

The effect of ER α and ER β specific agonists on cell proliferation and energy metabolism in human vascular smooth muscle cells

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

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

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

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

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

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Introduction: In type 2 diabetes hyperglycemia and elevated free-fatty acids (FFA) induce metabolic stress leading to beta-cell dysfunction and apoptosis. In the short-term, glucose augments proinsulin biosynthesis and insulin secretion, however, chronic exposure to high glucose increases beta-cell apoptosis. It is not clear whether sustained stimulation of proinsulin biosynthesis adversely affects beta-cell survival in type 2 diabetes. PERK, an endoplasmic reticulum (ER) membrane associated kinase, phosphorylates eIF2 α leading to transient attenuation of translation. Robust activation of PERK-eIF2 α by FFA causes apoptosis through activation of ATF4 and CHOP. However, ATF4 may have a pro-survival effect by amelioration of the cellular antioxidant capacity. We hypothesized that PERK-eIF2 α coordinate proinsulin biosynthesis and the beta-cell response to metabolic stress and that dysregulation of PERK activity under conditions of hyperglycemia results in beta-cell apoptosis.

Patients/ Methods: Human and *Psammomys obesus* (P. obesus) islets and INS-1E beta-cells were incubated overnight at 3.3, 16.7 or 22.2 mmol/l glucose with and without 0.5 mmol/l palmitate. PERK and eIF2 α expression and phosphorylation and ATF4 and CHOP expression were analyzed by Western blot. The role of PERK in the regulation of proinsulin biosynthesis and beta-cell apoptosis was studied by RNAi knockdown of Perk in INS-1E cells. Proinsulin biosynthesis was analyzed by incubating islets or INS-1E cells with 3.3 or 16.7 mmol/l glucose for 1 h followed by metabolic labeling with L-[2, 3, 4, 5-³H]leucine and immunoprecipitation using anti-insulin serum. beta-cell apoptosis was assessed using the Cell Death ELISA PLUS assay (Roche Diagnostics, Manheim Germany).

Results: Incubation of human and P. obesus islets at 16.7 or 22.2 mmol/l glucose decreased basal PERK and eIF2 α phosphorylation. Moreover, prolonged exposure to hyperglycemia attenuated FFA-stimulated PERK and eIF2 α phosphorylation indicating that glucose inhibited PERK activity under conditions of metabolic stress. To study the impact of PERK inhibition on beta-cell function and survival, we knocked down Perk in INS-1E cells. This resulted in a 3-fold increase of proinsulin biosynthesis and beta-cell apoptosis at all glucose concentrations. Reducing proinsulin synthesis to basal levels by cycloheximide reduced beta-cell apoptosis, suggesting that the induction of beta-cell apoptosis by PERK inhibition may be related to augmented proinsulin synthesis. Glucagon-like peptide 1 (GLP-1) was shown to alleviate ER stress induced by pharmacological agents and FFA. Perk knockdown INS-1E cells were treated with the GLP-1 analogue exendin 4. This increased the expression of ATF4, a downstream target of eIF2 α expression and decreased beta-cell apoptosis. Finally, treatment of Akita mice, an animal model of beta-cell ER stress with exendin 4 improved hyperglycemia, increased serum insulin levels and preserved the islet architecture and insulin content

Conclusions: Chronic hyperglycemia inhibits PERK in islets resulting in increased proinsulin biosynthesis, which in turn renders the beta-cell susceptible to stress and apoptosis. Thus, PERK might be an important link between hyperglycemia and beta-cell stress in type 2 diabetes. Reducing ER protein load under conditions in which PERK is down-regulated, such as hyperglycemia may ameliorate beta-cell survival. Moreover, GLP-1 signaling increases the expression of ATF4 despite of decreased eIF2 α activity, thereby protecting the beta-cells from apoptosis. GLP-1 based therapy prevents beta-cell ER stress in vivo leading to improvement of diabetes.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Clinical effects of moderately intensive glycemic control after cardiac surgery

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

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

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

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

Stress hyperglacemia and Mody 2

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

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

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

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

Permanent neonatal diabetes due to an INS gene mutation

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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