Abstract Code: A1

Protean manifestations of Adrenal Hypoplasia Congenita:Not just adrenal insufficiency

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Introduction: X-linked adrenal hypoplasia congenita (AHC) is a rare hereditary disorder affecting the hypothalamic-pituitary-adrenal and the hypothalamic-pituitary- gonadal axis and is caused by mutation or deletion of the NROB1 gene. This gene encodes the DAX-1 protein that has been classified as an orphan member of the nuclear receptor superfamily. AHC is characterized by adrenal insufficiency in infancy and early childhood and failure to undergo puberty because of hypogonadotropic hypogonadism.The aim of this study was to evaluate the clinical, endocrine and molecular characteristics of X-linked AHC patients, diagnosed between 1984 and 2007 in Israel.
Patients/ Methods: Twelve patients from 5 families who were followed up for up to 23 years were studied. The diagnosis of AHC was confirmed by hormonal and molecular studies in all patients.
Results: Most of the boys presented with signs of adrenal insufficiency such as salt wasting and failure to thrive during the neonatal period. Aldosterone deficiency usually preceded cortisol deficiency requiring early mineralocorticoid therapy. Serum cortisol levels performed in the first weeks of life ranged from very low to high levels (< 0.1 to < 64.4 mcg/dl). Five boys showed signs of precocious sexual development during infancy and childhood (e.g. enlargement of penis and testes). In 4 patients the initial diagnoses were erroneous. Molecular analysis of the NROB1 gene identified point mutations in 6 patients including a novel splice site mutation in one family (IVS1-1G→C). In six patients from two families who manifested impaired mental development, contiguous gene deletion was found.
Conclusions: This study highlights the protean manifestations of X-linked AHC due to different molecular defects and emphasizes the value of genetic testing in boys presenting with salt-wasting with or without cortisol deficiency. A high index of suspicion is required in order to avoid misdiagnosis and to facilitate appropriate management.

Abstract Code: A2

PPARalpha is essential for the development of aldosterone-induced hypertension in unilaterally nephrectomized mice

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Introduction: We previously reported that absence of PPARalpha gene abolished hypertension in the Tsukuba Hypertensive Mouse (THM), a model of hypertension due to transgenic expression and activation of the human renin-angiotensin system (RAS).In this study, the most striking feature had been a very significant reduction in plasma and tissue human renin levels. However, this decrease could not solely account for the total protection from hypertension in the PPARalpha -null THM animals. Aim of the present study: To further elucidate the role of PPARalpha in blood pressure (BP) regulation in mice using a paradigm that circumvents activation of the RAS: the unilateral nephrectomy-aldosterone-saline model.
Patients/ Methods: 10-12 weeks old C57 mice or PPARalpha-null (PPARKO, on the C57 background) were subjected to unilateral nephrectomy. An osmotic minipump was implanted a week later to deliver a continuous subcutaneous infusion of either aldosterone (0.12 mcg.h-1, Aldo), or saline for 4 weeks. Animals had access to 1% NaCl water. BP was measured non-invasively. During the last week of treatment, Aldo animals were studied in metabolic cages.
Results: Whereas, as expected, systolic BP in Aldo C57 mice significantly increased compared to baseline (135.5+/-2.7 vs. 108.7+/-2.8 mm Hg, P=0.0001, n=15) there was no such increase in Aldo PPARKO animals (n=15). Moreover, at the end of the experiment, BP in Aldo C57 animals was significantly higher than in PPARKO mice (135.5+/-2.7 vs. 108.8+/-3.5 mm Hg, P=0.0004). Although the compensatory kidney hypertrophy (kidney /body, mg/g) seen in control animals was identical, the hypertrophy seen in Aldo C57 was significantly greater than in Aldo PPARKO, 12.24+/-0.3 vs 10.97+/-0.4, P=0.026. Kidney histology, however, revealed no significant differences between the strains. Aldo C57 mice drank significantly more water than Aldo PPARKO mice 24.5+/-1.2 vs. 19.3+/-1.3 ml/20 g/24 h, P=0.01. Renal expression of the mineralocorticoid receptor (MR) mRNA was similar in both Aldo C57 and PPARKO mice.
Conclusions: As the lack of PPARalpha did not affect expression of renal MR, the differences observed under Aldo suggest a central mechanism whereby PPARalpha modulates dipsogenic signals. Thus PPARKO mice might have a decreased thirst drive, thereby leading to decreased salt load, less compensatory renal hypertrophy, and protection from hypertension which develops under these conditions in the PPARalpha wild type C57 mice. Study of the expression of brain factors likely to be involved in this model should help clarify the present findings.

Abstract Code: A3

Apparent isolated 17,20 lyase deficiency caused by the homozygous mutation

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Introduction: Very few patients have been described with isolated 17,20 lyase deficiency who have had their mutations in P450c17 (17□-hydroxylase/17,20 lyase) proven by DNA sequencing and in vitro characterization of the mutations. Most patients with 17,20 lyase deficiency have mutations in the domain of P450c17 that interacts with the electron-donating redox partner, P450 oxidoreductase (POR).Our objective was to clarify the genetic and functional basis of isolated 17,20 lyase deficiency in a small Bedouin family.
Patients/ Methods: Four members of an extended Bedouin family had clinical and serum hormonal findings suggestive of isolated 17,20 lyase deficiency. One of these has previously been reported to carry mutations in the CYP17 gene encoding P450c17. Serum hormones were evaluated before and after stimulation with ACTH. Urinary steroid metabolites were profiled by gas chromatography/mass spectrometry. Exons 1 and 8 of CYP17 previously reported to harbor mutations in one of these patients and all 15 coding exons of POR were sequenced
Results: Both the serum and urinary hormone studies suggested combined deficiencies of 17,20 lyase and 21-hydroxylase. Sequencing of exons 1 and 8 of CYP17 in two different laboratories showed no mutations. Sequencing of POR showed that all four patients were homozygous for G539R, a previously studied mutation that retains 46% of normal capacity to support the 17□-hydroxylase activity, but only 8% of the 17,20 lyase activity of P450c17.
Conclusions: Apparent 17,20 lyase deficiency can be caused by mutations in POR

Abstract Code: A4

Renin-Aldosterone Response is Dependent on the type of ENaC Subunit Gene Mutation in Multi-System Pseudohypoaldosteronism Patients

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Introduction: Multi-system pseudohypoaldosteronism (PHA) is a rare syndrome of aldosterone unresponsiveness characterized by symptoms of severe salt losing caused by mutations in one of the genes that encode alpha, beta or gamma subunit of epithelial sodium channels (ENaC). The objective of our study was to assess long-term changes in the renin-aldosterone system response in patients with different mutations.
Patients/ Methods: Four PHA patients were followed-up for 7-22 years. Patient A with a heterozygous Gly327Cys mutation in alpha ENaC is a mild case and patients B, C and D are severe cases. Two additional patients with renal PHA served as controls.
Results: In patient A, serum aldosterone and plasma renin activity (PRA) decreased with age, PRA reaching near normal values at age 11. In contrast, patients B-D showed a positive correlation between age and aldosterone ($r>0.86$ for all). In patient B with Arg508stop mutation, aldosterone reached 166 nmol/L at age 19 (up to 300 fold higher than normal). Urinary Na/K ratios decreased gradually with age in all patients. Growth curves of the patients were reflective of the severity of PHA and the degree of compliance with salt therapy. Functional expression studies in oocytes showed that ENaC with G327C mutation, as observed in patient A, showed nearly 40% activity of the wild type ENaC. In contrast, stop mutation as in patient B reduce ENaC activity to less than 5% of the normal.
Conclusions: Our results demonstrate distinct genotype-phenotype relationships in multi-system PHA patients. The degree of ENaC function impairment affects differently the renin-aldosterone system and urinary Na/K ratios. The differences observed are age dependent and PHA form specific.

Abstract Code: A5

Measurement of serum free cortisol and/or salivary cortisol along with serum total cortisol improves the interpretation of the low dose 1µg ACTH test

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Introduction: Serum free cortisol, rather than serum total cortisol determines endogenous glucocorticoid activity in vivo, but how the considerable inter-subject variation in ambient serum free cortisol (up to ~ 7 fold difference) affects the outcome of dynamic hypothalamic-pituitary-adrenal (HPA) assessment in non- critically ill subjects is unknown
Patients/ Methods: We performed the low dose 1µg ACTH test in 68 subjects referred for HPA evaluation. Serum total cortisol was determined by a chemiluminescence method (Roche, Cobas A 411) and serum free cortisol was measured by the same method following equilibrium dialysis.
Results: Using the previously determined peak total cortisol of 18µg/dl as the "pass" value for normalcy, 48 of these subjects were considered as having normal response whereas 20 subjects had a subnormal response, indicative of hypoadrenalism. In a subset of these patients, salivary cortisol was also measured (Table1)

	Normal ACTH test (N=48)		Pathological ACTH test (N=20)	
	Baseline	Peak post-ACTH	Baseline	Peak post-ACTH
Free cortisol µg/dl (±SD)	0.63±0.54	1.83±0.93	0.44±0.22	0.88±0.41
Total cortisol, µg/dl (±SD)	12.4±3.68	24.3±3.78	8.93±2.94	14.45±3.22
Salivary cortisol µg/dl (±SD)	0.34±0.19	1.52±0.68	0.44±0.41	1.08±0.76

Baseline total cortisol and free cortisol as well as serum free cortisol and salivary cortisol were positively related in normal responders (n=48, r=0.43, p=0.0025 and r=0.52, p=0.0068, respectively) but not in subjects showing subnormal response. Using the lowest attained peak serum free cortisol in the normal group, the minimal "pass" level for normal serum free cortisol response to 1 µg ACTH was set at 0.9µg/dl , nearly identical to the "pass" level we previously defined for ACTH- stimulated salivary cortisol (Clin Endocrinol. 2006,64:215-8). Nevertheless, peak response for serum free cortisol and salivary cortisol were not correlated in either normal or abnormal tests. Of particular significance is the observation that 6/20 of the patients showing subnormal total cortisol response had a normal serum free cortisol response to 1 µg ACTH. Nevertheless, the peak total cortisol in subjects showing subnormal (<0.9 µg/dl) free cortisol and subnormal total cortisol response was appreciably lower than in patients with decreased stimulated total cortisol but normal stimulated free cortisol (13.34±3.12 vs. 16.53±2.2 µg/dl, P<0.01).).
Conclusions: In summary, some subjects showing borderline subnormal total cortisol response to1 µg ACTH have normal serum free cortisol response. The measurement of serum free cortisol allows further refinement of the assessment of borderline responses to 1ug ACTH.

Abstract Code: A6

Is Bilateral Adrenalectomy the Treatment of Choice for ACTH-Independent- Macronodular-Adrenal-Hyperplasia ?

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Introduction: ACTH Independent Macronodular Adrenal Hyperplasia (AIMAH) is defined as Cushing's syndrome caused by enlarged adrenals composed of macronodules with surrounding active hyperplasia. There is no long term specific treatment for this disease and bilateral adrenalectomy is the treatment most commonly used. It is not clear if unilateral adrenalectomy can induce a long period of remission, avoiding hypoadrenalism for significant time.
Patients/ Methods: We present a case of AIMAH in which unilateral adrenalectomy induced a remission of 14 years. This long remission is supported by 4 other cases in which a remission was induced for years, without recurrence of Cushing's syndrome thus far.
Results: Presenting case: A 49 Y.O woman presented 16 years ago with classical symptoms and signs of Cushing's syndrome which developed within 2 years. UFC 450 µg/24h (n 20-90), ACTH level < 5.0 pg/ml (n 5-46). Abdominal CT demonstrated a 6.5 cm tumor in Lt adrenal gland. She underwent open adrenalectomy, pathological report was "An adrenal tumor of 7cm size". Glucocorticoid replacement was administered, tapered down and stopped after one year. After 14 years of being euadrenal, she gradually developed weight gain, hypertension, hyperglycemia and mild hypokalemia. UFC 390 µg/24h (n 20-90), ACTH level < 5.0 pg/ml (n 5-46). No suppression on high dose dexamethazone. CT demonstrated a 6cm Rt adrenal tumor. The patient underwent laparoscopic adrenalectomy and pathological report was "Adrenocortical nodular clear cell hyperplasia, 7.5x6.5x1.7cm in size". Revision of slides from the first surgery showed the adrenal tissue outside the adenoma to be hyperplastic. Further cases: In the last 8 years we encountered 5 other cases of AIMAH. All had Cushingoid features, elevated and unsuppressed cortisol levels, undetectable ACTH, and bilateral adrenal enlargement on CT. In each patient adrenal scintiscan showed isotope enhancement in one adrenal more the other. All 5 patients underwent laparoscopic adrenalectomy of the adrenal which showed isotope accumulation and histology in all patients was characteristic for AIMAH. In all but one patient did post surgical cortisol drop to very low levels, and adrenal functions returned to normal within 1-10 months. These 4 patients remain euadrenal for 2-8 years now. Only one patient sustained elevated cortisol levels after surgery and had the second adrenal removed laparoscopically 2 months later.
Conclusions: Laparoscopic adrenalectomy is a commonly used procedure nowadays. As bilateral adrenalectomy and consequent hypoadrenalism is both irreversible and debilitating, we suggest unilateral adrenalecomy, preferably scintiscan guided, as the treatment of choice for AIMAH.

Abstract Code: A7

p38MAPK-induced Disruption of Hsp90-ErbB2 Interaction Promotes Src-Dependent ErbB2 Transactivation and IRS-1 Serine Phosphorylation Under Stress Conditions

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Introduction: TNF is a key mediator of insulin resistance in infection, obesity and diabetes. In addition, other agents and conditions that induce cellular stress including the translational inhibitor anisomycin (AN) and oxidative stress, impair insulin action. Previously, we had demonstrated in the rat hepatoma Fao cells, that the stress kinase p38 is playing a pivotal role in a cytokine- and cellular stress-induced ErbB2/3 transactivation. This in turn triggers a PI3K cascade, which induces the serine phosphorylation of IRS proteins resulting in insulin signaling desensitization.
Patients/ Methods: To expand our knowledge on the mechanism by which p38 leads to transactivation of ErbB receptor in response to stress signals, we analyzed in Fao cells, by a combination of biochemical and molecular biology techniques, the role of p38 activation on the interaction of ErbB2 with the molecular chaperone Hsp90, a modulator of ErbB2 signaling.
Results: Coimmunoprecipitation studies revealed that p38 activation either by stress stimuli (AN) or by overexpression of constitutively active MKK6 (a p38 upstream kinase) promotes dissociation of Hsp90 from ErbB2 and in parallel enhanced its tyrosine phosphorylation. Correspondingly, dominate negative-p38 overexpression attenuated these processes. Interestingly, the disruption of HSP90/ErbB2 interaction was concomitant with dissociation of Hsp90 from Src and by enhanced ErbB2 phosphorylation on Tyr877, a Src phosphorylation site. Furthermore, treatment Fao cells with PP1, a Src-class kinase inhibitor resulted in prominent reductions of stress-induced ErbB2 phosphorylation that culminating with inhibition of its downstream kinases signaling and IRS-1 serine phosphorylation.
Conclusions: Taken together, these data suggest that p38 can regulate ErbB receptors transactivation by controlling the Hsp90 association with ErbB2 and Src. Stress-induced p38 activation lead to disruption of Hsp90 interactions with ErbB2 and Src and permits Src-dependent ErbB2 phosphorylation. This phosphorylation is necessary for ErbB receptors transactivation and mediates IRS-1 serine phosphorylation, culminating in insulin signaling impairment. These findings point to new potential sites for therapeutic intervention to correct insulin-resistance.

Abstract Code: A8

Role of the metabolic energy regulator AMP-activated protein kinase in skin keratinocytes proliferation and differentiation

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Introduction: AMP-activated protein kinase (AMPK) is a member of a large serine/threonine protein kinase family that monitors energy status and regulates the metabolic pathways in response to reduced cellular energy charge. As such, AMPK plays a central role in regulation of metabolic reactions in the cells. Recently it has been proposed that the metabolic state of cells affects cellular growth and cell fate. Thus, in the present study we investigated the effects of AMPK activation on skin cell growth, differentiation and apoptosis, in order to reveal a possible link between AMPK-signaling and skin cell fate.
Patients/ Methods: For this purpose we studied cultures of primary murine keratinocytes that were infected by adenovirus expressing constitutive active (CA) and dominant negative (DN) forms of AMPK.
Results: It was found that overexpression of CA-AMPK caused a decrease in the proliferation rate, measured by thymidine incorporation into DNA, while overexpression of DN-AMPK raised the proliferation rate. Next, we investigated the effect of over expressing the constitutively active- and dominant negative forms of AMPK on keratinocytes differentiation. Differentiation of keratinocytes was followed by measuring the induction of keratin1 (K1) which is an "early" marker of keratinocyte differentiation, by Western blot as well as by immunocytochemistry staining for K1. We have found that in cells infected with the CA-AMPK there was a marked decrease in the induction of keratin 1, in comparison with DN-AMPK. Interestingly, over expression of the CA AMPK protein promoted a significant increase of apoptosis.
Conclusions: Thus, our results demonstrate that in addition to its well-established role in maintenance of cellular energy balance, AMPK also functions to regulate keratinocyte proliferation, differentiation and apoptosis, directing the cells to decreased proliferation and to cell death. Further studies are required to further substantiate the interaction between the regulation of skin dietary state by AMPK, as well as by other metabolic regulators, and skin cell turnover.

Abstract Code: A9

Serine Phosphorylation of Insulin Receptor Substrate Proteins by Glycogen Synthase Kinase-3 and its Priming Kinases

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Introduction: Serine phosphorylation of insulin receptor substrate proteins (IRS) is a potential inhibitory mechanism in insulin signaling. Our work focuses on the role of serine phosphorylation of IRS-1/2 catalyzed by Glycogen Synthase Kinase 3 (GSK-3) in insulin signaling and its contribution to insulin resistance. In recent work we identified GSK-3 phosphorylation sites at IRS-1 as serine 332, however, the fact that GSK-3 requires pre-phosphorylation of its substrates prompted us to identify the 'priming' kinases that phosphorylates IRS-1 at serine 336. Interestingly, GSK-3 phosphorylation site in IRS-1 is absent in IRS-2. Therefore, it was not possible to predict the GSK-3-IRS-2 phosphorylation site(s) by similarity. In the present work we aimed to identify GSK-3-IRS-2 phosphorylation sites and IRS-1/2 respective priming kinases.
Patients/ Methods: We generated specific anti-phopsho IRS-1 antibody that recognizes IRS-1 phosphorylation at serine 332 or 336. Cells were treated with phorbol-estres or PKC inhibitors to verfy the role of PKC in the phosphorylation of IRS-1. Epidydimal fat tissue were obtained from high fat diet-fed C57BL/6J mice (HF) and ob/ob mice.Tissue extracts were prepared and immunoprecipitated proteins were subjected to Western blot analysis using the specific anti-phospho IRS-1 antibody (332, 336). To identiy IRS-2 phosphorylation sites, DNA constructs of IRS-2 fragments were generated and expressed in HEK-293 cells. The ability of GSK-3 to phosphorylate each fragment was examined by in vitro kinase assays. Heptoma H4IIE cells were transiently transfected with JNK and GSK-3 constructs and insulin-induced tyrosine phosphorylation of IRS-2 was determiend by immunoblot analysis using anti-phospho tyrosine antibody.
Results: We found that PKC primes IRS-1 at serine 336,which in turn enhanced GSK-3 ability to phosphorylate IRS-1 at serine 332. In vitro kinase assays and studies in cells overexpressed with various PKC isoforms, indicated that the conventional PKC's (alpha or beta) are likely the priming kinases. However, expression levels PKCbetaII but not PKCbetaI or PKCalpha were remarkably elevated in the fat tissue of ob/ob or HF mice as compared with fat tissue from lean animals. This was associated with increased activity of GSK-3beta, PKCbetaII, and enhnaced phosphorylation of IRS-1 at serines 336/332. Moreover we were able to demonstrate a direct modulation of serine 336 phosphorylation of IRS-1 by PKCbetaII using a recombinant adenovirus encoding PKCbetaII in adipocytes. To further identify GSK-3 phosphorylation sites in IRS-2, we first identified the priming kianse. It was found that among several stimulators, the stress activator anisomycin enhanced GSK-3's ability to phosphorylate IRS-2. Use of a selective c-jun-N-terminal kinase (JNK) inhibitor and cells over-expressing JNK implicated JNK as the priming kinase. Activation of JNK in H4IIE liver cells by anisomycin inhibited insulin-induced tyrosine phosphorylation of IRS-2, this inhibition was reversed by pre-treatment with JNK and GSK-3 inhibitors. Overexpression of JNK and GSK-3 in these cells reduced insulin-induced tyrosine phosphorylation of IRS-2 and its association with the p85 regulatory subunit of the PI3 kinase. Finally, both GSK-3beta and JNK were abnormally upregulated in the diabetic livers of ob/ob mice.
Conclusions: We suggest that sequential phosphorylation of IRS-1/2 by GSK-3 and its priming kinases, PKC or JNK, represent an important mechanism in the progression of insulin resistance and in the pathogenesis of type 2 diabetes.

Abstract Code: A10

A preformed signaling complex mediates GnRH activated ERK phosphorylation of paxillin and FAK at focal adhesions in LβT2 gonadotrope cells

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Introduction: Most receptor tyrosine kinases (RTKs) and G-protein coupled receptors (GPCRs) activate MAPK cascades but still exert diverse functions and therefore signal specificity remains an enigma. Also, most GPCR ligands utilize families of receptors for mediation of diverse biological actions, however the type I GnRH receptor (GnRHR) seems to be the sole receptor mediating GnRH-induced gonadotropin release. Signaling complexes associated with GPCRs may provide the means for signal specificity.
Patients/ Methods: We used an immunoprecipitation coupled to western blotting method to detect the signaling complex associated with the GnRHR, which seems to mediate GnRH-activation of ERK. To this end we utilized the gonadotrope cell line LβT2.
Results: Here we describe a signaling complex associated with GnRHR, a unique GPCR lacking a C-carboxy tail. Unlike in cases of other GPCRs this signaling complex is preformed and exposure of LβT2 gonadotropes to GnRH induces dynamic rearrangement of the complex. The signaling complex core is c-Src and its binding partners include PKC δ, ε and α, Ras, MEK1/2, ERK1/2, tubulin, FAK, paxillin, vinculin, caveolin-1, KSR-1 and the GnRHR. Exposure to GnRH (5 min) causes MEK1/2, ERK1/2, tubulin, vinculin and the GnRHR to detach from c-Src followed by their re-association with the complex by 30 min. On the other hand, FAK, paxillin, the PKCs and caveolin-1 stay bound to c-Src and KSR-1 appears in the complex only after 30 min of GnRH stimulus. GnRH activated ERK1/2 in the complex in a c-Src-dependent manner and the activated ERK1/2 phosphorylates FAK and paxillin, while caveolin-1 is phosphorylated on Tyr14 apparently by the activated c-Src.
Conclusions: RTKs and GPCRs translocate ERK1/2 to the nucleus to phosphorylate and activate transcription factors. We propose that the role of the multi-protein signaling complex identified here is to sequester a cytosolic pool of activated ERK1/2 to phosphorylate FAK and paxillin at focal adhesions apparently for cell migration and spreading.

Abstract Code: A11

Amino acids control mTOR signaling pathway in mammary epithelial cells

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Introduction: In addition to their role as protein building blocks, amino acids also function as signaling molecules that control the limiting step in protein production - translation initiation, through the mTOR signaling pathway. Recent studies with muscle, liver and heart tissues established a positive role for the branched-chain amino acids, especially leucine, on translation initiation. The present study focuses on the role of essential amino acids in regulating translation in differentiated mammary epithelial cells cultured under lactogenic conditions.
Patients/ Methods: Cell- culture: The murine CID-9 mammary epithelial cells were derived from continuously proliferating, non-transformed mammary epithelial cell populations that lack tumorigenic properties. To acquire lactogenic conditions, these cells were differentiated on Matrigel for 5 days in the presence of the lactogenic hormones insulin, hydrocortisone and prolactin. Consequently, proliferation is ceased, the cells aggregate to mammosoheres and synthesize milk proteins. Analysis of factors involved in translation initiation was performed by immunoblot assay. Protein synthesis was measured by metabolic labeling and immunoprecipitation.
Results: Re-addition of all the amino acids, or only leucine to amino acids-deprived cells augmented the phosphorylation of S6K1 and 4E-BP1 – signaling molecules that control translation initiation. Consequently, syntheses of total- and specific milk-proteins were recovered. Surprisingly, three amino acids exerted a negative, dose-dependent, effect on this signaling pathway. Lysine, histidine and threonine at concentration 5 times higher then in medium, inhibited phosphorylation of S6K1, 4E-BP1, as well as total and milk protein synthesis to levels lower than detected in the amino acids- deprived cells. Their repressive effects were additive and a combined addition of the three as a mix completely abolished phosphorylation of S6K1, 4E-BP1 and mTOR in the murine and bovine epithelial cells. Total protein and milk protein synthesis were significantly lower than in the amino acids-deprived cells. IRS-1 phosphorylation by S6K1 is a prior step for its inactivation. Addition of amino acids or leucine to the amino acids-deprived cells induced S6K1 and IRS-1 phosphorylation, thus repressing the activity of insulin-mediated pathway to mTOR. In amino acids-deprived cells S6K1 and IRS-1 phosphorylation was suppressed, thus allowing the insulin pathway to function while the amino acids-induced pathway activity is lowered. Surprisingly, a mix of the three negative amino acids induced IRS-1 phosphorylation while suppressing S6K1 activity. This finding suggests the involvement of additional regulator(s) in phosphorylating IRS-l -leading the shut down of both amino acids and insulin anabolic function of the cell, evidenced by the abrogation of total- and specific milk proteins synthesis.
Conclusions: Taken together, a mix of lysine, histidine and threonine blocks the amino acids-regulated pathway of mTOR signaling in a manner comparable to that of rapamycin. At high levels, it also suppressed the insulin-mediated anabolic wing of this pathway. Consequently, the synthesis of total- and specific milk proteins were significantly suppressed. .

Abstract Code: A12

A novel heart specific CRF receptor type 2β splice variant exhibits a dominant negative activity

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Introduction: Peptides of the corticotropin-releasing factor (CRF) family signal through the activation of two seven-transmembrane domain (7TMD) receptors, known as CRF receptor type 1 (CRFR1) and CRFR2, both of which exist as multiple splice variants. The CRFR2 has three functional splice variants in human (α, β, and γ) and two rodent variants (α and β). CRFR2α is the major splice variant expressed in the rodent brain, whereas CRFR2β is expressed in peripheral tissues, with highest levels of expression in the heart and skeletal muscle. Different studies have proposed an important endogenous cardioprotective role for CRFR2β in the heart. We have recently identified a novel heart specific CRFR2β splice variant derived from an insertion of additional, previously unknown exon, located in intron number 13 of the CRFR2 gene. This insertion cause a frame-shift of a single base-pair, resulting in additional neucleotide sequence and in-frame premature stop codon. This new mRNA variant encodes a 7TMD receptor, identical to CRFR2□ but carrying a novel cytoplasmic-tail.
Patients/ Methods: To further characterize this novel receptor splice variant and to try and elucidate its physiological functions we developed a specific antibody against its unique cytoplasmatic-tail, explored its mRNA distribution in peripheral tissues and studied his signaling and regulation in comparison with the known CRFR2□ isoform.
Results: The tissue distribution of the new splice variant transcript demonstrated a heart-specific expression, compared with wider tissue distribution of the known CRFR2□ isoform. The developed antibody was shown to be highly effective in recognizing the new splice variant both in transfected and in endogenous applications. Using epitope tagged-receptors we demonstrated that the new CRFR2□ splice variant have a very low cell-surface expression as compared with the wild-type receptor, and showed no activation of cAMP signaling when treated with his specific ligand, Urocortin-2. Moreover, when they were co-transfected, the new CRFR2β splice variant, dose-dependently, inhibited the cell surface expression of the wild-type receptor, acting as a dominant negative, preventing the wild-type receptor from signaling. Preliminary results regarding the regulation of this novel isoform suggest a differential regulation of the two isoform expressed in hearts obtained from mice undergo mild chronic stress paradigm, compared with control mice.
Conclusions: This study demonstrated, for the first time, the presence of a novel functional and heart specific splice variant of the CRFR2 gene. This novel receptor isoform may play an important role in heart physiology and in its response to stressful stimuli.

Abstract Code: A13

Five Novel Mutations, including insertion, identified in GCK in Patients with MODY in Israel

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Introduction: Maturity onset diabetes of the young (MODY) is characterized by hyperglycemia due to a primary defect in insulin secretion, nonketotic disease, monogenic autosomal dominant mode of inheritance, age at onset less than 25 years, and lack of auto-antibodies.
Patients/ Methods: In this study, 94 patients in Israel with a clinical presentation suggestive of MODY were screened for mutations in the gene encoding glucokinase (GCK, MODY2). Genomic DNA was isolated from peripheral blood lymphocytes, PCR amplified and analyzed by denaturing gradient gel electrophoresis (DGGE) and sequencing.
Results: Taking together, eleven mutations were found, with a relative frequency of 11.7% in this gene. Five mutations are novel: Four of them are missense mutations and one is an insertion of four nucleotides resulting in a premature stop codon.
Conclusions: The low overall mutations frequency found here indicate that mutations in glucokinase are not the main cause of MODY in Israel. It may suggest the involvement of other genes in the etiology of MODY in Israeli patients. These may be either other MODY genes such as HNF4α/MODY1, TCF1/MODY3 etc or other yet unidentified genes.

Abstract Code: A14

MODY Type 2 in Greig Cephalopolysyndactyly Syndrome (GCPS) as part of a Contiguous Gene Deletion Syndrome

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Introduction: GCPS (OMIM 175000) is a rare syndrome affecting limb formation and craniofacial development, with an autosomal mode of inheritance. The syndrome originates from a deletion in the 7p13 region, including the GLI3 gene. Atypical cases with additional symptoms including mental retardation (MR) are related to the loss of genes closely linked to the GLI3 locus.
Patients/ Methods: We describe a 7-year old girl with GCPS based on typical signs of macrocephaly, a broad nasal bridge, hypertelorism, syndactyly of hands and preaxial polysyndactyly of the feet, with MR. Following febrile convulsion-associated hyperglycemia, several fasting blood glucose measurements were in the range of 126-136 mg/dl.
Results: OGTT showed impaired glucose tolerance, and first-phase insulin response (1+3 min in IV-GTT) was 23 mU/L, compatible with impaired secretion of insulin. Her HgA1C was 6.9 (normal range 4.5-5.7), and Islet-cell, insulin and GAD autoantibodies were negative. At the time of the workup, the patient reported polyuria, polydypsia and a new onset of nocturnal enuresis. Glybenclamide was initiated, and over a period of 16 months of treatment her HgA1C was normalized but her fasting glucose levels are still elevated. Cytogenetic study has shown a deletion in 7p13-15 area that has been reported to include both GLI3 gene and the closely located glucokinase gene (GCK) , which accounts for the presentation of MODY type 2.
Conclusions: Although GCK gene was found to be deleted in five patients with atypical GCPS in a previous study, only one of them presented with borderline high blood glucose levels. We describe the first case of MODY type 2 in a patient with GCPS due to contiguous gene deletion syndrome

Abstract Code: A15

Adiponectin levels in adolescents with polycystic ovary syndrome

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Introduction: Adiponectin is an adipocytokine, its secretion and circulating levels are inversely proportional to body fat content. Adiponectin has a physiological role in the control of insulin sensitivity, some studies showed that high levels of adiponectin may protect against the development of type 2 diabetes. Polycystic ovary syndrome (PCOS) is a syndrome of ovarian dysfunction, with a prevalence of up to 10%. Its cardinal features are menstrual irregularities, hyperandrongenism and insulin resistance. Approximately half of the women with PCOS are overweight or obese. Among adult women with PCOS adiponectin levels were determined by several groups. Some investigators found that adiponectin levels correlated with degree of obesity but not with PCOS per se, while others claim that PCOS does have a role in determining adiponectin levels. The correlation between adiponectin levels and degree of obesity in adolescents with PCOS has not been investigated. The objective of this study was to determine whether Adiponectin levels are associated with PCOS per se or with the degree of obesity in adolescent girls.
Patients/ Methods: Forty four selected adolescent girls were classified as follows: 14 girls "at risk for overweight" and overweight (body mass index [BMI] standard deviation score >1.036) with PCOS, 16 lean (BMI SDS<1.036) with PCOS, and 14 lean (BMI SDS <1.036) without PCOS. Diagnosis of PCOS was based on the Revised Rotterdam criteria. Patients had to fulfill 2 out of the 3 following criteria: 1) oligo- or unovulation 2) clinical and/or biochemical signs of hyperandrogenism, after exclusion of other etiologies. 3) polycystic ovaries in ultrasound. Adolescents who used medication were excluded. The research protocol was approved by the ethical committee of the Sheba Medical Center.
Results: Adiponectin levels were significantly lower in obese adolescents with PCOS compared with normal weight adolescents (10.5±5.5 ng/ml vs. 18.0±7.4 p=0.03). Adiponectin level in normal weight PCOS adolescents (16.9 ±8.4 ng/ml) was higher than in obese adolescents with PCOS (10.5±5.5) however this did not reach statistical significance. There was no statistically significant difference between mean levels of androgens ie testosterone, androstendione, DHEAS, and 17-OH-progesterone between the groups. There was no difference in LH FSH and estrogen levels between the groups.
Conclusions: Our data show that adiponectin levels are associated with the degree of obesity in adolescent girls with PCOS. PCOS per se does not seem to play a pivotal role in determining adiponectin levels in adolescent girls. In addition adiponectin levels did not correlate with androgen levels.

Abstract Code: A16

The role of nitric oxide in beta-cell glucolipotoxicity

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Introduction: Cytokines induce beta-cell apoptosis via induction of inducible nitric oxide synthase (iNOS) with increased generation of nitric oxide (NO) leading to activation of cellular stress. Previous studies showed that high glucose and free-fatty acids (FFAs) increase NO production in islets, however, the role of NO as a mediator of glucolipotoxicity in type 2 diabetes is controversial.
Patients/ Methods: We studied the regulation of NOS expression in rat and P. obesus islets under in vitro conditions of glucolipotoxicity. In addition, we analyzed the effects of the NOS inhibitor L-NAME on beta-cell function and survival. Proinsulin biosynthesis was analyzed by tritiated-leucine labeling of islets followed by (pro)insulin immunoprecipitation. Insulin secretion was assessed by static incubations at 3.3 and 16.7 mM glucose and insulin content by RIA. The expression of NOS isoforms, JNK and cleaved Caspase 3 was analyzed by Western blot.
Results: Exposure of P. obesus islets to 22.2 mM glucose and 0.5 mM palmitate for 24 h and 5 days led to 60% depletion of islet insulin content at 5 days and impaired glucose-stimulated proinsulin biosynthesis and insulin secretion. Palmitate markedly increased nNOS protein expression at 3.3 and 22.2 mM glucose in both P. obesus and rat islets, however, it did not induce the expression of iNOS. L-NAME failed to improve beta-cell function in P. obesus islets at both time points. Surprisingly, treatment of islets with L-NAME increased the phosphorylation of the stress-responsive kinase JNK and of cleaved Caspase 3, indicating that inhibition of NOS resulted in cellular stress and apoptosis as a consequence. In P. obesus islets, treatment with FFAs and L-NAME augmented JNK and cleaved Caspase 3 activity in a synergistic manner. Moreover, preliminary experiments showed that adding DETA/NO, which generates a small amount of NO to INS-1E beta-cells treated with FFAs and L-NAME decreased the activation of JNK.
Conclusions: Taken together, our studies show that FFAs increase nNOS expression in islets and that the NO generated in response to treatment with FFAs protects the islets from cellular stress. Therefore, increased NOS expression and NO production in response to FFAs should be considered a protective adaptive response, rather than a mediator of glucolipotoxicity.

Abstract Code: A17

Systemic regulation of beta cell proliferation :a prabiosis approach

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Introduction: Multiple lines of evidence suggest that a set-point for tissue mass exists and is actively monitored, yet the signals that govern tissue homeostasis and regeneration are largely unknown. One basic question is whether tissue mass is regulated by local interactions or by systemic, blood-borne factors. To examine the contribution of systemic factors to tissue homeostasis and turnover we performed parabiosis experiments, where the circulatory systems of two mice are joined.We focus here on the systemic regulation of insulin-producing pancreatic beta cells. When beta cells are completely eliminated using the toxin streptozotocin, mice become irreversibly diabetic. By connecting the circulation of such mice to healthy mice, we ask if the blood of a diabetic mouse elicits a regenerative response in beta cells of a healthy partner.
Patients/ Methods: Two C57/BL6 mice are parabiosed. On post operative day (POD) 5 high dose (150 mg/kg) streptozotocin is injected to one mouse, rendering it diabetic. By POD 6-7 vascular connectivity is achieved. Blood samples for glucose and insulin measurements are routinely taken until sacrifice of the pair. We then examine pancreas gene expression, histology and beta cell proliferation rate.
Results: Parabiosis between healthy and diabetic mice significantly alters insulin-glucose physiology in both partners, including rapid oscillations of blood glucose levels in the diabetic partner. Healthy islets exposed to a diabetic circulation respond by increased insulin secretion and beta cell proliferation.
Conclusions: Parabiosis between healthy and diabetic mice significantly alters insulin-glucose physiology in both partners, including rapid oscillations of blood glucose levels in the diabetic partner. Healthy islets exposed to a diabetic circulation respond by increased insulin secretion and beta cell proliferation. These results indicate that beta cell proliferation is at least partially controlled by systemic factors. Ongoing experiments characterize the putative circulating beta cell mitogen, in particular the potential involvement of glucose and insulin.

Abstract Code: A18

Inhibition of diabetic nephropathy and reduction in oxidative damage in rats' kidneys by an anti diabetic material extracted from yeast

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Introduction: Diabetic nephropathy is one of the major complications of diabetes mellitus. Its pathogenesis includes: increased formation of glucose-derived glycated end products, activation of the aldose reductase pathway, increased formation of reactive oxygen species and decreased activity of antioxidant systems. The Glucose Tolerance Factor (GTF) is a dietary agent extracted from brewer's yeast. GTF reversed glucose intolerance in diabetic animals and humans. We found that GTF decreased blood glucose and lipids and reduced lipid peroxidation in both types of diabetes. We also found that GTF potentiated insulin action both in vivo and in vitro. The purpose of the present study was to examine the effects of GTF treatment on diabetic nephropathy in general, and on the antioxidant systems in diabetic kidneys, both in vivo and in vitro, in particular.
Patients/ Methods: Diabetes was induced by a single Streptozotocin injection. A group of diabetic animals was left untreated and additional group was orally treated with GTF for two weeks. Urine and blood were collected for biochemical determinations. The animals were sacrificed and their kidneys were removed for determination of peroxidation products, antioxidant enzymes activity and immuno histochemistry.
Results: Our results indicate that both urine volume and urine protein levels of diabetic rats were higher compared with control group, and were reduced following treatment with GTF. The higher levels of lipid peroxidation products measured in diabetic kidneys were remarkably reduced in kidneys of animals treated with GTF. The activity of key enzymes that was remarkably altered in kidneys of diabetic animals was positively affected by GTF treatment: aldose reductase activity was increased in diabetic kidneys, and significantly decreased by GTF treatment. Na/K ATPase activity was reduced in diabetic kidneys and significantly increased upon treatment with GTF. The antioxidant system (catalase, SOD, glutathione reductase), was also affected in diabetic kidneys and improved by treatment with GTF. In vitro studies were done with NRK (Normal Rat Kidney) cells grown in control and in high glucose medium, with or without the addition of GTF. The activity of Na/K ATPase and that of the antioxidant enzymes, significantly decreased in high glucose medium. Treatment with GTF significantly increased the activity of all these enzymes. We also found that the activity of aldose reductase that was significantly increased in high glucose conditions, was significantly decreased when GTF was added to the medium.
Conclusions: Our study demonstrates that GTF inhibits diabetic nephropathy and improves the activity of antioxidant systems in renal cells.

Abstract Code: A19

The role of hypothalamic CRF receptor type 2 in modulating metabolic and energy homeostasis during challenge

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Introduction: Corticotropin-releasing factor (CRF) and its related Urocortin's are key regulators of energy balance. The function of these peptides is mediated via the activation of CRF receptors type 1 (CRFR1) and CRFR2. Stressful stimuli, food deprivation and leptin administration, remarkably alter hypothalamic CRFR2 expression, suggesting an important role for this receptor in regulating energy homeostasis during challenge.
Patients/ Methods: To examine the role of CRFR2, expressed by the ventro-medial hypothalamus (VMH), in modulating energy balance, we used two complimentary in vivo models, consists of either VMH-CRFR2 knockdown (KD) or VMH-CRFR2 over-activation. Lentiviruses expressing CRFR2 shRNA were generated and validated in vitro. C57B/6 mice were bilaterally injected with either CRFR2-shRNA or control lentiviruses directly into the VMH. To establish a CRFR2 over-activation model we generated transgenic mice that over-express Urocortin-3 (a CRFR2-specific ligand) in inducible manner using the Tet-On system. Transgenic mice, carrying the tetracycline responsive element sequence upstream to the Urocortin-3 coding sequence, were injected with lentiviruses expressing the reverse transactivator protein, specifically into the rostral perifornical area, which was previously demonstrated to projects to the VMH. These mice were tested before and after dox administration. All mice were tested both on basal conditions and following exposure to a variety of physiological perturbations to homeostasis.
Results: Under basal conditions, no significant metabolic differences were detected in the VMH-KD injected mice as compared with the control-injected group. In contrast, over-activation of VMH-CRFR2 neurons led to increased respiratory exchange ratio (RER) and increased heat production (HP) as measured by indirect calorimetry. Following 24h food deprivation challenge, VMH-KD mice showed reduced RER during the light phase and a tendency toward lower HP, compared to control group. VMH-KD mice consumed 85% of control food levels in the 24h period following the food deprivation. Glucose or insulin tolerance test (GTT and ITT, respectively) performed in mice with over-activation of VMH-CRFR2, while kept for three weeks on high fat diet, showed improved glucose tolerance and increased insulin sensitivity compared to control mice. GTT profile of CRFR2-KD mice was similar to that of the control mice while, ITT profile revealed reduced insulin sensitivity in these mice compared to control.
Conclusions: The results obtained in this study support an important role for VMH CRFR2 neurons in the control of food intake and energy expenditure in response to homeostatic challenge and suggest a role for these neurons in glucose sensing.

Abstract Code: A20

mTOR Inhibitors Rapamycin and RAD001 (Everolimus) Induce Antiproliferative Effects in GH3 Cell Line and Human Pituitary Adenomas

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Introduction: The effect of mTOR inhibitors on pituitary tumors is unknown. Akt overexpression was demonstrated in pituitary adenomas, which may render them sensitive to the antiproliferative effects of these drugs
Patients/ Methods: To evaluate the in vitro effects of mTOR inhibitor rapamycin, and its orally-bioavailable analog RAD001 on pituitary cells, GH3 cells, a mammosomatotroph rat pituitary tumor cell line, and human GH-secreting and non-functioning pituitary adenoma (NFPA) cells were used
Results: Treatment of GH3 cells, cultured GH-secreting adenomas and NFPAs with rapamycin or RAD001 induced a significant dose- and time-dependent inhibition of cell viability. The inhibition of GH3 cell viability involved G0/G1 cell cycle arrest associated with cyclin D3 suppression. Expression of phosphorylated-p70S6K in GH3 cells, GH-secreting adenoma and NFPA cells was significantly reduced by rapamycin and RAD001. mTOR phosphorylation was significantly decreased by rapamycin and RAD001 in GH3 cells, while Akt phosphorylation was unchanged
Conclusions: Our results showed that mTOR inhibitors potently inhibit pituitary cell proliferation suggesting that mTOR inhibition may be a promising antiproliferative therapy for pituitary adenomas. This therapeutic manipulation may have beneficial effects particularly for patients harboring invasive pituitary tumors unresponsive to current treatments

Abstract Code: A21

siRNA targeting ECE-1 as a tool to inhibit tumorogenesis of ovarian carcinoma

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Introduction: Endothelin-1 (ET1) is produced by endothelial cells (EC), and is overexpressed in various tumors including ovarian carcinoma (OVC), where it advances tumor progression and metastasis. EC-derived ET1 may also directly affect angiogenesis essential for tumor growth. The active form of ET1 is generated by endothelin-converting enzyme-1 (ECE-1) thus determining its levels. This study therefore examined the use of siRNA targeting ECE-1 as a tool to silence ECE-1 expression and ET-1 production in EC and in OVC and its ability to inhibit tumorogenesis.
Patients/ Methods: Selective 21-mer siRNAs targeting a sequence common to all ECE-1 isoforms, were transfected into OVC cell lines – OVCAR3 and ES2 and bovine aortic EC (BAEC). mRNA levels were measured by Real Time PCR and protein levels were evaluated by Western analysis using specific antibodies. Peptide concentrations of ET1 were measured by EIA. Chemoinvasion was studied using Boyden chamber and MMP activity by gel zymography.
Results: OVC cell lines and patients' OVC samples expressed high levels of ECE-1. Moreover, ECE-1 was 2.5 fold higher in metastatic OVC as compared with the primary tumors. Transfection of siRNA targeting ECE-1 into ES2 and OVCAR3 cell lines decreased its mRNA levels to 88 % and 94 % and protein levels were reduced to 40% and 12%, compared to scrambled siRNA, respectively. ET1 concentrations in culture media were compatible to ECE-1 silencing, showing >80% inhibition. Next we examined the invasiveness of the silenced cells. Ablating ECE-1 in OVC caused a two-fold decrease in tumor cell invasiveness as compared to negative controls. The invasiveness was dependent on MMP activity as in both cell lines there was a significant reduction of MMP-2 activity in the ECE-1 silenced cells. Notably, addition of exogenous ET1 reversed these effects. Invasiveness is associated with loss of the epithelial features and gain of a mesenchymal phenotype, a process known as epithelial-mesenchymal transition (EMT). In ECE-1 silenced cells we observed an increase in E-cadherin (epithelial marker) that was accompanied by a reduction of N-cadherin (mesenchymal marker). ECE-1 siRNA was also efficient in targeting the enzyme in BAEC. Transfection of ECE-1 siRNA into BAEC reduced its mRNA to levels 35% as compared to controls. Western analysis showed that the amount of ECE-1 protein levels was reduced to 20-40% of original values and the concentrations of ET1 peptide were also significantly lowered
Conclusions: Collectively, these studies show that siRNA is an effective tool for manipulating ECE-1 expression and ET-1 biosynthesis in EC and in OVC. As it may simultaneously target cancer cells and the growing blood vessels, ECE-1 silencing may develop into a novel cancer therapy.

Abstract Code: A22

Klotho: a tumor suppressor and a modulator of the IGF-1 pathway in human breast cancer

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Introduction: The aging suppressor klotho is a transmembrane protein, which can be shed and act as a circulating hormone. It is mainly expressed in the brain and kidney, but also in endocrine-related tissues, including testes, ovaries and placenta. Klotho has been shown recently to inhibit the insulin and insulin-like growth factor 1 (IGF-1) pathways in hepatocytes and myocytes. Since IGF-1 and insulin regulate proliferation, survival and metastasis of breast cancer we studied klotho expression and activities in human breast cancer.
Patients/ Methods: Klotho expression was analyzed, using immunohistochemistry studies, in breast tissue arrays. Klotho effects on growth and signaling were studied, following klotho overexpression or downregulation, in the breast cancer cells MCF-7 and MDA-MB-231 and in the non-tumorous HEK293 cells. Soluble klotho was produced and purified in Drosophila cells. MTT assays were used to assess proliferation and Western blotting to evaluate expression and phosphorylation of signaling pathways proteins. Co-immunoprecipitation (Co-IP) was used to identify protein-protein interactions and confocal microscopy was used for localization studies of GFP-tagged klotho.
Results: Immunohistochemistry analysis of klotho expression on breast tissue arrays, consisting of 116 samples, revealed high klotho expression in normal breast samples, but very low expression in invasive cancer and in ductal carcinoma in situ (DCIS). In the cancer samples, high klotho expression was associated with smaller tumor size and reduced KI67 staining. Klotho overexpression and soluble klotho reduced, while downregulation enhanced, breast cancer cell proliferation, but had no effect on HEK293 cells. Klotho overexpression in breast cancer cells inhibited IGF-1-induced phosphorylation of the IGF-1 receptor (IGFR) and its downstream targets, activated GSK3β, which is negatively regulated by the IGF pathway, and increased expression of p53 and the tumor suppressor gene C/EBPβ. Co-IP studies revealed, for the first time, a direct association between klotho and the IGFR. Localization studies revealed klotho expression in the cell membrane and cytoplasm and altered localization following IGF-1 stimulation. Importantly, klotho did not inhibit IGF-1-induced activation in HEK293 cells, and did not affect activation of the epidermal growth factor (EGF) pathway in neither cell line.
Conclusions: In conclusion, we show, for the first time, high klotho expression in normal breast and reduced expression in breast cancer, discover klotho as a growth inhibitor of breast cancer cells and identify it as a potent inhibitor of the IGF-1 pathway in these cells. These data suggest that klotho is a novel tumor suppressor in breast cancer.

Abstract Code: A23

Differential regulation of IGF-IR gene expression by androgen receptor in benign versus transformed prostate cancer cells

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Introduction: The progression of prostate cancer from an organ-confined, androgen-sensitive disease to a metastatic one is associated with dysregulation of androgen receptor (AR)-regulated target genes and with a decrease in insulin-like growth factor-I receptor (IGF-IR) expression. The molecular mechanisms that are responsible for regulation of the IGF-IR gene in prostate cancer, however, remain largely unidentified. Previous studies have established that an active AR leads to increased IGF-IR levels. The aim of this study was to investigate the differential effects of wild type (wt) and mutant AR on IGF-IR gene expression in benign and transformed prostate cancer cells. To this end, we employed the P69 cell line, a benign primary prostatic cell line with high IGF-IR levels, and its metastatic derivative, M12, with low IGF-IR levels.
Patients/ Methods: P69 and M12 cells were stably transfected with expression vectors encoding the full-length wt AR, or mutant AR-T857A, that includes a mutation in the ligand-binding domain and, therefore, shows a promiscuous AR ligand response, or mutant AR-E231G, that includes a mutation in the highly conserved N-terminal domain (involved in interactions with co-regulators) and, as a consequence, shows oncogenic potential when expressed in transgenic mice. Wt and mutant AR mRNA expression in stable transfected P69-and M12-derived clones was monitored by semiquantitative RT-PCR and the transcriptional effects of AR were measured by coexpression experiments. In addition, methylation of the IGF-IR and AR promoters was evaluated by sodium bisulfite-DNA sequencing and 5-aza-deoxycytidine (a methylation inhibitor) treatment.
Results: Results of Western immunoblots showed that wt AR enhanced IGF-IR levels in tumorigenic M12 cells at the transcriptional level, while mutant AR expression vectors had no effect. Conversely, wt AR diminished IGF-IR levels in benign P69 cells, while mutant AR versions had no effect. Combined, these results show that the oncogenic status of prostate cells is a critical determinant of AR action. Next, we evaluate the methylation status of the IGF-IR and AR promoters in P69 and M12 cells. We were unable to detect methylation in the IGF-IR promoter in neither P69 nor M12 cells. In contrast, we showed that the AR promoter is methylated in M12, but not in P69, cells.
Conclusion: In summary, our results suggest that progression from early prostate cancer (P69 cells) to advanced stage disease (M12 cells) is associated with a decrease in IGF-IR expression, AR promoter methylation, and differential response to AR action.

Abstract Code: A24

Long acting somatostatin analogues are effective for treatment of type 1 gastric carcinoids

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Introduction: The enterochromaffin like (ECL) cells originating in the gastric mucosa may develop into endocrine tumours (gastric carcinoids), which are divided into three distinct categories: type 1 (GCA1), associated with chronic atrophic gastritis, type 2, associated with the Zollinger-Ellison syndrome (in the context of MEN1) and type 3, which is sporadic. ECL cell hyperplasia in GCA1 and eventual tumor formation results from the chronic hypergastrinemia secondary to achlorhydria. In this setting, treatment with a somatostatin analogue might impede ECL hyperplasia by suppressing gastrin secretion, by a direct anti-proliferative effect on the ECL cells, or by both mechanisms together. We have conducted a prospective study to assess the effects of long-acting somatostatin analogues (SSA) on the hypergastrinemia and related growth of enterochromaffin-like cells in patients with GCA1.
Aim: To confirm the suppressive effect of the SSAs on gastrin secretion and their ability to induce regression or complete disappearance of macroscopic tumors and the associated intestinal metaplasia in GCA1.
Patients/ Methods: Eight patients with GCA1 were treated with Sandostatin LAR (20 mg to 30 mg) or Somatuline Autogel 90mg given at monthly intervals for a period of at least 2 years. Biopsies from tumours and from surrounding mucosa were done before treatment and every 6-12 months during treatment. Sections were immunostained for chromogranin A (CgA) and Ki-67. Serum gastrin and CgA were measured. To determine association with pernicious anemia, vitamin B12 levels and serum anti-parietal cell, anti-intrinsic factor and anti proton-pump antibody titers were determined.
Results: In all patients the treatment was well tolerated, without any serious side effects and with alleviation of symptoms (e.g., abdominal pain, weight loss and soft stools), pernicious anemia was diagnosed in all of the study subjects (based on low vitamin B12 levels and at least one positive antibody). Periodic endoscopic surveillance over a 2-year period showed complete resolution or partial regression (a 50% or greater reduction in all measurable tumour, with no appearance of new lesions) of the tumors in 1/8 (12.5%) and 7/8 (87.5%) patients, respectively. The total number of visible tumours was reduced by more than 93%. Sections from both tumours and flat mucosa showed a reduced number of CgA immunoreactive cells. Gastrin levels were normalized in 25% and reduced by > 80% in the remaining 75% of patients, mean±SD serum gastrin decreased from 991.3±216 mI/L to 313± 43mI/L ($p<0.005$, normal range 40-108 mI/L).
Conclusions: Treatment of GCA1 patients with SSAs caused diminished tumor load and reduced ECL cell density with a concomitant decrease of serum gastrin levels. Our data indicate an important anti-proliferative effect of somatostatin analogues on ECL cells, providing clinical benefit with respect to disease symptoms and perhaps obviating the need for invasive therapies for GCA1 tumors.

Abstract Code: A25

The treatment of the neck in Differentiated Thyroid Cancer

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Introduction: Treatment of the neck in patients with differentiated thyroid cancer remains an issue of controversy. The purpose of the current study was to review the current policy for the elective and therapeutic treatment of the neck for thyroid cancer.
Patients/ Methods: The various regions of the neck are described. The treatment of the neck is divided into elective and therapeutic as well as treatment of the lateral and Paratracheal regions of the neck. Patients that were diagnosed with thyroid carcinoma and that were treated within the last 7 years in Tel-Aviv Medical Center were enrolled. The literature regarding the treatment of the neck for differentiated thyroid cancer was thoroughly reviewed.
Results: Neck dissection for thyroid cancer is a safe practice including surgery done for recurrent disease. Elective treatment of the lateral neck (the jugular chain) is not indicated for patients with well differentiated thyroid cancer. Elective treatment of the Paratracheal region, however, seems indicated in high risk patients (i.e. male patients or patients >45) or high risk tumors (aggressive types or aggressive behavior). When the jugular chain is involved with disease, there is enough data to support elective treatment of the Paratracheal region even if no metastases are noted on physical examination or imaging. Radio-guided surgery or "Berry picking" do not seem to offer a reasonable alternative to a comprehensive neck dissection in patients with nodal metastases from differentiated thyroid cancer.
Conclusions: A more aggressive treatment of the neck in patients with thyroid cancer may be indicated in selected patients. Comprehensive treatment of the neck and not solitary picking of involved nodes is the standard of care and the post-operative morbidity is minimal.

Abstract Code: A26

Micropapillary Carcinoma of Thyroid: How aggressive should we be and what is the price?

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Introduction: Thyroid microcarcinoma is defined by World Health Organization as carcinoma of 1.0 cm or less in greatest dimension. No increased mortality has been reported, however recurrences in the neck and distal metastases have been described. The study aim was to assess patients' outcomes after two different types of surgical therapy
Patients/ Methods: Data were retrieved from the charts of patients who were diagnosed with Papillary Microcarcinoma (PMC), and treated between 1978 and 2007 at the Department of Endocrinology, Lin Medical Center. Kaplan-Meier method was used to estimate cumulative incidence of recurrence
Results: 113 patients were diagnosed with PMC. The mean follow-up period was 7.8 years (range 0.5 to 29 years). The mean tumor size of unifocal tumors was 5.5±2.9 mm. 40 patients (35.4%) presented with multifocal tumors. 58 patients underwent total or near total thyroidectomy (TT) and 55 patients partial thyroidectomy (PT). 12 patients were included in the PT group due to high postoperative I131 uptake, although the surgical procedure was initially defined as total thyroidectomy according to a surgical report. The number of multifocal tumors was greater in TT group (p=0.031), 57.7% of them were bilateral. Neither distant metastases nor death were reported during the follow up period. All patients who experienced a recurrence presented with thyroid bed or lymph node recurrence. In PT group 4 patients experienced a tumor recurrence. The cumulative incidence of recurrence at 10 years was 6.6% and at 12 years – 14.4%. All recurrences in this group were detected by ultrasonography. In TT group 5 patients experienced a tumor recurrence. The cumulative incidence of recurrence at 10 years and at 12 years was 10.1%. All 5 recurrences were detected by unsuppressed Tg elevation without ultrasonographic evidence of tumor. The incidence of recurrence was higher in patients with multifocal tumors compared to patients with single tumor in both groups. 12 patients experienced permanent complications in TT group (8 patients - hypoparathyroidism and 7 patients - vocal cord paralysis) vs. 3 patients in PT group (vocal cord paralysis) (p=0.017).
Conclusions: TT is the treatment of choice in patients with multiple tumor foci or patients with known lymph node involvement. This approach allows unsuppressed thyroglobulin assessment, which is a sensitive marker for microscopic tumor recurrence. TT leads to a higher post surgical complication rate. Although the incidence of local recurrence is higher after PT, it is safe approach to treatment of patients with unifocal papillary microcarcinoma. Macroscopic tumor recurrence in patients after partial surgery can be easily detected by ultrasonography.

Abstract Code: A27

Well-Differentiated Thyroid Cancer: A comparison between microscopic and macroscopic disease

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Introduction: The rapidly increasing incidence of well-differentiated thyroid cancer is the result of smaller thyroid tumors (1 cm or less) being diagnosed more frequently. Few studies are available regarding the appropriate approach to this previously known postmortem incidental finding, and their results remain controversial

Patients/ Methods: In 2005 we started a registry of all non-medullar thyroid cancer patients followed at our institute. Inclusion is both prospective and retrospective regarding year of diagnosis. Here we analyzed the data for 217 patients with microscopic disease (MIC) compared to 540 patients with macroscopic disease (MAC).

Results: Patients in the MIC group were slightly older (50.8 vs 47.5 y.o., p=0.003), had a higher F:M ratio (189:37 vs 419:123, p=0.06), and were more affected by PTC type (98.2% vs 85.5%, p<0.001). Total/subtotal thyroidectomy was performed in 76.8% and 97.8%, MIC and MAC groups respectively. The disease was multicentric in 50.2% vs 46.8% (p=0.4) and bilateral in 42% vs 36.8% (p=0.272) (MIC vs MAC respectively). At initial treatment, lymph nodes were involved in 26% vs 29.8% (p=0.4), and distant metastases were found in 2.8% vs 5.6% (p= 0.222) (MIC and MAC, respectively). Persistent/recurrent disease was seen in 15.6% vs 31.6% (p<0.001) and new distant metastases in 3.2% vs 6.8% (p=0.105) (MIC vs MAC, respectively). With a median follow up of 5 years for each group, 93.5% in the MIC group were disease-free, compared to 77.3% in the MAC group (p<0.001)

Conclusions: The small differences seen in patients with microscopic and macroscopic disease may not justify a different therapeutic approach.

Abstract Code: A28

Clinical Use of rhTSH as an Adjunct to Radioiodine Therapy to

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Introduction: Age-standardized incidence of thyroid cancer is increasing among the population in Israel reaching 12.45 in Jewish women and 3.68 among Jewish men. These high rates of cancer are due to an increase in the detection of low risk cancers, probably secondary to an increase in diagnostic vigilance. Radioiodine is currently used for ablation of residual thyroid gland post total thyroidectomy and to treat persistent, recurrent or metastatic disease. Recently, preparation of patients for thyroid ablation with recombinant human TSH (rhTSH) and 100 mci 131I on L-T4 therapy has been approved in Europe and by the FDA as an alternative to thyroid hormone withdrawal based on studies demonstrating equivalent results and lower total body irradiation and better quality of life experienced by those who were not rendered hypothyroid. We report the treatment of 5 patients with well differentiated thyroid cancer with of rhTSH as an adjunct to radioiodine
Patients/ Methods: 3 men and 2 women, mean age 50.8 (25-77). Two patients were treated for ablation post-operation and 3 patients for residual disease after prior radioiodine. Patients who were treated for ablation were started with suppressive doses of L-T4 the day following the thyroidectomy and those who were treated for residual disease continued their suppressive dose throughout the treatment. . In both groups, rhTSH treatment consisted of one injection of 0.9 mg rhTSH IM for 2 consecutive days, followed by the therapeutic dose of 100 mci 131I for ablation and 112, 150 , 260 mci for residual disease treatment, 24 h after the last injection. Post therapeutic scans were performed 7 days after treatment. All patients signed an informed consent for unlabeled use of rhTSH. Outcome measures were: serum thyroglobulin levels on suppressive therapy and during TSH stimulation, clinical examination and neck US.
Results: No apparent disease was noted in the two patients treated for ablation. For 2 patients treated for residual disease – normalization of thyroglobulin and no apparent disease was noted. One patient has not yet completed the follow-up.
Conclusions: The small number of patients treated by rhTSH-aided postsurgical thyroid ablation demonstrated successful outcome similar to published larger series (Pilli et al JCEM 2007, Pacini et al JCEM 2006) .The procedure avoids the need for hypothyroidism and decreases whole body exposure to radiation.

Abstract Code: A29

Disease perception of patients with differentiated thyroid cancer

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Introduction: Differentiated thyroid cancer (DTC) has a very good prognosis and the overall long-term survival rate of patients with DTC is more than 90%. Nevertheless, it is well-recognized that diagnosis and management of cancer can have a major impact on patient's quality of life, and this is influenced by the patient's perception of the disease. The objective of the study was to investigate the DTC patients' illness perception in correlation with demographic parameters as well as with objective indices of disease severity.
Patients/ Methods: A self-administered questionnaire was filled by consecutive patients with DTC after signing an informed consent during their routine follow-up visit in the endocrine clinic. The patients' medical records were reviewed for data including gender, date of birth, date of diagnosis of DTC, staging of the disease, number of operations and number of radioactive iodine treatments the patient underwent, and evidence of disease persistence/recurrence. The patients were asked for additional demographic data including their family status, education and employment status. Patient's illness perception was measured by the Illness Perception Questionnaire – Revised (IPQ-R), yielding 7 sub-scales that represent different aspects of the illness perception, such as the extent to which the illness affects the patient's daily lives and their emotional well-being as well as their understanding of the illness. Two additional scales measure the illness identity: the number of symptoms that the patient feels, and the number of symptoms that the patient relates to the illness. The illness severity was measured by staging of the DTC, the number of operations and iodine treatments the patient had, and evidence for persistence or recurrence of the disease. Pearson or Spearman correlations between the demographic characteristics, illness perception sub-scales and the severity of the ilness were performed.
Results: One hundred and nine patients were enrolled (91 females, mean age 53.5 years). No correlation was found between patients' disease perception and the staging of their cancer. The patients' education level was found to be the most influential demographic factor affecting their illness perception. Patients that are relatively highly educated feel and relate less symptoms to their illness. They also consider the consequences of their illness and the way it affects their daily lives as less severe, and have a higher sense of knowledge and understanding regarding the illness. The number of iodine treatments was the only parameter of disease severity that significantly correlated with the patient's distress and negative perception of the illness. Patients that have undergone more radioactive iodine treatments were proved to find more symptoms related to the illness, and perceived the illness as having severe consequences and negative effects both on their daily lives and their emotional state. Illness perception sub-scales were highly correlated among themselves. A clear correlation was found between the emotional perception and all other sub-scales.
Conclusions: Emotional illness perception and not objective disease severity is the major factor inflicting on all aspects of the DTC patients perception. Patient's education is the strongest demographic factor and number of radioactive iodine treatments is the only medical factor affecting disease perception of DTC patients

Abstract Code: A30

Construction of gene therapy vector(s) targeting thyroid carcinoma cells

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Introduction: Thyroid cancer is one of the most common endocrine malignancies. As a step towards the development of gene therapy vectors directed toward human thyroid carcinoma, we set upon defining a minimal thyroid cancer-specific enhancer/promoter. Thyroglobulin (Tg) is a glycoprotein precursor for thyroid hormone biosynthesis, while the Tg promoter has been employed in gene therapy strategies for thyroid cancer, further substantial tissue-specific enhancement with a minimal Tg enhancer has yet to be established.
Patients/ Methods: To enhance Tg promoter transcriptional activity by the addition of a minimal Tg enhancer that will allow maximal thyroid-specific transcriptional activity for our future viral vectors. This minimal Tg enhancer is derived from deletion analysis of the human Tg upstream enhancer: size 1.4 kbp, localized between -3.6 and -2.2 kbp upstream from the transcription start site. The CAT (chloramphenicol acetyltransferase) reporter gene was used to measure Tg enhancer/promoter transcriptional activity in human thyroid carcinoma cell lines (NPA-papillary, MRO-follicular and ARO-anaplastic) as well as primary human thyroid and nonthyroid cells.
Results: The full length of the upstream enhancer (1.4 kbp) gave 13 and 2% CAT converted in follicular and papillary thyroid carcinoma cells, respectively. Deletion analysis of the 1.4 kbp enhancer revealed a Tg enhancer, "ΔPstI-EcoRV", 624 bp fragment, which is specific to human follicular and papillary thyroid carcinoma cells and yields maximal transcriptional activity (38 and 28% CAT converted, respectively, as compared to <1% CAT converted for anaplastic and primary human thyroid cells).
Conclusions: The Tg enhancer/promoter construct achieved a level of transcriptional activity which was directly correlated with the differentiation stage of the thyroid carcinoma cells: follicular 38%, papillary 28%, anaplastic <1% CAT conversion. The Tg enhancer/promoter construct we developed may serve as a template for future thyroid-specific gene therapy. Such a model could be used in exploring target gene manipulations aimed at thyroid cancer therapy.

Abstract Code: A31

Up-regulation of the epidermal vitamin D endocrine system by the inflammatory cytokine TNF

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Introduction: Calcitriol, the hormonal metabolite of vitamin D, is known as an anti- inflammatory agent in the epidermis. The epidermis contains an autonomous vitamin D endocrine system that has the ability to produce and degrade calcitriol. Calcitriol binds to its nuclear receptor VDR that forms a heterodimer with RXR to modulate the expression of vitamin D responsive genes. TNF is a universal inflammatory cytokine that is up-regulated in the epidermis following skin exposure to UV radiation, injury and viral or bacterial infection. In view of the well-established anti-inflammatory role of vitamin D in the skin, we aimed to examine the notion that the efficacy of the epidermal vitamin D endocrine system is up-regulated during epidermal inflammation as part of the self-limiting nature of the keratinocyte inflammatory response.
Patients/ Methods: Our experimental model were immortalized HaCaT cells cultured in the absence of exogenous growth factors or active mediators that present the mitotic population of basal keratinocytes. The inflammatory state was simulated by exposure to TNF (10 ng/ml) for 2 to 24 hours. Level of proteins was quantified by inmmunoblotting and level of mRNA by real-time PCR.
Results: Treatment with TNF up-regulated the expression of both the vitamin D receptor – VDR and the rate-limiting enzyme of calcitriol synthesis 25-OH vitamin D 1 a hydroxylase (1-OHase). The effect of TNF on 1-OHase mRNA was detectable after 8 hours, peaked at 12 hours and declined at 24 hours of exposure to the cytokine. The increase in the level of VDR mRNA was detectable after 8 hours and maximal between 16 and 24 hours. The increase of the in-situ efficacy of calcitriol signaling due to up-regulation of the VDR was demonstrated by increased mRNA level of the VDR target gene 25-OH vitamin D 24 hydroxylase following treatment with sub-maximal concentrations of calcitriol. By using specific inhibitors we show that the Jun kinase cascade is involved in the upregulaion of both VDR and 1-OHase. The PKA pathway participates in the up-regulation of 1-OHase, but not in up-regulation of VDR.
Conclusions: The up-regulation of the epidermal vitamin D endocrine system by the ubiquitous inflammatory cytokine TNF taken together with the known anti- inflammatory action of the hormone is in line with the notion that this system is an essential participant in the resolution of epidermal inflammation.

Abstract Code: A32

Growth without growth hormone in 3 sporadic patients with the same mutation in the HESX1 gene

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Introduction: The HESX1 gene is a transcriptional repressor which plays a role in the development of the optic nerves and the anterior pituitary during embryogenesis. Mutations in HESX1 gene are associated with a broad spectrum of phenotypes – septo-optic dysplasia (SOD), midline defects, and pituitary abnormalities with consequent hypopituitarism either isolated growth hormone deficiency (IGHD) or combined pituitary hormone deficiencies (CPHD). Furthermore, some of the mutations have been found in the homozygous state and others in the heterozygous state
Patients/ Methods: A total of 50 patients with CPHD and IGHD without SOD were screened for mutations at the HESX1 locus using PCR-DGGE-sequencing methodology
Results: Three CPHD patients (GH, TSH, ACTH and gonadotropines), out of the whole cohort, were found to have the same Asn125Ser mutation in HESX1. None had a family history of consanguinity or CPHD. Two patients presented at the neonatal period (severe hypoglycemia and prolonged jaundice), while the 3rd patient was referred at the age of 13.5 years because of lack of pubertal signs. Their physical examination was remarkable for coarse face and prominent big ears. MRI performed in two patients revealed hypoplastic anterior and ectopic posterior pituitary without other midline malformations. Despite persistent GHD and undetectable IGF1, all exhibited normal linear growth along the 10-25th percentile with no GH therapy.
Conclusions: Normal growth has been observed in patients with SOD and GHD. Our findings of growth without GH in patients with HESX1 mutation suggest that these previous cases might have had HESX1 mutations as well. It is possible that in patients with GHD and HESX1 mutation other mechanisms may compensate the GHD and enable normal linear growth. Yet, the identity of such factors is still not resolved.

Abstract Code: A33

Physical activity is associated with increased bone mass in female adolescents despite vitamin D deficiency

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Introduction: Major factors affecting bone mineral density (BMD), other than genetics and age, include physical activity (PA), estrogen, smoking, sun exposure, and calcium and vitamin D intakes. Studies have shown that PA is superior to other factors in determining BMD, but none have examined the relationship between PA and vitamin D in this matter. This was the purpose of this study.
Patients/ Methods: The study population consisted of 51 female adolescent ballet dancers and 97 female sedentary adolescents from a previous study, aged 15.1±0.8 years, grouped together due to similarities in biological and dietary characteristics. This grouping allowed for a wide range of PA (1-30 hours of PA/week) in an otherwise homogenous population. BMD was measured by DEXA. 25(OH)D3, PTH, and bone turnover markers were assessed. Subjects were grouped by serum 25(OH)D3 tertiles: less than 11.3, 11.3-14.9, and ≥15 ng/ml.
Results: Mean 25(OH)D3 level of the entire cohort was 13.4±4.5 ng/ml (median 12.8 ng/ml, range 3.2-28.8), and mean PTH levels were 31.5±12.9 pg/ml (median 30 pg/ml, range 11-81). Across 25(OH)D3 tertiles, there were no differences in age, weight, height, PA, calcium and energy intake, BMD, or parathyroid hormone. Multivariate regression analysis, controlling for age, body mass index, PTH and bone turnover markers, showed that total-body, femoral neck and lumbar spine BMDs were all positively related to PA, with regression coefficients increasing as vitamin D levels dropped across tertiles (each daily hour of PA increased total-body BMD by 0.28, 0.14 and 0.14 gr/cm2 across decreasing tertiles, femoral neck BMD by 0.56, 0.35, and 0.07gr/cm2, lumbar spine BMD by 0.35, 0.14 and 0.07 gr/cm2, respectively).
Conclusions: We conclude that vitamin D deficiency is frequent in adolescent girls. BMD is positively related to PA, and with increasing magnitude as serum vitamin D levels drop. These findings imply that PA can enhance bone mass even in the presence of marked vitamin D deficiency.

Abstract Code: A34

Is vitamin D anti-atherogenic? A pilot animal study

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Introduction: The pleiotropic effects of calcitriol, 1,25(OH)vitamin D3, outside of calcium metabolism are increasingly recognized. Epidemiologic evidence suggests vitamin D is inversely correlated with blood pressure. Recent data have indicated that calcitriol suppresses the renin gene, the rate limiting step of the renin angiotensin system (RAS).As suppression of renin leads to reduction in angiotensin II (AII), a potent pro-atherogenic cytokine, we anticipated that vitamin D treatment would reduce blood pressure and atherosclerosis in the Tsukuba Hypertensive Mouse (THM), a model of high AII hypertension and atherosclerosis. As hypercalcemia is a limiting factor to calcitriol treatment , in this study we also evaluated the effects of a less calcemic analog QW-1624-F22.
Patients/ Methods: At 6 weeks, THM animals were switched to an atherogenic diet, and calcitriol (n=35) or QW-1624-F22 (n=26) treatment was given for 12 weeks.The dosage was 0.5 ng/g body weight in 0.1% ethanol as intraperitoneal injections every other day.Control animals (n=43), received vehicle only. The blood pressure of the mice was measured non invasively at the beginning and at the end of the study. Blood glucose levels, BUN, triglycerides, cholesterol and calcium were taken. The extent of atherosclerosis was evaluated at the aortic sinus by serial sections of the area and quantification of the plaques stained by oil-red-o. The expression of renin, angiotensinogen, ATR-1 at the atherosclerotic lesions was examined by semiquantative RT-PCR of mRNA extracted from the aortas.
Results: By the end of the study, both treatment groups had normalized blood pressure (106.2±9.6, 105.7±9.8 mm Hg for calcitriol, and QW-1624-F22 respectively compared to 145.4±17 in controls, $P<0.0001$). The blood calcium levels of the mice treated with vitamin D were significantly higher Cholesterol levels were higher in both treatment groups.In PCR both treatment modalities tended to decrease the expression of mRNA of h-angiotensinogen Treatment with the analog decreased the expression of renin and of ATR-1 in the aortas of the mice. In contrast to our expectations, neither of the treatments reduced the development of atherosclerosis, most probably because the control mice did not develop significant atherosclerosis.
Conclusions: In this study we have shown that vitamin D and its analog lower blood pressure significantly. This effect which is probably due to reduction of renin levels and down regulation of the RAS. We were unable to evaluate the effect of vitamin D on atherosclerosis in this study, since the control mice did not develop significant atherosclerosis. Further studies- with older mice or a prolonged study period or the use of a lipid solvent instead of ethanol might be needed. The findings that vitamin D and its analog reduce the expression of mRNA of renin and ATR-1, support our assumption that vitamin D and its analog may have an a beneficial effect on the atherogenic process.

Abstract Code: A35

Eating Salad, Is It Good For Your Bones? Plant-Derived Micronutrients Inhibit Estrogen Signaling in Breast Cancer Cells But Support Estrogen Activity in Bone Cells.

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Introduction: The advantages of fruit and vegetable rich diet for human health are well known. Micronutrients from such diet were found by us to inhibit sex steroid signaling, the major risk factor in breast and endometrial cancer. In addition, carotenoid derivatives, polyphenols and isothiocyanate stimulate the electrophile/antioxidant response element (EpRE/ARE) transcription system and the Nrf2 transcription factor. Moreover, using siRNA for Nrf2 and chromatin immuno-precipitation (ChIP) assay we showed that Nrf2 is involved in the inhibition of estrogen activity in breast cancer cells. Although estrogen effect in breast and endometrial cancer is harmful, it is beneficial for bone formation. Thus, the aim of the present work was to determine whether micronutrients, which inhibit the harmful signals of estrogen in cancer cells, do not inhibit its positive action in bone cells.
Results: We used MC3T3-E1 mouse calvaria osteoblast like cells which respond to estrogen and MG-63 human osteosarcoma cells stably transfected with estrogen receptor alpha (ERα). Breast cancer T47D cells were used for comparison. Three major groups of plant derived micronutrients were used in this study: carotenoids – lycopene active derivates, polyphenols – carnosic acid, curcumin and resveratrol, and isothiocyanate - sulforaphane. We found that these micronutrients do not inhibit estrogen activity in bone cell lines. Moreover, some of them stimulated the estrogen induced reporter gene activity. However, these micronutrients activated the Nrf2 transcription system in bone cells similar to the activation of this system in breast cancer cells. Using real-time PCR we examined transcription of various markers of bone differentiation such as Osteocalcin, Osteoprotegerin and Alkaline Phosphatase. We found that the combination of micronutrients with estrogen up-regulated transcription of these genes, in contrast to the inhibition of estrogen-induced gene transcription in breast cancer cells. Nrf2 is involved in the inhibition of estrogen activity in breast cancer cells, whereas in bone cells, we found that reduction of Nrf2 level, by siRNA, leads to a decrease in micronutrient supported activity of estrogen.
Conclusions: Dietary micronutrients, which inhibit estrogenic activity in cancer cells, do not inhibit and even stimulate estrogen signaling in bone cells. The results suggest that the Nrf2 transcription factor is involved in this differential activity of plant-derived micronutrients. The mechanism for stimulation of estrogenic activity in bone cells is under study.

Abstract Code: A36

Treatment-resistant auto-immune hypoparathyroidism associated with celiac disease

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Introduction: A 32 year old female patient was referred to our endocrinology unit for evaluation of severe treatment-resistant hypocalcemia.
Patients/ Methods: She was diagnosed 5 years earlier with idiopathic hypoparathyroidism after presenting with a seizure 2 weeks after her first delivery. Since then, she was treated with oral elemental calcium, 1 alpha-OH-vitamin D3, and oral magnesium supplementation due to hypomagnesemia. In the year before our assessment, the patient had several hypocalcemic crises. Doses of oral medications were increased and twice-weekly intravenous treatment of calcium and magnesium were administered. Despite this, calcium levels remained low (mean 1.5 mmol/l, 2<N<2.5), with elevated levels of phosphorus (mean 2.3 mmol/l, 08<N<1.4) and low levels of magnesium (mean 0.5 mmol/l, 0.8<N<1.2). The physical examination was normal. An intra-venous magnesium infusion test failed to increase plasma PTH or calcium levels.
Results: The calcium-sensing receptor (CaSR) gene was normal by direct sequencing. An immune screening panel revealed antibodies positive for ANA. To determine if the patient's serum contains antibodies against the CaSR, we expressed hCaSR in human embryonic kidney 293 cells and used a flow cytometry assay to determine the effect of the patient's serum on the binding of a monoclonal antibody against the CaSR (ADD, Novus Biologicals). Addition of the patient's serum markedly reduced binding of the monoclonal antibody to the cell membranes when compared to control serum. To confirm this finding, we used immunohistochemical staining and determined that the binding of the monoclonal antibody to rat parathyroid tissue was blocked by the patient's serum but not by control serum. These results suggested that the patient had auto-immune hypoparathyroidism. We then searched for an explanation for her resistance to oral therapy. The patient had chronic diarrhea, which was thought to be related to oral magnesium supplementation. However, Celiac disease was diagnosed based on the presence of serum antibodies as well as a suggestive duodenal biopsy which showed lymphocytic infiltration. A gluten-free diet trial led to improvement of her diarrhea and stabilized her blood calcium (mean 1.9 mmol/l) without need for further intra-venous supplementation.
Conclusions: Although the association of idiopathic hypoparathyroidism and celiac disease has already been described, this is the first reported case with evidence that antibodies to the CaSR are directly involved

Abstract Code: A37

AMH is a better predictor than Inhibins and gonadotropins of ovarian function in survivors of chemotherapy

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Introduction: Women exposed to gonadotoxic chemotherapy suffer frequently premature ovarian failure [POF]. To evaluate the odds of continued cyclic ovarian function [COF] vs POF we have measured the concentrations of AMH, Inhibin B, Inhibin A, FSH, LH, and Estradiol in young women with or without cotreatment with GnRH-a in an attempt to minimize the gonadotoxic effect of chemotherapy.
Patients/ Methods: More than 150 women, 14-40 years old, have been treated with GnRH-a in parallel to chemotherapy for various indications [lymphoma, SLE, leukemia, breast cancer, and other], study group, and compared to 125 patients of similar age, diagnoses, and chemotherapy protocols who did not receive the GnRH-a cotreatment. The sera of a minority of these patients have been tested for AMH and Inhibins, in addition to , FSH, LH and sex steroids.
Results: Less than 7% of the patients who have been treated with GnRH-a in addition to chemotherapy suffered POF, as compared to almost half of the controls, who did not receive the GnRH-a cotreatment [$P < 0.01$]. The AMH had a better predictive value than Inhibin B or all the other hormones for subsequent ovarian function vs POF. AMH had a sensitivity of 81% and specificity of 78% for the correct prediction of COF or POF at 188.5 pg/mL, whereas Inhibin B had a sensitivity of 75% and specificity of 64% at 57 pg/mL.
Conclusions: AMH is a better predictor of COF vs POF in young women exposed to gonadotoxic chemotherapy. GnRH-a may minimize the gonadotoxic effect of chemotherapy.

Abstract Code: A38

Hormonal regulation of lutenizing hormone(LH)-beta and gonadotropin releasing hormone (GnRH) mRNA expression in cultured granulosa cells

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Introduction: Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are synthesized and released by the anterior pituitary and function to stimulate various gonadal activities. Lately, these glycoprotein hormones were found to be produced also in the mammalian testes and fish ovary. Recently we found that the rat ovary expresses LHbeta, FSHbeta and the common alpha subunit mRNA.
Patients/ Methods: In the present study we examined the regulation of LHbeta and of GnRH mRNA expression in rat ovarian granulosa cells that were isolated from either: (1) estrogen treated rats, a treatment that induces proliferation of the granulosa cells without affecting the maturation of the follicles, or (2) rats that were treated with pregnant mare serum gonadotropin (PMSG) that leads to maturation of the follicles towards ovulation, including proliferation and maturation of granulosa cells.
Results: We found that granulosa cells purified from PMSG primed rats expressed lower amounts of LHbeta and GnRH mRNA as compared to cells purified from estrogen primed rats. In both cell types, GnRH agonist treatment resulted in a decrease in LHbeta expression. However only in mature granulosa cells derived from PMSG treated rats, GnRH agonist treatment produced an increase in GnRH mRNA expression. The expression of GnRH mRNA was vastly increased by LH treatment of cells from PMSG primed rats, whereas the expression of LHbeta did not change. In contrast, FSH reduced the expression of LHbeta mRNA in granulosa cells from estrogen primed rats, but had no effect on the expression of GnRH mRNA.
Conclusions: These results indicate that the expression of LHbeta in the ovary is dependent on the maturation state of the follicle and is regulated by locally produced GnRH and by pituitary/ovarian LH. Thus, the hormonal regulation of gene expression in granulosa cell culture depends both on the expression of the relevant receptor and on the maturational state of the cells.

Abstract Code: A39

The secretion of the equine LH/CGbeta subunit combines different characteristics of the human LHbeta and CGbeta subunits

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Introduction: The gonadotropins LH, FSH and CG are non-covalent heterodimers composed of the common alpha and the hormone specific beta (b) subunits. The human (h) CGb genes have presumably evolved from an ancestral LHb gene and as a result a short carboxyl sequence of hLHb is replaced by a longer O-glycosylated carboxyl terminal peptide (CTP) in hCGb. While the short carboxy end of the hLHb subunit is involved in the intracellular retention of the protein, the CTP stretch of hCGb contains determinants for release. In vivo, the secretion profiles of the hormones differ. There is data showing that hLH is secreted from the basolateral surface of the pituitary gonadotrope. In contrast, hCG is released to the maternal circulation through the villi, at the apical side of the trophoblast cells to maintain the corpus luteum of pregnancy in humans. Recent studies, utilizing the Madin-Darby canine kidney (MDCK) model, identified sorting determinants in the b subunit of the hormones. While the secretion of the hLHb subunit from transfected MDCK cells was basolateral, the hCGb subunit was routed to the apical secretion pathway. This was primarily due to the O-glycosylated CTP. In contrast to primates, the placental CGb and pituitary LHb subunits in the equine (e), are products of the same gene (eLH/CGb) and both contain a glycosylated CTP. The distinguished expression pattern of this gene in horses compared to humans intrigued us to study (I) whether the secretion of the eLH/CGb subunit is analogous to that of hLHb or to the hCGb case and (II) to what secretion pathway the equine b subunit and eLH/CG heterodimer are sorted in polarized cells.
Patients/ Methods: To address these issues, we transfected the eLH/CGb cDNA alone and in combination with th alpha subunit into CHO and MDCK cells and the secretion into the media was examined by SDS-PAGE.
Results: In continuous labeling and pulse chase experiments, the secretion of the eLH/CGb subunit from the transfected CHO cells was inefficient (media recovery of 16-25%) and slow (t1/2 >6.5 hrs). This indicated that, in accordance with previous studies, the release of the eLH/CGb subunit resembles that of hLHb rather than hCGb. In polarized MDCK cells, grown on Transwell membranes, the eLH/CGb subunit was preferentially secreted from the apical compartment, similar to the hCGb subunit route (66.2±9% of the total apical plus basolateral secreted). However, the eLH/CG heterodimer seemed to exhibit only a modest apical preference (54.2±11%) over the basolateral compartment (45.8±11%), suggesting that unlike hLH (mainly basolateral secretion) and hCG (apical release), eLH/CG did not have a strong preference to a certain compartment.
Conclusions: Taken together, these data suggest that the intracellular behavior of the eLH/CGb subunit is unique and integrates features of the hLHb and hCGb subunits. The apparent random-like tendency to release the heterodimeric eLH/CG from polarized cells, rather than a vigorous exocytosis through a certain compartment, may fulfill the physiological needs for secretion of the hormone from the pituitary as well as the placenta.

Abstract Code: A40

Fatty Acyl CoA Reductase1: New Potential Regulator of the Ovulatory Process

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Introduction: The suppression subtractive hybridization (SSH) method was previously applied in order to systematically isolate genes with an ovulation-selective pattern of expression in the mouse. One of these genes: fatty acyl CoA reductase 1 (FAR1) which participates in the synthesis of ether lipids (EL) was characterized in this study. We aimed at characterizing FAR1 expression in ovulation, unveil the signaling cascade leading to its up-regulation and elucidate its role in ovulation.
Patients/ Methods: In order to examine the temporal expression pattern of FAR1, we analyzed cDNA of mouse ovaries at different time points after human chorionic gonadotropin (hCG) administration and of mouse follicles incubated with luteinizing hormone (LH) by qPCR. Localization of these genes was analyzed by in situ hybridization and confirmed by qPCR of RNA from isolated cumulus oocyte complexes (COCs) and granulosa cells. Different inhibitors/activators such as RU486, a progesterone receptor antagonist, indomthacin a prostaglandin synthesis inhibitor, UO126 a MEK inhibitor and forskolin an activator of adenylyl cyclase were used in order to unveil the signaling cascade leading to gene up-regulation.
Results: Our study demonstrates a temporal hCG/LH-induced expression of FAR1 in mouse ovaries in vivo and in vitro. We further localized FAR1 expression to cumulus and granulosa cells compartments of the ovarian follicle. Our experiments revealed that the LH-induced expression is mediated by cAMP and that prostaglandins and MAPK also play a crucial role in LH-mediated up-regulation of FAR1.
Conclusions: We conclude that LH-induced FAR1 elevation may imply on EL involvement in different processes in ovulation such as prostaglandin synthesis.

Abstract Code: A41

Uterine Dendritic cells are crucial for Decidua Formation during Embryo Implantation

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Introduction: The fate of the embryo is frequently decided upon its first contact with the maternal endometrium, at implantation. Dendritic cells (DC) accumulate in the uterus around this time. However, their function in pregnancy and specifically during implantation is not yet understood.
Patients/ Methods: To investigate the function of uterine DC (uDC) during embryo implantation, we used the CD11c:DTR mouse model that allows the conditional ablation of CD11c high DC by Diphtheria toxin (DTx) administration. Pregnant mice were treated by intra-peritoneal DTx injection at E.3.5, i.e. just before implantation and analyzed on E4.5 and E5.5. DTx injection led to the depletion of CD11c high DC, which covered the two day implantation window. uDC- depleted mouse embryo implantation sites were examined using flow cytometry, histology and immunohistochemistry complemented with two photon microscopy and macromolecular contrast- enhanced magnetic resonance imaging (MRI).
Results: We show that conditional depletion of uDC resulted in a severe impairment of the implantation process and led to embryo resorption. Arguing against their role in tolerance establishment, uDC depletion caused resorptions also in syngeneic and T cell-deficient pregnancies. Moreover, ovaries of DTx- injected mice exhibited normal corpora lutea and progesterone administration was unable to rescue the impaired implantation phenotype. uDC depletion was associated with impaired proliferation and differentiation of the decidua. Furthermore, using MRI-assisted studies we found decidual angiogenesis to be perturbed in uDC absence.
Conclusions: Collectively, our data suggest a novel role for uDC directly linked to tissue remodeling associated with decidualization, which governs uterine receptivity.

Abstract Code: A42

Involvement of PKCβII and RACK1 in cortical granules exocytosis

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Introduction: Fusion of the mammalian sperm with the metaphase II (MII) arrested egg, results in a rise in intracellular calcium concentration ([Ca2+]i), resumption of the second meiotic division and cortical granules exocytosis (CGE). The pathway leading to CGE is a network of interacting proteins affecting each others activity. In a previous study we demonstrated that CGE can be triggered by an increase in [Ca2+]i, as well as by activation of protein kinase C (PKC). We have also demonstrated activation of PKCα, PKCβI and PKCβII during in-vivo fertilization and following parthenogenetic activation, which lead to their translocation and to CGE. All 3 PKCs translocated from cytosol to cortex, with PKCβII demonstrating the most rapid translocation. A group of lipid receptors, named receptors for activated C kinases (RACKs), located at the cytoskeleton and the cell membrane may enhance and prolong PKC activity and may serve as shuttling proteins to the cell membrane. RACK1, the founding member of this family, has been found to be specific for PKCα and PKCβII. The aim of our study was to examine the possible interaction between PKCβII and RACK1 and their involvement in egg activation, particularly in the signal transduction pathway leading to CGE.
Patients/ Methods: Rat eggs at the MII stage were exposed for various time intervals to the parthenogenetic activator, TPA (12-O-tetradecanoyl phorbol-13-acetate). Expression of PKCβII and RACK1 in MII eggs and in eggs parthenogenetically activated by TPA was demonstrated by western blot anaylsis. PKCβII and RACK1 localization and translocation within the eggs were imaged using immunohistochemistry and confocal scanning microscopy. Eggs were fixed, labeled for CGE with LCA-biotin and Texas-red-Streptavidin, or labeled with either anti-PKCβII or anti-RACK1 antibodies and the appropriate fluorescent-labeled Cy secondary antibodies. DNA was labeled by Hoechst.
Results: We have demonstrated the expression and localization of PKCβII and RACK1 in MII and TPA activated rat eggs. We demonstrated a concomitant translocation pattern upon activation for both proteins, from the cytosol to both cortex and spindle, as well as a decrease in the amount of PKCβII protein and an increase in the amount of RACK1 protein.
Conclusions: Our results imply a mechanism in which RACK1 may serve as a shuttling and anchoring protein for PKCβII, positioning the signaling enzyme at the egg's cortex, in close proximity with its specific substrates, resulting in CGE.