

Abstract Code: P43

A homozygous mutation R496C mutation in the CYP17A1 gene causes a severe disorder of sexual development

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Introduction: 17 hydroxylase/17,20-lyase (CYP17A1) deficiency is a rare autosomal recessive disorder of steroidogenesis, characterized by various degrees of glucocorticoid and androgen deficiencies. The resultant presentation may include relative cortisol deficiency and female external genitalia in 46 XY genotypic patients due to impairment of embryonic androgen synthesis. Both enzymatic activities are catalyzed by a single microsomal cytochrome P450c17 enzyme. Here we describe 2 cases with isolated 17,20-lyase deficiency caused by a homozygous gene mutation.

Patients/ Methods: Two cousins from 2 consanguineous Palestinian families, presented neonatally with a female phenotype and 46XY karyotype following extremely low gestational estriol levels. On ACTH stimulation 17OH pregnenolone, 17OH progesterone (17OHP), DHEAS and testosterone were low at 0 time and following stimulation. Basal aldosterone and PRA were initially high but decreased to low levels at 2.3y & 4.1y respectively. Blood pressure and electrolytes were initially and consistently normal. Cortisol levels stayed in the lower side of the normal range without replacement therapy. Both patients reared as females underwent gonadectomy at 1.5y and 3y of age respectively. Gonads of the younger sister were testes showing occasional PLAP and C-KIT positive germ cells in tubular lumina and along basement membranes, consistent with maturation delay. In the older sister, most tubules contained Sertoli cells only, with occasional, weakly positive C-kit cells.

Results: DNA was extracted from the index cases, their parents and healthy siblings. Using chromosome 10 microsatellite markers – D10S1709, D10S192 and D10S597 we found that both patients were homozygous at the CYP17A gene locus while their parents were heterozygous. Sequencing of the gene revealed a homozygous mutation in exon 8 of the CYP17A1 gene in both patients predicting a substitution of arginine (CGC) to cysteine (TGC) at the 496 amino acid (R496C). Parents were heterozygous as expected.

Conclusions: We describe a severe deficiency of the 17,20-lyase activity causing a male to female phenotypic change caused by the R496C homozygous mutation. The total absence of 17OHP and the presence of cortisol indicate both glucocorticoid activity by mineralocorticoid precursors and cortisol production by alternative pathways bypassing 17OHP. The initial high aldosterone and PRA decreasing later to low levels may support the hypothesis that a gradual increase in deoxycorticosterone eventually suppresses PRA, aldosterone and ACTH secretions. Finally, the

significance of C-kit positive germ cells in the gonads in our 3-year old patient is unknown. Early gonadectomy may be warranted.

Abstract Code: P44

Adrenal Cushing's Syndrome in Pregnancy: Laparoscopic resection in the third Trimester

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Introduction: Cushing's Syndrome (CS) in pregnancy is rare. In about 70% of cases it is associated with significant maternal and fetal morbidity and mortality. In pregnancy, adrenal adenomas underlie a disproportionately high proportion of CS cases. In pregnant women CS is often not detected until 12–26 wk of gestation, in part because changes in physical appearance are often ascribed to pregnancy itself. In pregnant women with confirmed CS, a low ACTH is a sign for an adrenal cause for CS and should prompt imaging of the adrenals. In most cases adrenal Ultrasound and Magnetic Resonance Imaging has been used effectively. Without proper treatment, live births are 76% compared to 89% in women in whom treatment is instituted. For adrenal CS in pregnancy, laparoscopic adrenalectomy is the treatment of choice. In the third trimester, fear of surgical complications makes medical treatment the preferred choice in many centers. Metyrapone, although effective, has the potential of exacerbation of hypertension and progression to preeclampsia, which may limit its use. In the rat, ketoconazole was found to cross the placenta and to be teratogenic and abortifacient, so that the drug is FDA category C. Similarly, mitotane is contraindicated as it has teratogenic effects.

Patients/ Methods: A 32 Y.O woman developed cushingoid signs for the last two years. She underwent fertility treatment and got pregnant. She presented on 28 week of gestation with legs and face swelling and an abnormal glucose tolerance test. UFC 1900 nmol/24h (n 55-299), 8am cortisol after 1 mg dex at midnight was 50 µg/dl (n 5-25) and basal ACTH level < 5.0 pg/ml (n 5-46). Adrenal Ultrasound and Magnetic Resonance Imaging revealed Rt adrenal mass 4x3cm.

Results: On week 32 of gestation the patient underwent laparoscopic Rt adrenalectomy by an experienced laparoscopic surgeon. No peri-operative maternal or fetal complications were encountered. After surgery the patient became hypoadrenal and was given glucocorticoid replacement therapy. The fetus was born on term, with a mildly low birth weight (2.480kg). On follow up, 2 month after the surgical treatment the patient still showing suppressed ACTH and cortisol levels, without stimulation on both low (1.0 µg) and high dose ACTH tests.

Conclusions: In the third trimester of pregnancy, for unilateral adrenal cortisol secreting adenoma, laparoscopic adrenalectomy if done by an experienced surgeon, should be considered as a possible mode of treatment.

Abstract Code: P45

Evaluation of the hypothalamic-pituitary-adrenal axis in patients with antiphospholipid syndrome

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Introduction: Insufficiency of the hypothalamic-pituitary-adrenal (HPA) axis is associated with the antiphospholipid syndrome (APS), being the commonest endocrinologic manifestation in this disorder. Adrenal vein thrombosis and/or hemorrhage leading to primary adrenal failure are the leading diagnoses, while another possible mechanism is autoimmune adrenal failure. Pituitary or hypothalamic insufficiency leading to secondary adrenal failure has been anecdotally reported. Prospective evaluation of the HPA axis in patients with APS was not performed before. **OBJECTIVE:** To evaluate the HPA axis in patients with APS.

Patients/ Methods: Ambulatory adult patients (older than 18 yrs) with APS were recruited and their clinical data obtained. Baseline labs included full chemistry, cortisol, corticotropin (ACTH), and aldosterone levels. Cortisol was also measured 30 and 60 minutes after a low dose (1 mcg) cosyntropin (synthetic ACTH) administration. Patients with a present or 6-months prior steroid treatment for more than 2-weeks period were excluded.

Results: 16 patients (11F/5M, mean age 43.4±15.6 years) participated in the study, 13 of whom had primary APS. Lupus anticoagulant and anticardiolipin antibodies were positive in 81 and 63%, respectively, with median duration of disease 3.5±5.9 years. Weakness, dizziness and nausea were reported by 50, 31 and 31% of the patients, respectively). However, weight loss, darkening of the skin and low blood pressure were reported by less than 20% of the patients. Sodium and potassium levels were 138.8±2mU/L and 4.5±0.4mU/L, respectively. 27% of the patients had fasting blood glucose below 80mg/dL. Baseline cortisol level was 13.1±4.7mg/dL (normal 7-25), and after cosyntropin stimulation 24.6±4.4mg/dL and 21.7± 6.4mg/dL (at 30, 60 minutes, respectively). One patient, that was treated with inhaled steroids 6 months prior to the test had baseline cortisol level of 3.9mg/dL, and stimulated cortisol of 18><20mg/dL. Another patient had stimulated cortisol of 18><20mg/dL. Aldosterone level was 59±59.5pg/ml (normal 10-160) and ACTH level was pg/ml 18.4± 9.7 (normal 9-52).

Conclusions: In our cohort patients with APS did not have insufficiency of the HPA axis. Partial adrenal insufficiency could not be excluded in two patients. Larger scale longitudinal studies are needed to define whether these patients will progress over time to overt insufficiency of the HPA axis.

Abstract Code: P46

Non classical 21-hydroxylase Deficiency - Prevalence in Males with Abnormal Sperm Analysis- A Case Control Study

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Introduction: Male factor accounts for about 40% of all cases of infertility and in about 50%, the cause is unknown. Non-classical 21-hydroxylase deficiency (NC-21-OHD) is a common autosomal recessive disorder associated with elevated adrenal-source androgens. High androgen levels cause gonadotropin suppression, leading to suppressed spermatogenesis, lower sperm concentration, and oligospermia. The aim of the study was to evaluate the prevalence of NC-21OHD in men with abnormal sperm parameters of unexplained etiology compared to normal controls.

Patients/ Methods: Design: Case control Setting: Major tertiary medical center. Participants: 515 healthy men being followed at a fertility clinic: 253 (mean age 34.0±6.3 years) with abnormal findings on sperm analysis (1999 WHO criteria) of unknown cause and 262 (mean age 34.8±6.4 years) with a normal sperm analysis. Interventions: Random mid-morning blood sampling to test for 17-hydroxyprogesterone (17-OHP) levels. Subjects with levels of >6 nmol/L underwent standard adrenocorticotrophic hormone (ACTH) stimulation test. Outcome measure: NC-21-OHD, defined as a stimulated ACTH level of >45 nmol/L.

Results: A serum 17-OHP level of >6 nmol/L was detected in 11 study patients (4.3%) and 14 control subjects (5.3%) (p=NS). Seven study patients and 8 controls subsequently underwent ACTH stimulation test, and none had levels compatible with a diagnosis of NC-21OHD. Mean 17OHP levels were similar in the two groups (3.3±1.4 nmol/L and 3.3±1.3 nmol/L, respectively). There was no correlation between sperm parameters and serum 17-OHP levels.

Conclusions: Until larger studies are performed, the routine measurement of 17-OHP in the evaluation of male infertility is not recommended.

Abstract Code: P47

The use of Metyrosine (Demser) in The Pre-operative Management of Pheochromocytoma

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Introduction: Patients with pheochromocytoma may develop life threatening cardiovascular and other complications, especially on induction of anesthesia or during surgery. The main goal of preoperative management of a pheochromocytoma patient is to normalize blood pressure, heart rate and restore volume depletion. Alpha and β adrenoreceptor antagonists, calcium channel and angiotensin receptor blockers in different combinations is frequently used. However, they do not reduce catecholamine production and release. This release during surgery, especially in very large tumors, may be dangerous despite adequate preparation. Continuous administration of saline before surgery is needed for expansion of intravascular volume and reduction of severity of postoperative hypotension. Alpha-Methyl-L-tyrosine or Metyrosine (Demser) is an analog of tyrosine which competitively inhibits tyrosine hydroxylase, the rate-limiting step in catecholamine biosynthesis. It significantly depletes catecholamine stores and is particularly used preoperatively in patients with biochemically large and active pheochromocytomas. Demser facilitates blood pressure control both before and during surgery. When used in combination, Demser results in less labile blood pressure during anesthesia, reduced intra-operative blood loss, and reduced volume replacement during surgery compared with the use of α - and β adrenoreceptor blockers alone. Despite the well-documented efficacy of Demser, its use has been adopted by only a few institutions. This is partially due to the limited availability of this drug and its side effects - most commonly sedation.

Patients/ Methods: 43 Y.O man, two years history of sustained hypertension, headache and weakness. Urinary free catecholamine 460 μ Gr/GCreat (n 15-115), urinary normetanephrine 30075 μ Gr/GCreat (n 0-600), urinary metanephrine 3977 μ Gr/GCreat (n 22-220). CT of the abdomen revealed 15cm Rt adrenal mass, MIBG scan showed contrast pooling in the adrenal mass. The diagnosis of pheochromocytoma was thus confirmed. The patient was put on pre-operative α , β , and calcium receptor blockade and two weeks before surgery Demser 250mg tid was added and was gradually titrated to 500mg tid. On the evening before surgery, the patient received 2000 cc saline 0.9% IV.

Results: The patient underwent thoraco-abdominal adrenalectomy. During surgery a short period of elevation of BP and pulse to 210/110 and 105 respectively was documented. Postoperatively, the patient suffered of a few hours of hypotension, treated successfully with saline. Histopathological examination confirmed the diagnosis of pheochromocytoma, without features of malignancy.

Conclusions: The use of Demser adjvantly to α and β blockers appears to result in better peri-operative blood pressure control.

Abstract Code: P48

A clinical test for DHEA sulfotransferase activity

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Introduction: DHEA and its sulfate ester are the most abundant hormones in the body. In its sulfated form, DHEAS is a product of DHEA sulfotransferase, which turns DHEA into DHEAS. Our objective was to develop a clinical test to assess the activity of DHEA sulfotransferase. The test is based on suppression of endogenous adrenal androgens by dexamethasone (DEX), providing DHEA as a substrate and measuring the product DHEAS.

Patients/ Methods: 15 young healthy women, age range 18-32 yrs, participated in the study during their follicular phase. They received 2 mg of DEX 24 hours prior to the activity test. A blood sample for DHEAS was taken at 0900 as a baseline. DHEA was orally administered at 50 mg /sqm. Blood samples for DHEAS were taken 1 and 2 hours later.

Results: Following a 24 HPA axis suppression, serum DHEAS was 2.8 ± 1.9 nmol/l. It increased to 6.0 ± 2.4 nmol/l by 1 hr and 10.7 ± 2.6 nmol/l by 2 hr, increases by 3.2 ± 1.9 and 7.9 ± 2.6 nmol/l, respectively. Peak generated DHEAS levels correlated negatively with the age ($r=-0.419$, $p<0.07$), degree of DHEAS suppression by dexamethasone ($r=-0.355$, $p<0.09$) and cortisol suppression by DEX ($r=-0.473$, $p<0.05$). It did not correlate with the BMI or with the in vivo activity of 11beta-hydroxysteroid dehydrogenase, as measured by cortisol generation from an administered cortisone. Preliminary results show enhanced DHEA sulfotransferase activity in patients with PCOS.

Conclusions: We offer a simple clinical test to evaluate DHEA sulfotransferase activity.

Abstract Code: P49

The IVS14 + 1G >A splice mutation in the AAAS gene causes Triple A Syndrome in a Palestinian Child - Clinical and Genetic Characteristics

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Introduction: Triple A (Allgrove Syndrome) is a rare autosomal recessive syndrome characterized by Alacrima, Achalasia and Adrenal insufficiency. Other features may include autonomic instability, neurological symptoms and hyperkeratosis of palms and soles. The recently identified AAAS gene located on chromosome 12 is considered responsible for the disease phenotype. Several mutations have been so far identified mainly in the Western European descent.

Patients/ Methods: A 9 years old Palestinian boy, born to consanguineous parents, was diagnosed with glucocorticoid insufficiency at 7.6 y of age following a severe hypoglycemic event. On evaluation his mother recalled the absence of tears (alacrima) since birth. Upper GI series revealed moderate achalasia. Histamine test showed a significantly delayed wheal and flare response. Family history was remarkable for a brother who had alacrima and died from pneumonia at 2 y of age. Genetic analysis of DNA from the proband and his parents using microsatellite markers on chromosome 12q13 (D12S368 and D12S83) was done.

Results: Genetic analysis of DNA from the proband and his parents using microsatellite markers on chromosome 12 flanking the AAAs gene (D12S368 and D12S83) found our patient to be homozygous and the parents heterozygous in the gene locus. AAAS gene sequencing revealed the patient to carry a homozygous mutation in the splicing site following exon 14 IVS14+1G >A.

Conclusions: Although Triple A syndrome is rare it should be considered in patients with adrenal insufficiency, to facilitate early treatment of other affected systems (GI) and enable prenatal and early clinical diagnosis in siblings prior to devastating consequences. The mutation in the splicing site following exon 14 is found for the first time in Palestinians which may indicate an early founder effect reported previously in families from Tunisia and Algeria.

Abstract Code: P50

Isoflavones treatment for familial hypercholesterolemia (FH) in children

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Introduction: There are contradictory reports on the effect of soy proteins on lipid profile, but few studies in children have consistently shown a positive, cholesterol-lowering effect. It has been postulated that this effect is mediated through the soy-containing isoflavones. In order to elucidate this issue, we evaluated the effects of soy-derived isoflavones in children with FH.

Patients/ Methods: 15 children with FH were recruited to the study. Inclusion criteria were total cholesterol (TC) above 200 mg/dl and fasting LDL-C above 130 mg/dl on two measurements. Children with metabolic and endocrine diseases and those with BMI > 95th percentile for age were excluded from the study. Isoflavones' containing candies with HPLC-approved concentrations were used to improve compliance of the children. After an intensive dietary treatment based on AHA step-1 diet over 12 weeks, participants with persistent elevated TC and LDL-C were randomly assigned to three 8-weeks long interventions: placebo, 16 mg/d and 48 mg/d of isoflavones. There was a washout time of two weeks between the intervention periods. Throughout the study, a monthly report of intake was obtained from each participant, and dietician instructions were delivered in line with AHA step-1 diet.

Results: One patient achieved normal TC after dietary period, and two others were withdrawn on their will. 12 children (8 F/4M) aged 8.8 ± 2.0 y (range 5.3-11.2 y) have completed the study, 11 of them were prepubertal and one girl was in early puberty. An adherence to the study protocol was assured by candies counting. Isoflavones had no effect on thyroid hormones, gonadotropins, sex-hormones and sex-hormone binding globulin. There was however a significant reduction in mean TSH levels in 16mg-isoflavones compared with the placebo. Neither low nor high dosage of isoflavones had any effect on lipid profile of the children (Table).

	Diet only	Placebo	16 mg/d isoflavones	48 mg/d isoflavones	p-value
Cholesterol	-4 ± 2	1 ± 4	0 ± 2	0 ± 2	ns
LDL-C	-4 ± 3	2 ± 5	0 ± 3	0 ± 3	ns
HDL-C	-4 ± 4	5 ± 4	2 ± 5	-3 ± 3	ns
Triglycerides	-9 ± 7	-4 ± 11	15 ± 12	12 ± 16	ns
APO-B	-3 ± 5	4 ± 4	2 ± 6	1 ± 5	ns
LP(a)	5 ± 10	13 ± 6	-5 ± 9	11 ± 6	ns

Conclusions: Isoflavones have no effect on lipid profile in children with hypercholesterolemia. Furthermore, although considered the preferred approach in these children, intensive dietary intervention yielded no benefit in our study.

Abstract Code: P51

Conservative treatment of L-asparaginase-associated lipid abnormalities in children with acute lymphoblastic leukemia

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Introduction: L- asparaginase is an important component of pediatric acute lymphoblastic leukemia (ALL) treatment protocols. Significant systemic adverse reactions of L-asparaginase have been described, with scarce reports of abnormalities in lipid metabolism. Severe hypertriglyceridemia (>1000 mg/dl) can cause acute pancreatitis and intermittent neurological manifestations. Lipid abnormalities, are generally transient nonetheless, extremely elevated triglycerides levels may necessitate immediate therapeutic intervention to prevent complications. The aim of this study was to determine the incidence and clinical consequences of lipids profiles abnormalities in children with ALL treated with L-asparaginase.

Patients/ Methods: The study population constituted 65 (39 males) newly diagnosed children and adolescents aged 0.4-22 years with ALL (N=61) or Lymphoblastic Lymphoma (N=4) which were treated according to ALLIC-BFM 2002 protocol between 2002-2005. 49 (75%) were from Jewish origin, 15 (23%) from Arabic origin and 1 from Cyprus. Fasting plasma lipids including cholesterol [C], low-density lipoprotein [LDL], liver functions and glucose levels were obtained. Triglycerides [TG] were measured in 42 subjects.

Results: Mean post treatment TG levels were 459±526 mg/dl (range 54-3009). 12 (28%) subjects had TG levels<200 mg/dl, 18 (43%) between 200-400 mg/dl, 3 (7%) between 400-600 mg/dl, 4 (10%) between 600-1000 mg/dl, and 5 (12%) >1000 mg/dl. No association was found between age, gender and TG level. 5/15 of the subjects with elevated TG were from Arabic origin compared with 7/49 from Jewish Origin. Children with TG levels between 400-600 mg/dl were fasted. Fibrates and heparin were given in those with levels>600 mg/d. One child developed parasthesias, decreased strength in his right arm, dysarthria and facial nerve palsy 3 weeks into the induction phase. MRI revealed left saggital sinus thrombosis and left frontal lobe infarct. TG level at the time of event was 2640 mg/dl. He was treated with low-molecular heparin, fasting and bezafibrate with complete recovery of neurological signs and normalization of TG levels. He did not received L-asparaginase during the reinduction phase due to this severe complication. In the study period of 36 months, we found no association between high TG levels and acute pancreatitis. Subsequent to the completion of chemotherapy, lipid profiles normalized in all the children with ALL.

Conclusions: Abnormalities in lipid profile in children with ALL during L-asparaginase therapy are relativity common. Early recognition and conservative

treatment may prevent significant complications necessitating either discontinuation of L-asparaginase therapy or major therapeutic interventions.

Abstract Code: P52

Osteoporosis in elderly fallers- underdiagnosed and undertreated

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Introduction: Falls are a major risk factor for osteoporotic fractures in the elderly. The use of diagnostic and therapeutic modalities in elderly fallers in Israel has not been assessed. Aims: to assess the use of bone densitometry (DXA) and osteoporosis (OP) treatment in elderly women with propensity to fall, to detect factors interfering with implementation of diagnostic and therapeutic procedures in this population.

Patients/ Methods: 242 community dwelling women, aged 65-91, that were hospitalized in the Western Galilee hospital after a fall. Methods: At hospitalization the patients (pts) were interviewed regarding medical history, previous fractures, nutritional status, DXA and the use of treatment for OP. Three months after hospital discharge a phone interview was performed with all participants. The pts were asked about diagnostic and therapeutic procedures for OP after hospital discharge

Results: 50 pts (20.6%) were diagnosed with OP before hospitalization (group A), 192 pts (79.4%) were not (group B), 164 (85%) of them received a written recommendation to perform DXA examination (group B1), 28 (15%) had not (group B2). There was no significant difference between the groups in socio-economic and nutritional status, health insurance and use of medications. 157 (95) of group B1 pts underwent post hospital discharge phone interview: 46 (24%) performed DXA, 24 (52%) were diagnosed with OP, 6 (13%) with osteopenia. Of 24 OP pts 14 (58%) were treated with calcium + D before the diagnosis of OP, and all of them after diagnosis. After diagnosis 14 pts (58%) were treated with alendronate, 10 (42%) with risedronate. Of 6 osteopenic pts, 3 (50%) received calcium + D, none was treated for OP although two (30%) had previous OP fractures and according to WHO criteria had severe OP. Of group B2 one pt (3.5%) performed DXA and was diagnosed with OP, 8 (28.5%) pts received calcium + D. Reasons for not performing DXA: 50 (26%) pts didn't ask, 24 (12.5%) not referred after asking, 28 (14.1%) refused: 13 (6.8%) due to poor health, 13 (6.8%) due to distance, 2 (1%) due to economic problems.

Conclusions: In spite of high rate of OP and increased fracture risk, the disease in elderly fallers is underdiagnosed and undertreated. Recommendation in hospital discharge letter may improve the rate of diagnosis and promote treatment initiation.

Abstract Code: P53

Endocrine Complications in Adult β -Thalassemia Major Patients

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Introduction: Endocrine complications in β -Thalassemia Major (TM) are classically considered to be the result of iron overload and deposition in the endocrine glands. Consequently to improved transfusion and chelating therapy in recent years, patients with TM survive longer, and more complications become clinically significant. Osteoporosis is very common among adult TM patients. The pathogenesis of bone disease is multifactorial, including environmental as well as genetic factors. Bone marrow expansion, direct iron toxic effect on osteoblasts, iron-chelation therapy, hepatitis, deficiency of growth hormone or insulin growth factor 1, and hypogonadism have been reported to be risk factors in these patients. We present an analysis of the endocrine status in adult TM patients. The aim of the analysis was to identify new possible risk factors contributing to endocrine complications, especially osteoporosis, in adult TM patients.

Patients/ Methods: Twenty-five TM patients from the Clinic of Thalassemia and Hemoglobinopathies, Institute of Hematology, followed at the Endocrinology Institute, in Rabin Medical Center, Beilinson Hospital, were included in this study. The medical records were reviewed for data including gender, date of birth, ethnicity, familial status, age of menarche in women, presence of hypogonadism, hypothyroidism, hypoparathyroidism, and diabetes mellitus, vitamin D level, and bone mineral density (BMD). Compliance to hormone replacement therapy was assessed by history and plasma hormone measurements.

Results: Of the 25 patients, 15 were males and 10 females. The median age was 29 years (range 19-38). Twenty-one patients were of Arab origin, 3 were Sephardic Jews, and one was of Jewish Yemenite origin. Twenty-one patients (84%) had at least one endocrine-related complication, 16 (64%) had more than one complication. The most common endocrine complication was osteoporosis, found in 15 out of 20 patients (75%). Three patients had osteopenia, and only two patients had normal BMD results. BMD data of additional 5 patients were not available.

Hypogonadotropic hypogonadism was diagnosed in 12 out of 15 males (80%) and in 7 out of 10 females (70%), four women had primary and 3 had secondary amenorrhea. Non-compliance to testosterone replacement treatment was found in at least 6 of the 12 hypogonadal men based on history and/or repeated plasma testosterone measurements. The mean random plasma testosterone level in the hypogonadal men group was 4.85 nmol/L (normal range: 9.9-27.8 nmol/L). Among the 10 hypogonadal women, non-compliance to estrogen-progestin treatment was detected in 3 per history. Additional endocrinopathies were diagnosed in the minority of the patients: 5 patients (20%) had primary hypothyroidism and 4 patients (16%) had hypoparathyroidism. Abnormal glucose metabolism was found in 9 patients (36%): one had insulin-treated diabetes, 3 had non-insulin treated diabetes and an additional 5 patients had impaired fasting glucose and/or impaired glucose tolerance. Low level of vitamin D was

detected in the majority of the patients. Among 23 patients in whom this data was available, 21(91.3%) had low vitamin D level (mean: 19.3ng/ml, normal range: 30-58ng/ml). Of the 21 patients, 9 had vitamin D deficiency (defined as <15ng/ml) and 12 had vitamin D insufficiency (defined as 15-30ng/ml), with an average level of 9.9 and 21.3ng/ml, respectively. Patients with vitamin D deficiency had lower BMD results than patients with vitamin D insufficiency.

Conclusions: The most common endocrine complication in adult Israeli TM patients is osteoporosis. Vitamin D deficiency and non-compliance to sex hormones replacement therapy are common and possibly treatable risk factors for osteoporosis in this population.

Abstract Code: P54

The modulation of the keratinocyte inflammatory response by vitamin D is trigger- specific and time-dependent.

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Introduction: The skin is the barrier between the host and the external environment. A threat to the integrity of the barrier leads to the cutaneous inflammatory response. Epidermal keratinocytes play an important role in the induction and maintenance of this response. Initially chemokines, adhesion molecules and cytokines induced in the keratinocytes are responsible for the recruitment and activation of immune cells. The immune cells in turn secrete pro-inflammatory cytokines that reinforce the keratinocyte inflammatory response. An efficient inflammatory response should be vigorous in its initial stages but should be self-limited to avoid damage to the healthy epidermis. Vitamin D metabolites and analogs are known for their anti inflammatory effects in the skin. The aim of this study was to examine the effect of calcitriol, the hormonal metabolite of vitamin D, on an in-vitro simulation of the various stages of epidermal inflammatory response.

Patients/ Methods: Human HaCaT keratinocytes cultured in the absence of any active mediator were exposed to the TLR3 agonist Poly IC to simulate viral infection initiating the inflammatory response and to TNF to simulate cytokines secreted by the recruited immune cells that are responsible for the maintenance of the response. The keratinocyte inflammatory response was assessed by measuring mRNA levels of 3 key inflammatory genes using real-time PCR: the chemokine IL-8, the adhesion molecule ICAM-1 and the cytokine TNF.

Results: All 3 genes exhibited similar induction pattern in response to challenge with Poly IC. The genes were induced rapidly peaking after 2h (TNF & IL-8) or 4h (ICAM-1) and decreasing thereafter, returning to their baseline by 12-24h after exposure. 24h pretreatment with calcitriol slightly inhibited IL-8 induction (35%), enhanced ICAM-1 induction but did not affect the induction of TNF. A short, 2h exposure to TNF increased the mRNA levels of the three genes. Only the induction of IL-8 was inhibited by calcitriol (70%), whereas the hormone did not affect the induction of ICAM-1 and TNF. A longer, 16h exposure to TNF revealed a different pattern: the mRNA level of the three genes increased and this increase was markedly inhibited by calcitriol (85%, 70% & 50%, respectively).

Conclusions: Epidermal keratinocytes contain an autonomous vitamin D endocrine system, they synthesize the hormone from its parent compound and react to it through the nuclear receptor, VDR. Our data suggest a novel role for this compound in regulation of the epidermal inflammatory response to a viral threat. Calcitriol allows it to proceed and even enhance its initial stages but attenuate the late stages and thus contribute to its timely termination.

Abstract Code: P55

Interrelation between Fibroblast Growth Factor (FGF) and Osteoprotegerin (OPG) in regulation of skeletal growth

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Introduction: Achondroplasia (ACH), a syndrome of asymmetric dwarfism which involves a fail in endochondral ossification (EO). The etiology of the syndrome is a point mutation in FGFR3, which causes a constitutive activation of the receptor. However, the precise mechanism leading to atypic chondrogenesis leading to ACH is not fully understood, nor there is any efficient treatment. Using FGF variants that specifically activate certain FGFR (produced by Prochon Ltd), we mimicked ACH physiologic state, and thereby unraveled the FGF regulatory effect on EO. Osteoprotegerin (OPG) which locally produced during EO, has been shown by us to act as a potent chondrogenic factor. In the currant study, we also examine the effects of OPG on ACH-derived primary chndrocytes, and its effects on FGF variants-treated WT-derived cells. Research hypothesis: we hypothesize OPG might reverse the FGF-induced antichondrogenic activities, in both FGFR3 over expression (achondroplasia) and FGF over-treatment.

Patients/ Methods: Mice-derived mandibular condyle primary chondrocyte (MCDC) cultures originated from wild type (WT) mice are treated with FGF variants or OPG, and ACH-derived cultures with OPG solely. Proliferation activity is assessed by MTT, PCNA and Thymidine incorporation into DNA. Quantitative IHC analysis of Type I, II, X collagens and aggrecan, Alcian blue assay and sulfate incorporation into proteoglycans served to determine differentiation. Effects on apoptosis are examined using TUNEL and active CASPASE 3 assays.

Results: FGF variants (FGF2v & FGF9v) increase proliferation rate. Sulfate uptake was increased by FGF9v and decreased by FGF2v. Total proteoglycans is lower in both FGFv-treated cultures. Proliferation rate in ACH-derived cultures is higher than that of WT. OPG seems to restrain this tendency. Sulfate incorporation is enhanced in ACH cultures, at earlier stage and declines earlier, thus causing a premature chondrogenesis. OPG reverses Sulfate incorporation pattern of the ACH cultures at early stages (up to 8 days).

Conclusions: FGF9v treated cultures resemble ACH derived cultures by presenting accelerated proliferation rate and a premature differentiation. OPG restrains accelerated proliferation activity of ACH and shift back its chondrogenesis rate thus might prevent early exhaustion of growth plate potential in ACH growth plate leading eventually to delayed skeletal growth. OPG seems to have an ameliorative effect on the accelerated differentiation rate of heterozygous ACH cultures, at an early chondrogenesis stages.

Abstract Code: P56

Severe PTHrP-Induced Postpartum Osteoporosis in a Patient after Total Thyroidectomy and Parathyroidectomy

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Introduction: Pregnancy and postpartum-associated osteoporosis is an uncommon condition characterized by the occurrence of fractures during late pregnancy or the puerperium. We present a patient with hypoparathyroidism following total thyroidectomy for papillary carcinoma, who presented with severe postpartum osteoporosis, as well as hypercalcemia and hypercalciuria

Patients/ Methods: A case of severe postpartum osteoporosis with hypercalcemia and hypercalciuria in a hypoparathyroid patient

Results: Metabolic evaluation performed at presentation revealed hypercalcemia of 11 ng/ml, hypercalciuria of 469 mg/24h, ALP of 122 mg/dl (normal 54-117), 25OHD3 -15 ng/ml (normal >30), normal serum protein immunoelectrophoresis and a detectable level of PTHrP (> 1 ng/ml, normal <0.5 ng/ml). Serum levels of bone turnover markers were markedly above premenopausal range: total procollagen type I amino-terminal (P1NP) 98 ng/ml (normal 15-58), collagen beta cross-laps (CTX) 1.2 ng/ml (normal <0.57). Bone mineral density at the lumbar spine was 3.8 SD below age matched mean and 2.3 SD below age matched mean at the femoral neck. After cessation of lactation, the PTHrP level became undetectable. Bone turnover markers gradually decreased into normal premenopausal range: P1NP 37, CTX 0.29 ng/ml.

Conclusions: This patient presented with severe postpartum osteoporosis, hypercalcemia and hypercalciuria. Such a combination of signs in a hypoparathyroid patient is unusual and has not yet been reported. Several factors may have contributed to the reduced bone mass in this patient, including increased PTHrP, deficiency of calcitonin, hypoparathyroidism, secondary amenorrhea and suppressive levothyroxine treatment.

Abstract Code: P57

Vitamin D3 derivatives and rosemary antioxidants cooperate in the antileukemic effects in a mouse model of acute myeloid leukemia

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Introduction: 1 α ,25-dihydroxyvitamin D3 (1,25D3) is a well-known differentiation inducer which have potential as an anticancer agent. However, it has a marked toxicity at pharmacologically active doses. Attempts to overcome this problem have recently focused on the synthesis of vitamin D analogs with lower calcemic effects. Alternatively, we have shown that in human myeloid leukemia cells the effects of low, non-toxic doses of 1,25D3 can be synergistically enhanced by plant polyphenols, e.g., carnosic acid from rosemary. The major aim of this study was to determine whether carnosic acid-rich rosemary extract (RE) and the low-calcemic 1,25D3 analog (19-nor-Gemini Ro27-5646) can cooperate in the antileukemic effect using a mouse systemic leukemia model in vivo.

Patients/ Methods: Proliferation and viability of murine myelomonocytic leukemia cells (WEHI-3B D-) was determined by Vi-Cell XR cell viability analyzer. Cell cycle progression was measured by DNA staining with propidium iodide and FACS. Differentiation was measured using non-specific esterase (NSE) staining (monocytic differentiation marker). The intracellular levels of reactive oxygen species (ROS) were measured with oxidation-sensitive fluorescent probe DCFH-DA by FACS. Glutathion levels were measured by the glutathion reductase recycling assay. NADP(H)-quinone oxidoreductase (NQO1) levels were evaluated by Western blotting. Leukemia was induced by intravenous inoculation of WEHI-3B D- cells in Balb/c mice. Separate groups of mice were treated with (i) vehicle, (ii) Ro27-5646 (intraperitoneally, 3 times a week), (iii) RE (mixed with food), and (iv) their combinations.

Results: When added alone, 1,25D3, its analog Ro27-5646, carnosic acid and RE inhibited WEHI-3B D- cell proliferation in vitro. When combined at low concentrations, rosemary agents and vitamin D derivatives cooperated in growth inhibition and differentiation. This was accompanied by G1 cell cycle arrest, without a cytotoxic effect. CA or RE reduced ROS levels and elevated glutathion levels. Combinations with 1,25D3 or Ro27-5646 did not increase this effect, but caused synergistic induction of NQO1. Mice inoculated with WEHI-3B D- cells developed systemic leukemia. Ro27-5646 and RE alone had slight effects while the combined treatment resulted in a substantial synergistic increase in the life span of leukemia-bearing mice (up to 80%), as compared to untreated animals.

Conclusions: These results indicate that vitamin D3 derivatives and plant polyphenolic antioxidants cooperate in the anti-leukemic effect not only in cell culture but also in the animal model, without toxicity. Thus, these combinations may be used as an alternative to conventional cytotoxic chemotherapy of myeloid leukemias.

Abstract Code: P58

Clinical characteristics and outcomes in patients with post transplant diabetes mellitus.

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Introduction: Post transplant diabetes mellitus (PTDM) is a well-known complication of immunosuppression in solid organ recipients. The "natural history" of diabetes in this population in Israel and its impact on overall patient outcome has not yet been described.

Patients/ Methods: We retrospectively reviewed the charts of 54 patients(mean age 53 years, 38% female) who received renal (50%), liver (35%), lung (10%) or heart (5%) transplants with subsequent development of post transplant diabetes mellitus at a single tertiary medical center between 2000 and 2005.

Results: During a period of 5 years 4/54 patients died (7.4%), half of them from sepsis. There were 45 hospitalizations throughout the post transplant period: 4 patients were hospitalized more than 3 times, 9 patients were hospitalized twice. Bacterial infections comprised the most common cause of hospitalization (47%). 36 % of patients were treated with insulin one year after the diagnosis. 72 % were compensated (HBA1C less than 7%) at last visit. The rate of macrovascular complications was 11%. 79.6% received antihypertensive treatment, 43% received lipid-lowering agents, and 24 % were treated with aspirin

Conclusions: PTDM is associated with high rate of bacterial infections that comprise the most common cause of patient morbidity and mortality at our tertiary medical center.

Abstract Code: P59

High glucose causes direct impairment of cellular activities in vitro associated with wound healing - Implication on skin pathologies in diabetes.

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Introduction: Diabetes mellitus is one of the most severe metabolic diseases affecting people worldwide. One major complication of diabetes mellitus is impaired wound healing leading to amputations and even death. Therefore, it is crucial to understand the mechanisms which are involved in the pathogenesis of this complication. In the present study we examined how the high glucose affects in vitro cellular activities that participate in the wound healing process.

Patients/ Methods: The studies were carried on skin epidermal keratinocytes, which are central players in the wound healing process, following cellular parameters of adhesion, migration, proliferation and apoptosis. In addition, we explored some of the molecular pathways involved in those parameters. Finally, we examined whether glucose has any effect on skin organization in a three dimensional skin co-culture model.

Results: The results demonstrate that high glucose has a negative effect on keratinocytes adhesion when both cells were pre-incubated and extracellular matrix (ECM) was produced in high glucose. Adhesion is also decreased when cells were plated onto ECM produced by cells grown in high glucose. Furthermore, cellular proliferation was decreased when cells were plated on this ECM. Moreover, adhesion is also decreased when only ECM proteins-collagen, laminin and fibronectin were directly exposed to high glucose environment, which is suggestive of a possible direct reaction between glucose and those ECM proteins. On the other hand, cells that were pre-incubated in high glucose attached more rapidly to various surfaces. Molecular analysis of cells-ECM interactions has demonstrated that in ECM produced under high glucose there was a lower expression of laminin 5, but a higher expression of cell's integrin alpha 3 – the receptor for laminin, collagen and fibronectin. These changes could explain the results in keratinocytes adhesion. Our studies further showed that insulin increases keratinocytes attachment ability, but only in the control group and not in the high glucose environment. Molecular analysis of insulin signaling at high glucose has revealed abnormalities in ERK and AKT activation. Furthermore, high glucose decreases keratinocytes proliferation but has no effect on migration and apoptosis. Finally, we followed skin organization using the 3D skin organotypic coculture. Our results demonstrated that high glucose leads to abnormal skin organization including several dividing layers and thinner suprabasal layers.

Conclusions: In summary, our results demonstrate that high glucose might cause direct impairment in wound healing. Therefore, new therapeutic approaches for diabetes should focus on the pathophysiology of the skin.

Abstract Code: P60

Imprinted Genes on Ch11p15.5 are Potential Regulators of Beta-Cell Replication

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Introduction: Beta-cell proliferation has been the subject of intensive research in the recent years because of its relevance to the treatment of diabetes. We utilized the rare genetic disorder "Focal Hyperinsulinism of Infancy (Focal-HI)" to expand our knowledge about beta-cell turnover. Focal-HI is caused by benign proliferation of beta-cells within a limited region of the pancreas resulting in adenomatous hyperplasia. The genetic etiology of focal-HI includes loss of the maternal allele of a small region of chromosome 11 (Ch11p15.5) and duplication of the paternal allele. We previously demonstrated increased beta-cell proliferation in focal HI [Kassem, Diabetes 49(8): 1325-33, 2000] suggesting that this phenomenon could be attributed to the loss of paternally imprinted tumor suppressing genes that are normally expressed only from the maternal allele. A large number of genes exist on the involved region of Ch11p15.5, however, only a few are candidate tumor suppressors and also paternally imprinted. P57kip2 is one such gene that is expressed in normal human beta-cells and lost in focal HI [Kassem, Diabetes 50(12): 2763-9, 2001]. In this study we examined the expression of other genes on Ch11p15.5 in human beta-cells and in focal HI. These included 2 candidate tumor suppressor genes (PHLDA2 & SLC22A18), one gene associated with intestinal neoplasia (ASCL2) and one gene encoding a chaperone protein associated with chromatin assembly (NAP1L4).

Patients/ Methods: Archival pancreatic tissue from 2 focal-HI patients and 5 controls obtained at autopsy were used. Each Focal-HI sample included pancreatic tissue from inside and outside the focal lesion. Tissue sections were double stained for insulin, using guinea pig anti human insulin antibody visualized with anti guinea pig FITC labeled secondary antibody and for each of the 4 genes, using commercially available primary antibodies visualized by the streptavidin biotin-peroxidase system.

Results: Normal human islets stained positive for PHLDA2, ASCL2 and SLC22A18. Staining was restricted to insulin-positive cells, however distributed differently from insulin within beta-cells. Staining for NAP1L4 was seen only in insulin negative cells outside the islets. The staining within the focal lesion for PHLDA2, ASCL2 and SLC22A18 was markedly reduced compared to normal islets outside the lesion, confirming paternal imprinting.

Conclusions: PHLDA2, ASCL2 and SLC22A18 are expressed and paternally imprinted in human beta-cells. The loss of PHLDA2 and SLC22A18 may contribute to the increased beta-cell proliferation seen in focal HI, and thus may be important regulators of beta-cell replication.

Abstract Code: P61

Impaired Insulin Signaling is Associated with Decreased Incidence of Experimental Skin Cancer.

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Introduction: It has been shown that diabetes mellitus is associated with an elevated cancer risk, which is attributed to the hyperinsulinemia associated with the pre and early diabetic stages. This fact suggests that over-activation of insulin signaling might lead to cellular transformation, and that disruption of insulin signaling might result in reduced carcinogenic potential. Previously we have demonstrated that insulin plays a direct role in normal skin turnover, namely the balance between proliferation, differentiation and cell death. Therefore, disruption of insulin signaling might lead to an imbalance between these processes and contribute directly to the incidence of skin carcinogenesis.

Patients/ Methods: This hypothesis was tested in skin-specific IR knockout mice (SIRKO), where disruption of IR expression is restricted to the epidermal layer of the skin. Skin tumors were induced according to the initiation-promotion carcinogenesis model.

Results: We examined the kinetics of tumor formation (tumor multiplicity and incidence), histopathology and expression of specific epidermal markers, and found that lack of IR expression in skin resulted in a marked decrease in tumor induction efficiency and tumor proliferation capacity. Next we studied which stage in the carcinogenesis process was disrupted by the ablation of the IR. While there was no difference in the initiation stage, the promotion stage of the carcinogenesis was inefficient in SIRKO mice compared to the control group. In order to reveal the mechanism underlying the decreased carcinogenic potential of the skin associated with the lack of IR, we followed the turnover rate of the epidermis, as well as the transformation potential of the keratinocytes in vitro. We found that on one hand, the exit of keratinocytes from the basal layer was accelerated in SIRKO epidermis, suggesting that the initiated cells do not remain in the tissue for the sufficient time period in order to undergo the molecular events necessary for the promotion stage of skin carcinogenesis. On the other hand, expression of Ha-Ras oncogene in primary cultured keratinocytes transformed the control, but not the IRKO cells. Ha-Ras transformed keratinocytes lacking the expression of IR exhibited different morphology compared to the control cells and failed to proliferate further in response to the promotion agent.

Conclusions: Our data provide evidence that complete ablation of the IR in the epidermis reduces tumor potential of the skin, in a mechanism that combines both turnover rate of the epidermis and intrinsic properties of the keratinocytes. Further understanding the regulation of skin turnover by insulin signaling will hopefully lead to development of new treatments for various skin pathologies associated with impaired insulin signaling.

Abstract Code: P62

Insulin Increases PKC Delta in Isolated Nuclei from L6 Skeletal Muscle Cells

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Introduction: Protein kinase C (PKC) isoforms are involved in the transduction of a number of signals important for the regulation of cell growth, differentiation, apoptosis as well as effects of drugs and hormones such as insulin. PKC Delta was found to be a key molecule in the insulin signaling pathway. PKC proteins are believed to reside in the cytoplasm in an inactive state, and after phosphorylation, translocate to the plasma membrane or to membranes of cytoplasmic organelles to become fully activated. Evidence has accumulated that PKC isoforms may also have an important role in the nucleus. We recently showed that insulin induces a rapid increase in PKC delta RNA and protein in various cell fractions including the nucleus. In this study we attempted to elucidate the mechanism of the insulin-induced increase in nuclear PKC Delta and to investigate the involvement of Insulin receptor (IR) in this process .

Patients/ Methods: Studies were performed on L6 skeletal myoblasts. Nuclei were isolated from L6 cells and then were treated with insulin. Purification of the nuclear preparation was verified by SEM and by Western blotting for tubulin and other cell fraction markers. Standard procedures were used for Western blotting and immunostaining.

Results: insulin induced an increase in PKC Delta protein from isolated nuclei as it did in nuclear extracts from whole cells. This elevation was reduced by pretreatment with transcription and translation inhibitors. Insulin also induced an increase in IR protein levels in the nucleus, but not in isolated nuclei. The elevation in IR in the nucleus was due to translocation, as pretreatment with the import inhibitor-WGA blocked this elevation in IR protein. On the other hand, pretreatment with WGA did not block insulin-induced increase in PKC Delta protein levels. Moreover, pretreatment with export inhibitor-LMB did not reduce the insulin-induced increase in nuclear PKC Delta protein levels. Inhibition of nuclear IR by incubation with IR antibody before addition of insulin blocked the insulin-induced increase in nuclear PKC Delta protein and RNA.

Conclusions: This study suggests the possibility that PKC delta protein may be translated in the nuclei of skeletal muscle cells in response to insulin, closely time-linked to its transcription in the nucleus and IR might be involved in this process. This possible mechanism might explain the rapid increase in PKC delta protein in the nuclear fraction, which appears to be unique to insulin. The idea of rapid transcription and translation of proteins is highly unconventional and runs counter to current concepts of protein synthesis. However, this mechanism may be unique for insulin and PKC.

Abstract Code: P63

Conserved Charged Residues Essential for the Function of Human Epithelial Sodium Channel (ENaC) alpha subunit

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Introduction: Molecular analyses of epithelial sodium channel (ENaC) genes in multi-system pseudohypoaldosteronism patients as well as in vitro studies have demonstrated that the three subunits of ENaC are essential for ENaC activity in humans. All three subunits share sequence and structural homology with two transmembrane segments embedded in the membrane and a large (455-465 amino acids) extracellular domain. Previous site-directed mutagenesis studies have identified amiloride binding sites in ENaC subunits and additional residues involved in ion transfer. However, we have little knowledge of the structure and function of the largest extracellular domain of ENaC subunits and how they interact with each other in the final assembly of the channel. By inter-species sequence comparisons we have selected 16 conserved charged residues in the extracellular domain of the human alpha ENaC subunit, to examine their roles by site-directed mutagenesis studies.

Patients/ Methods: We have generated three cDNAs encoding for the alpha, beta and gamma subunits of human ENaC and subcloned these in plasmid pGEM-HJ. The mutated forms of the alpha subunit were generated using a modification of the Stratagene site-directed mutagenesis method. Both the wild type and the mutated forms were completely sequenced using an ABI 310 Genetic Analyzer to ascertain that the sequences carried the introduced mutations without any additional change. In vitro transcriptions of the cDNAs were carried out using T7-RNA polymerase. The cRNAs (3 ng for each subunit) were micro-injected into immature stage V-VI *Xenopus* oocytes that were dissociated with 0.3mg/ml type 1A collagenase. The oocytes were incubated at 18°C in ND-96 medium (in mM: 96 NaCl, 2 KCl, 1 CaCl₂, 1 MgCl₂, and 5 HEPES, pH 7.4) containing 2.5 mM sodium pyruvate, 50 µg/ml gentamicin and 10 mM amiloride. ENaC dependent amiloride-sensitive whole-cell inward Na⁺ current was measured 2-3 days after cRNA injection using the two-electrode voltage-clamp method while oocytes were clamped at -80 mV and continuously superfused with ND-96 +10 mM amiloride and ND-96 alternately at 22-25°C. Data were collected and analyzed using pClamp software.

Results: To examine the importance of the charged residues we generated 16 different constructs where alanine (that carries a neutral side chain) was substituted instead of the selected charged residues. Alanine substitution resulted in up to 60% inhibition of the ENaC activity. But conservative mutations keeping the charge of the residue, i.e. Asp for Glu, Glu for Asp, Lys for Arg and Arg for Lys in seven of the selected sites did not significantly inhibit ENaC activity.

Conclusions: The lack of an inhibitory effect of the conservative substitutions indicated changing amino acid while maintaining charge did not perturb the function of the channel. In contrast Ala substitution results revealed that most but not all charged residues examined are essential for the activity of the ENaC complex. We are currently examining the possible roles of some of these sites in subunit interactions and generating mutants in the other two subunits of ENaC.

Abstract Code: P64

The first description of a syndrome consisting of severe FTT and GH resistance due to a deletion of POU1F1, CHMP2B and VGL-3 genes

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Introduction: This is the first description a new syndrome in a girl with combined pituitary hormones deficiency (CPHD) and unresponsiveness to growth hormone (GH) treatment

Patients/ Methods: A 4 years old girl born at 28th week of gestation, with a birth weight of 994 gr (AGA), to healthy 1st degree cousins of Israeli-Arab origin. Central hypothyroidism (fT4 - 0.7 ng/ml and thyroid-stimulating hormone (TSH) - 0.05 mIU/L) and undetectable prolactin levels (< 5ng/ml) were diagnosed shortly after delivery and L-thyroxine treatment was initiated. Adrenal axis was intact. No hypoglycemic episodes were noted during the perinatal period. GH deficiency (<0.05 ng/ml) was diagnosed at the age of 6 months because of severe failure to thrive and physical findings (prominent forehead, depressed nasal bridge, micrognathia, and small hands and feet) characteristic to GH deficiency. Despite adequate GH and L-thyroxine treatment, and relatively appropriate caloric intake for BMR, her growth rate remained very disappointing and her IGF1 levels were undetectable (< 25 ng/ml). Chromosomal analysis revealed a 46XX karyotype. Because of the combination of GH, TSH, and prolactin deficiencies genetic analysis was performed by PCR and sequencing

Results: Cytological analysis of chromosome 3 did not reveal any gross abnormalities. PCR amplification of the POU1F1 gene suggested complete deletion of this gene. Further analysis showed a gross deletion consisting of 3 genes: Vestigial like 3 (VGLL-3), Chromatin modifying protein 2B (CHMP2B) and POU1F1 in the chromosomal region: 3p11.2-3p12.1.

Conclusions: POU1F1 is crucial for the development and differentiation of the anterior pituitary gland and mutations have been shown to be responsible for a syndrome of combined pituitary hormone deficiency (CPHD), including prolactin, GH and TSH deficiencies. CHMP2B gene, encodes a component of the endosomal ESCRTIII complex, and aberration were recently identified as in autosomal dominant frontotemporal dementia (FTD). VGL-3 is enriched in the placenta, however its exact role is yet unknown. We describe a new syndrome, associated with GH deficiency and resistance. These findings may suggest a new role for POU1F1, CHMP2B or VGLL-3 genes, either alone or in combination, in the GH response.

Abstract Code: P65

LDL Receptor Mutation in a Druze Kindred – Clinical, Biochemical and Genetic Characteristics

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Introduction: Familial hypercholesterolemia (FH) is an autosomal dominant disease caused by mutation in the LDL receptor gene. The heterozygous frequency is about 1/500 and the homozygous frequency is about 1/1,000,000. Three siblings aged 6, 7 and 9 years from a Druze kindred presented with cutaneous and tuberous xanthomas, and with failure to thrive. LDL-cholesterol levels ranged between 800-900 mg/dl. Analysis of mutation in the LDL receptor gene was done for 48 members of the extended family.

Patients/ Methods: Genomic DNA was extracted from the family member's peripheral blood, and from cord blood of a new-born sibling. LDLR exon 4 was sequenced directly.

Results: The Y188X mutation was detected in three index patients. Restriction enzyme analysis confirmed the DNA sequence in these patients. We identified other heterozygous family members as Y188X carriers. Using the cord blood of the new-born sibling we diagnosed him as heterozygous for the LDL receptor mutation. Interestingly 3 infants with normal LDL-cholesterol levels were diagnosed as heterozygous carrier based on DNA analysis.

Conclusions: Identifying the mutation in this large Druze family enabled us to diagnose carrier children who would otherwise be missed because of phenotype and genotype discrepancy and who are prone to develop symptoms of atherosclerotic cardiovascular disease in the third or fourth decade of their life. To the best of our knowledge this is the first report of using cord blood for DNA identification of FH and it is a possible tool for early diagnosis.

Abstract Code: P66

A study of RAD001 (everolimus) 10 mg/day in two patients with malignant pheochromocytoma

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Introduction: Malignant pheochromocytoma (MP) is an extremely rare neuroendocrine tumor. Other than complete surgical resection in selected patients, there is no known effective cure for the disease. in-vitro studies suggest that the mTOR pathway plays an important role in the pathogenesis of MP, and thus supported investigating whether RAD001 (everolimus), a new mTOR inhibitor, is effective in patients with MP

Patients/ Methods: Two patients with recurrent MP with measurable disease received RAD001 10 mg P.O./day and I.M. Sandostatin LAR 30 mg once a month, continuously until tumor progression, in the absence of any safety concerns.

Results: Patient 1 is a 40 year-old man diagnosed with locally invasive MP of the right adrenal gland. Following tumor resection there was evidence of local tumor recurrence and a second extensive surgery was performed followed by chemotherapy. After nearly 3 months of study therapy there was clinical improvement in the patients' condition and stable disease by CT. However, CT evaluation after 6 months showed progressive disease and the study medication was stopped. Currently 3 months after cessation of therapy the patient is clinically stable but has further radiologic progression of his disease. Patient 2 was a 24 year-old woman who had MP originating in the left adrenal, metastatic to bone and lung. Following resection of the primary tumor she received radioactive MIBG to which she did not respond. Chemotherapy induced partial response but was myelotoxic. To ameliorate toxicity and maintain stable disease we decided to try combining limited chemotherapy with study protocol. Her chromogranin A which was 3000 ng/ml at entry to the study, increased to 5000 after 3 months of therapy simultaneously radiologic evaluation showed disease progression both by CT and by 18FDG-PET and study protocol was discontinued. 3 months later in spite of full dose chemotherapy, she died of disease.

Conclusions: Based on our very limited experience, one patient experienced initial clinical response and radiological stabilization while the second patient did not show a clear-cut benefit from therapy with RAD001. Further studies are necessary for assessment of the role of rapamycin derivatives in the treatment of patients with MP.

Abstract Code: P67

Regulation of MS-KIF18A kinesin cellular distribution and estrogen-dependent expression

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Introduction: MS-KIF18A is a member of the kinesin super family, which possesses microtubules binding and ATPase domains and facilitates trafficking of cargo proteins within the cell. We identified that estrogen receptor alpha (ERalpha) is a cargo protein of MS-KIF18A. Estrogen (17beta-E2) role in regulation of MS-KIF18A message and protein expression was elucidated in this study.

Patients/ Methods: The impact of estrogen on MS-KIF18A expression was analyzed at the promoter, mRNA and protein levels. (i) We constructed 1.3 kb segment of MS-KIF18A promoter upstream to the TSS. This putative promoter region includes the binding sites for NFκB, AP-1 and ERα transcription factors (TF). This genomic segment was cloned in pGLuc vector upstream to luciferase reporter gene (pGLuc-K), thus luciferase activity served as a read out for promoter activity. (ii) TF binding to endogenous MS-KIF18A promoter was analyzed by ChIP assay. (iii) mRNA was analyzed under estrogen paradigm using qPCR. (iv) MS-KIF18A protein cellular localization, posttranscriptional modifications, turnover and degradation were studied by bioinformatics, metabolic labeling with Met/Cis-S35 and immunological assays [immunoprecipitation (IP) and western blot (WB)].

Results: (i) 17beta-E2 and ICI 182,780 challenge resulted with activation of MS-KIF18A promoter which was measured by luciferase activity in cells transfected with pGLuc-K plasmid (ii) ChIP assay revealed binding of ERalpha and AP-1 on endogenous kinesin promoter, (iii) These modulators induced the expression of MS-KIF18A mRNA. (iv) Metabolic labeling detected a faster turnover of MS-KIF18A in cells treated with 17betaE2 then in untreated ones. (v) We identified that diverse forms of MS-KIF18A in cytoplasm and membrane/nucleus is influenced by serum starvation, indicating the involvement of posttranscriptional modifications. We have shown that MS-KIF18A undergoes phosphorylation on serine and tyrosine residues and GlcNAc glycosylation. (vi) Accumulation of MS-KIF18A in cells treated with proteasome inhibitor MG132 and its' interaction with ubiquitin ligase E6-AP confirm the role of ubiquitin-proteasome pathway in the kinesin degradation.

Conclusions: We have demonstrated that 17beta-E2 modulates MS-KIF18A gene at the promoter and transcription level. Moreover, it accelerates the MS-KIF18A protein turnover. The cellular distribution of the analyzed protein is likely to be regulated by posttranscriptional modifications phosphorylation and glycosylation. The kinesin degradation employs ubiquitin-proteasome pathway. This study contributes to the understanding of the regulation of MS-KIF18A expression and function.

Abstract Code: P68

Vitamin D inhibits the induction of matrix metalloproteinase-7 by TNF in keratinocytes

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Introduction: There is growing evidence that matrix metalloproteinases (MMPs) secreted from the tumor or stromal cells surrounding the tumor are necessary for the creation and maintenance of a microenvironment that facilitates tumor growth and invasiveness and angiogenesis at primary and metastatic sites. MMP-7 (Matrilysin-1) is frequently overexpressed in human cancer tissues, has been shown to activate autocrine growth factors to promote proliferation, degrade ECM components and convert E-cadherin to soluble E-cadherin to promote invasion. Tumor necrosis factor- α (TNF α) is secreted by tumor infiltrating immune cells and its levels are often high in the tumor microenvironment. The aim of this study was to assess the notion that the anti cancer activity of calcitriol, the hormonal form of vitamin D, may be due not only to its known antiproliferative and proapoptotic activities, but also to modulation of MMP-7 activity in the microenvironment of cutaneous tumors.

Patients/ Methods: HaCaT keratinocytes cultured in the absence of exogenous growth factors or active ingredients are thought to represent the population of basal keratinocytes adjacent to the basal membrane, the barrier to invasiveness of cutaneous tumors. HaCaT cells were exposed to TNF (10-20 ng/ml, 2-16 hours). MMP-7 mRNA levels were quantified by real-time PCR (TaqMan methodology).

Results: MMP-7 mRNA levels in HaCaT cells were highly elevated after exposure to TNF: 10-fold after 2 hours, 20-fold after 8 hours and 16-fold after 16 hours. Pretreatment with calcitriol (100 nM, 24 hours) reduced the levels of TNF-induced MMP-7 mRNA by 85%. The EGF receptor, Jun kinase and NF κ B cascades are not involved in the induction of MMP-7 as attested by the lack of effect of their specific inhibitors, AG1478, SP600125 and BMS345541, respectively. On the other hand treatment with the p38 MAPK inhibitor, SB203580, decreased the induction of MMP-7 by 50% and with the ERK1/2 inhibitor, U0126 by 30%. Calcitriol did not affect ERK activation by TNF, but inhibited p38 activation.

Conclusions: Calcitriol inhibits the induction of MMP-7 gene expression by TNF in keratinocytes. This effect is at least partially due to inhibition p38 MAPK activation. This attenuation of the potential pro-metastatic activity may contribute to the anti cancer activity of the hormone.

Abstract Code: P69

Leptin Is Pro-Apoptotic and Regulates Androgen Receptors in Human Prostate Cancer Cells

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Introduction: Prostate cancer (PCa) progression is known to depend on various hormones and growth factors, but their role and underlying molecular mechanisms remain poorly understood. We recently presented preliminary findings indicating that leptin causes a greater level of activation of the JAK2/STAT3 and MAPK (ERK1/2) pathways, as well as transactivation of HER2, in androgen-sensitive LNCaP cells than in androgen-insensitive PC3 and DU145 human PCa cell lines. We and others were previously unable to demonstrate proliferative effects of leptin in LNCaP cells.

Patients/ Methods: We now studied the effects of leptin on LNCaP cell apoptotic proteins induced by serum starvation (intrinsic pathway) as well as on androgen receptor (AR) levels (Western analysis). Apoptosis was also studied by staining nuclei of damaged cells with Hoescht stain.

Results: Leptin, caused clear dose- and time-dependent (0.1-10 ng/ml, 6-72 h) stimulation of the serum-induced apoptosis: it caused an increase in the downstream apoptotic effector caspase 3 protein expression ($\leq 1.86 \pm 0.16$ -fold, av. \pm SE) and in cleaved (inactivated) poly-(ADP-ribose)-polymerase (cPARP89), an enzyme normally responsible for DNA repair and a downstream substrate of caspase 3 ($\leq 1.60 \pm 0.45$ -fold, av. \pm SE). All of these leptin effects were mostly maximal at 0.1-1.0 ng/ml and already at 6h and generally still maintained at 10 ng/ml and for up to 72h or gradually reduced (caspase 3) from 24 to 72h. The apoptotic effects of leptin were confirmed in the study of damaged DNA: 24 h exposure to 1 ng/ml leptin caused a maximal increase of 4.17-fold versus control in the number of stained cells/well. Since the mutant AR found in LNCaP cells as well as membrane AR were reported to mediate proapoptotic effects of androgens, we determined leptin effects on AR protein. Leptin also caused increased expression of AR protein ($\leq 1.96 \pm 0.59$ -fold, av. \pm SE). With view to understanding the mechanism of these leptin effects, we studied the effects of the JAK2 inhibitor AG490 on leptin-induced signalling and apoptotic and AR proteins. In a preliminary experiment, leptin-induced pJAK2 (10 min) was clearly inhibited by AG490 (50 μ M), while the inhibitor only partially inhibited leptin-induced apoptotic and AR proteins (6 h).

Conclusions: Clearly further studies with other kinase and AR inhibitors will be needed to delineate the mechanism of leptin-induced apoptosis in human PCa and its relationship with the effects of leptin on AR. These studies are expected to provide new insights into the possible role of leptin, presumably together with other hormones and growth factors, in progression of human PCa or its delay and may provide a basis for discovery of new drugs for therapy of PCa.

Abstract Code: P70

Thyroid Cancer Growth is Estrogen-Sensitive and t-Boc Derivatives of Carboxy-Alkyl Isoflavones Can Serve As Novel Anti-Cancer Agents for NPA, MRO and ARO cells in vitro

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Introduction: The incidence of thyroid cancer is 3 folds higher in women than in men, suggesting that estrogenic effects may be involved in the pathogenesis of this malignancy.

Patients/ Methods: Here we explore whether or not human thyroid cancer cell growth, can be curbed by novel phytoestrogen derivatives generated in our laboratory, which we previously found to possess potent anti-cancer effects in human ovarian cancer cells through interaction with ERb.

Results: First, although both ERA and ERb mRNA (quantified by Real Time PCR) are expressed in cell lines derived from papillary (NPA), follicular (MRO) and anaplastic thyroid carcinoma (ARO), expression of ERb is more abundant in these cell types. Second, estradiol-17b (E2, 0.03-300nM) per se increased cell proliferation in all three cell types as measured by 3[H]thymidine incorporation. The ERb specific agonist DPN increased 3[H]thymidine incorporation in all three thyroid cancer cell types, whereas the ERA specific agonist PPT increased growth only in NPA. The ERA antagonist raloxifene (Ral) abolished the stimulatory effects on cell proliferation induced by E2 and PPT, but did not modify DPN (ERb-dependent)-induced cell growth. Third, the phytoestrogen derivatives carboxy- Daidzain-tBoc (cD-tboc) and, to a lesser extent, carboxy-Biocanin-tBoc (cBA-tboc), dose-dependently diminished cell growth in all three cell lines as assessed by 3[H]thymidine incorporation (ranging from 70-85% inhibition), the XTT assay (75-90% inhibition) and microscopic analysis of the culture wells. Fourth, cD-tboc dose-dependently increased apoptosis in all three cell lines, as quantified by a Roche assay measuring the release of histone-DNA fragments (500-1500% stimulation). Further, the anti-apoptosis agent ZV (Z-VAD-FMK) reversed the growth inhibitory effect elicited by cD-tboc, and the magnitude of this salvage was cell type- and dose-dependent. Finally, cD-tboc did not induce necrosis in either of the three thyroid cancer cell lines since inhibition of growth and induction of cell death was not accompanied by increased release of LDH to the culture medium.

Conclusions: These results suggest that estrogens are involved in thyroid cancer cell growth, and that this property can be utilized to design highly effective anti-cancer drugs. The in vivo effects of t-boc derivatives of carboxy- alkyl isoflavones is subject to ongoing investigation.

Abstract Code: P71

BRCA1 controls IGF-IR gene expression in uterine serous papillary carcinoma (USPC) cell lines

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Introduction: IGF-1 and IGF-2 are a family of mitogenic polypeptides with important roles in growth and differentiation. The biological actions of the IGFs are mediated mainly through the IGF-I receptor (IGF-IR). Evidence is mounting for a pivotal role of the IGF system in cellular proliferation and apoptosis as well as in cancer transformation. BRCA1 is a tumor suppressor gene, which participates in multiple biological pathways including DNA damage repair, transcriptional control, cell growth, and apoptosis. Little information is available regarding the impact of BRCA1 action on the IGF signaling pathway. Germline mutations in the BRCA1 gene cause genetic predisposition towards breast and ovarian serous papillary carcinoma (OSPC). Uterine serous papillary carcinoma (USPC) is a rare, aggressive endometrial tumor that resembles OSPC clinically and histologically. It is unclear, however, whether BRCA1 mutation carriers are also predisposed to USPC. The aim of this study was to evaluate the effect of BRCA1 on IGF-IR gene expression in USPC-derived cell lines.

Patients/ Methods: USPC1 and USPC2 cell lines were transiently cotransfected with an IGF-IR promoter construct driving a luciferase reporter gene, along with a BRCA1 expression plasmid. For normalization purposes, cells were cotransfected with a β -gal vector. In addition, USPC2 cells were transiently and stably transfected with a BRCA1 expression plasmid, and levels of endogenous IGF-IR were evaluated by western immunoblotting.

Results: BRCA1 expression led to a 35% reduction in IGF-IR promoter activity in the USPC1 cell line, and to a 54% decline in the USPC2 cell line. Results from western immunoblotting showed a decline in the levels of phosphorylated IGF-IR both in transiently and stably transfected cells. In addition, the stably transfected cells also showed a decline in p-AKT levels, an important downstream protein in the IGF signaling pathway.

Conclusions: Our data showed that BRCA1 suppresses IGF-IR gene expression and IGF-I action, suggesting a correlation between BRCA1 status and the IGF system in USPC cell lines. Further research is needed in order to fully understand the mechanisms responsible for the interplay between the BRCA1 system and the IGF-I signaling pathway. This research will shed light on the possible connection between germline BRCA1 mutations and USPC. Current studies are addressing the impact of BRCA1 mutations on IGF-IR levels in primary USPC specimens

Abstract Code: P72

The effect of ionizing radiation on prostate cancer cells is effectively potentiated by pretreatment with 1,25(OH)2D3 and Sodium Valproate

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Introduction: Radiotherapy is known to be an effective treatment of prostate cancer (PCa). However, many complications are involved including rectal bleeding, erectile dysfunction and urinary incontinence. Therefore, it is important to develop PCa-sensitizing pretreatments that could potentiate the therapeutic effect of radiation, allow the use of lower radiation doses and limit the side effects. Toward these aims, we suggest combining the anticancerogenic vitamin D active metabolite, 1,25(OH)2D3, and sodium valproate (an antiepileptic drug that possesses histone deacetylase activity and therefore has an anticancer effect) in order to sensitize PCa cells to radiation. The rationale of this notion is based on preclinical studies reporting increased efficiency of radiation after 1,25(OH)2D3 or sodium valproate pretreatment (Dunlap et al., 2003, Camphausen et al., 2005).

Patients/ Methods: Androgen-refractory PCa cell line DU145 was grown in RPMI-1640 medium containing 10% FCS. Cancer cells were pretreated for three days with 100 nM 1,25(OH)2D3 or 1 mM sodium valproate, or their combination. After that, cancer cells were irradiated with a dose of 4 Gy and grown for an additional four days. The effect of the suggested treatment on DU145 cells proliferation, cell cycle and apoptosis was evaluated by crystal violet test and propidium iodide FACS procedure.

Results: Irradiation by itself decreased DU145 cell growth by 30.6% ($p < 0.0001$), but affected cancer cell growth more effectively after pretreatment with 1,25(OH)2D3, sodium valproate and a combination of both drugs and decreased PCa cell growth by 46.4%, 83.0% and 87.9%, respectively ($p < 0.0001$, compared to control, and between different treatments). Cell-cycle analysis showed that the tumor cell growth-inhibiting effect of our treatment was a result of apoptosis and altered cell-cycle distribution. Irradiation induced apoptosis and caused accumulation of DU145 cells mostly in the S phase and to a lesser extent in the G2-M phase of the cell-cycle in untreated and pretreated cells. These changes were maximal in the cells pretreated with sodium valproate alone. However, after combined pretreatment with 1,25(OH)2D3 and sodium valproate, irradiation had the greatest effect in suppressing PCa cell growth. Therefore, we suggest that irradiation after combined pretreatment is especially effective in decreasing the rate of cell progression through the cell-cycle.

Conclusions: The results support our hypothesis that a combination of 1,25(OH)2D3 and sodium valproate is highly efficient in potentiating the anticancer activity of ionizing radiation. As such, we believe that this combined pretreatment may provide the basis for the clinical application of radiotherapy for the treatment of hormone-refractory prostate cancer.

Abstract Code: P73

The Effect of Burn Injury in Children on the Secretion of Leptin, Adiponectin and Ghrelin

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Introduction: Burn injury induces a systemic inflammatory response as well as a hypermetabolic state. These processes are mediated by multiple cytokines. In this study we focused on leptin, adiponectin and ghrelin, which have both metabolic and inflammatory roles. Leptin is anorexigenic and proinflammatory, whereas, adiponectin and ghrelin are orexigenic and anti-inflammatory. We looked for the dynamics of the secretion of these cytokines in pediatric patients with burns and investigated the correlation with other inflammatory markers.

Patients/ Methods: Six patients (4M/2F) with burn injury (20%-85%), aged 21-108 months, were followed for 32-87 days. One of the patients died. A longitudinal record of each patient included: weight, body temperature, blood count and chemistry, insulin growth factor 1 (IGF-1) tumor necrosis factor alpha (TNF α), interleukin-6 (IL-6), leptin, adiponectin, and ghrelin. The hormones and interleukins samples were taken at days 1,2,3 and subsequently every week during the hospitalization period.

Results: The mean, trough and peak concentrations of leptin, adiponectin and ghrelin were not correlated with weight, age or size of the burn. Their mean concentration had a similar pattern: low concentration upon admission and a rise along the first weeks up to a peak at the third week. The trajectory of IGF-1 concentrations had a similar pattern, whereas, the trajectory of TNF α and IL-6 was different, with an earlier peak at the end of the first week and a trough at the third week. The most striking result was the loss of inverse correlation between leptin and adiponectin, as well as between leptin and ghrelin. Moreover, a positive correlation was found between the concentrations of leptin and adiponectin, in 4/6 patients. Leptin also had a positive correlation with IGF-1.

Conclusions: Burn injury initiates both metabolic and inflammatory process. Leptin, ghrelin and adiponectin are both inflammatory and metabolic mediators. Our results show that concentrations of these hormones as well as IGF-1 had a similar pattern, which was different from IL-6 and TNF α . We hypothesize that the dominance of the initial acute inflammation causes a relative inhibition of the three hormones, as was shown previously for IGF-1. Furthermore, the inverse relationship between leptin and adiponectin or ghrelin, which is related to the metabolic state of the patient, was either lost or altered to a positive correlation, for the same reason.

Abstract Code: P74

Urocortin-1 deficient mice exhibit a robust anxiogenic phenotype: the role of Edinger-Wesphal Urocortin-1 neurons.

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Introduction: Urocortin-1, a corticotropin releasing factor (CRF) related neuropeptide, is highly expressed by the midbrain Edinger-Wesphal (EW) nucleus. Urocortin-1 positive neurons innervate the lateral septum, dorsal raphe and the bed nucleus of the stria terminalis nuclei, which are known to express high levels of CRF receptors and are involved in modulating mood and stress-related behaviors (Hammack et al., 2003, Henry et al., 2006). Urocortin-1 can bind, with high affinity, to both CRF receptors, CRF receptors type 1 and 2 (CRFR1, CRFR2). It was previously demonstrated that different types of stressors cause activation of Urocortin-1 neurons in the EW (Gaszner et al., 2004) and that CRFR2-deficient mice are hypersensitive to stress and display increased anxiety-like behaviors (Bale et al 2002).

Patients/ Methods: To further explore the involvement of Urocortin-1 in mediating stress-induced behavioral changes we studied the anxiety-like behavior phenotype of Urocortin-1 knockout mice as compared with their wild-type littermates. We used a battery of behavioral tests, including open-field, dark-light transfer and elevated plus maze. To further support the role of EW-Urocortin-1 neurons in mediating the anxiety-like behavior, we are in a process of performing a "rescue" study, in which lentiviruses expressing Urocortin-1, we recently generated, were injected into the EW nucleus of the Urocortin-1 deficient mice followed by behavioral measurements.

Results: Urocortin-1 deficient mice showed a robust anxiogenic phenotype, compared with wild-type littermates. In the "rescue" study, preliminary results indicate a reduction in anxiety-like behaviors in Urocortin-1 knockout mice over-expressing Urocortin-1 in the EW, as compared with Urocortin-1 knockout mice injected with control lentiviruses.

Conclusions: In light of this data, we suggest that Urocortin-1 expressed by the EW, plays a major role in modulating anxiety-like behavior and stress-coping responses, possibly through activation of CRFR2, and may be involved in the pathogenesis of anxiety disorders.

Abstract Code: P75

Chronic over-expression of CRF in mice central amygdala attenuates stress-induced anxiogenic phenotype

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Introduction: The corticotropin-releasing factor (CRF) neuropeptide is essential regulator of the neuroendocrine stress response and is implicated in the control and maintenance of homeostasis. Flaws in the regulation of the stress-response can have severe psychological and physiological consequences. Dysregulation of the CRF system has been proposed to be involved in the development of anxiety disorders and depression. Central administration of CRF was demonstrated to increase anxiogenic-like behavior in rodents, affect that could be blocked by specific CRF receptor antagonist, strengthening the importance of the CRF as central players in modulating anxiety-like behavior.

Patients/ Methods: To further study the relative contribution of CRF, endogenously expressed by anxiety-linked brain structures, to anxiety-like behavior in mice, we designed and generated lentiviruses over-expressing mouse CRF. In-vitro validation of these lentiviruses using biochemical and immunocytochemistry techniques demonstrated the ability of these viruses to express high levels of CRF. Following in vitro validation, lentiviruses expressing CRF and GFP or control lentiviruses, expressing only GFP, were injected bilaterally to the central nucleus of the amygdala (CeA), or the bed nucleus of the stria terminalis (BNST), of male C57B/6 mice. Anxiety-like behavior tests were performed, with or without pre-exposure to acute stress, four months following virus injection

Results: Results obtained from this study demonstrated that chronic expression of CRF in CeA, but not in BNST, significantly attenuated the anxiogenic effect of acute stress, as compared with control groups. Chronic expression of CRF in CeA or in BNST did not affect the basal (non-stressed) anxiety-like behavior in these mice, compared with their respective control groups

Conclusions: Our findings suggests that while CRF expression in CeA and in BNST is known to mediate anxiety-like behavior in mice, chronic expression of this peptide in the CeA resulted in the attenuation of the anxiogenic consequence of acute stress. The effects of site-specific inducible-over-expression or knockdown of CRF expression are currently evaluated and efforts are made to explore the possible molecular mechanism mediating the effects of CRF on anxiety-like behavior.

Abstract Code: P76

Establishment and behavioral phenotype of Urocortin-1 and Urocortin-2 double knockout mice: possible involvement of the serotonergic system

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Introduction: The mammalian corticotropin releasing factor (CRF)/Urocortin peptide family, which comprises of CRF, Urocortin-1, 2 and 3 is proposed to integrate the endocrine, autonomic and behavioral responses to stressors. The effects of CRF-related peptides are mediated through activation of two known G protein-coupled receptors, CRF receptor type-1 and type-2 (CRFR2), both of which are widely expressed in stress-related regions in the brain. Part of the physiological and behavioral effects of CRF-related peptides may be mediated via the CRFR2 expressed by the serotonergic cells of the dorsal raphe nucleus (DRN). Both Urocortin-1 and Urocortin-2, highly expressed by the Edinger-Westphal and the Locus Ceruleus nuclei, respectively, may serve as the endogenous ligands, via known neuroanatomical connections, for the CRFR2-DRN-expressing neurons.

Patients/ Methods: To further explore the possible involvement of endogenous Urocortin-1 and Urocortin-2 in mediating stress related behaviors we established a double Urocortin-1/Urocortin-2 knockout mice. Individual Urocortin-1 and Urocortin-2 knockout mice were crossbred to generate a double Urocortin-1/Urocortin-2 knockout mice model. Male and female double Urocortin-1/Urocortin-2 knockout mice and wild-type mice, from the same colony, were tested in variety of anxiety-like behavior paradigm.

Results: Double Urocortin-1/Urocortin-2 knockout mice, both male and female, demonstrated a robust anxiolytic phenotype compared with wild type mice. Double mutant mice showed a significant reduction in anxiety-like behavior tested using the Open Field, Dark/Light Transfer and Elevated Plus Maze tests. We are currently conducting a battery of depression-like behavior tests and analyzing the expression levels of other CRF-family members in the double knockout brains.

Conclusions: Anxiolytic effect observed in double Urocortin-1/Urocortin-2 knockout mice suggests a role of both peptides in mediating anxiety related behavior and may be involve in the pathophysiology of this disorder.

Abstract Code: P78

Macroprolactin test: Does it improve reliability of prolactin results and treatment quality?

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Introduction: Macroprolactin is a high molecular mass complex of prolactin and antiprolactin antibody that has no known bioactivity. Most of the immunometric prolactin assays currently used detect macroprolactin in variable degrees, resulting in pseudo-hyperprolactinemia and potential misdiagnosis. Maccabi laboratories use the Bayer-Advia/Centaur prolactin assay which has a low detection rate of Macroprolactin (5-10%). Recently, we introduced a routine test for macroprolactin in samples with elevated prolactin concentrations. Objectives: 1. To evaluate the prevalence of hyperprolactinemia due to macroprolactinemia in the Centaur prolactin assay. 2. To investigate the physicians' approach: Does macroprolactin test change the course of diagnosis and treatment?

Patients/ Methods: Routine samples with elevated prolactin concentrations (>700mIU/L) were tested for macroprolactin by polyethyleneglycol (PEG) precipitation method in accordance with Bayer recommendation. Prolactin and macroprolactin results were collected from Maccabi's central laboratory database during an 11 months period. Physicians were inquired regarding the influence of the macroprolactin test on the clinical approach.

Results: During the investigation period 29000 prolactin tests were performed (82% females 18% males). Most patients were referred by general practitioners and gynecologists. Elevated prolactin concentrations (>700mIU/L) were found in 1460 (5%) samples, that were then tested for macroprolactin. Of the samples tested for macroprolactin, 151 samples (10%) were positive for macroprolactin. The estimated concentration of the monomeric prolactin in these samples revealed that in 103 (7%) samples the elevated prolactin concentrations were due to the presence of macroprolactin. 48 (3%) samples were found to have hypermacroprolactinemia as well as high values of monomeric prolactin. All samples in which the elevated prolactin concentrations were due to the presence of macroprolactin had initial prolactin concentrations below 2800mIU/L. Physicians' approach: Most endocrinologists and few gynecologists stated that in the absence of clinical symptoms, hyperprolactinemia due to macroprolactinemia need no further evaluation.

Conclusions: 1. Elevated prolactin samples should be tested for macroprolactin. 2. Macroprolactin should also be tested routinely when using assays declaring low sensitivity for macroprolactin. 3. Very high prolactin concentrations are probably not related to the presence of macroprolactin. 4. The macroprolactin test has a potential to improve the specificity of prolactin assays thus improving quality of treatment and reducing the necessity of additional expensive tests. However, the clinical impact of the macroprolactin test should be further investigated.

Abstract Code: P79

Adiponectin as an inflammatory marker following pediatric open-heart surgery with cardiopulmonary bypass

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Introduction: Open heart surgery (OHS) and cardiopulmonary bypass (CPB) are well-recognized inducers of a systemic inflammatory response, with endothelial cell injury, neutrophil activation, increased synthesis of multiple cytokines, adhesion molecule up-regulation, and initiation of the coagulation cascade. Adiponectin, which is expressed exclusively in adipose tissue and is abundant in human plasma, has prominent anti-inflammatory effects, and is negatively correlated with markers of inflammation such as CRP. Our aim was to investigate changes in adiponectin in pediatric patients undergoing open heart surgery (OHS) with CPB, and to relate them to post-operative changes in other inflammatory markers and clinical parameters.

Patients/ Methods: 24 patients (M=15), aged 18.5±30.1 months, undergoing OHS for congenital heart disease were studied. Serial blood samples were collected 24-hours prior to surgery, and up to 96 hours after surgery, and assayed for adiponectin, CRP, sP selectin, tPA, MCP1, sCD40, and sVCAM1.

Results: Preoperative adiponectin levels were inversely correlated with the patients' weight and age. There was no significant difference in adiponectin levels between males and females. Adiponectin levels decreased to 49.4±19.4% of baseline levels (from 33.1±1.8 to 13.3±6.1 micg/ml) during the first 12 hours following CPB. Subsequently, adiponectin increased, but did not reach baseline levels up to 96h after surgery. CRP levels decreased from 0.88±1.57 to 0.73±1.04 mg/L during CPB, and then increased, remaining significantly elevated at 3.59±0.86 mg/L 96 hours after surgery. Patients with a more complicated peri-operative course had a more pronounced decrease in adiponectin during the first 6 hours following CPB. Adiponectin was positively correlated with sVCAM1 levels. There was no correlation between adiponectin levels and the other inflammatory markers studied.

Conclusions: Following CPB, the usual negative correlation between adiponectin and CRP was dissociated. The decreased adiponectin following OHS may be attributed to inhibition by proinflammatory factors, and may in turn perpetuate inflammation. Since adiponectin is anti-inflammatory and cardio-protective, we hypothesize that it could be a therapeutic target following cardiac surgery in pediatric patients.

Abstract Code: P80

Induction of apoptosis in 3T3-L1 fat cell line by antisense knockout of 12-lipoxygenase gene expression

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Introduction: Obesity is a rapidly spreading health hazard contributing to multiple chronic diseases such as diabetes, hyperlipidemia, hypertension, heart disease, renal failure and cancer. Recent evidence suggests that adipocyte number is not constant throughout human life. Hence the induction of adipocytes apoptosis may be a therapeutic way for the long term treatment of obesity. Arachidonate Lipoxygenases and their products can play an important role in mediating cell survival/apoptosis. The current study set out to characterize 3T3-L1 preadipocytes following the genetic downregulation of endogenous 12-LOX (lipoxygenase).

Patients/ Methods: This project attempted to establish whether or not adipocyte death can be induced through 'Antisense Knockout' of 12-LOX RNA expression in cultured 3T3-L1 preadipocytes. Cell death was measured by trypan blue exclusion and cell necrosis by LDH (lactate dehydrogenase) release into the medium. Apoptosis was assessed by DNA fragmentation, AnnexinV/ Propidium Iodide and the induction of activated Caspase-3 by western blot.

Results: Trypan blue exclusion assays and Annexin V staining after transient transfection showed a significantly higher death rate in 12-LOX knockdown cells compared with control cells. Assessment of relative DNA fragmentation of stably transfected 12-LOX knockdown cells showed a relative apoptotic rate >1 vs. control. The increased death rate in cloned 12-LOX knockdown transfectants could be reduced by the addition of the 12-LOX product, 12(S)-HETE ("rescue").

Conclusions: 'Antisense Knockout' of the platelet-type 12-LOX gene product induces apoptosis in 3T3-L1 preadipocytes. Hence, platelet-type 12-LOX appears essential for preadipocyte survival. Addition of 12(S)-HETES "rescues" the 12-LOX knockdown cells from apoptosis, thus highlighting the essential function of 12-LOX gene product in preadipocytes.

Abstract Code: P81

The prevalence of thyroid test dysfunction in obese children and adolescence before and after weight reduction programs and their relations to other metabolic parameters

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Introduction: In recent years, abnormalities in thyroid function have been described in individuals with obesity. The aim of our study was to establish the prevalence of elevated TSH levels in obese children and adolescents, and to find the relationship between changes in TSH levels and other metabolic and hormonal parameters before and after weight reduction programs.

Patients/ Methods: 207 obese participants aged 5-18 years who were referred for evaluation and were enrolled into different weight reduction programs participated in the study. Anthropometric, biochemical, metabolic and hormonal parameters including thyroid functions were measured at baseline and at the end of the intervention.

Results: At baseline 46 participants (22.2%) had elevated TSH levels (≥ 4.0 mIU/L). FT4 levels were within the normal range in all participants, without significant difference between the group with elevated TSH and that with normal TSH. Obese boys with hyperthyrotropinemia had significantly elevated triglycerides levels compared to obese boys with normal thyroid functions ($p=0.047$). No significant differences in other anthropometric or laboratory parameters were found between the group with hyperthyrotropinemia and the group with normal TSH. We found a significant positive correlation between baseline TSH level and triglyceride levels ($r=0.261$, $p<0.001$). No significant correlation was found between baseline TSH level and age, BMI-SDS, body fat percent, waist circumference, REE, or other laboratory parameters. Thirty (20 %) out of 147 participants who completed the different intervention programs had elevated TSH levels. There were no significant differences in TSH level changes during the interventions in relation to changes in BMI-SDS. At the end of the interventions, comparison between the group with normalization of TSH and the group with hyperthyrotropinemia did not find significant differences in change in BMI-SDS, body fat percent or waist circumference. A significant positive correlation was found between TSH level at the end of the intervention and triglyceride levels ($r=0.167$, $p=0.045$), and a positive correlation between the decrease in TSH levels and decrease in waist circumference ($r=0.291$, $p=0.013$).

Conclusions: the presence of elevated TSH levels with normal FT4 levels and absence of thyroid antibodies in obese children seems to be frequent and is correlated with higher triglyceride levels, raising the question of the necessity to treat the elevated TSH levels in obese children.

Abstract Code: P82

Insulin Secretion and Resistance in Severely Obese Children Due to Melanocortin-4 Receptor Gene Mutation

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Introduction: The melanocortin signalling pathway plays an essential role in energy homeostasis. Mutations in the melanocortin 4 receptor gene (MC4R) are a common cause of monogenic human obesity. MC4R knockout mice models suggest that MC4R dysfunction causes insulin resistance. Aim: To study insulin secretion and sensitivity in young severely obese children carrying a mutation in the MC4R gene.

Patients/ Methods: Standard intravenous (IVGTT) and oral (OGTT) glucose tolerance tests were performed on three members of a consanguineous Palestinian family carrying a homozygous mutation in the MC4R gene.

Results: The patients aged 13, 7, and 8 years old, were severely obese for several years and at admission had BMI's of 51, 48 and 43.4 kg/m², respectively. Only one patient aged 13, developed clinical evidence of diabetes mellitus unsatisfactorily controlled by either oral metformin and/or by Glargine insulin injections (HbA1C 10.34%). OGTT suggested overt diabetes and during the initial IVGTT, insulin-secreting capacity was diminished (maximal insulin 372 pmol/l at 14.1 mmol/l glucose). During his hospital admission a strict diet resulted in significant weight reduction (BMI decreased to 43.7 kg/m²). Subsequently the diabetes resolved: OGTT and HbA1C (5.39%) normalized, and peak insulin secretion in IVGTT increased to 1,216 pmol/l at 10.8 mmol/l glucose, indicating good insulin secretion with some residual insulin resistance. The younger two patients did not develop diabetes as demonstrated by OGTT in spite of several years of severe obesity. Both patients demonstrated extremely high quantities of insulin in response to IVGTT (peak levels over 100,000 pmol/l) indicating significant insulin resistance.

Conclusions: Severely obese children carrying MC4R mutations maintain large insulin secretion capacity for years in spite of chronic major insulin resistance. This exaggerated secretion protects them from the development of diabetes. The diabetes that develops subsequent to beta cell failure in these severely obese patients may be reversed by diet therapy. The insulin resistance observed is only partially corrected by diet and may be also related to the MC4R genotype.

Abstract Code: P83

Testosterone is a major determinant of ghrelin levels in men and post-menopausal women

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Introduction: The secretion and regulation of several hormones including leptin and growth hormone (GH) is sexually dimorphic. Gender effects on ghrelin, a hormone involved in the regulation of GH secretion and appetite control is controversial. Our aim was to study the relationship between plasma ghrelin and serum sex steroid hormone concentrations.

Patients/ Methods: Forty five subjects (19 men, 12 pre-menopausal and 14 post-menopausal women) were evaluated at the Institute of Endocrinology and Metabolism, Tel Aviv-Sourasky Medical Center, Israel. Blood samples were collected after an overnight fast for measurements of ghrelin, testosterone, bioavailable testosterone (BT) and estradiol. Statistical analyses were performed with adjustments for age and body mass index.

Results: Ghrelin levels were significantly higher in women (510 +- 489 pg/ml) than in men (319 +- 237 pg/ml, $p=0.02$). There was a positive correlation between ghrelin and both total testosterone ($r = 0.5$, $p = 0.039$) and BT ($r = 0.719$, $p = 0.0011$) in male subjects. In pre-menopausal women there was a negative correlation between ghrelin and BT ($r = - 0.39$, $p = 0.2$). In contrast, ghrelin strongly and positively correlated with total testosterone ($r = 0.7$, $p = 0.01$), and BT ($r = 0.821$, $p = 0.001$) in post-menopausal women. There was a positive correlation between estradiol and ghrelin levels in the study population ($r = 0.356$, $p = 0.019$) that lost its significance when analyzed separately by gender.

Conclusions: Ghrelin secretion in humans is sexually dimorphic. Testosterone is a major determinant of ghrelin levels in men and post-menopausal women.

Abstract Code: P84

Premature thelarche- Characteristics at presentation and clinical follow-up

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Introduction: Premature thelarche (PT) is defined as isolated breast development without any other signs of sexual maturation. The possible progression of PT towards true precocious puberty (PP) is not well established. The study aimed at investigating the clinical course and growth pattern of girls with PT.

Patients/ Methods: The charts of 140 girls with PT followed in our clinic between 1995 and 2005 were reviewed. Data on general and endocrine evaluation, course of growth and puberty, and the outcome of PT were collected. Analysis was conducted by age at appearance of PT: birth (n=58), 1-24 months (n=62), or 2-8 years (n=20).

Results: PT was diagnosed at mean age of 2.1 ± 1.8 years. It was bilateral in 108 girls (93 – Tanner stage 2, 15 – Tanner stage 3), and unilateral in 32 girls (all - Tanner stage 2), ($p=0.02$). The prevalence of unilateral PT was similar in all age groups. Anthropometric, bone age and laboratory parameters on admission were comparable in the 3 age groups, except for a lower weight SDS ($p<0.05$) on admission, significant increase in height (Ht) SDS during follow-up, (0.06 ± 0.75 to 0.36 ± 0.87 $p<0.04$), and higher FSH (basal and GnRH stimulated) ($p<0.05$), in girls with PT who presented before the age of 2 years. Mean duration of follow-up was 3.0 ± 2.5 years (0.2-9.3). PT regressed in 51 girls, persisted in 46, progressed in 6 and had a cyclic pattern in 13. A progressive or cyclic course was significantly more prevalent among girls with PT presented after the age of 2 year (55%) compared to girls who presented at birth (13.3%) or at age 1- 24 months (5.9%), ($p<0.001$). Only 7 girls (5.9%) progressed to PP: three with PT appearing at birth, one - at 6 months, and three between 4.4-6.08 years.

Conclusions: Our data confirm the benign nature of PT. Late presentation (after age 2 years) is associated with a higher rate of either progressive or cyclic course. Neither age at presentation nor course of thelarche appears to be associated with different anthropometric, biochemical or bone age characteristics.

Abstract Code: P85

Mullerian inhibiting factor assay- a helpful diagnostic test for Intersex disorders and ambiguous genitalia evaluation

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Introduction: Mullerian inhibiting factor (MIF) is a hormone secreted by Sertoli cells in the testes during embryogenesis. Its role is to suppress the development of the mullerian ducts into female internal organs. In males, the testes continue to produce MIF at high levels during the prepubertal period, and in females, MIF is secreted from the ovarian granulosa cells during the reproductive age. Because levels of MIF do not overlap between males and females throughout childhood, MIF may serve as a marker of testicular presence and function in children with cryptorchidism, intersex disorders, and ambiguous genitalia. The MIF assay was only recently introduced for use in Israel. The aim of the study was to evaluate MIF levels in normal prepubertal children in Israel and in children with various syndromes of abnormal sexual differentiation using the newly introduced MIF assay.

Patients/ Methods: The study was conducted at a major pediatric tertiary medical center in Israel. Serum levels of MIF were measured by ELISA (Immunotech-Beckman Coulter kit) in 67 healthy male and female children and in 57 children with a diagnosis of ambiguous genitalia (n=24), cryptorchidism (n=15), hypospadias (n=5), micropenis and hypotrophic testis (n=4), anorchia (n=7), and complete sex reversal (n=2).

Results: In the control group, MIF levels were significantly higher in the males (median 56.1, range 20.8-132.3ng/ml) than the females (median 1.1, range 0-6.4 ng/ml) ages 0-6 years (p<0.05). There was no overlap in levels. MIF levels were low and close to female levels in the anorchic children (median 0.9 ng/ml, range 0-3.1). In the children with intersex disorder, the level of MIF was lower than that in normal males but higher than in females (median 15.2 ng/ml, range 0-33.4). MIF levels were high in XY females with complete androgen resistance before gonadectomy and low in children with dysgenetic gonads and true hermaphrodites.

Conclusions: The data collected in the present study may serve as a reference for use of the MIF assay by pediatric endocrinologists in Israel and the formulation of a local normogram for the males and females prepubertal population. The findings suggest

that nondetectable levels of MIF, along with a negative human chorionic gonadotropin (HCG) test and high levels of gonadotropins, might replace the need in the future for surgical intervention in patients with anorchia. They also demonstrated the contribution of MIF in the differential diagnosis of intersex disorders. We suggest that the MIF assay be routinely used in the evaluation of anorchia, cryptorchidism and ambiguous genitalia.

Abstract Code: P86

Thyroid autoimmunity testing- is it worthwhile as a routine cost-effective investigation in infertility workup?

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Introduction: The indication for routine thyroid antibody testing in infertility is equivocal. It has been hypothesized that thyroid ab's may indicate subclinical hypothyroidism. Repeated IVF failures, and premature ovarian failure [POF] were associated with autoantibodies. We have, therefore, examined the presence of anti-TPO ab's in infertile women and correlated it with clinical parameters.

Patients/ Methods: 306 women attending the gynecology-endocrine and infertility clinic were tested for TPO ab's. Among these 210 were younger than 40, 98 were infertile, 79 had thyroid dysfunction, 25 had PCOS, and 16 had POF.

Results: Overall 27.4% had TPO ab's. The prevalence of TPO ab's was age correlated: 16.6% below 20 years, 26% at 20-40 years, and 35.4% above 40. 14.3% of the habitual aborters and 18.8% of the sporadic aborters had TPO ab's [NS from controls]. However, 56.3% of the POF patients and 69.2% of patients with thyroid dysfunction experienced anti-thyroid or TPO ab's. Of the infertile patients, 29.2% had anti-thyroid and/or TPO ab's, vs 13.6% in the fertile patients. Whereas 45.3% of the TPO positive patients between 20-40 years were infertile, only 19.6% of the anti-thyroid ab's came for infertility. Infertility treatment was successful in 46% of the TPO positive vs 28% of the TPO negative patients.

Conclusions: Anti TPO ab's are significantly correlated with POF and thyroid dysfunction but insignificantly increased in infertile patients. Anti TPO ab's did not affect the success of infertility treatment.

Abstract Code: P87

Chemotherapy-induced ovarian toxicity

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Introduction: Advances in cancer therapy have improved the long-term survival of cancer patients who are then commonly faced with infertility and premature gonadal failure. Due to physiological traits, germ cells are susceptible to chemotherapy-induced-apoptosis, raising concern and potential health risks for women surviving diseases such as breast cancer, Hodgkin's disease and leukemia. Nevertheless, the pathway by which germ cells commit apoptosis remains obscure. The chemotherapeutic agent Doxorubicin (DXR) has been demonstrated in various cell types to initiate an apoptotic pathway via several mechanisms of action as: induction of DNA damage, activation of the mitochondria and activation of the caspase family. Our study aims to characterize the possible mechanism by which DXR exerts the gonadotoxic effect in a mouse model. Ovarian tissue was examined following in vivo exposure to DXR using apoptosis assays as TUNNEL and caspase-3.

Patients/ Methods: Ovulated or ovarian mouse oocytes which were exposed to DXR in vitro were examined using fluorescent immunohistochemistry, Specific activity assays were used to detect potential effectors such as caspase-12 and caspase-3.

Results: Our results suggest that DXR simultaneously stimulates two apoptotic pathways: firstly, through the nucleus (being its known original target site, as expected) where it induces acute obliteration of the DNA, secondly, through the mitochondria, where co-localization of DXR with the mitochondria was observed. In ovarian sections, apoptotic signal was observed in ovarian follicles as soon as 12 hours after exposure to DXR.

Conclusions: Our results imply that doxorubicin elicits a unique apoptotic signal transduction pathway within the oocyte via direct co-localization within the mitochondria and its activation. Possible cellular effectors appeared to be caspase12 and caspase-3. Exploring the cellular mechanisms by which doxorubicin executes germ cells destruction might elucidate chemotherapy- induced infertility and be useful in discovering biological keys needed for protecting the germ cells against damage from chemotherapy.

Abstract Code: P88

A Contrary Effect of Magnesium Sulfate on Interleukin-1 Receptor Antagonist Release by Normotensive compared to Preeclamptic Human Placenta

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Introduction: To examine the effect of magnesium sulfate (MgSO₄) on interleukin-1 receptor antagonist (IL-1Ra) release by normotensive and preeclamptic placentae.

Patients/ Methods: Cotyledons of term normotensive and preeclamptic placentas were dually perfused for 6 hours (6h), with MgSO₄ (6-7 mg %) in the maternal reservoir [normotensive (n=3), Preeclamptic (n=4)] and with control medium (without MgSO₄) [normotensive (n=4), Preeclamptic (n=4)]. Perfusate samples from the maternal and the fetal circulations were collected every 1h for 6h of perfusion, and examined for IL-1Ra levels by ELISA. Statistical significance was determined using 2-way analysis of variance (ANOVA).

Results: Preeclamptic placenta perfused with control medium released significantly higher levels of IL-1Ra into the fetal circulation as compared to normotensive placenta, after 4h of perfusion (82+6 pg/ml and 154+42 pg/ml, respectively), 5h (95+60 pg/ml and 207+35 pg/ml, respectively), and 6h (78+3 pg/ml and 225+92 pg/ml, respectively) (p<0.05). However, no significant differences in IL-1Ra levels were detected in the maternal circulation of preeclamptic placenta as compared to normotensive placenta. Addition of MgSO₄ to the maternal circulation of normal placenta increased IL-1Ra release into both the fetal and the maternal circulations (p<0.05). Exposure of preeclamptic placenta to MgSO₄ decreased IL-1Ra release only into the fetal circulation (p<0.05), but not into the maternal circulation.

Conclusions: We show that preeclamptic placenta releases higher levels of IL-1Ra as compared to normotensive placenta. Moreover, MgSO₄ may differently affect IL-1Ra release by normotensive and preeclamptic placentae. Down-regulation of IL-1Ra release by preeclamptic placenta may contribute to the mechanism of action of MgSO₄.

Abstract Code: P89

A novel ovary-specific and ovulation-associated variant

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Introduction: The release of the female gamete from the ovary, known as ovulation, is a key event in mammalian reproduction. LH acts on mature follicles to down-regulate the expression of genes associated with folliculogenesis and stimulates the up-regulation of other specific genes involved in ovulation. We have previously applied the SSH method in order to systematically isolate genes with an ovulation-selective pattern of expression. We herein report of identification of an ovary-restricted ovulation-associated novel isoform of Ephx2, Ephx2C.

Patients/ Methods: In our experimental model, human chorionic gonadotropin (hCG) that binds to the LH receptors served as the ovulation trigger, while the actual follicular rupture occurs approximately 12-14 hr after the injection of this gonadotropin to sexually immature C57BL/6 female mice, primed 48 hr earlier with pregnant mare's serum gonadotropin (PMSG), an FSH-like gonadotropin. In the present work, we employed bioinformatics search tools in combination with molecular techniques such as RACE, real-time PCR, tissue array and in-vitro analysis of the follicular culture.

Results: 1. The expression of the 4E4 clone is transiently elevated in an ovulation-associated manner 2. Ephx2C is a novel isoform of Ephx2 3. We examined the tissue expression profile of Ephx2, Ephx2B and Ephx2C isoforms. Ephx2C expression was detected exclusively in ovarian tissue with a significant increase following hCG administration, whereas the Ephx2, as expected, had a wide range of tissue expression. Ephx2B was highly abundant in ovarian tissue, however, its expression in other tissues as well. 4. The message encoding Ephx2C is expressed in both granulosa and cumulus cells of the large antral follicles, with no detectable signal in either the oocyte or the theca cells. Time course analysis confirmed that Ephx2C mRNA expression increases from undetectable levels at 48 h after PMSG to high levels at 8 h after hCG followed by a drop to undetectable levels at 24 h after hCG administration. 5. Our results indicate that the LH-induced mRNA expression of Ephx2 isoforms is mediated by various intracellular signaling molecules, including AC, PKA, MEK, PKC and p38 kinase. However, there are differences between the various isoforms: Ephx2 expression is not stimulated at all by LH, Ephx2B expression is stimulated by the PKA pathway, and to a much lesser extent by p38 MAPK, Ephx2C expression is stimulated equally by PKA and p38 MAPK, and in addition, it is partially stimulated by PKC.

Conclusions: To conclude, Ephx2C is a novel ovary specific isoform of Ephx2 that is distinct from both Ephx2 and Ephx2B and is highly expressed in response to the ovulatory stimulus. Its LH-induced mRNA expression involves the PKA and p38

MAPK pathways, and to a lesser extent PKC. More studies are needed to elucidate the exact role of this novel isoform in ovulation.

Abstract Code: P90

Expression of mMagoh in mammalian ovary: characterization, regulation and function

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Introduction: Ovulation is a key event in female reproduction. The release of the oocyte represents the end point of a series of intrinsic and extrinsic ovarian processes, which are closely regulated, both spatially and temporally. The Lutenizing hormone (LH) is a major regulator of ovulation, which triggers massive modulation of gene expression in the ovarian follicle, affecting oocyte maturation, cumulus cells expansion, follicular rupture and lutenization. One of the genes that we found to be upregulated by LH is Mago nashi homolog (Magoh). Magoh is a highly preserved protein, expressed in various eukaryotes, from protozoa to human. Magoh functions as a regulator of mRNA metabolism, as a part of exon-junction complex (EJC). This complex is involved in diverse, spliced-mRNA-related processes, such as quality control, transport, intracellular localization, and translation efficiency.

Patients/ Methods: Magoh and the EJC were found to be major contributors to development, germ cell formation and reproduction, in various systems such as flies, nematodes and plants.

Results: Our results indicate that Magoh has two isoforms in the mouse genome, which might have different roles in reproduction and/or different regulation and expression patterns. Magoh mRNA and protein accumulate in mice ovary following PMSG/hCG treatment. Magoh protein was also found to accumulate in oocytes during maturation, and immunofluorescence staining indicates a unique pattern of cytoplasmic speckle staining in oocytes..

Conclusions: We hypothesize that in the somatic compartment of the ovary, Magoh may have a role in ovulation as mediator of LH-induced gene expression. In oocytes, Magoh may be involved in control of meiosis, possibly through mediating polyadenylation and/or translation of bound mRNAs. Magoh itself is thought to be regulated by cytoplasmic polyadenylation, a unique mechanism for gene expression in the transcriptionally dormant oocyte. Our results suggest for the first time that Magoh may have an important role in regulating multiple aspects of mammalian reproduction and development

Abstract Code: P91

Estradiol- 17 β an unexpected regulator of lipooxygenase enzyme expression and activity in human vascular smooth muscle cells

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Introduction: Why estrogens fail to positively affect cardiovascular outcome in post-menopausal women despite their multiple experimentally proven vascular protective effects remains enigmatic.

Patients/ Methods: In the present study we set out to determine whether or not estradiol-17 β (E2) regulates lipoxygenase (LO) expression / activity, thereby potentially negating some of their "positive" arterial effects.

Results: First, E2 induced a ~ 9 fold increase in platelet-type 12LO mRNA expression (Real Time PCR) and an 8 fold increase in 15LO type 2 mRNA in cultured vascular smooth muscle cells (VSMC). Second, E2 increased the production rate of both 12- and 15- hydroxyeicosatetraenoic acid (HETE), the arachidonic acid derived metabolites of these enzymes by 60% and 30%, respectively. Third, by comparison, several phytoestrogens had a relatively minor effect on LO mRNA expression, except for biochanin A and its synthetic carboxy-derivative, which also induced a 2-4 fold increase in both 12- and 15 LO mRNA. Fourth, induction of 12- and / or 15LO expression required the entry of these hormones into the cell, as the stimulatory effects of E2, biochanin A and carboxybiochanin A were not seen once these hormones were conjugated to macropoteins, which precluded their trans-membrane transport through the membrane into the cytoplasm. Fifth, using specific estrogen receptor (ER) α and ER β agonists we observed that the stimulatory effects of estrogens on LO expression could not be simply attributed to either one of these receptor subtypes: the ER α agonist PPT (1,3,5-tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole) inhibited 12LO and did not affect 15LO type 2 expression, whereas the ER β agonist DPN (diarylpropionitrile) inhibited 12LO mRNA and moderately stimulated 15LO mRNA. Both PPT and DPN increased 12-and 15HETE, though the effect of DPN was larger. In conclusion, E2, but not several phytoestrogenic compounds, increases 12- and 15LO mRNA expression as well as 12- and 15HETE production in human VSMC through mechanisms, which require cytoplasmatic entry and are not specifically and/or exclusively linked to either ER α or ER β in VSMC.

Conclusions: The most serious potential hazard of these effects could be unwanted increase in 12/15HETE-mediated VSMC proliferation, contractile responses and transition of VSMC to foam cells secondary to increased lipoxygenase-driven lipid oxidation.

Abstract Code: P92

Effect of alpha linolenic acid and linoleic acid on the IGF-I-mediated signal transduction pathways in colorectal cancer cells

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Introduction: The insulin-like growth factor (IGF) system regulates cells growth and transformation and therefore plays a critical role in normal growth and development as well as in malignant states. Most of the biological activities of the IGFs are mediated by the IGF-IR, which is overexpressed in most tumors and cancer cell lines. Fatty acids have critical roles in both systemic physiological processes like metabolism, and also in cellular events such as proliferation, apoptosis, signal transduction, and gene expression. Alpha-linolenic acid (ALA) and linoleic acid (LA) are essential fatty acids from the omega-3 and omega-6 families, respectively. Many laboratory and clinical studies examined the involvement of these fatty acids in cell survival and malignancy, leading to controversial results. The aim of this study was to examine the effect of ALA and LA on colon cancer cells survival and proliferation, and to investigate the signal transduction pathways involved in these processes.

Patients/ Methods: The research was performed on the colorectal cancer derived HCT116++ cell line. The effect of ALA and LA on cell proliferation was examined by FACS analysis and by cell counting experiments. The effect on IGF-IR protein expression and activation of its phosphorylation cascade were assessed by Western immunoblotting. Transcriptional changes were tested by transient transfection experiments.

Results: Our results showed that treatment with both ALA and LA for 6, 12, and 24 h caused a significant increase in the portion of cells in S phase. Also, ALA treatment for 96 h caused a 30% increase in cell proliferation. Fatty acids treatment at doses between 0.01 mM and 2 mM had no major impact on IGF-IR promoter activity and on protein expression. However, 24 h treatment with either fatty acid led to IGF-IR activation. Furthermore, both fatty acids enhanced the ligand-induced phosphorylation of the receptor. In addition, the different treatments caused an increased phosphorylation of downstream proteins in the IGF signal transduction pathway, including PKB/Akt and MAPK/Erk.

Conclusions: our results suggest that both ALA and LA have a mitogenic effect in HCT116++ cells. This effect is reflected by an increase in the portion of cells in S phase, and by the activation of IGF-mediated signal transduction pathways that lead to cell proliferation and anti-apoptotic activities.

Abstract Code: P93

Leptin Up-regulates the Lactogenic Effect of Prolactin in the Bovine Mammary Gland In Vitro

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Introduction: ABSTRACT Leptin up-regulate the action of prolactin in the mammary gland. The effect of leptin and prolactin on mammary gland parameters such as albumin-synthesis, proliferation (Thymidine incorporation), amino acid metabolism (APN expression), and apoptosis (caspase-3 expression) is shown. Leptin enhanced the effect of prolactin on all of these processes in bovine mammary explants. It is also shown that leptin and prolactin regulate mTOR (mammalian target of rapamycin) expression in these explants. mTOR is one of the signal-transduction junctions involved in the regulation of DNA synthesis, apoptosis and protein-synthesis regulation.

Patients/ Methods: Mammary tissue and mammary fat tissue were obtained from cows in the slaughterhouse and transferred to the lab in M-199 medium containing 100 U penicillin, 100 µg streptomycin, 0.25 µg fungizone and 1 µg/mL insulin. Explants were prepared, and were placed on an impregnated lens paper floating in 5 mL of M-199 medium, in the first three days the mammary explants were incubated in medium supplemented with 1 µg/mL insulin, 0.5 µg/mL cortisol then, prolactin and leptin were added according to the experimental protocol. The medium was changed every 24 h, for 5 days. At the end of the incubation tissue was analyzed for the parameter checked.

Results: Effect of Leptin and Prolactin on Albumin Synthesis in Bovine Mammary Gland Explants Explants from lactating bovine mammary gland were incubated with insulin and cortisol for three days, after three days the medium was supplemented with leptin, prolactin, or both. Leptin alone at concentrations of 1 and 10 ng/mL in the culture medium did not affect albumin expression in the explants, whereas 100 ng/mL leptin elevated albumin expression (Fig. 1). Prolactin alone did not have a dramatic effect on albumin expression however, 1 µg/mL prolactin together with 100 ng/mL leptin elevated albumin accumulation to its highest level (fig1). Effect of Leptin and Prolactin on Proliferation in Bovine Mammary Gland Explants Cell proliferation was evaluated by [³H] Thymidine incorporation. Explants from lactating bovine mammary gland were incubated with leptin, prolactin, or both. Prolactin at the concentration of 0.1 µg/mL in the medium did not affect proliferation in the mammary explants, whereas at a concentration of 1 µg/mL, it upregulated proliferation (Fig. 2). Leptin at a concentration of 50 ng/mL did not affect cell proliferation in the explants. However, together with prolactin, leptin elevated the cells' proliferation in a dose-responsive manner, with the highest rate of proliferation being observed when 100 ng/mL leptin was added to medium containing 1 µg/mL prolactin (Fig. 2). Effect of Leptin and Prolactin on Apoptosis in Bovine Mammary Gland Explants Explants from lactating bovine mammary gland were incubated with leptin, prolactin, or both for 24 h, after 3 days in medium containing insulin and cortisol alone. Caspase-3 cleaved and uncleaved protein expression was then analyzed

by western blot (Fig. 3). Leptin down regulated caspase-3 expression at concentrations of 10 and 100 ng/mL in the medium. Addition of 1 μ g/mL prolactin to medium containing 10 or 100 ng/mL leptin decreased caspase-3 expression compared to treatments with leptin alone. Prolactin alone at a concentration of 1 μ g/mL reduced caspase-3 expression relative to the control and leptin treatments. Leptin and Prolactin Regulate APN (Aminopeptidase N/CD13) Expression in Bovine Mammary Gland Explants Explants from lactating bovine mammary gland were incubated leptin, prolactin, or both. APN protein expression, as determined by western blot analysis, after 24-h incubation with the different treatments is shown in Figure 4. Leptin alone upregulated the expression of APN, a further increase in APN expression was achieved by prolactin alone. Addition of leptin to medium containing prolactin upregulated APN expression to its highest level in these experiments. Leptin and Prolactin Regulate mTOR protein level in Bovine Mammary Gland Explants Explants from lactating bovine mammary gland were incubated with leptin, prolactin, or both for 24 h. Western blot analysis of mTOR protein level is shown in Figure 5. Leptin alone had a minor effect on mTOR protein level in the explants. Medium containing 1 μ g/mL prolactin elevated mTOR protein level slightly above that induced by leptin alone. The highest expression of mTOR was achieved when leptin and prolactin were introduced together into the medium.

Conclusions: In this study, we showed that the interaction between leptin and prolactin is extended to parameters other than milk-proteins expression and fat synthesis, which indicate up-regulation of lactogenesis. We showed that the interaction between leptin and prolactin also affect protein synthesis, such as that of albumin. We also demonstrated the effect of leptin and prolactin on apoptosis and proliferation. All of the above confirm and give evidence to our hypothesis that leptin and prolactin have de novo effects on bovine lactogenesis.

Abstract Code: P94

Elucidation of the signaling pathways elicited by the long-acting insulin analogues Glargine and Detemir

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Introduction: Long-acting insulin analogues are characterized by their slow and constant release into the circulation, mimicking basal insulin secretion. This feature is due to modifications introduced into the C-terminus of the insulin beta chain. An emerging question is whether these modifications affect the binding properties of the analogues to the insulin-like growth factor-I receptor (IGF-IR) and/or the insulin receptor (IR), and confer upon the analogues different biological properties than those of insulin, including an enhanced mitogenic potential. Recent in vitro studies in our laboratory revealed that two long-acting analogues, Glargine (G1, Lantus®, Sanofi Aventis) and Detemir (Dt, Levemir®, Novo Nordisk), have increased mitogenic capacity in various cancer cell lines, strongly resembling those of IGF-I. The aim of this study was to investigate the mechanisms that mediate the actions of G1 and Dt in comparison to regular human insulin (rIns) and IGF-I in the colon cancer-derived HCT116 cell line.

Patients/ Methods: The ability of the analogues to activate the IGF-IR and IR was assessed by immunoprecipitation assays. The expression levels of the receptors and the ability of the analogues to activate specific signaling pathways were measured by western immunoblotting. Finally, antibody arrays were used to identify novel genes activated by G1.

Results: Results of immunoprecipitation assays using specific anti-receptor antibodies along with phosphotyrosine antibodies showed that both analogues mediate their effects mainly through the IR, and not through the IGF-IR. Furthermore, the ability of the analogues to activate the major signaling pathways, PI3K and MAPK, in terms of kinetics and intensity, are essentially different from those of insulin. Part of the effects of the analogues resembled those of IGF-I. In addition, we found that, in contrast to insulin, the analogues have a relatively strong antiapoptotic effect, similar to that of IGF-I. Finally, the antibody array experiment showed many similarities between G1 and IGF-I regarding activation of downstream signaling molecules.

Conclusions: G1 and Dt, the two long-acting analogues tested, exhibit different binding characteristics to the IR, which may lead to differences in their activation of signaling pathways and in end-point biological activities. In particular, we found that, in contrast to rIns, the analogues have an increased IGF-I-like mitogenic activity, which is mediated through the IR. Further research is needed in order to fully characterize the mechanisms of action of the insulin analogues and to assess the possible clinical implications of these findings.

Abstract Code: P95

Involvement of sphingolipids metabolism in oocyte ageing and apoptosis

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Introduction: Under normal physiological conditions, more than 85-90% of mammalian oocytes succumb to apoptosis. Meiosis of mammalian oocytes arrests during embryonic life at the diplotene of the first meiotic prophase. Resumption of the first meiotic division leads to extrusion of the first polar body and to an arrest at the metaphase of the second meiotic division (MII) prior to ovulation. Ovulated MII oocytes are fertilizable for a limited time. If not fertilized, the oocytes age and proceed through cell death processes. Sphingolipids are essential second messengers in a variety of signal transduction pathways, including apoptosis. Ceramide is a sphingolipid that serves as a precursor for all glycosphingolipids. It is composed of a long sphingoid base chain, usually sphingosine, linked to a fatty acid via an amide bond. Acid ceramidase (AC) functions as an “amidase”, and breaks down ceramide. Acid sphingomyelinase (ASM) is a sphingolipid hydrolase that hydrolyses sphingomyelin at the plasma membrane to produce ceramide, following cell stimulation. ASM and AC form a tight complex within cells and their activities are carefully regulated and coordinated. Our working hypothesis was that an increase in ceramide level causes apoptosis in aged, unfertilized oocytes.

Patients/ Methods: MII oocytes were “aged” in vitro after 27 hours incubation. To establish that the death of aging oocytes is caused by apoptosis, we employed immunohistochemistry for identifying breakdown of poly ADP ribose polymerase (PARP) by caspase 3, a marker indicating early stages of apoptosis. We followed changes in the amount of ASM and AC during the “aging” process of unfertilized MII oocytes by means of immunohistochemistry, confocal microscopy and western blot analysis.

Results: Our results suggest that “aged” oocytes die in the process of apoptosis. We showed that ASM level remains constant and that AC level decreases throughout the “aging” process of MII oocytes, indicating a continuous production of ceramide accompanied by its accumulation in “aged” oocytes.

Conclusions: We suggest that ceramide is produced but not degraded in aged oocytes, hence its level rises and causes apoptosis.

Abstract Code: P96

The involvement of insulin and insulin signaling pathway in the skin cell death

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Introduction: Maintaining skin integrity and proper wound healing is essential for life. This is achieved by continuous skin cell proliferation, differentiation and programmed cell death. In our previous studies we have demonstrated that insulin is a regulator of skin proliferation and differentiation. In the current work we have investigated the effects of insulin and its downstream signaling on skin apoptosis. We have focused on UVB induced pathological skin cell death.

Patients/ Methods: In order to identify specific pathways involved in these effects, we studied genetically manipulated skin cells. These primary keratinocytes were genetically manipulated to under- or over-express insulin receptor (IR), insulin receptor substrates 1 and 2 (IRS-1 and IRS-2 respectively), Phosphatidylinositol- 3-Kinase (PI3K) or HaRas. These proteins are known to mediate insulin signaling in two main downstream pathways.

Results: First we found that lack of IR, the protein which initiate the intracellular signaling pathway upon insulin binding caused a dramatic increase of keratinocytes cell death after UVB. Next we studied the IRS's family which are play as docking proteins and are the first to get phosphorylated by the IR. Surprisingly, while manipulating IRS-2 expression had no significant effects on skin cell death, over-expression of IRS-1 resulted in a marked increase in the apoptotic cells population after UVB irradiation. Unexpectedly, lack of IRS-1 expression had no significant effect, probably due to redundancy between the members of the IRS family. Also while lack or over- expression of PI3K had no effect on keratinocytes apoptosis induced by UVB, we found that Ras which represent one of the main signaling pathways of IR is involved in cell death after UVB, and that overexpression of constitutively active of Ras caused a significant increase in apoptosis.

Conclusions: In conclusion, these data provide evidence that insulin participate in programmed cell death in skin cells after UVB radiation. This signal in keratinocytes is mediated by IRS-1-, Ras pathway and causes a silencing of the IR. Unveiling the mechanism of insulin involvement in skin turnover is of major importance for our understanding of normal skin physiology and skin wound healing process and hopefully will lead to new insights on the pathogenesis of skin diseases.

Abstract Code: P97

Utilization of Serum Thyrotropin (TSH) Measurements in the Pediatric Population

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Introduction: Objective: 1. To study the utilization of serum TSH by primary care physicians (PCPs) in the pediatric population. 2. To define populations at risk for sustained abnormal TSH level based on the initial TSH determination and patient characteristics.

Patients/ Methods: We used the database of all outpatients aged 0.5 to 16 years without previous thyroid disease insured by Clalit Health Services (CHS) who had a TSH determination in 2002, and follow-up measurements during a 5-year period. Exclusion criteria: Patients with documented thyroid disease diagnosed prior to 2002 or an abnormal serum TSH during 2001, or patients treated with lithium, amiodarone or interferon during the 5-year interval of the study. Pregnant adolescents identified by a delivery between January 2002 and December 2006, subjects lost to follow up and those deceased until December 2006 were also excluded. TSH determinations were performed using the Immulite 2000 (Diagnostic Products Corp. Los Angeles, CA) and Centaur (Bayer Health Care) apparatus. TSH values (mIU/L) were: limits 0.35-5.5, decreased <0.35, elevated >5.5<10 or highly elevated >10.

Results: 121,052 children (11.6% of the cohort) performed at least one TSH measurement between the years 2002-2006. Initial TSH concentrations were normal, elevated, highly elevated and suppressed in 96.4%, 3.1%, 0.3%, 0.2%, respectively. The frequency of TSH testing increased with age and female gender. Despite a normal initial TSH, a second, third and four or more TSH tests were performed in 26.1%, 10.7% and 8.4% of cases, respectively, during the 5 year follow up period. Forty percent of initially highly elevated and 73.5% of elevated TSH levels normalized in the second TSH determination. Predictive factors for a second highly elevated TSH: were a higher initial TSH and female gender.

Conclusions: TSH measurement is used extensively and repeatedly by general pediatricians as part of the work up for non specific complaints. Normal TSH levels used as a in this population are likely to remain normal over a 5 year period. Mild TSH elevation will most likely normalize over a 5 year period. These findings should be taken into consideration when using thyroid function in children and adolescents

Abstract Code: P98

The Effects of Engineered Thyrotropin Antagonists on Mutated Constitutively-Active Thyrotropin Receptors

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Introduction: Thyrotropin (TSH) is a glycoprotein hormone which affects the thyroid gland through a specific receptor and induces production of the thyroid hormones, T3 and T4. Somatic and germline activating mutations of the TSH receptor are identified as a molecular cause of toxic adenomas and inherited hyperthyroidism. The hetero-dimeric TSH was previously converted in our lab to a single-peptide chain (chimera) by fusing the genes encoding the α and the β subunits of TSH, between which a C-terminal peptide of hCG β hormone (CTP) served as a linker. The chimeric hormone was found to be biologically active. Two other variants were also developed using the single chain variant. One is lacking both N-linked oligosaccharide chains on the α subunit, hTSH β -CTP- α deg, and the other lacking the N-linked chain also on the β subunit: hTSH β deg-CTP- α deg. Both variants showed inhibition of TSH and TSI activity in human thyroid cells with regard to cAMP and T3 formation.

Patients/ Methods: CHO cells were transfected with the pCDNA vectors coding for the different TSH receptor mutations (Ile486Met, Ser505Arg, Ala623Ile, Pro639Ser). Transfected cells were screened to express the human thyrotropin-receptor using RT-PCR and to have constitutively-active receptors with regard to cAMP formation. In order to determine the antagonistic effects of TSH variants on the mutated receptors, cells expressing these mutations were exposed to increasing concentrations of both TSH variants, and cAMP levels were measured using ¹²⁵I in a RIA assay.

Results: Mutated TSH receptors revealed high constitutive activity in the absence of TSH stimulus. Exposure of these mutations to the deglycosylated TSH antagonists showed no significant inhibition of the constitutive activities. In addition, the two hTSH variants stimulated cAMP formation on CHO cells expressing the TSH wild type receptor. These findings do not support previous main report, in which the variants significantly reduced cAMP formation in primary cultured human thyroid follicles.

Conclusions: It seems that hTSH receptor behaves differently in CHO cells comparing to human thyroid follicle cells. Therefore, further studies can be performed on primary human thyrocytes of normal thyroid tissue to be transfected with the mutant TSH-receptor genes and used for testing the effects of hTSH variants.