalization, and IGF-IR and downstream mediators activation.

Results: A12 effectively inhibited IGF-IR activity in ECC-1, Ishikawa, USPC-1 and USPC-2 cells, whereas it abolished AKT and ERK activity only in ECC-1 and USPC-1 cells. In addition, treatment with A12 on top of IGF-I exhibited a pro-apoptotic activity, as demonstrated by PARP and Caspase-3 cleavage. Furthermore, proliferation assays showed that the inhibitor caused a significant decrease in proliferation rate in ECC-1 and USPC-1 cells. Cell cycle analyses revealed that the antibody caused a progressive accumulation of ECC-1 cells in G0/G1 phases compared to IGF-I-treated cells, with a marked decrease in the percentage of cells in S and G2/M phases. Results of internalization assays revealed that A12 treatment shifted the distribution of IGF-IR from the cell membrane periphery to the cytoplasm in ECC-1 and USPC-2 cells. Furthermore, treatment with the antibody caused a reduction in IGF-IR expression after 24 h and 48 h in all cell lines. Finally, immunoprecipitation analysis revealed that A12 blocked IGF-I-signaling without detectable effects on insulin receptor activation.

Conclusions: Taken together, our results demonstrate that A12 may be an effective therapeutic tool for the treatment of endometrial cancer in which deregulated expression of the IGF-IR plays a critical role. Receptor internalization and degradation seem to be important aspects of the mechanism of action of A12 in these tumors.

Carotenoid derivatives prevent cancer and improve bone health by Inhibition of NFkB and induction of Nrf2 transcription systems

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Introduction: Nrf2 mediates induction of phase II detoxifying and antioxidant enzymes which are responsible for reducing the mutagenic effects of carcinogens and reactive oxygen species. In contrast, activation of the NFkB transcription system increases cancer cell proliferation and tumor metastasis and decreases apoptosis, all of which lead to cancer progression. Recently, a harmful effect of NFkB on bone health has also been shown. Interestingly, under un-stimulated conditions, both Nrf2 and NFkB transcription factors are retained in the cytoplasm by their inhibitory proteins, Keap1 and IkB, respectively. Phosphorylation of IkB by the IkB kinase (IKK) complex is an obligatory step prior to IkB degradation and activation of NFkB. In addition, both Keap1 (Nrf2 system) and IKK (NFkB system) have been shown to harbor cysteine thiols that are critical for their activity. Various electrophyles have been shown to interact with these cysteines, resulting in activation of Nrf2 and inhibition of the NFkB system. Intact carotenoids such as lycopene and beta-carotene lack such electrophylic groups and we have recently demonstrated that carotenoid derivatives, but not the intact carotenoids, activate the Nrf2 transcription system. The aim of the current study was to examine whether carotenoid derivatives and not the intact carotenoid molecule prevent cancer and improve bone health by stimulating Nrf2 and inhibiting NFkB transcription systems in cancer as well as in bone cells.

Methods: We analyzed the structure-activity relationship of a series of dialdehyde carotenoid derivatives in NFkB inhibition.

Results: diapocarotene-dials inhibited NFkB-driven reporter gene expression in both, cancer and bone cells. Moreover, similar to our previous findings regarding the Nrf2 system, we found that the activity of the carotenoid derivatives depends on the relative position of the methyl group to the terminal aldehyde, which determines the reactivity of the conjugated double bond in reactions such as Michael addition to SH groups in proteins (e.g. Keap1, IKK). Importantly, these derivatives also attenuated IKK activity as seen in western blot analysis of its product: phosphorylated-IkB. In addition, the carotenoid derivatives inhibited NFkB nuclear translocation and reduced mRNA level of the target gene TNF alpha. These results suggest a novel mechanism for carotenoid beneficial effect on bone health through inhibition of NFkB activity in osteoblast cells.

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

The histone deacetylase inhibitor vorinostat exhibits a potent pro-apoptotic activity in endometrial cancer cell lines

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Introduction: Endometrial cancer is the most common gynecologic cancer in the Western world. Several studies have been shown a correlation between components of the insulin-like growth factor (IGF) system and endometrial cancer risk. Vorinostat (Merck Oncology) is a novel histone deacetylase inhibitor which induces growth arrest, differentiation and/or apoptosis in a variety of transformed cells, including prostate, leukaemia, breast, and colon cancers. The mechanism underlying the antitumour action of vorinostat is not entirely clear but may involve changes in the expression of specific genes via acetylation of histones and transcription factors as well as nontranscriptional effects such as inhibition of mitosis. The aim of this study was to establish whether vorinostat can modify the expression of specific genes related to the IGF-IR signaling pathway and revert the transformed phenotype.

Patients/ Methods: To investigate the effect of vorinostat on the IGF-I signaling pathway, human endometrioid Ishikawa (Type I) and serous papillary (USPC-2, Type II) endometrial cancer cell lines were treated with vorinostat (5uM) for 24 h, in the presence or absence of IGF-I during the last 10 min of the incubation period. The expression and activation (phosphorylation) of specific genes involved in IGF signaling was evaluated by Western blots. Apoptosis was evaluated by cleavage of PARP and caspase-3 measurements.

Results: Vorinostat increased IGF-IR phosphorylation, BRCA1, pTEN, and p21 expression, and reduced Sp1 and p53 protein levels in Ishikawa cells. In addition, vorinostat up-regulated the expression of total IGF-IR, p21 and down-regulated the expression of total AKT, BRCA1, Sp1, p53, and pTEN in USPC-2 cells. Vorinostat did not alter the expression of ERK1/2 in neither cell line. Of interest, IGF-IR activation was associated with a major elevation in IGF-IR promoter activity. In addition, vorinostat treatment induced apoptosis in both cells lines and abolished the anti-apoptotic activity of IGF-I. Next, we investigated whether the pro-apoptotic effect of vorinostat is connected with IGF-IR levels. For this purpose, cells were treated with vorinostat for 24 h, separately and in combination with MK-0646, a humanized monoclonal anti-IGF-IR antibody (Merck Oncology), in the presence or absence of IGF-I. Western blot analysis revealed that vorinostat abolished the anti-apoptotic activity of IGF-I both in the absence or presence of MK-0646, thus suggesting that the pro-apoptotic action of vorinostat is not correlated with IGF-IR levels.

Conclusions: In summary our studies demonstrate that vorinostat exhibits a potent pro-apoptotic effect in both Type I and Type II endometrial cancer cell lines. The mechanism of action of vorinostat, at least in the specific context of endometrial cancer, is most probably an IGF-IR independent mechanism. Future studies will address the molecular nature of these biological effects.

Differential effects of plant polyphenols on leukemia cell differentiation are associated with distinctive changes in Nrf2/ARE and VDR-RXR/VDRE transcription systems

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Introduction: Acute myeloid leukemia (AML) is a hematological malignancy with poor prognosis. Differentiation therapy is an alternative to cytotoxic therapy of AML. 1alpha,25-dihydroxyvitamin D3 (1,25D) is a strong differentiation inducer in AML cells, but it causes severe hypercalcemia at pharmacologic doses in vivo. We have shown that plant polyphenols, e.g. carnosic acid (CA), curcumin (CUR) and silibinin (SIL), at non-toxic concentrations synergistically enhance the differentiation effects of physiologic concentrations of 1,25D in myeloblastic leukemia cells (HL60). Since CA, CUR and SIL are known antioxidants, here we determined whether this enhancement is associated with activation of the Nrf2/antioxidant response element (Nrf2/ARE) transcription system and/or changes in the levels and activity of the nuclear receptor for 1,25D (VDR/RXR).

Patients/ Methods: Cell proliferation and viability were determined by the trypan blue exclusion assay. Cell differentiation was measured by the expression of CD11b and CD14 markers using flow cytometry and real-time RT-PCR. The levels of VDR and RXRalpha as well as Nrf2/ARE responsive genes NADP(H)-quinone oxireductase and gamma-glutamylcysteine synthetase were examined using real-time RT-PCR and Western blotting. Activity of vitamin D responsive element (VDRE) and ARE was assessed by the luciferase reporter gene assay.

Results: Plant polyphenols CA (10 microM), CUR (10 microM) and SIL (60 microM) strongly enhanced HL60 cell differentiation induced by 1 nM 1,25D. This was associated with a strong increase in both VDR and RXRalpha protein levels and VDRE activity. However, in U937 promonocytic leukemia cells, CA and CUR showed only a moderate enhancement of differentiation, and SIL even had an inhibitory effect. Accordingly, CA hardly affected and SIL strongly reduced both 1,25D-stimulated VDRE activity and RXRalpha levels, though VDR levels tended to increase. Consistent with their antioxidant features, CA and CUR induced ARE transactivation in both HL60 and U937 cells. On the other hand, the antioxidant SIL only moderately activated ARE in HL60 cells and was without effect in U937 cells.

Conclusions: Our results demonstrate that various polyphenols differentially affect cell maturation, depending on AML subtype. Furthermore, polyphenol-induced changes in VDR and RXRalpha levels are associated with the extent of Nrf2/ARE activation. These findings suggest that Nrf2/ARE activation is required for the upregulation of VDR and RXRalpha and, thus, for the differentiation-enhancing effect of plant polyphenols. On the other hand, the lack of Nrf2/ARE activation may result in a decline in cellular RXRalpha levels, leading to inhibition of 1,25D-induced differentiation.

Pathways involved in 1,25(OH)2D3 and valproic acid – induced enhanced response of prostate cancer cells to ionizing radiation

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Introduction: We have previously shown that pre-treatment of prostate cancer (PCa) cells with a combination of the active metabolite of vitamin D (1,25(OH)2D3) and the sodium salt of valproic acid (VPA) markedly enhanced the damaging effect of ionizing radiation (IR) resulting in increased apoptosis and cell-cycle progression delay. The aim of the present investigation was to study in PCa cells the effect of this combinatorial pre-treatment on intracellular pathways involved in the enhanced response to IR. For this purpose, generation of DNA double-strand breaks (DSBs), activation of DNA-damage checkpoint kinases Chk1 and Chk2, and levels of cell-cycle inhibitory proteins p21Cip1/Waf1 and p27Kip1 were assessed.

Patients/ Methods: Androgen-refractory PCa DU145 cells were grown in RPMI-1640 medium containing 10% FCS. Cancer cells were seeded into two 96-well tissue culture plates. Twenty four hours later, cells were treated with 100 nM 1,25(OH)2D3 or 1 mM VPA, or their combination. Control cells were treated with medium containing vehicle 0.1% ethanol. Seventy-two hours later, a test plate was irradiated with a dose of 4 Gy and both irradiated and non-irradiated PCa cells were incubated for additional 3 hours. Induction of DSBs, expressed as levels of phosphorylated histone H2AX, activation (phosphorylation) of Chk1 and Chk2, and expression of p21Cip1/Waf1 and p27Kip1 were assessed by cell-based ELISA.

Results: IR caused a significant generation of DSBs. However, DNA damage in pre-treated cells was greater and reached a maximum in cells pretreated with both VPA and 1,25(OH)2D3. DSBs level after IR treatment was increased by 11.7% ($p<0.001$), and by 18.7%, 20.6% and 34.7% in cells pretreated with VPA ($p<0.006$), or 1,25(OH)2D3 ($p<0.03$) or both drugs ($p<0.001$) respectively. Although no effect on Chk1 activity was found following IR treatment, a significant increase in Chk2 activity was observed, Chk2 activity was increased in non-pretreated cells by 23.8% and by 33%-39% in pretreated cells ($p<0.05$, compared to cells exposed to IR only). IR and VPA alone raised p21Cip1/Waf1 levels by 19.0% ($p=0.01$) and 32.7% ($p<0.005$) respectively, while VPA treatment followed by IR increased p21Cip1/Waf1 level by 54.9% ($p<0.001$). The dual pre-treatment with VPA and 1,25(OH)2D3 followed by IR was most effective and increased p21Cip1/Waf1 level by 94.0% ($p<0.02$). The p27Kip1 expression level was increased by VPA alone by 34.7% ($p<0.003$), and by VPA followed with IR by 52.6% ($p<0.0001$). Here again, the most prominent increase (86.5%, $p<0.0001$) was observed in cells irradiated after combinatorial pretreatment with VPA and 1,25(OH)2D3.

Conclusions: The results show that a combination of 1,25(OH)2D3 and VPA efficiently sensitizes PCa cells to radiation-induced damage through enhanced activation of intracellular pathways comprising DNA-damage checkpoint kinase Chk2, and cyclin-dependent kinase inhibitors p21Cip1/Waf1 and p27Kip1. The present results suggest the possible use of such pretreatments to enhance IR effect in the treatment of prostate cancer.

Endothelin converting enzyme (ECE)-1: a plausible target gene for hypoxia inducible factor (HIF)

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Introduction: Experimental diabetes is characterized by diminished renal parenchymal oxygenation, particularly at the medulla, with enhanced expression of HIF. This condition, as well as ambient hypoxia per se, triggers ET-1 synthesis. We have recently reported that diabetes augment the expression of ECE-1. The aim of the study is to explore the potential role of HIF as the link between evolving renal tissue hypoxia and the regulation of ECE-1 expression, by the inhibition of HIF-degradation.

Patients/ Methods: Rats were subjected to the prolyl-hydroxylase inhibitor L-mimosine (600 mg/kg), or to vehicle, and sacrificed 6h later. The right kidney was perfusion-fixed for immunostaining, while the left kidney was dissected and samples of cortex, outer medulla and inner medulla were analyzed for prepro ET-1 and ECE-1 mRNA, and for ET-1 and ECE-1 protein.

Results: Mimosine led to HIF-1 accumulation mainly in S3 segments of the outer stripe of the outer medulla. This was associated by enhanced pSTAT-3 expression, principally in distal nephron segments both in the cortex and in the outer medulla, and with a 51% and 66% increase in pSTAT-3 in the outer and inner medulla respectively, without a significant effect in the cortex. Both induction of HIF-1 and pSTAT-3 were associated by a three folds increase in ECE-1 protein expression in the inner and outer medulla.

Conclusions: Enhanced ECE-1 expression in the hypoxic kidney might be triggered by HIF through pSTAT-3.

Identification and characterization of novel pate-like genes that code for secreted, cysteine-rich proteins expressed in reproductive and nervous systems

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Introduction: We previously reported on gene clusters in both humans and mice that code for secreted proteins each comprising ten cysteine residues (Levitin JBC 2008) . These conform to TFP/Ly-6/uPAR domains that shape three-fingered proteins. The founding gene is PATE, expressed primarily in prostate and less in testis. We identified 3 additional similar human PATE-like genes that co-localize with the PATE locus. Our study elucidates tissue expression of the human PATE-like genes, and determines their function and expression level in some pathological states. Finally, as PATE-M protein interacts with nicotinic acetylcholine receptors (nAChRs), PATE gene expression in Alzheimer's disease (AD), was investigated.

Patients/ Methods: Anti sera and mAbs were generated in Tel Aviv University. Effect of PATE proteins at the nAChRs were evaluated using the Xenopus oocyte cell expression system. In mice, hormonal effect of murine Pate expression was evaluated in the prostate following castration and in the mammary gland during pregnancy. Expression of Pate genes in the mammary glands of pregnant and/or lactating mice was examined by immunohistochemical staining of the relevant tissues. Similar staining was employed for evaluation of human tissues and sperm cells. Expression level of PATE-M gene in Alzheimer's disease patients was determined by using real-time PCR of cDNA from brain samples obtained from normal and AD patients. The results were confirmed at the protein level by immunohistochemical staining in relation to amyloid plaques stainable by the anti-amyloid mAbs.

Results: PATE-like proteins show selective expression in prostatic neuroendocrine cells (Fig. 1 A, black arrows) and human sperm (Fig. 1 B, white arrows). In male mice, the expression of syntenic Pate-like genes was modulated by the androgenic status. However, in ventral lobes the expression was reversibly suppressed in an androgen-deprived milieu. In female mice, expression of certain Pate-like genes was observed only in mammary glands from pregnant or lactating mice and not from virgin mice. Various forms of nAChRs expressed in the Xenopus expression system revealed modulation of the nAChRs by the PATE-like proteins. Low expression of PATE-M gene was found in temporal lobes of AD patients (Fig 2, I), as compared to normal controls. It co-localized with the characteristic AD Abeta-containing plaques (Fig 2, II).

Conclusions: 1.PATE –like genes are expressed in the reproductive system and are modified by sex hormones. 2. PATE –like genes are also expressed in the nervous system and modulate the activity of nAChRs. 3. PATE-M may have a role in the pathogenesis of AD.

Construction of gene therapy viral vectors targeting thyroid cells: infection of thyroid cancer cells with Adeno-associated viral (AAV) vector serotypes

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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. Thyroglobulin (Tg) is a thyroid-specific protein that is expressed in the normal thyroid and a majority of thyroid tumors. As a step towards the development of gene therapy vectors directed toward human thyroid carcinoma, we previously characterized a minimal Tg enhancer fragment which provided maximal, thyroid-specific, transcriptional activity from the Tg promoter in human thyroid carcinoma cells. Furthermore, we clarified the mechanism of thyroid specificity for the Tg minimal enhancer at the level of thyroid-specific transcription factors and with agents modulating the cAMP pathway. In the present study, we screened different serotypes of recombinant adeno-associated viral (rAAV) gutless vectors to define a relevant AAV viral envelope for potential thyroid cancer therapy. We compared these rAAV gutless serotypes expressing green fluorescent protein (GFP) under the control of the CMV promoter/enhancer. Additionally, we compared two forms of the GFP protein, eGFP and the nuclear localization signal (nls) GFP. Gutless rAAV vectors are considered safe for in vivo use for both animals and humans. In fact, numerous human clinical trials are currently in progress using different rAAV serotypes. Our aim was to screen four serotypes of rAAV vectors in order to determine the serotype which is the most efficient and effective for infecting human thyroid carcinoma cells

Patients/ Methods: Four gutless rAAV serotype (2,4,5 and 12) vectors expressing eGFP or nlsGFP protein were prepared. Their infection of human thyroid carcinoma cells, papillary (NPA) and follicular (WRO), were characterized in a time- and concentration-dependent manner. Infection efficiency was measured by calculating the percentage of green fluorescent cells following different time periods.

Results: Thyroid carcinoma cells were infected at a virus/cell ratio of 2000 (modality of infection, MOI): AAV12nlsGFP, follicular carcinoma cells 92+/-5.9%, papillary carcinoma cells 82.7+/-3.6%. This peak response was seen 3 days post-infection. AAV12CMVGFP, follicular carcinoma cells 47.8+/-10.5%, papillary carcinoma cells 1.4%. This peak response was also seen 3 days post-infection. AAV4nlsGFP, follicular carcinoma cells 4.8+/-1.14%, papillary carcinoma cells 27.9+/-9.1%. This peak response was seen 19 days post-infection. AAV5CMVGFP, follicular carcinoma cells 26+/-6.6%, papillary carcinoma cells 18.4+/-4.9%. This peak response was seen 3 days post-infection. For the virus, AAV2CMVGFP, which was infected a virus/cell ratio of 4000: follicular carcinoma cells 32.3+/-6%. This peak response was seen 6 days post-infection. Papillary carcinoma cells 27.9+/-9.6%. This response was seen 9 days post-infection.

Conclusions: The rAAV12 data obtained will permit the completion of the AAV-Tg enhancer/promoter driven vector with and without GFP and/or an appropriate killing gene construct(s). Furthermore, these constructs will allow us to confirm thyroid tissue specificity and hopefully be the basis for future gene therapy trials for the treatment of thyroid cancer.

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

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Introduction: Normal adult lungs contain scattered pulmonary neuroendocrine cells (PNEC). Reactive PNEC hyperplasia is commonly observed in persons who live at high altitude, in cigarette smokers, and in numerous lung diseases. DIPNECH is a rare entity in which PNEC hyperplasia appears without predisposing conditions. According to the 1999 WHO lung tumor classification, DIPNECH is thought to be primarily a neuroendocrine proliferative process, which can be associated with carcinoid tumors and with a clinical picture of constrictive obliterative bronchiolitis. The available data regarding the treatment and the prognosis of this rare condition is very limited.

Aims: To describe the clinical, radiological and pathological characteristics of patients with DIPNECH, and the effect of different therapeutic modalities on disease progression and patient well-being.

Methods: We have retrospectively studied 9 consecutive patients with DIPNECH followed at two referral centres in Israel between 2001 and 2009. Clinical, biochemical, pathological and imaging data were collected from the medical files.

Results: All patients were female, with a mean age of 62.5 years (range 53-74). Seven patients were lifetime nonsmokers, and two patients had quitted smoking more than 10 years prior to the diagnosis. Five patients presented with respiratory symptoms, such as prolonged dyspnea, wheezing and cough, while in the other four the disease was incidentally diagnosed by thoracic imaging. The mean delay in the diagnosis of DIPNECH in symptomatic patients was 15.8 years (range 2-25 years). All patients had carcinoid tumor together with multiple small pulmonary nodules on thoracic HRCT examinations. The mean size of the dominant lesion was 18.7±9.6 mm. Seven patients underwent thoracotomy and resection of the dominant lesion, while in the other two the diagnosis was made using biopsy. Ki-67 proliferation index was less than 5% in 6 patients in which it was available. The disease was stable in the 6/9 patients. In 3/9 patients it progressed, and treatment with Sandostatin LAR (30-40 mg/month) was administrated, inducing disease stabilization in 2/3 patients, in 1/3 patients the treatment was terminated due to diarrhea. Metastatic disease has been diagnosed in 2/9 patients (22.2%) (to hilar lymph nodes and to adrenal gland). All patients were alive during the follow-up period (1.9±0.76 years, ongoing).

Conclusions: The association of carcinoid tumor with multiple lung nodules in female patients, together with complains of chronic cough and wheezing, shall raise suspicion of DIPNECH. While rare albeit important entity, DIPNECH seems to present with a slow-progressive course. Whenever possible, surgical excision shall be performed to resect the dominant lesion, while somatostatin analogues may be considered for the symptomatic improvement in patients unresponsive to anti-asthmatic medications.

Chronic complications of peptide receptor radionuclide therapy (PRRT)- A single center experience

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Introduction: Peptide receptor radionuclide therapy (PPRT), is an established form of treatment for gastro-entero-pancreatic neuroendocrine tumor (GEPNET) patients. PRRT has been associated with several complications including bone marrow suppression, renal toxicity and hepatic damage.

Patients/ Methods: We retrospectively analyzed our data of 90 consecutive patients with different GEPNETs who received PRRT. CBC and renal function tests were performed prior to treatment and every 2 weeks following each treatment cycle for a minimum of 8 weeks. Possible risk factors were identified. Only patients with such complications that persisted for over 8 weeks were included. Three women who developed premature ovarian failure (POF) were assessed for pituitary gonadal axis function. The CTCAE version 3 NIH/NCI adverse event grading system was used to grade adverse events

Results: Of 90 patients, 13 were excluded due to lack of follow-up. 29 (37%) of these 77 patients developed late hematologic complications: mean age 58 years, 17 men and 12 women, 19 (66%) PNET and 10 (34%) carcinoids, 55% received Y90 DOTATOC, 28% received Lu177 DOTANOC/TATE and 17% received both isotopes, mean cumulative dose 531±265 mCi. 13 (44%) developed anemia (G1-2), 21 (72%) developed leucopenia (G1-3), and 18 (62%) developed thrombocytopenia (G1-4). During follow-up (range 1-60 months, median 18 months), of patients with anemia 30% improved and 70% remained unchanged, 67% of leucopenia improved and 33% remained unchanged, and 44% of thrombocytopenia improved while 44% remained unchanged and only 7% (one patient) worsened. 12 (41%) had monocytopenia, 13 (45%) bicytopenia and 4 (14%) pancytopenia. There were a total of 8 complications secondary to hematologic toxicity: bleeding - 2, infections- 2, blood transfusions-4. Three women (mean age 41) who had prior regular menses developed POF. A total of 6 patients (~8%) developed renal impairment: mean age 66±8 yrs, 2 (33%) had carcinoid and (66%) PNET. 5 had prior renal compromise with risk factors for renal disease. 3 (50%) received Y90DOTATOC 1 (17%) received Lu177 DOTANOC/TATE and 2 (33%) received both, with a mean cumulative dose of 747±547 mCi. Only one patient had worsening of renal function 8-12 weeks from the last treatment but during follow up ranging between 2-24 months (median 16.5 month) 5 of 6 patients had further deterioration of their renal function. Only one patient required dialysis.

Conclusions: In patients with GEPNETs PRRT is a safe treatment modality. The major side effects in our cohort were bone marrow suppression that improved in the long-term in most patients. Young women may be at risk for POF. Deterioration in renal function was uncommon and usually appeared late after treatment, therefore requiring prolonged follow up.

Metastatic growth hormone secreting pituitary carcinoma treated with peptide receptor radionuclide therapy

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Introduction: Pituitary carcinoma (PC) is an extremely rare condition defined by the presence of adenohypophyseal neoplastic tissue outside the pituitary. Clinical experience regarding diagnosis, management and prognosis of PC is very limited. Growth hormone (GH) secreting PC is even rarer and represents a particular challenge to clinical practice. Therapeutic modalities utilized to treat PC include surgery, radiation, hormonal therapy, and cytotoxic drugs. Peptide Receptor Radionuclide Therapy (PRRT) is an emerging therapeutic modality that involves the targeted delivery of an ablative dose of radiolabelled somatostatin analog. PRRT has been applied to various neuroendocrine tumors and results in prolonged survival and enhanced quality of life. As yet, this therapy has not been applied to malignant pituitary tumors. We present a case of GH secreting PC and suggest PRRT as an apparently effective therapeutic option.

Patients/ Methods: A 56 year-old female with a 9 year history of GH secreting pituitary macroadenoma presented with worsening headache and marked acromegalic features. Surgical removal of the pituitary tumor was attempted twice, but GH and IGF-1 levels remained elevated and the pituitary mass re-expanded despite medical therapy with Octreotide and Cabergoline. The patient became severely debilitated with increasing fatigue, musculoskeletal pain, weight loss, and uncontrolled diabetes. Serum IGF-1 and GH levels were 135 nmol/L and 241 mcg/L, respectively. Whole body CT exam demonstrated numerous osteoblastic bone lesions. A CT guided biopsy obtained from a bone lesion confirmed the diagnosis of metastatic GH secreting PC. Octreoscan demonstrated numerous somatostatin avid lesions in the pituitary and the skeleton. Pasireotide therapy was attempted but not tolerated, Temozolamide was not available due to cost.

Results: Three courses of PRRT (117Lutetium-DOTATOC and 90Yttrium-DOTATOC, 200 mCi each) were administered. Post treatment scan after the 3rd PRRT course demonstrated a decrease in uptake intensity in several bone lesions with other metastases stable-appearing. However, the GH level continued to rise and palliative external radiation therapy to skeletal lesions was administered for local relief. The patient died 18 months after the initial PRRT and survived significantly longer than previously reported in PC patients.

Conclusions: The clinical course in this patient favors an aggressive therapeutic approach early in the management of malignant pituitary tumors. PRRT may be an effective therapeutic modality in PC. Additional studies are needed to examine the benefit of PRRT in selected cases of somatostatin receptor positive, unresectable, radiation resistant pituitary tumors.

Metformin displays antiproliferative activities in endometrial cancer cells via interaction with the IGF-IR signaling axis

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Introduction

Accumulating epidemiological evidence shows that obesity is associated with an increased risk of several types of adult cancers. Chronic hyperinsulinemia, a typical hallmark of diabetes, is one of the leading factors responsible for the obesity-cancer connection. Numerous cellular and circulating factors are involved in the biochemical chain of events leading from hyperinsulinemia and insulin resistance to increased cancer risk and, eventually, tumor development. Metformin is an oral anti-diabetic drug of the biguanide family. It is the first-line drug of choice for the treatment of type 2 diabetes, particularly in overweight and obese people. Metformin lowers glucose levels by reducing glucose production in liver cells *via* activation of AMPK and by increasing insulin sensitivity. Recently, metformin was shown to exert an anti-neoplastic effect in ovarian cancer cells, although the mechanism/s responsible for this non-classical metformin action remain unclear. The insulin-like growth factors (IGFs) play a prominent role in cancer biology and their mechanisms of action are tightly interconnected to the insulin signaling pathways. Given the cross-talk between the insulin and IGF signaling pathways, the aim of this study was to examine the hypothesis that the anti-proliferative actions of metformin are potentially mediated via suppression of the IGF-I receptor (IGF-IR) pathway.

Materials and Methods

To address the effect of metformin on IGF-IR activation, human endometroid (ECC-1, Ishikawa) and serous papillary (USPC-1, USPC-2) endometrial cancer cell lines were treated with metformin (10 mmol/L) for various periods of time in the absence or presence of IGF-I during the last 20 min of the incubation. IGF-IR expression and activation, as well as the activation of downstream mediators, was assessed by Western immunoblots. Apoptosis was evaluated by PARP cleavage and cell proliferation by MTT assays. The effect of metformin on IGF-IR promoter activity was assessed by transient transfection assays.

Results

Results of Western blots with anti-phospho antibodies revealed that metformin abrogated the IGF-I-induced IGF-IR phosphorylation. This effect was associated with a reduction in Akt phosphorylation. In addition, metformin activated AMPK and reduced mTOR phosphorylation. Of interest, metformin was able to abrogate the anti-apoptotic action of IGF-I, as measured by PARP cleavage. Furthermore, metformin had a potent inhibitory effect on cell proliferation. Finally, metformin treatment led to a significant reduction in IGF-IR promoter activity.

Conclusions

Taken together, our data indicates that metformin displays potent apoptotic and anti-mitogenic actions in endometrial cancer cells that are mediated, at least in part, via interaction with the IGF-IR axis.

Long-term low dose calcitriol treatment reduces blood pressure and decreases diet-induced atherosclerosis in Tsukuba hypertensive mice (THM)

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Introduction: Epidemiologic studies have suggested vitamin D might have favorable cardiovascular effects. Indeed, there is evidence that vitamin Dlevels are inversely correlated with blood pressure, and with cardiovascular mortality. Suppression of the renin-angiotensin system (RAS), through downregulation of renin, its rate-limiting step, has been proposed as one of several possible mechanisms. We reported that calcitriol administered for 3 weeks to young Tsukuba Hypertensive Mice (THM) prevented hypertension in this model of hypertension and atherosclerosis secondary to the transgenic expression of the human renin (hRen) and angiotensinogen (hAGT) genes. We questioned whether long-term calcitriol teatment would have a sustained effect on blood pressure ,and whether it would affect the atherosclerosis that develops in these mice.

Patients/ Methods: Starting at 7-9 weeks, THM animals were fed an atherogenic Western diet. 17 animals received calcitriol as an intraperitoneal injection at a dose of 0.25 ng/g body weight every other day for 12 weeks (1/2 the dose used in our previous study, and shown not to cause hypercalcemia in the short-term). 19 control mice received the vehicle only. BP was measured noninvasively. The extent of atherosclerosis at the aortic sinus was assessed by quantification of Oil-Red-O-stained lesions. Expression of the RAS in the aorta was studied by real-time PCR.

Results: BP was unchanged in control animals but was significantly lower in calcitriol-treated mice: systolic 142.7+1.8 vs 111.9+3.2 mm Hg, diastolic 88.6+1.4 vs 69.6+2.5, $P<0.0001$ for both. Calcitriol treatment significantly increased cholesterol concentrations 186.4+8.7 vs. 144.8+8.3 mg/dl, $P=0.0028$, and serum calcium 10.3+0.2 vs 8.9+0.3 mg/dl, $P<0.001$. In addition, calcitriol-treated animals weighed more. Nonetheless, the extent of atherosclerosis was reduced by 42.9% with calcitriol, $P=0.015$. This was accompanied by a significant 77% suppression of the hRen gene at the aorta, $P=0.005$. hAGT, ACE and the angiotensin II type 1 receptor were not affected.

Conclusions: Long-term calcitriol treatment provided sustained BP reduction as well as a significant attenuation of atherosclerosis in THM animals. The concomitant suppression of hRen is in agreement with the notion that the beneficial effect is mediated by downregulation of the RAS. However, even at this lower dose which had no untoward effect in the short-term, prolonged treatment resulted in hypercalcemia. Moreover, the unfavorable metabolic profile seen with treatment is a major concern. Animal studies with analogs devoid of these effects will have to confirm the above findings before clinical trials can be contemplated.

The mechanism of the regulation of the epidermal vitamin D endocrine system by inflammatory cytokines

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Introduction: The epidermal keratinocyte contains the machinery for the production of the hormonal form of vitamin D, calcitriol, from 7-dehydrocholesterol, and a vitamin D response system.Our previous findings indicate that calcitriol takes part in the stress and inflammatory responses in the skin and we argue that to fulfill these roles the epidermal vitamin D endocrine system should be unregulated when exposed to inflammatory stresses. The vitamin D endocrine system includes the enzymes responsible for the two hydroxylations of vitamin D at position 25 and 1 alpha, the vitamin D receptor (VDR). The in situ overall activity of this system can be assessed by exposing the keratinocyte to the parent compound vitamin D and monitoring the up-regulation of the sensitive calcitriol target gene CYP24A1. We aimed to examine the effect of inflammatory agents on the epidermal vitamin D endocrine system and to identify the signaling pathways involved in its regulation.

Patients/ Methods: HaCaT keratinocytes, cultured in the absence of exogenous growth factors or active mediators, were our experimental model. Cultures were exposed to TNF or interferon gamma (IFN) for 24 hours. mRNA levels were quantified by real time PCR. The overall activity of the vitamin D endocrine system was assayed by exposing the cultures to vitamin D3 for 5 hours and assaying CYP24A1 mRNA levels. 25-hydroxy 1a hydroxylase (CYP27B1) and VDR mRNA levels were quantified following exposure to the cytokines. This was done in the presence of inhibitors of the following signaling pathways: ERK(U0126), c-Jun N-terminal kinase (SP600125), p38 MAPK(SB203580), NF kappaB(BMS) and PKA (4C3M).

Results: Exposure to TNF (10 ng/ml) and IFN (15 ng/ml) for 24 hours resulted in a marked increase in the overall activity of the keratinocyte vitamin D endocrine system as demonstrated by the induction of CYP24A1 by vitamin D. Upregulation of CYP27B1 by both cytokines and upregulation of the VDR by TNF underlie this effect. The signaling pathways involved are shown in the table.

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

Vitamin D increased E-cadherin synthesis and inhibited E-cadherin cleavage by TNF in epidermal keratinocytes

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Introduction: Cell-cell adhesion plays an important role in tissue and organ pattern formation, maintenance of specific tissue architecture and in the regulation of cell migration and proliferation. E-cadherin, which is the prototypical member of the classic cadherin family, is a major component of adherens junctions, at which it provides cell-cell adhesion through Ca+2-dependent, homophilic binding between molecules on adjacent epithelial cells. E-cadherin is trafficked to and from the cell surface by various pathways. E-cadherin can be cleaved by different metalloproteases induced under various pathological conditions including skin inflammation. It was previously shown that treatment with the hormonal form of vitamin D increased the formation of adherens junctions in cultured keratinocytes. This work was undertaken in order to explore the effect of calcitriol on E-cadherin turnover and cleavage by pro-inflammatory cytokines.

Patients/ Methods: The non-tumorigenic immortal HaCaT keratinocytes were employed as an experimental model, and cultures in the absence of serum, exogenous growth factors and active mediators were treated with calcitriol (100 nM) for 24 and 48 hours. mRNA levels were quantified by real time PCR and protein levels by western blot analysis. E-cadherin fraction present in adherens junctions was quantified by Triton-X100 solubilization. E-cadherin fraction present on cell surface was distinguished from the intracellular fraction by the trypsin protection assay.

Results: Treatment with calcitriol increased E-cadherin mRNA levels in HaCaT cells. In accordance, calcitriol increased the level of an intracellular protein with a higher molecular weight recognized by E-cadherin antibody. This protein almost disappeared following a 2h treatment with cycloheximide. These features support the identification of this protein with the short-lived pro-E-cadherin that is processed in the Golgi apparatus to the mature protein. In addition, immunobloting with E-cadherin antibody revealed a calcitriol inducible protein, of a slightly lower molecular weight, which may be an E-cadherin variant as it co-localized with the wild type protein in the different cellular fractions. Simulation of the inflammatory state by exposure to TNF brought about cleavage of E-cadherin, which was markedly inhibited by pretreatment with calcitriol.

Conclusions: Vitamin D increased E-cadherin synthesis and inhibited its cleavage by a pro-inflammatory cytokine. These effects could contribute to its known anti inflammatory action and suggest novel therapeutic modalities in ailments involving defects in epidermal cell adhesion.

Vitamin D up-regulates the expression of MMP-1 in human keratinocytes

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Introduction: The collagenase, matrix metalloproteinase 1 (MMP-1) plays a major role in wound healing by mediating keratinocyte migration and remodeling scar tissue. Low expression of MMP-1 may result in delaying the first stage of wound healing on the one hand and is associated with fibroproliferative disorders such as keloid and hypertrophic scars on the other hand. Calcitriol, the hormonally active form of vitamin D, enhances wound healing by an unknown mechanism. We aimed to examine the effect of calcitriol on MMP-1 expression in human keratinocytes and to identify the signaling pathways involved in this process.

Patients/ Methods: The immortalized, non-tumorigenic HaCaT keratinocytes, cultured in the absence of exogenous growth factors or active ingredients served as an experimental model. These cells represent the mitotic basal keratinocyte layer. MMP-1 mRNA levels were quantified by real-time PCR and protein levels in the culture medium by western blotting. Rates of mRNA decay were determined following 5h treatment with the transcription inhibitor actinomycin D (1 mic.M).

Results: Treatment with calcitriol (1-100 nM for 6-24 hours) increased MMP-1 mRNA and protein levels in a dose and time dependent manner. The increase in mRNA was significant at a concentration of 1nM and detectable already following 6h treatment. This effect was partially due to increase in mRNA stability. The increase in MMP-1 expression by calcitriol was significantly higher than that due to treatment with the pro-inflammatory cytokine TNF, a classical inducer of MMP-1. The effect of co-treatment with the two agents was additive. The involvement of intracellular signaling pathways in the up-regulation of MMP-1 by calcitriol was examined by using pharmacological inhibitors. The signaling pathways examined are known participants in the regulation of MMP-1 expression and also known to be affected by calcitiriol in keratinocytes. The up-regulation of MMP-1 mRNA by calcitriol was found to be fully inhibited by a Src kinase inhibitor (PP1), and partially inhibited by the ERK inhibitor (U0126) and EGFR inhibitor (AG1478). In contrast, a pan-PKC inhibitor (GF109203x), PLC inhibitor (U73122) and PI3K inhibitor (Wortmannin) had no inhibitory effect on MMP-1 mRNA levels in calcitriol-treated cultures.

Conclusions: Taken together, these results indicate that calcitriol is a potent regulator of MMP-1 expression in human keratinocytes. Src kinase, EGFR, and ERK are involved in the up-regulation of MMP-1 by calcitriol. This finding supports the notion that treatment with hormonally active vitamin D derivatives may enhance the initial steps of wound healing while reducing the risk of hypertrophic scars.

Angiotensin 1-7 prevents the metabolic syndrome, hepatosteatosis and adipose tissue inflammation (Adipositis) in the fructose-fed rat

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Introduction: The metabolic syndrome (MetSyn) usually evolves in the context of visceral adiposity which fosters insulin resistance, hypertension, dyslipidemia, fatty liver and white adipose tissue (WAT) inflammation induced by adipocyte enlargement and macrophage infiltration. We have previously shown that the receptor for angiotensin 1-7 (Ang 1 -7), Mas, is expressed in the adipose tissue. Mas knockout mice were shown to feature MetSyn, we therefore reasoned that treatment with Ang 1-7 may prevent the development of MetSyn in animals fed on high-fructose/low magnesium diet over 24 wks.

Patients/ Methods: The experimental setting was as follows: one group of animals (n= 6) received a continuous infusion of Ang 1-7 (576 μg/kg/day, s.c., via an Aldzet pump for 6 months) and the other served as a control group (n=9, no treatment).

Results: By the end of the treatment period, Ang 1-7 -treated animals had lower final body weight (457+/-8.9 vs 483+/-7.8g, $p=0.03$), lower fat mass (detected by MRI , t-test $p<0.05$) and lower serum triglycerides (97.1±16.3 vs 227.5±21.7 mg/dl, t-test $p<0.001$). Additionally, Ang 1-7 treatment markedly lowered serum aldosterone levels (11.1+/- 2.2 vs 19.1+/-2.1 ng/dl, $p<0.01$). Ang 1-7 treatment did not induced changes in basal serum glucose or insulin, while it did attenuate increase in serum glucose ($P<0.05$) that normally occurs in response to acute intraperitoneal glucose challenge (2 grams/kg). Histological examination of the liver revealed that fructose-fed rats developed hepatosteatosis which was nearly absent in the fructose fed, Ang1-7-treated rats. Mean adipocyte size in epididymal fat sections was significantly larger in untreated than in Ang1-7 treated, fructose fed rats (4133+/-729 vs 8370+/-3934 μm2, respectively, $p=0.008$). Additionally, macrophage infiltration was present in white adipose tissue (WAT) from untreated, but not from Ang1-7 treated rats. This was associated with reduced epididymal fat tissue pP65 protein expression ($p<0.05$), suggesting lower activation of the NFkB pathway in Ang1-7-treated rats. Finally, based on lucigenin-enhanced chemiluminescence, WAT from Ang1-7 treated rats showed reduced NADPH stimulated superoxide production.

Conclusions: We show that Ang 1-7 had an ameliorating effect on insulin resistance, hypertriglyceridemia, fatty liver, obesity and adipositis in the high fructose fed rats. These beneficial effects could be related, at least partially, to the anti-oxidative and anti-inflammatory influence in adipose tissue and to the prevention of hepatosteatosis by Ang1-7.

Differential effect of antidepressants on body mass regulation and food intake in rats exposed to chronic mild stress as compared to unstressed - evidence for a role of leptin

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Introduction: Depression is associated with alteration in food intake and in body mass regulation. Treatment with different antidepressants (AD) such as selective serotonin reuptake inhibitors (SSRIs), noroadrenaline reuptake inhibitors (NRIs) and NE and DA reuptake inhibitor (NDRIs) was reported to modify appetite and body weight.

Patients/ Methods: Male SD rats were exposed to 8 weeks of unpredictable chronic milled stress (Ucms) or to normal conditions (unstressed). Animals (8/group) were treated daily with vehicle, reboxetine (NRI), paroxetine (SSRI), or bupropion (NDRI) (5, 5 and 20 mg/kg respectively). Body weight and food intake were followed weekly. After sacrificing, trunk blood was collected for leptin determination (ELISA), and brains were dissected for neurotrophic protein determination using western blot analysis.

Results: Body weight gain was significantly suppressed in all the UCMS groups as compared to unstressed rats. In the stressed group, the SSRI paroxetine showed impaired body weight gain on the 7th and the 9th week. Whereas, in the unstressed rats food intake and body weight gain was significantly suppressed by bupropion. Plasma leptin levels did not differ between the vehicle groups of stressed and unstressed rats, however, bupropion caused a significant decease in plasma leptin levels in both UCMS and unstressed groups. Paroxetine also caused a significant decrease in leptin levels in the stressed rats.

Conclusions: Stress caused a marked decease in body weight gain and in food intake. The AD bupropion seems to inhibit appetite and body weight gain mainly in unstressed conditions.This effect is accompanied by a decrease in plasma leptin levels. Bupropion, therefore might have a role in the therapy of obesity and hyperphagia in humans.

Effect of weight loss maintenance on arterial compliance, metabolic and inflammatory parameters

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Introduction: Data related the long-term vascular impacts of purpose weight loss are limited. Even less information is available on whether the positive vascular effects of weight reduction are maintained after weight regain following treatment termination. The aim of the present study was to evaluate the effect of long-term (three years) weight loss maintenance on arterial compliance, metabolic and inflammatory parameters in obese patients who participated in a 6-month weight loss program featuring nutritional and exercise intervention.

Patients/ Methods: Open prospective study, 67 obese subjects who participated in a 6-month weight loss program featuring nutritional and exercise intervention followed additional 30 months. The 47 patients that fully completed the three year follow-up were divided into two groups according BMI: group 1 included 22 patients which decreased or did not change BMI after weight loss program discontinuation, group 2 included 25 patients which increased BMI from visit 3 to 4. Arterial compliance as well as metabolic parameters were evaluated at baseline, 3-, 6- and 36-months of follow up.

Results: BMI changed from 35.4 ± 6.9 kg/m2 to 33.0 ± 6.5 kg/m2 after 3 months, to 32.6 ± 6.6 kg/m2 after 6 months and to 33.4 ± 7.0 kg/m2 after 36 months. While 53 % of participants regained weight after 6-month initial weight loss program discontinuation, the mean weight at 3 years remained lower than mean weight at entry into the study (p=0.01). Although SAEI did not differ significantly between the groups at baseline, at the end of the study SAEI was greater in patients who decreased or did not change BMI than in subjects who gained weight (p=0.025). LAEI was greater in group 2 compared to group 1 at baseline, after 3 and 6 months of follow-up, however, at the end of the study no significant difference in LAEI was detected. Repeated measures analysis indicated that LAEI changed significantly over time (p=0.022).

Conclusions: Obese subjects who completed behavioral weight loss program and decreased or maintained weight during 30 additional months future improved arterial stiffness in comparison to subjects who regained weight.

Prevalence of polycystic ovary syndrome in non-classical 21 hydroxylase deficiency females by age at initiation of glucocorticoids therapy and genotype

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Introduction: Non-classical 21-hydroxylase deficiency (NC21OHD) is a mild form of congenital adrenal hyperplasia 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). Compound heterozygosity has been associated with an earlier and more severe presentation: females with NC21OHD might develop polycystic ovarian syndrome (PCOS) secondary to chronic adrenal androgen hypersecretion. Aims: To determine the prevalence of secondary PCOS and identify clinical parameters associated with the development of PCOS in females with NC21OHD.

Patients/ Methods: Medical records of females with NC-21OHD were retrospectively reviewed for presenting signs, age at diagnosis, timing of puberty, age at initiation of therapy, CYP21 genotype and pelvic ultrasound reports. PCOS was diagnosed according to clinical signs of hyperandrogenism and ultrasound findings. The ultrasound criteria included the presence of ≥12 follicles in each ovary, or increased ovarian volume.

Results: Ultrasound results were available for 52 females [mean age at diagnosis (SD): 10.5 (7.6) years]. Thirteen (25%) had clinical signs and ultrasound findings compatible with PCOS. PCOS was more prevalent (67%) in girls who were diagnosed and started on therapy after the age of 12 years compared with those diagnosed and started on therapy earlier (33%, $p=0.02$ and $p=0.04$, respectively). PCOS was less prevalent in girls with presenting signs of precocious pubarche or precocious puberty, compared with girls presenting with post-pubertal virilization ($p<0.001$). There was no significant correlation or association between PCOS and other clinical parameters, including genotype.

Conclusions: The prevalence of PCOS is higher in females with NC21OHD compared to the normal population. Early diagnosis and initiation of therapy in subjects with NC21OHD might prevent the development of postpubertal PCOS. The lack of correlation with genotype might be due to our small sample size.

Recurrent episodes of iatrogenic Cushing's syndrome due to inhaled steroids in ritonavir-treated HIV-infected child

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Background: Ritonavir is protease inhibitor used in the treatment of HIV infection. It is an extremely potent inhibitor of cytochrome P450 3A4, and may increase the bioavailability of drugs metabolized by this pathway such as glucocorticoids. Although inhaled steroids have allegedly local effects, they may cause systemic complications when administer with ritonavir. We describe the hormonal and auxological effects of inhaled steroids over seven years in a ritonavir-treated HIV-infected child.

Case report: The patient is a 12.5 y.o. girl of Ethiopian origin who was diagnosed with AIDS at 2.5 years of age. Since 1999 she has been treated with Zidovudine and Lamivoudine with Lopenavir, boosted with ritonavir. Additionally, she was also treated with inhaled bodesonide and fluticasone as preventive therapy for asthma. In her last admission for asthma exacerbation, remarkable Cushingoid features were noticed and her hormonal profile was compatible with iatrogenic Cushing's syndrome, with suppression of the hypothalamic-pituitary-adrenal axis. Her morning cortisol level was <20 nmol/L and raised to a peak of 70 nmol/L and 99 nmol/l in response to low and high dose Synacten, respectively. Her corresponding ACTH level was 4.7 pmol/L (N 2.2-11.0) and urinary free cortisol was remarkably suppressed at a levels of 13 nmol/L (N 42-254). Two months after cessation of inhaled steroids, her morning cortisol and ACTH levels were raised to 307 nmol/L and 18 pmol/L, respectively. Notably, the patient initially demonstrated severe insulin resistance (glucose 93 mg%; insulin 152.9 mU/L) that was resolved following cessation of inhaled steroids (glucose 64 mg%; insulin 12.4 mU/L). Height and weight measurements recorded over the last 7 years have shown periods of remarkable growth decelerations (Fig 1) and reciprocally opposite weight gain (Fig 2), in association with inhaled steroids treatment (at 6, 8, & 12 years of age).

Conclusion: In ritonavir-treated HIV-infected children, inhaled steroids should be avoided as they may cause iatrogenic Cushing's syndrome with suppressing effect on growth.

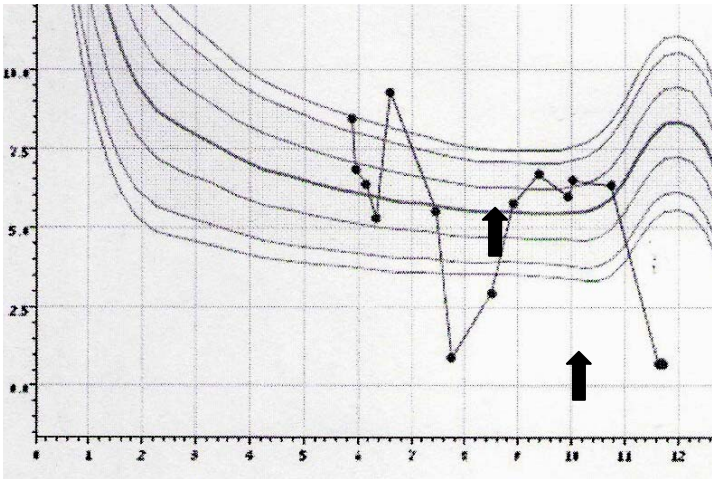

Fig 1: Growth velocity (cm/y)

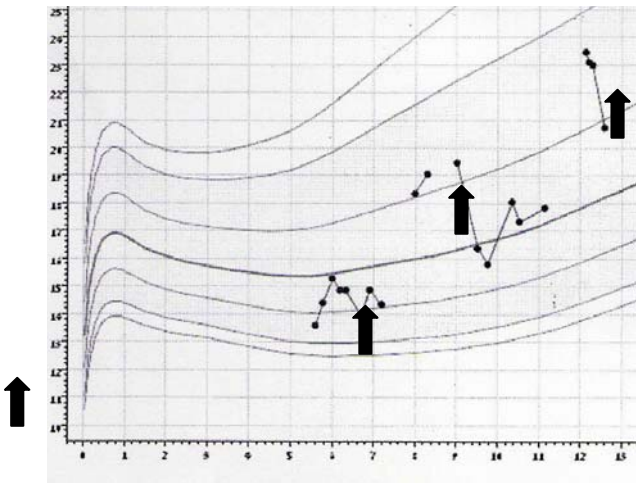

Fig 2: Body mass index (BMI)

Effect of treatment with insulin sensitizer on arterial properties, metabolic parameters and liver function in patients with nonalcoholic fatty liver disease: A randomized, placebo-controlled trial

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Introduction: Insulin resistance has an important role in the development of nonalcoholic fatty liver disease (NAFLD) and is involved in both pathological processes: hepatic steatosis and atherosclerosis. Therefore, treatment of NAFLD with insulin sensitizers is likely to have a favorable effect towards hepatic steatosis and cardiovascular outcomes. Measurement of arterial stiffness provides useful information regarding vascular health and may serve as a marker of atherosclerosis as well as a surrogate for cardiovascular morbidity and mortality risk. The present study investigated the effect of metformin on arterial properties, metabolic parameters and liver function in patients with NAFLD.

Patients/ Methods: In randomized, placebo controlled study, 63 patients with NAFLD were assigned to one of two groups: Group 1 received daily metformin, Group 2 received placebo. Pulse wave velocity (PWV) and augmentation index (AI) and were performed using SphygmoCor (version 7.1, AtCor Medical, Sydney, Australia). Metabolic measures were determined at baseline and at the end of 4-month treatment period.

Results: Among metformin treated patients: PWV and AI decreased significantly during the study. Significant declines in fasting glucose, triglyceride, alkaline phosphatase and a significant increase in HDL-cholesterol were observed. CRP, ALT and GGT decreased and serum adiponectin increased marginally. In placebo group: neither PWV nor AI improved significantly during the treatment period. ALT, AST and adiponectin did not change in placebo group.

Conclusions: Metformin treatment was associated with significant decrease in PWV and AI in NAFLD patients. This beneficial vascular effect was accompanied by an improvement in glucose and lipid metabolism as well as liver function.

Implications of vitamin D status in patients with primary hyperparathyroidism

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Introduction: Primary hyperparathyroidism (PHP) and vitamin D deficiency are connected on several levels. Epidemiological data suggests that vitamin D deficiency is more prevalent in PHP patients than in matched control subjects. Moreover, there is data indicating, that PHP manifestations are more severe in patients with vitamin D deficiency. This is probably true in respect to the severity of the bone involvement and parathyroid adenoma weight. Scant data exists regarding vitamin D status normalization in this patient population, which shows that PTH might even be lowered by vitamin D supplementation. Little is known about the safety aspects of vitamin D replenishment in PHP patients, and there has been some concern about worsening hypercalcemia and hypercalciuria.

Patients/ Methods: We retrospectively examined the electronic database of patients treated in our department since 2005. Medical records of patients with a diagnosis of "hyperparathyroidism" were reviewed. Normocalcemic patients, those with impaired kidney function and patients with only one measurement of 25-OH- D or without increment of vitamin D during the years of follow up were excluded from the analysis. The remaining cohort was analyzed for calcium metabolism parameters at two different time points: lowest and highest vitamin D measurement. The parameters selected were blood calcium, phosphorus, albumin, PTH concentration, and 24-hour urinary calcium and creatinine.

Results: Thirty-three patients met the sample criteria. The mean age was 63 and eighty-nine percent were women. Vitamin D status changed significantly during the follow up years, due to supplementation. Mean vitamin D level at the lowest point was 16.4 + 6 ng/ml. The mean 25-OH-D concentration was 33.8+8.9 ng/ml at the highest measurement. Neither of the parameters examined was influenced by vitamin D status. Serum calcium, phosphorus, and PTH were not significantly changed by vitamin D replenishment: 10.7+ 0.4 mg/dl, 3+0.4 mg/dl and 130+39.5 pg/ml at lowest vitamin D value as compared with 10.7+0.36, 2.9 +0.4 and 133+55, at the highest, respectively. Twenty-four hour urinary calcium excretion, calculated as 24-hour calcium/24-hour creatinine, had not significantly changed between the two time points (0.2+0.1 vs. 0.26 + 0.13 mg Ca/mg Cr, respectively).

Conclusions: Vitamin D replenishment in patients with primary hyperparathyroidism does not cause worsening of hypercalcemia and hypercalciuria, and thus, is safe. As opposed to previous reports, we did not find significant improvement in the degree of PTH elevation.

Neonatal panhypopituitarism: A unique presentation

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Introduction: Infantile cholestasis should be evaluated completely to exclude genetic, metabolic, infectious, obstructive and endocrine causes. Congenital hypopituitarism is an uncommon cause of neonatal cholestasis, and can present with hypoglycaemia. Treatment with glucocorticoid and thyroid hormones, play a significant role in the resolution of cholestasis and hepatosplenomegaly. we report on an infant with panhypopituitarism, presenting with cholestatic jaundice, hypoglycaemia and high serum ferritin level suggesting neonatal hemochromatosis.

Patients/ Methods: Comprehensive clinical and laboratory investigations were performed to establish the etiology of the presenting complaints. This included genetic, metabolic, infectious , as well as thorough hormonal profile (LH, FSH, PRL, TSH, FT4, ACTH and growth hormone stimulation tests).

Results: Hormonal evaluation revealed cortisol and growth hormone deficiency with central hypothyroidism. Other causes of cholestasis were ruled out. In addition there was a high serum ferritin level of 2315ng/ml suggesting neonatal hemochromatosis that was excluded by the absence of hemosidrin deposition in buccal mucosal biopsy. Treatment with cortisol and eltroxin resulted in dramatic improvement of the liver function tests, resolution of cholestatic jaundice and significant reduction of serum ferritin level.

Conclusions: To our knowledge this is the first description of an infant with congenital panhypopituitarism, presenting with cholestasis, hypoglycaemia and high serum ferritin level. Panhypopituitarism should be considered in any infant who presents with cholestasis, hypoglycaemia, and other manifestations of pituitary malfunction. High serum ferritin level most probably suggests acute phase reactant.

Single intrauterine L-Thyroxin treatment of fetal goiter secondary to fetal dyshormonogenesis and maternal hypothyroidism

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Introduction: Fetal goitrous hypothyroidism is rare. Only few cases of fetal goitrous hypothyroidism are described in the English literature.

Patients/ Methods: The diagnosis of fetal goiter was made in the 32th week of geststion by sonography. Size of the goiter was 3.3X2X1 cm with calculated volume of 5.7 ml (normal 0.4). Apparent hypereflexion of the neck with severe polyhidramnion were noted. Fetal blood sampling showed TSH 744 mIU/L(normal 8-10) , free T4 7.1 pmol/L(normal 8-10). The common therapeutic regime of repeated fetal blood sampling for determination of thyroid hormones and intra-amniotic administrations of 250-300 µg levothyroxine (LT4) weekly was not used. A single intramniotic LT4 dose of 500 µg was injected . Maternal hypothyroidism was treated by oral LT4 treatment.

Results: During the 3 following weeks there was no change in fetal condition but after the 3rd week the fetal goiter size, hypereflexion of neck and polyhidramnion were all reduced as seen by repeated fetal sonography . Normal fetal growth and an uncomplicated course of pregnancy between the 32th and 39th week of gestation were observed. After elective Cesarean birth small goiter was observed without any respiratory distress. Neonatal thyroid function tests showed TSH 924 mIU/L(normal 1-20) , free T4 6.8 pmol/L(normal 7-15). and oral L T4 treatment was initiated.

Conclusions: Fetal goiter secondary to fetal dyshormonogenesis and maternal hypothyroidism was treated by a single higher LT4 and careful sonography followup. Monitoring of repeated intramniotic LT4 therapy by determination of TSH in fetal serum or TSH in amniotic fluid may not be necessary,as the change in fetal goiter size was observed only 3 weeks after therapy.

Clinical consequences of prolonged subclinical hypothyroidism

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Introduction: Subclinical hypothyroidism is typically considered a benign condition, whose main importance lies in progression to overt thyroid disease. Data supporting associations of subclinical thyroid disease with symptoms or adverse clinical outcomes are few. Whether subclinical thyroid disease should be treated is controversial, there is little robust evidence that treatment is beneficial and it is not routinely recommended as long as TSH is <10 mU/l. Recently is has been suggested that overweight and obesity may be associated with sub-clinical hypothyroidism in pediatric patients

Patients/ Methods: We describe a patient with prolonged iatrogenic subclinical hypothyroidism associated with growth deceleration and overweight, reversed after correction of thyroid function.

Results: The patient was diagnosed with brain stem glioma at age 2 years. He was treated with Temozolomide and CNS irradiation and was subsequently put on a PTU induced hypothyroidism protocol. As his tumor was stable, treatment was continued for 7 years. Thyroid function was maintained for most of the time in the mild sub-clinical range. Over the past 5 years, TSH ranged from 6 to10 mIU/L and FT4 range from 10 to13 pmol/L. During the treatment period, height percentile decreased from the 75th to the 10th percentile, weight increased from the 50th to the 75th percentile, and BMI increased from 5th to the 95th percentile. IGF-I was consistently low. PTU treatment was discontinued at age 9 years. Shortly after, precocious puberty was noted and treatment with a GnRH agonist was started. Subsequently, growth acceleration and a decrease in weight and BMI were noted. At last measurement (one year after discontinuation of PTU and 9 months after starting GnRH agonist) height was at the 25th percentile, weight at the 60th percentile and BMI at the 75th percentile. IGF-I levels are now normal.

Conclusions: This case offers a unique opportunity to observe the clinical implications of sustained sub-clinical hypothyroidism. Contrary to current thinking, subclinical hypothyroidism per se may be associated with growth retardation and weight gain.

Vitamin D status, calcium intake and bone density in young HIV infected Israeli women

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Background: Decreased bone mineral density (BMD) was reported in HIV infected patients. Mechanisms leading to this decrease are poorly understood.

Aim: To assess sun exposure, clothing habits, vitamin D status and BMD in young HIV infected Israeli women of Ethiopian (ET) and Caucasian (CA) origin.

Patients and Methods: 75 HIV infected women aged 34.5±8.5 years with regular menses who were followed up at the Institute of Allergy, Clinical Immunology & AIDS. Data about the HIV status and treatment was collected from the patients' charts; the patients filled a questionnaire about sun exposure, daily calcium intake and dress habits. Laboratory evaluation: routine chemistry, 25(OH)D by ^{125}I-radioimmunoassay, PTH (Intact) by STAT; bone turnover by plasma total procollagen type1amino-terminal peptide (P1NP) and collagen beta cross-laps (CTX). BMD measurements of the lumbar spine (LS), femoral neck (FN) and total hip (TH) by using dual energy X-ray absorptiometry (Lunar DPX scanner). The BMD results were expressed in comparison to aged matched (Z-scores).

Results: 43 (57.3%) patients were Ethiopian (ET) and 32 (42.6%) Caucasian (CA). There were no significant differences in demographics, actual and past HIV status, antiretroviral treatment and bone turnover markers between the groups.
25(OH)D serum levels<10 ng/ml (severe vitamin D deficiency),were observed in 28(66.7%) of ET vs 2 (6.5%) of CA, p =0.001. Plasma PTH was72.14±57.37 ng/l (normal 12-65), in ET vs 31.23±14.21 in CA, p<0.001. 17 (40.4%) of the ET had sun exposure <1 hour/day, vs 6 (19.4%) of CA patients, p= 0.07; daily calcium intake was 514 vs 164 mg, p=0.001. Avoidance of sun exposure was observed in 21 (67.7%) ET, vs 16 (39%) CA, p= 0.019.
Z-scores in ET and CA were: at LS -1.8±1.1 vs -0.79±0.88, respectively, p=0.001; at FN -1.12±1.1 vs -0.59±0.87, p=0.02, at TH -0.94±1.1 vs -0.25±1.1, p=0.007. BMD Z scores<-1 at LS were observed in26 (89.7%) vs 20 (48.8%), p<0.01, at FN- 20(69%) vs 17 (41.5%), p<0.03, at TH 17 (58.6%) vs 9 (22%), p<0.001 of severely vitamin D deficient pts vs pts with 25(OH)D >10 ng/ml respectively. Logistic regression: risk for LS Z-scores <-1 SD was 5.74-fold higher in pts with vitamin D levels <10 ng/ml.

Conclusion: Osteopenia is frequent in young HIV infected women. Vitamin D deficiency, low calcium intake, limited sun exposure and clothing habits might affect

Comparative genomics, epigenomics, and nutrition in perinatal health: Keys to understanding the molecular basis of the developmental origins of adult disease

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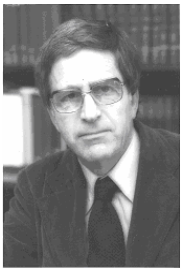
Our lab is dedicated to studying the effects of the *in utero* milieu, especially with regards to maternal high fat diet, on epigenetic changes in the fetus which could help to potentially elucidate the mechanisms involved in the fetal origins of adult disease hypothesis. We have a well established non-human primate (NHP) model of obesity, now in its seventh year, to study the changes in histone modifications and DNA methylation in the fetal liver tissue upon exposure to a maternal high fat (MHF) diet. In our model of maternal obesity, we have observed a profound affect of *in utero* high fat diet exposure on the developing fetus which is independent of maternal obese phenotype. We have demonstrated that a majority (but not all) of the macaque dams chronically consuming a 35% high fat diet become obese and insulin resistant. However, regardless of maternal obesity and insulin resistance, all fetal offspring of high fat fed dams develop non-alcoholic fatty liver disease, hypertriglyceridemia, and premature gluconeogenic gene activation. Over the 180 day postnatal period, the high fat exposed fetuses demonstrate a 2-fold increase in body fat composition. Of interest, we have demonstrated that these fetal and postnatal alterations are accompanied by fetal chromatin modifications, epigenetically regulated alterations in fetal gene expression, and ensuing alterations in the fetal metabolome.

We are highly enthused by our recent significant successes in the laboratory with regards to the development and successful utilization of four high throughput modalities in our model system: exon-hybrid capture arrays for the identification of *de novo* SNPs, whole genome custom primate CpG gene arrays for the identification of gene-specific alterations in DNA methylation, chromatin immunoprecipitation with genome-wide sequencing (ChIP-Seq) for the identification of modified chromatin specific DNA sequences, and metabolomics for the tight correlation of phenotype to metabolites. Of particular interest, a characteristic fetal hepatic epigenomic signature (*i.e.,* hyperacetylation of H3K14 on the histone tail) does appear in offspring destined to obesity regardless of the maternal obese phenotype and is the focus of much of our research.

Taken together, our emerging data suggests that fetal epigenetic signatures may primarily reflective of the maternal diet, and not maternal obesity *per se*. Conversely, the metabolomic signatures in the fetus appear to be related predominantly to the obese maternal phenotype. Full interrogation of these observations will be instrumental in efforts aimed at deciphering the effect of the gestational milieu on the fetal epigenome, transcriptome and metabolome in relation to metabolic disorders in later life.

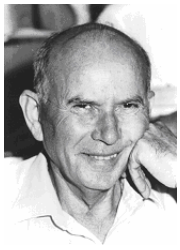


פרופ' הנס יוחנן לינדנר ז"ל

הנס יוחנן לינדנר נולד בשנת 1922 בגרמניה ועלה ארצה עם הוריו בשנת 1936. לאחר מלחמת השחרור הוא למד רפואה וטרינארית בסידני (אוסטרליה) וסיים בהצטיינות.
את לימודיו לתואר .Ph.D הוא השלים באוניברסיטת קיימברידג' שבאנגליה. עם תום לימודיו, חזר לינדנר לאוסטרליה, התמנה כחוקר בכיר ב-(CSIRO) Commonwealth Scientific Research Organization והתרכז בחקר פיטואסטרוגנים.
בשנת 1964, הגיע ארצה למכון ויצמן כחוקר אורח במח' לביודינמיקה, כעבור שנה הוא קודם לדרגת פרופ' חבר ובשנת 1967 הוא מונה לראשות המחלקה.
פרופ' לינדנר בנה מחלקה מולטידיסיפלינארית שעסקה בחקר הפוריות ושינה את שמה ל: "חקר הורמונים".
בזכות תכונותיו התרומיות כאינטלקטואל וכמדען, נשא פרופ' לינדנר תפקידים רבים נוספים: הוא מונה במכון ויצמן כדיקן הפקולטה לביולוגיה, לראשות הועדה לקידום מדענים ולוועדה המייעצת של נשיא המכון.
בנוסף לכך, הוא היה חבר בחבר הנאמנים של ביה"ח הדסה בירושלים, היה פעיל בהקמת הפקולטה לווטרינריה ואף היה נשיא האגודה הישראלית לאנדוקרינולוגיה. בתקופת כהונתו החלה מסורת קיום הכנסים השנתיים.
פרופ' לינדנר היה פעיל גם בארגונים בינל"א: חבר בועדות של WHO, של מכון מקס-פלאנק בגרמניה, של INSERM בצרפת, של ארגונים אנדוקריניים בינל"א וב-Editorial Board של עיתונים מדעיים. הוענקו לו תארי כבוד במס' אוניברסיטאות בעולם.
בשנת 1979 הוענק לו פרס ישראל במדעי החיים והוא נבחר כחבר באקדמיה הישראלית למדעים.
בשנת 1982 הוענקו לו פרס רוטשילד בביולוגיה וכמו כן, פרס Axel- Munthe בשטח הביולוגיה של הפוריות.
פרופ' הנס יוחנן לינדנר נפטר בשנת 1982 עקב מחלה קשה.
כראש המחלקה לחקר ההורמונים הכשיר פרופ' לינדנר דורות של חוקרים בתחום האנדוקרינולוגיה.
הפרס ע"ש פרופ' לינדנר הוא הפרס היוקרתי ביותר של האגודה הישראלית לאנדוקרינולגיה. הפרס ניתן לחוקר/ת, מתחת לגיל 50, עבור הישגים מדעיים בתחום האנדוקרינולוגיה במהלך חמש השנים האחרונות.

זוכי פרס לינדנר

1989- ישראל חנוקוגלו
1990- מרדכי ליסקוביץ
1991- ראובן רייך
1992- אבי קרסיק
1993- רוני זגר
1994- עירית גרנות
1995- אורי פלס
1996- דורית אהרוני
1997- חנה קנטי
1998- בנימין גלזר
1999- מיכל נאמן
2000- רינה מידן
2001- חיים ורנר
2002- משה פיליפ
2003- שרה פרבר
2004- פואד פארס
2006- איתן גרוס
2007- אילן שמעון
2008- חגית אלדר-פינקלמן
2009- אסף רודיך
2010- גיל לייבוביץ



פרופ' ישראל חוברס ז"ל

פרופ' חוברס נולד בפולין ב- 1923 והגיע לארץ בגיל חצי שנה. את חינוכו היסודי קיבל בביה"ס החקלאי ע"ש מאיר שפיה. הוא היה פעיל במשך תקופה ארוכה בשורות ההגנה, בהבאת יהודים ארצה ובצה"ל. הוא התקבל ללימודי הרפואה בשוויץ, אך בינתיים פרצה מלחמת העצמאות והוא החליט להישאר בארץ ולהשתתף בה באופן פעיל, בעיקר בהגנת אזור ירושלים. עם גמר המלחמה, סיים את לימודי הרפואה באוניברסיטה העברית בירושלים.
פרופ' חוברס שרת כרופא בית במחלקת עצבים ולאחר מכן השלים את התמחותו כרופא פנימי במחלקה פנימית בהדסה. מתוך עבודתו ברפואה פנימית ובנוירולוגיה, החל פרופ' חוברס להתעניין באנדוקרינולוגיה ואף היה בין הראשונים שקיבל תואר רופא מומחה בשטח זה בארץ. הוא התעניין במיוחד בתחום הנוירואנדוקרינולוגיה שבו תרם רבות מבחינה עיונית ומחקרית.
בשנת 1962 יצא פרופ' חוברס מטעם NIH להשתלמות באוניברסיטה של פנסילבניה, שם עבד בשיתוף עם פרופ' McCann שעבודתו הקנתה לו מעמד של חלוץ במחקר הנוירואנדוקריני בתחום הקשר בין ההיפותלמוס והורמוני יותרת המוח, ובעיקר בגילוי ובאפיון של הפקטור ההיפותלמי המזרז את הפרשת הגונדוטרופינים מיתרת המוח (מאוחר יותר, זיהוי סופי של פקטור זה כ- LHRH ע"י Shally הקנה לו פרס נובל).
עם שובו ארצה המשיך פרופ' חוברס את עבודתו במח' פנימית בביה"ח הדסה והועלה לדרגת פרופסור. במקביל לעבודתו כרופא, הוא הקים מעבדת מחקר לאנדוקרינולוגיה ניסויית במסגרת מחלקת עצבים.
פרופ' חוברס וקבוצתו עסקו בחקר מנגנונים עצביים ואנדוקריניים הקשורים בויסות חום הגוף ובתפקיד מערכת העצבים המרכזית בויסות הפעלת הורמוני הדחק. כמו כן, עסקה מעבדתו בחקר יחסי הגומלין בין ההיפותלמוס האינסולין ורמת הגלוקוז בדם. מחקריו של פרופ' חוברס הקנו לו שם בינלאומי בתחום הנוירואנדוקרינולוגיה. הוא הוזמן להציג את מחקריו בפני כנסים בינלאומיים ושהה כמדען אורח באוניברסיטאות ובמכוני מחקר מהחשובים בעולם.
לצד עיסוקו ברפואה, במחקר ובהוראה, מצא פרופ' חוברס זמן לתת שירותים רפואיים ללא תמורה לאוכלוסיה מעוטת יכולת בירושלים.
ב-1975 מונה פרופ' חוברס כמנהל המח' האנדוקרינית ומכון המחקר ע"ש רוגוף בביה"ח בילינסון. עם זאת, אהבתו לירושלים ולביתו בבית-זית ושאיפתו לעסוק ברפואה פנימית על כל היבטיה, הביאו אותו לקבל את הצעת ביה"ח "ביקור חולים" לנהל את המח' הפנימית. על אף הקשיים הרבים שבהם היה נתון ביה"ח, ובמיוחד המח' הפנימית, הצליח פרופ' חוברס, בזמן קצר יחסית, לארגן צוות רופאים ועובדים ולשנות כליל את פני המחלקה. ביוזמתו עבר ביה"ח שינויים ניכרים לקראת הפיכתו לבית-חולים מודרני ואוניברסיטאי. במסגרת שיקום המחלקה, הקדיש פרופ' חוברס תשומת לב רבה לשטח האנדוקרינולוגיה ובמיוחד לנושא הסוכרת. הוא הקים יחידת סוכרת עם ציוד מודרני וייחודי להדרכה, אבחון, טיפול ומחקר קליני.
במקביל לעבודתו בביה"ח "ביקור חולים", מונה פרופ' חוברס כמנהל השירות האנדוקריני של קופ"ח הכללית בירושלים. במסגרת זו הוא ארגן וניהל את מרפאת הסוכרת של קופ"ח בפרוז'ינין אשר סיפקה את שירותיה לאלפי חולי סוכרת במחוז י-ם.
פרופ' חוברס הקים וחינך דור של רופאים וחוקרים העוסקים ברפואה פנימית, אנדוקרינולוגיה וסוכרת. הוא הדגיש תמיד את חשיבות הגישה החמה לחולה ובמיוחד לחולה הבודד והקשה.
פרופ' חוברס, שהיה מותיקי האגודה הישראלית לאנדוקרינולוגיה, נפטר באופן פתאומי ב- 3.2.89. לאחר מותו, יסדה משפחתו פרס לזכרו לשם קידום המחקר האנדוקריני בישראל. הפרס מוענק לחוקר צעיר, מתחת לגיל 45 עבור עבודה בתחום האנדוקרינולוגיה שפורסמה בשנה האחרונה (או עומדת להתפרסם).

זוכי פרס חוברס

1992- דניאל מלול
1993 - טלי נוה-מני
1994- ליאורה שוקובסקי
1995 - איריס קרן-טל
1996- קרן פז
1997 - פואד פארס
1998 - אסף רודיך
1999- סיגל כורם
2000 - אפרת וורטהיימר
2001 - אלון חן
2002- רינה המי
2003 - יעל קלמה
2004 - שלומי לזר
2006- אמיר תירוש
2007- נועה שר וערן גרשון
2008- עירית מיבר-לוי
2009- עידו וולף
2010- מוריר חמאיסי

IES Hans Lindner Award 2010

The recipient of the Hans Lindner Prize 2010 is Dr Gil Leibowitz, Associate Professor at the Department of Endocrinology and Metabolism at the Hadassah Hebrew University Medical Center.

Prof. Leibowitz is a fine example of the clinician-scientist. Over the last 5 years, he published 10 original articles, 3 reviews, and a chapter in The Joslin's Diabetes Mellitus, the most prestigious textbook of clinical diabetes. His research interests are divided between clinical and basic. The clinical aspects of his contributions include a protocol design for in-hospital management of diabetic patients, and design of an intensive insulin therapy protocol that improves glycemic control and clinical outcomes after heart surgery.

In his basic research, Prof. Leibowitz recent studies unraveled rather counter-dogma insights on the signaling mechanism by which glucose regulates insulin biosythesis; he studied the effect of insulin signaling on regulation of the beta-cell mass; revealed a potential involvement of mTOR on beta-cells under hyperglycemic stress, and the role of mTOR in hyperglycemic induced dysfunction of beta-cell under oxidative and endoplasmic stresses. The latter two pathways are also in the center of Prof. Leibowitz's cutting-edge studies on the mechanism of beta-cell apoptotic death. The Israel Endocrine Society is blessed to have an excelling physician-investigator like Prof. Leibowitz, and found him most worthy of The Hans Lindner Prize.

IES Israel Chowers Award 2010

The recipient of the Israel Chowers Prize this year is Dr Mogher Khamaisi from the Rappaport Faculty of Medicine, Technion, Haifa. Dr Khamaisi was selected for the Prize for his outstanding publication during 2009, entitled "Role of Protein Kinase C in the Expression of Endothelin Converting Enzyme-1", published in the March issue of Endocrinology 150:1440–1449, 2009.

Doctor Khamaisi received all his degrees- B.Sc. to MD/PhD- in Ben-Gurion University of the Negev. After postdoctoral studies and Residency in Internal Medicine at the Hebrew University-Hadassah Hospital, he moved to Rambam Medical Center where he is now Senior Lecturer and Internal Medicine Specialist with Sub-Specialty in Endocrinology. Dr. Khamaisi is senior author in over 20 peer-reviewed publications and reviews, and in the past 5 years he received several Young Investigator Awards and presented his studies in high profile meetings. The Israel Society of Endocrinlogy appreciates Dr. Khamaisi contribution, motivation and perseverance that led him to the excellent published study chosen for this Prize.

Glucocorticoids and developmental 'programming' of brain and body

Jonathan Seckl

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Epidemiological evidence suggests that an adverse fetal environment permanently programmes physiology leading to increased risks of cardiometabolic, neuroendocrine and psychiatric disorders in adulthood [1]. We originally hypothesised that prenatal stress via fetal glucocorticoid excess might explain this link [2]. Indeed, in rodents, prenatal stress, glucocorticoid exposure or inhibition/knockout of 11ß-hydroxysteroid dehydrogenase type 2 (11□-HSD2), the feto-placental 'barrier' to maternal glucocorticoids, reduces birth weight and causes permanent hypertension, hyperglycaemia, increased hypothalamic-pituitary-adrenal (HPA) axis activity and anxiety-related behaviours in adult offspring [3]. The phenotype persists into a second generation and transmits via male and female lines. This implies epigenetic mediation, a mechanism emerging for at least HPA axis programming. This also appears of potential clinical relevance. Thus, in a singleton-bearing, non-human primate model, exposure to glucocorticoids in the second half of gestation programmes cardiometabolic, HPA and behavioural parameters in 1-year old offspring. In humans, placental 11□-HSD2 activity correlates directly with birth weight and inversely with infant blood pressure. Moreover, low birth weight babies have higher plasma cortisol levels throughout adult life, indicating HPA programming [4]. Indeed, maternal glucocorticoid therapy or ingestion of liquorice (which inhibits 11β-HSD) alters offspring cognition and affect [5]. Stress has similar effects since pregnant women exposed to the 9.11.2001 atrocity and who developed PTSD 'transmit' neuroendocrine changes to their one-year old offspring, but confined to third trimester exposure. Furthermore, exposure to the Nazi Holocaust exerted permanent effects upon glucocorticoid levels and steroid metabolism, effects dependent upon the age at exposure [6]. Second generation effects also occur. Overall, the data suggest that developmental exposure to excess glucocorticoids/stress programmes peripheral and CNS functions in adult life, predisposing to affective and other pathology, and may be transmitted into at least one subsequent generation.

The genomics and epigenomics of smoking in pregnancy

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Over the past century, studies of development and reproductive biology have transcended our understandings of what constitutes heritability and the acquisition of phenotypic traits from one generation to the next. In recent years such investigations have evolved to focus on the role of *epigenetic modifications* to DNA and core histones in higher mammalian developmental processes. Two decades of research has led us to appreciate that one's epigenetic signatures are the net outcome of genotype, developmental lineage, and environmental exposures.

Our lab is dedicated to studying the effects of the *in utero* milieu on epigenetic changes in the human and non-human primate fetus in order to elucidate the mechanisms involved in aberrant regulation of fetal growth potential leading to risk of adult disease. In our human translational studies, we have focused our efforts on discerning the effects of a specific environmental exposure *in utero* (maternal tobacco use) on a single adverse fetal outcome (small for gestational age; SGA).

The metabolic pathways utilized by higher eukaryotic organisms to deal with potentially carcinogenic xenobiotic compounds from tobacco smoke have been well characterized. Carcinogenic compounds such as polycyclic aromatic hydrocarbons are metabolized sequentially in two-phases: in phase I *CYP1A1* catalyzes conversion into harmful hydrophilic DNA adducts, while in phase II *GSTT1* enables excretion via conjugation into polar electrophiles. In an effort to understand susceptibility to *in utero* tobacco exposure, we first characterized known metabolic functional polymorphisms and demonstrated that while deletion of fetal *GSTT1* significantly modified birth weight in smokers, no polymorphism fully accounted for fetal growth restriction. Since smoking upregulates *CYP1A1* expression, we hypothesized that non-allelic (epigenetic) dysregulation of placental *CYP1A1* expression via alterations in DNA methylation (meCpG) may further modify fetal growth. In the present manuscript, we compared placental expression of multiple CYP family members among gravidae, and observed significantly increased *CYP1A1* expression among smokers relative to controls (4.4-fold, $p<0.05$). To fully characterize *CYP1A1* meCpG status, bisulfite modification and sequencing of the entire proximal 1 kb promoter (containing 59 CpG sites) was performed. CpG sites immediately proximal to the 5'-XRE transcription factor binding element were significantly hypomethylated among smokers (55.6% vs 45.9% meCpG, $p=0.027$), a finding which uniquely correlated with placental gene expression ($r=0.737$, $p=0.007$). Thus *in utero* tobacco exposure significantly increases placental *CYP1A1* expression in association with differential methylation at a critical XRE element.

Epigenetic plasticity in embryonic stem cells

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Normal development appears to take place through a unidirectional process characterized by a step-wise decrease in cell potency, and it is presumably this phenomenon that is mainly responsible for the difficulty in reprogramming of differentiated somatic cells in vivo. We showed that the histone methylase G9a is a master structural regulator that plays an important role in early development by targeting a wide network of embryonic genes for post-implantation repression. This silencing process includes key genes, such as Oct-3/4, Nanog and Dnmt3L that are intimately involved in maintaining the embryonic stem cell phenotype and for establishing maternal imprints in mammalian germ cells. As for the Oct-3/4 gene, it undergoes a novel multi-step program of inactivation that involves direct inhibition of transcription, heterochromatinization through the tri-methylation of H3K9 and subsequent DNA methylation that represents the major barrier to embryonic reprogramming.

microRNAs (miRs) are important regulators of post-transcriptional gene expression and are critical for stem cell maintenance and differentiation. miRs have also been shown to be involved in cancer pathogenesis as well. Most recently, we have shown that G9a also regulates the expression of a number of miRs both in undifferentiated embryonic stem (ES) cells and in early differentiated cells. Moreover, Gene Ontology analysis showed that these miRs are involved in several cellular and developmental functions and aberrantly regulated in various types of cancer. This work suggests a global mechanism for regulation of miRs during early differentiation of ES cells. This mechanism seems to imitate the silencing of many oncogenic miRs during early development whose de-repression in somatic cells may contribute to the development of cancer.

DNA methylation and regulation of IGF1R expression in cancer progression

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The involvement of the insulin-like growth factors (IGF1, IGF2) in cancer biology has been the focus of extensive research. Epidemiological studies revealed that elevated serum IGF1 is associated with increased occurrence of various tumors, including breast, prostate, and colorectal cancer. The IGF1 receptor (IGF1R) is overexpressed in most malignant cells, where it displays a potent antiapoptotic activity. Regulation of IGF1R gene expression is an important mechanism that allows the cell to 'decide' whether to proliferate, go into arrest, or apoptose. Control of IGF1R gene expression at the transcriptional level has been identified as a key regulatory level. Using a novel DNA affinity chromatography protocol linked to mass spectroscopic proteomic analyses we identified a number of nuclear proteins with oncogenic or antioncogenic properties that regulate IGF1R gene transcription. Transcription factors with tumor suppressor activity, including p53, BRCA1, Von-Hippel Lindau (VHL), Wilms' tumor-1 (WT1), and others, were shown to negatively regulate IGF1R 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 their molecular targets, including the IGF1R gene. On the other hand, gain-of-function mutations of oncogenes are usually associated with increased transactivation of the IGF1R promoter and/or augmented phosphorylation of its cytoplasmic domain and downstream signaling molecules. In addition, we evaluated the methylation status of the IGF1R and androgen receptor genes in a series of prostate cancer cell lines corresponding to early (benign) and advanced (metastatic) stages of the disease. Results of 5-Aza-2'-deoxycytidine experiments, methylation specific PCR, and sodium bisulfite-direct DNA sequencing revealed that the androgen receptor promoter is hypermethylated in metastatic, but not in benign, prostate cancer cell lines. On the other hand, no methylation was seen in the IGF1R promoter at any stage of the disease. In summary, interactions between transcriptional and epigenetic mechanisms may ultimately determine the level of expression of the IGF1R gene and, consequently, the proliferative status of the cell. Understanding the molecular basis of these interactions will be of significant value both in basic as well as translational terms.

The gender-specific approach to diabetes: what is known and what is yet to learn

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Service for Endocrinology, Hadassah Medical School, The Hebrew University

Gender-specific care of diabetic patients is poorly developed, with few applications in the daily practice of endocrinologists and diabetologists. The large, prospective trials made in the past 20 years have assumed that results related to efficiency of glucose-lowering therapies as well as management of hyperglycaemic-related complications could be applied without distinction to men and women. Whereas a much higher number of men than women were included in these trials, no gender-specific analysis of the results was made. Actually, beyond obvious gynecologic considerations (gestational diabetes mellitus and pre-conception care), which concern diabetic women solely, the *Standards of Medical Care in Diabetes Mellitus* annually published by the American Diabetes Association gives little or no consideration to gender-specific recommendations. However there are significant differences between diabetic women and men that can be found in the medical literature. In this presentation, I will review and discuss the most relevant topics: some known risk factors for developing diabetes mellitus differ between men and women; different sensitivity of screening and diagnostic tests (fasting glucose, oral glucose tolerance test) for diabetes mellitus in men and women; all cardiovascular risk factors (hypertension, hyperlipidemia, obesity, inflammation) in diabetic women have worst effects on mortality and morbidity than in diabetic men; some medications, such as thiazolidinediones, do not have the same safety profile in diabetic women and men (significantly more fractures in the former than in the later) whereas another drug such as aspirin has a different gender-efficacy profile in primary cardiovascular prevention.

These observations could be regarded as trivial, were it not for a distressing clinical endpoint observed in a recent meta-analysis: diabetic men benefitted from a significant improvement in total cardiovascular and all-cause mortality in the last 30 years, whereas diabetic women had no improvement at all. So what is wrong in the standard management of diabetic women which can explain such inequality? Answering this question is challenging. For example, data suggest differences in pathophysiology of insulin resistance and endothelial dysfunction between men and women, thus partly explaining increased cardiovascular morbidity mortality. Furthermore, it appears that women are usually less aggressively treated than men. The combination of both factors may be particularly deleterious to diabetic women.

The concept of *gender-medicine* is gaining in popularity and influence in many fields, but is only at its infancy in regard to management of diabetes mellitus. The first lesson we have to learn is that women should be treated at least as well as men, and reach the same therapeutic targets. Only then we may identify the critical factors that need to be addressed to improve gender-specific care of patients with diabetes mellitus, and maybe change the therapeutic goals for women.

One system, two genders: bone and gonadal steroids

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Mammalian bones cells express low concentrations of specific intracellular and membranal receptors for estrogens and androgens. Those cells also respond to the hormones by modulating different parameters. In our studies we demonstrated that: rat bone *in vivo* and rat bone cells *in vitro* responded sex-specifically to gonadal steroids in stimulation of both cell proliferation as measured by monitoring DNA synthesis and the specific activity of the BB isozyme of creatine kinase, a marker for hormonal responsiveness. Similar results were also demonstrated *in vivo* in mouse bone and in primary cultures of human osteoblast-like cells *in vitro* and in cell lines from both rat and human bone. In these systems female- derived bone responded only to estradiol-17β (E2) and male- derived bone cells responded only to dihydrotestosterone (DHT). The epiphyseal cartilage cells both *in vivo* and *in vitro*, on the other hand, responded to both E2 and DHT. The sex-specific response of bone cells to gonadal steroids was modified by manipulation of the endocrine environment in early development as was demonstrated in young rats or mice after gonadectomy, in pre-natally or neo-natally androgenized female rats and in androgen- receptor deficient (Tfm) male rats. Vitamin D and its non-hypercalcemic analogs up-regulated the sex-specific response of skeletal tissues both *in vivo* and *in vitro* from rat, mice and human. Bone marrow (BM) which contains committed osteo-progenitor cells, when transplanted into mice under the renal capsule formed after 21 days bone ossicles originated from the donor cells. The response of this new bone to E2 or DHT was according to the gender of the donor. On the other hand demineralized bone or tooth matrix (DTM) particles implanted under the skin induce bone formation by the pluripotent mesenchymal cells of the recipient, which responded to E2 and DHT according to the gender of the host. BM from femoral rat bone in culture was differentiated into bone cells which responded to the gonadal steroids sex- specifically according to the origin of the donor, with the loss of this specificity in bone cells originated from BM from gonadectomized rats. Human osteoblasts showed also sex-specific responsiveness not only to E2 but also to the estrogen mimetic anti-idiotypic antibody and to different native and synthetic phytoestrogens. The sex-specific response was also demonstrated in both human and rat bone cells by measuring membranal originated responses such as [Ca+2] mobilization. Of interest is the fact that male bone cells which did not respond to estrogenic compounds expressed estrogen receptors similar to female derived bone cells, suggesting post- receptor mechanism(s). It is also important to note that the "less differentiated" cells of the epiphyseal cartilage which can be considered as "pro-osteoblasts" and the "de-differentiated" bone cells derived from gonadectomized animals lost their sex-specific response to gonadal steroids. In conclusion cultured bone cells from different origins and mammals *in vivo* respond sex-specifically to gonadal-steroids by changes in both intracellular and membranal parameters, in a mechanism which is still not known. Whether this implies also for human bone *in vivo* is yet to be established.

From gender to transgender: sex hormones and the brain

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The brain is a sexually dimorphic organ. Brain structure differences that result from the interaction between sex chromosome genes, gonadal hormones and developing brain cells are thought to be the basis of sex differences in a wide spectrum of behavioral and cognitive characteristics. Important insights on the hormonal effects on gender identity and sexual orientation have been obtained from patients with disorders of sexual development. Nevertheless, the degree of masculinization of the external genitalia may not reflect the degree of masculinization of the brain and its impact on gender identity. Finally, transsexual people have genital differentiation concordant with chromosomal sex, with normal circulating levels of sex hormone steroids, suggesting that other as yet unidentified mechanisms are involved in gender identity disorders.