

## MALIGNANCY PREDICTORS OF ADRENAL INCIDENTALOMAS

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

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

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

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

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

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

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

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

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

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

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

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

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

\* Mayo criteria

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

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

## PREVALENCE OF METABOLIC SYNDROME IN PATIENTS WITH ADRENAL INCIDENTALOMAS

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

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

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

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

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

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

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

## MULTIDISCIPLINARY APPROACH TO A PATIENT WITH HURTHLE CELL CARCINOMA

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

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

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

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

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

## **BILATERAL CORTISOL SECRETING ADENOMAS TREATED BY PARTIAL ADRENALECTOMY**

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

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

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

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

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

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

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

## CONGENITAL ADRENAL HYPERPLASIA IN DUE TO HSD3B2 MUTATION

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

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

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

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

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

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

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

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

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

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



## POSTTRANSCRIPTIONAL REGULATION OF HUMAN ADIPONECTIN RECEPTOR 2

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

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

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

# CELLULAR MECHANISM OF INSULIN MIMETIC MATERIAL DERIVED FROM YEAST

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

298 HCP (46% of HCP), age above 50 (43%) answered the questionnaire (56% female, 21% male, 23% missing data). Risk score for diabetes was 1.792(SD 1.08). BMI < 25kg/m<sup>2</sup>, between 25 BMI 30 kg/m<sup>2</sup>, between 31 and 40kg/m<sup>2</sup>, above 40kg/m<sup>2</sup> was found in 37.4%, 36.6%, 23.6% and 2.4% of the HCP respectively. The waist circumference was normal in 47.6% of HCP and 39.6% performed physical activity. In 2010, 106 of the HCP (11% response rate) rate 7.8 on a scale of 1 to 10, their satisfaction from their work in the field of diabetes. Sixty eight percent of HCP consider that work load impede diabetes care, 92% that individual treatment is the best way to diabetes care, 88% that group treatment should be part of diabetes care , 68% that group treatment could decrease work load, 31% already participated in group therapy and 59% would like to start group treatment for diabetic patients.

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

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

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

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

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

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

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

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

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

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

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



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

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

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

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

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

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

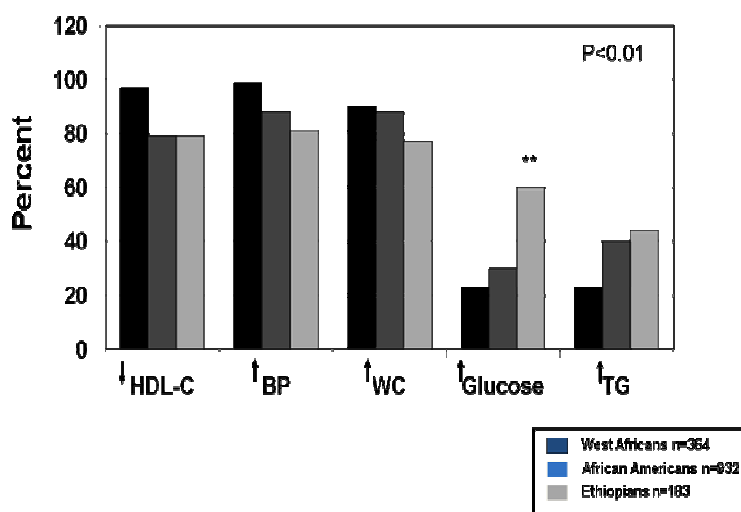
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Jewish Ethiopians lived for centuries as an isolated, rural community. Mass migration from sub-Saharan Africa to Israel occurred in the latter decades of the 20th century. On arrival in Israel, undernourishment was common, and type 2 diabetes mellitus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

**Variable Distribution in Metabolic Syndrome**



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

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

In a 13-week, multi-centre, open-label, non-randomized, uncontrolled, observational study including 338 Israeli patients with T2DM already treated with insulin, we evaluated the safety and efficacy of BIAsp (NovoMix®30, NovoMix®50, NovoMix®70 or combinations) in routine clinical practice. The dose and choice of BIAsp was by the discretion of physician. The patients (50.3% male) had an average age of 62.1±10.8 years (Mean±SD) with a BMI of 32.4±6.3 kg/m<sup>2</sup> and diabetes duration of 15.0±8.3 years. Overall, the rate of hypoglycaemia (episodes per patient year) was significantly reduced from baseline to end-of-treatment in total cohort for both symptomatic and major hypoglycaemia (Table). A total of 13 serious adverse events (SAEs) and one non-serious adverse drug reaction (ADR) were reported. SAEs were not related to the trial drug. HbA<sub>1c</sub>, FPG and PPPG were lowered after 13-week treatment with different BIAsp regimens (p<0.0001). No clinically relevant weight gain was observed. To conclude, in routine clinical practice in Israel, BIAsp treatment in patients with T2DM is safe and well tolerated, and is associated with a significant improvement in glycaemic control.

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

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