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Enhanced lipid peroxidation and platelet activation in the early phase of type 1 diabetes mellitus.

Role of interleukin-6 and disease duration

Francesca Santilli, Francesco Chiarelli, Carlo Patrono, Giovanni Davi
Departments of Pediatrics and Medicine, University of Chieti, Italy

Background: To investigate early events possibly related to the development of diabetic angiopathy, we examined whether 8-iso-PGF_{2α} formation, a marker of *in vivo* oxidant stress, is altered in different stages of type 1 diabetes (T1DM) and whether it correlates with the rate of TXA₂ biosynthesis, a marker of *in vivo* platelet activation. We also investigated the relationship between inflammatory markers and F₂-isoprostane formation in this setting.

Methods: A cross-sectional study was performed in 23 insulin-treated patients aged <18 yrs with new onset T1DM (≤6 weeks, group A), matched for age and gender with 23 patients with stable disease (>1 yr, group B). Urinary 8-iso-PGF_{2α} and 11-dehydro-TXB₂ were measured in all patients and in age- and gender-matched controls. Circulating IL-6, TNF-α, and CRP were also determined as markers of the inflammatory response. Fifteen of the 23 children in group A were reexamined after 12 months.

Results: Compared with either controls or group B, diabetic children in group A showed significantly higher levels of 8-iso-PGF_{2α}, 11-dehydro-TXB₂, IL-6, TNF-α and CRP. Statistically significant correlations between IL-6 and both 8-iso-PGF_{2α} (Rho 0.63, p<0.001) and 11-dehydro-TXB₂ (Rho 0.51, p<0.01) were observed. The 15 patients reexamined after 1 year showed a significant reduction in lipid peroxidation and platelet activation (p<0.02 and p<0.001, respectively), consistent with reduced levels of IL-6 and TNF-α.

Conclusions: These results demonstrate that enhanced lipid peroxidation and platelet activation represent early events in T1DM, possibly related to an acute inflammatory response. These non-invasive indexes may help in further examining T1DM pathophysiology and monitoring pharmacologic interventions interfering with disease development and progression.

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Effects of vitamin E supplementation on intracellular antioxidant enzyme production in adolescents with early diabetic angiopathy

Francesco Chiarelli, Francesca Santilli, Cosimo Giannini, Stefano Tumini
Department of Pediatrics, University of Chieti, Italy

Background: Defective intracellular antioxidant enzyme production (IAP) has been demonstrated in adults with diabetic nephropathy. Primary objective was to evaluate the effects on IAP of vitamin E administration in adolescents with type 1 diabetes mellitus (T1DM) and early signs of retinopathy and nephropathy.

Methods: 12 adolescents (11-21 yr, diabetes duration 10-18 yr) participated. Eight of them had retinopathy (background, preproliferative or proliferative), 4 had persistent microalbuminuria. Skin fibroblasts were obtained by skin biopsies from the anterior forearm and cultured in Dulbecco's modified Eagle's medium. CuZnSuperoxide-dismutase (SOD), MnSOD, catalase (CAT), and glutathione-peroxidase (GPX) and mRNA expression were measured before and after 3 months of high-dose (600 mg b.i.d.) vitamin E supplementation; on both occasions antioxidant enzyme activity was evaluated at different glucose concentrations (5 mmol/l and 22 mmol/l). Ten adolescents with T1DM (age 12-20 yr) without diabetic angiopathy and 8 healthy volunteers (age 15-22 yr) participated in the study as control groups.

Results: In normal glucose concentration, CuZnSOD (0.54±0.21 U/mg protein; 4.3±1.4 mRNA/GAPDH), MnSOD (0.29±0.06; 0.8±0.3), CAT (0.34±0.09; 4.1±1.2), and GPX (0.50±0.12; 2.3±0.8) activity and mRNA expression were not different among the three groups (values of diabetics with angiopathy). In high glucose concentration, CuZnSOD activity and mRNA increased similarly in all groups (in angiopathics: 0.96±0.30; 9.9±3.2). CAT and GPX activity and mRNA did not increase in high glucose conditions only in the adolescents with diabetic angiopathy (0.35±0.09; 4.2±0.1, and 0.52±0.14; 2.4±0.9, respectively). MnSOD did not change in any group. Vitamin E supplementation had no effect on any enzymatic activity and mRNA in both normal and hyperglycemic conditions.

Conclusion: Adolescents with early signs of diabetic angiopathy have defective intracellular antioxidant enzyme production and activity, which is not modified by vitamin E.

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Increased cholesterol absorption and decreased biliary excretion in streptozotocin-diabetic rats are associated with decreased ABCG5 and ABCG8 expression

Willie M Bakker-van Waarde, Vincent W Bloks, Henkjan J Verkade,
Henk Wolters, Pieter JJ Sauer, Folkert Kuipers
University Hospital Groningen, The Netherlands

Background: Type 1 diabetes is associated with marked alterations in hepatic bile formation and with increased intestinal cholesterol absorption. The ATP Binding Cassette (ABC) halftransporters ABCG5 and ABCG8 have been implicated in control of both hepatobiliary cholesterol secretion and intestinal cholesterol absorption.

Methods: We have evaluated the expression of *Abcg5* and *Abcg8* in liver and intestine of rats with streptozotocin (STZ)-induced diabetes, insulin-treated-STZ rats and controls, in relation to relevant metabolic parameters.

Results: Realtime PCR analysis revealed that hepatic mRNA levels of both *Abcg5* (-76%) and *Abcg8* (-71%) were significantly reduced in STZ-diabetic rats when compared to controls. In spite of elevated levels of HDL cholesterol, considered a major source of biliary cholesterol, secretion of cholesterol into bile relative to that of bile salts was reduced by 65% in the diabetic animals. Intestinal mRNA levels of *Abcg5* (-47%) and *Abcg8* (-43%) as well as *Abcg5* protein levels were also reduced in insulin-deficient animals. This was accompanied by a 3-fold increase in plasma β -sitosterol levels and by a doubling of the calculated apparent cholesterol absorption. These effects partially normalized upon insulin supplementation.

Conclusions: Our data indicate that effects of insulin deficiency on bile composition and cholesterol absorption in rats are, at least in part, attributable to changes in hepatic and intestinal *Abcg5* and *Abcg8* expression.

**QTc interval prolongation and QTc dispersion
in type 1 diabetic children and adolescents**

Bert Suys, Daniel De Wolf, Sylvie Huybrechts, Bart Van Overmeire,
Dirk Matthys, Raoul Rومان
University Hospitals Antwerp and Ghent, Belgium

Background: Several recent studies describe a relation between QTc prolongation, diabetic complications and increased mortality in adults with diabetes.

Objective: To evaluate whether QT, QTc and QTc dispersion changes are already present in diabetic children and adolescents.

Method: QT, QTc and QTc dispersion were measured on a 12 lead surface ECG in 60 children and adolescents with stable type 1 diabetes, and in 63 sex- and age-matched controls. Differences were evaluated using the Kolmogorov-Smirnov Z test. The number of patients with QTc > 440 ms was compared in the two groups. The possible influence of age, gender, diabetes duration and HbA1c was examined using Spearman correlation analysis.

Results: Diabetic children had significantly longer QTc intervals and a significantly larger QTc dispersion. The number of individuals with a QTc > 440 ms was significantly higher in the diabetic group (14/60) than in the control group (2/63). The effect of age on RR interval and QTc dispersion in normal children was much less pronounced in diabetic children. HbA1c values did not significantly correlate with any of the parameters.

Conclusion: QTc prolongation and a larger QTc dispersion are already present in a significant proportion of children and adolescents with diabetes. Prospective follow-up is needed to determine whether these findings imply a higher risk of arrhythmias or sudden death, as mentioned in adult diabetic patients.

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Atherosclerosis in coronary arteries and right common carotid artery in type 1 diabetes are strongly associated in women

Jakob R Larsen, Magne Brekke, Lise Bergengen, Leiv Sandvik,
Harald Arnesen, Kristian F Hanssen, Knut Dahl-Jørgensen
Aker and Ullevål University Hospitals, Oslo, Norway

Background: Atherosclerosis develops at an early age in type 1 diabetes. Intima media thickness (IMT) measurements are now being used more actively in assessing atherosclerosis in young diabetics.

Objective: To test the association between coronary atherosclerosis and carotid artery atherosclerosis in type 1 diabetes patients with no history of cardiovascular disease.

Methods: Measurements of early development of atherosclerosis were performed with intracoronary ultrasound examinations (IVUS) and B-mode ultrasound measurement of IMT of the common carotid artery (CCA). Spearman's correlation coefficient was used to express associations between IMT and IVUS. Analysis was done for the whole group and separately for each gender.

Results: IVUS examinations were performed in 29 patients and CCA IMT measurements in 39 patients. Mean age was 43 years and mean duration of diabetes was 30 years. Cardiovascular risk factors were similar for males and females. For the whole group there was a significant association between max IMT and IVUS ($r=0.36$, $p=0.048$). This correlation was 0.74 ($p=0.009$) for women and 0.07 ($p=0.79$) for men.

Conclusions: Degree of atherosclerosis found in the carotid arteries is strongly associated with coronary atherosclerosis in women with type 1 diabetes mellitus.

**Are young females with type 1 diabetes at risk
for developing diabetic cardiomyopathy?**

Bert Suys, Nienke Katier, Dirk Matthys, Marc Du Caju,
Daniel De Wolf, Raoul Rooman

University Hospitals of Antwerp and Ghent, Belgium

Background: Several studies in adults have established diabetes mellitus as a strong risk factor for cardiovascular morbidity and mortality, especially in women. This study was designed to assess whether children and adolescents with type 1 diabetes have early echocardiographic signs of systolic and or diastolic dysfunction and whether tissue Doppler provides additional information.

Methods: Eighty unselected children and adolescents with stable type 1 diabetes and fifty-two age- and sex-matched controls underwent a complete echocardiography, including tissue Doppler measurements of the septal mitral annulus.

Results: Diabetic children had significantly higher body mass index standard deviation scores (BMI-SDS) compared to controls (0.57 vs -0.28, $p < 0.0005$). Female diabetic patients showed significantly larger left ventricular wall dimensions ($p < 0.05$) and signs of significant diastolic filling abnormalities ($p < 0.05$) on conventional and tissue Doppler echocardiography (increased mitral and tricuspid peak A velocity with decreased E/A ratio, higher E/E' ratio, longer isovolumic relaxation time-IVRT), suggesting delayed myocardial relaxation. Male diabetic patients differed significantly from their controls only for IVRT ($p < 0.005$). Correlation analysis showed an important influence of age and BMI-SDS on these parameters in the control group, less present in the diabetic group. Only a weak correlation was found for diabetes duration and glycosylated hemoglobin levels.

Autonomic and blood pressure pattern in a pediatric population affected by type 2 diabetes

Adriana Bobbio, Carla Sacchetti, Michela Trada, Barbara De Vito, Sonia Aguzzi, Marianna Michelazzo, Giannina Leotta, Mirko Tredici, Sara Del Colle, Ivana Rabbone, Franco Rabbia, Franco Cerutti
S Vito Hospital, University of Turin, Turin, Italy

Background: Type 2 diabetes mellitus (T2DM) is an emerging public health problem in the pediatric population, often associated with other cardiovascular risk factors such as hypertension, dyslipidemia and proteinuria. Our aim was to investigate the cardiovascular autonomic function and 24-hour BP pattern in a sample of children with T2DM.

Methods: 6 girls with T2DM (13.1 ± 1.2 yr) were compared with 8 healthy lean girls (12.9 ± 1.6 yr) and with 12 obese (OB) non-diabetic insulin resistant girls (13.6 ± 1.7 yr). In all participants we performed BP measurements, laboratory tests and 24-h ECG/ambulatory BP monitoring. 24-hour ABPM was obtained using Spacelabs 90207. Recording was programmed every 15 min over a 24-hour period. BP values were averaged to obtain 24-h, daytime (7 am to 8 pm) and night-time (midnight to 7 am) BP data. BP dipping and BP variability were calculated. The spectral power was quantified in total power (TP), low frequency power (LF, index of sympathetic tone), high frequency power (HF, index of vagal tone) and LF/HF ratio. Total, long-term and short-term time domain HRV were calculated.

Results: T2DM and OB had BMI significantly higher than healthy girls (27.6 ± 0.9 vs 29.7 ± 4.7 , vs 18.6 ± 0.4). Casual, 24 h, daytime and night-time BP values were similar in T2DM and healthy girls, whereas OB showed higher daytime SBP values. BP dipping and variability was similar in the 3 groups. All parameters reflecting parasympathetic tone (HF band, RMSSD, PNN50) were significantly reduced in the T2DM group compared to control and OB groups, particularly during night-time. Mean time LF and LF/HF (overall, daytime and night-time) were significantly increased in T2DM groups.

Conclusions: T2DM in pediatric patients is characterized by profound autonomic nervous system changes that are more pronounced than in subjects with insulin resistance but without diabetes. These alterations may provide important insights in prognostic terms.

Genetic predisposition to insulin resistance/sensitivity and cardiovascular/metabolic risk factors in obese children

David Meyre, Stéphanie Mouton, Iva Georgieva,
Philippe Froguel, Jacques Weill

CNRS, Biology Institute, University Hospital, Lille, France
Genome Center, Hammersmith Hospital, Imperial College, London, UK

Background: The aim of the present study is to compare cardiovascular metabolic risk factors between different classes of genetic predisposition to insulin resistance/sensitivity in obese children.

Methods: Predisposition to insulin resistance was characterized by comparing the distribution of standardized insulin sensitivity index ISI, corrected for gender, age, BMI Z-score and pubertal stage, between 225 obese children and their 59 non-obese sibs from 115 families.

Results: Percentiles of ISI for obese and non-obese children became significantly different below the 5th percentile and beyond the 95th percentile for non-obese, allowing the definition of 3 classes of obese children: insulin-resistant (IR, n=18); neutral (IN, n=142); insulin-hypersensitive (IS, n=26). Concerning obese children, there was no difference between the 3 classes for BMI Z-score, blood pressure, fasting glycemia, total cholesterolemia or plasma level of adiponectin hormone. There was a difference between IR and IS classes for waist circumference SDS (p=0.04), quotient waist/hip circumference (p=0.03), HDL-cholesterol (p=0.02) and leptin (p=0.005) plasma levels. There was a difference between the 3 classes for area under the curve (AUC) of glycemia during OGTT, for fasting value and AUC of insulinemia and for triglyceridemia (p<0.001). Their parents also presented a difference of insulin resistance/sensitivity and triglyceridemia (p<0.001).

Conclusions: 1) Correction of ISI for main environmental determinants and comparison of parents allow the definition of 3 classes of genetic predisposition regarding insulin resistance/sensitivity in obese children.

2) Well known heritability of triglyceridemia proceeds through insulin resistance.

3) Genetic predisposition to insulin resistance/sensitivity may be, at least partially, mediated by leptin.

**Comparison of different insulin sensitivity indices,
calculated from basal and OGTT-induced
insulin and glucose levels, in obese children**

Malgorzata Zawodniak-Szalapska, Renata Stawerska,
Urszula Pytasz, Jolanta Lukamowicz, Andrzej Lewinski
*Institute of Endocrinology, Medical University of Lodz,
Polish Mother's Memorial Hospital, Research Institute, Lodz, Poland*

Background: In industrialized countries obesity is an increasing problem. It represents the main risk factor for the development of insulin resistance. Several simple methods exist for calculating indices of hepatic and whole-body insulin sensitivity (IS) from measurements of plasma glucose (G) and insulin (I) concentrations, fasting and during the oral glucose tolerance test (OGTT). In this study, we compared different methods of assessment of IS.

Methods: 62 obese children with normal OGTT were enrolled in the study. The controls were 20 healthy, normal weight children. We calculated BMI and standard deviation scores of BMI (BMI SDS) for all examined children. The obese children were divided into two groups: moderate obesity (BMI >1.75 and <2.5 SD for height and age) and severe obesity (BMI \geq 2.5 SD for height and age). After an overnight fast the children received 1.75 g/kg glucose *per os* and the G and I levels were estimated at 0, 60 and 120 min after glucose administration. Based on the fasting G and I, we calculated a hepatic IS as a G/I index and insulin sensitivity index homeostasis model assessment: ISI (HOMA). According to data derived from OGTT, we assessed the whole-body IS as a ISI (Composite) and ISI (acc. Belfiore). These different IS indices were compared and correlated with BMI SDS.

Results: Positive correlations were found between ISI (HOMA) and ISI (acc. Composite) ($r=0.90$), between ISI (HOMA) and ISI (acc. Belfiore) ($r=0.49$), between G/I index and ISI (acc. Composite) ($r=0.89$), and between G/I index and ISI (acc. Belfiore) ($r=0.49$). Statistical differences among the groups (normal, moderately obese and severely obese) with respect to ISI (HOMA) and G/I index were noticed.

Conclusions: Positive correlations were found between calculated indices of ISI. Insulin sensitivity was significantly lower in children with severe obesity than in children with moderate obesity and children with normal weight.

**Risk of metabolic disturbance and diabetes development
in Thai obese children**

Chantraporn Keamseng, Supawadee Likitmaskul,
Pornpimol Kiattisakthavee, Linda Weerakulwatana,
Saroj Nimkarn, Pairunyar Sawathiparnich, Chanika Tuchinda
Siriraj Hospital, Mahidol University, Thailand

Background: The primary objective of the present study was to investigate changes in biochemical parameters in obese youngsters who came to our service at Siriraj Hospital and the secondary objective was to investigate the association of insulin resistance with indices of obesity and to identify the predictors for glucose intolerance in obese children .

Methods: Of the 77 obese patients enrolled whose age ranged from 3.1-19.0 yrs (mean 11±3 yrs), 40 were female and 37 male. Baseline fasting biochemical testing was composed of glucose, insulin, lipid profile. Insulin resistance was determined by homeostatic model assessment (insulin resistance index). Then a standard oral glucose load was given and blood sample for plasma glucose was drawn at 120 minutes.

Results: We demonstrated high percentages of hypercholesterolemia, hypertriglyceridemia and high LDL-C (45.4%, 46.7% and 33.8% respectively) and 46.7% had low HDL-C. 88.3% had high basal insulin levels. Impaired glucose tolerance test (IGTT) was identified in 33.8%. Silent type 2 diabetes mellitus was found in 2.6%. We found that children who had glucose intolerance have age and HOMA_{IR} significantly higher than the normal glucose tolerance group. One-fourth of prepubertal children had glucose intolerance especially those who were in late prepubertal and had high HOMA score. We also demonstrated a positive correlation between insulin resistance index and two-hour plasma glucose and triglyceride level, and a negative correlation between insulin resistance index and HDL-C.

Conclusion: These results showed an increased risk of metabolic disturbances among obese children, especially with regard to diabetes development. Impaired glucose tolerance was associated with insulin resistance and older age. Screening for metabolic disturbance should be considered among obese children not only in adolescence but also in late prepubertal age group in favor of early intervention and prevention of chronic complications in late adulthood.

**Abnormalities of glucose tolerance
in severely obese Singapore children**

Warren Lee, Jenny Tang, Hamidah Bte Karim, Fabian Yap,
Vera Oh, Stephanie Ng, Anne Goh
KK Women's and Children's Hospital, Singapore

Background: The prevalence of diabetes mellitus in Singapore is 9% of the adult population. Type 2 diabetes and IGT are increasingly seen in adolescents. We investigated prevalence of obstructive sleep apnoea and metabolic abnormalities in 60 severely obese (>170% of IBW) children.

Method: Investigations included poly-somnography, an oral glucose tolerance test, liver function, lipid and HbA1c profiles. Children with pathological results were referred for appropriate management, including surgical referrals, dietary counseling, psychological testing, medication, and an exercise regime as needed.

Results: There were 30 males and 30 females, mean age 12.6 (6-17.5 yrs), mean BMI $36.5 \pm$ SD 5 kg/m², mean weight 85.5 kg (range 42-125 kg) or 192% IBW (170-266), mean body fat 50.4% (32-73%). Obstructive sleep apnoea syndrome was found in 37 subjects, 50% of whom had abnormal OGTTs, unlike those without OSAS (38%).

	Chinese	Malay	Indian	Others	Overall
% in gen. population	77%	14%	7%	2%	
% of sample	47%	43%	7%	3%	
% above expected no.	61%	310%	100%	150%	
% with abnormal OGTT	40%	54%	50%	0%	45%
% with diabetes	3.6%	12%	0%	0	6.7%
% with IGT	36%	42%	50 %	0	38%

Conclusions: In 60 consecutively studied severely obese Singapore children (>170% IBW), 6.7% had biochemical diabetes mellitus and 38.3% had impaired glucose tolerance. Concurrent obstructive sleep apnoea and ethnicity predisposed to abnormal glucose tolerance.

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Waist circumference does not predict metabolic disturbance in children (The EarlyBird Study)

Terence Wilkin, Linda Voss, Jo Kirkby, Alison Jeffery, Brad Metcalf
Peninsula Medical School, Plymouth, UK

Background: Visceral fat mass is the principal contributor to metabolic risk in adults, for which girth is a useful surrogate. Percentile charts for waist circumference have recently been published for use in children, but their relationship to health risk has not been established. We have assessed the value of waist circumference in the prediction of insulin resistance and triglycerides in young children.

Methods: BMI, skinfold thickness at five sites and circumferences (waist, hip, midarm), HOMA-IR and triglycerides were measured in 307 randomly selected children (170 boys, 137 girls, mean age 4.9 y) at school entry. BMI, waist circumference, HOMA-IR and triglycerides were measured in their parents.

Results: 1) Waist and BMI both predicted insulin resistance in the parents (mothers $r=0.62$ and $r=0.64$, $p<0.001$, respectively; fathers $r=0.57$ and $r=0.53$, $p<0.001$). 2) The same measures, however, were poor predictors of insulin resistance in children (girls waist: $r=0.31$, $p<0.001$, BMI: $r=0.24$, $p=0.01$; boys waist: $r=0.21$, $p=0.01$, BMI: $r=0.15$, $p=0.06$). 3) The addition (or substitution) of waist circumference or waist/height to (in) predictive models of insulin resistance did not improve their R^2 value. 4) Waist circumference and BMI were equally predictive of triglycerides in the parents (mothers $r=0.36$, $p<0.001$; fathers $r=0.42$, $p<0.001$) but were co-correlated ($r=0.92$, $p<0.001$). (5) BMI was a weak predictor of triglycerides in the boys ($r=0.27$, $p<0.001$), but waist circumference was unrelated to triglycerides in either sex.

Conclusion: Waist circumference can be used to identify adults with metabolic risk factors (insulin resistance and triglycerides). However, its poor clinical performance in children questions the validity of waist circumference percentiles.

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Insulin resistance and infertility: Implications in the search for diabetes genes (The EarlyBird Study)

Terry Wilkin, Jonathan Lord, Brad Metcalf, William Atiomo,
Michael Murphy, Jo Kirkby, Alison Jeffery, Linda Voss
Peninsula Medical School, Plymouth, UK

Background: Insulin resistance increases with body mass, and young girls in particular are getting fatter. Insulin resistance is also the principal cause of anovulatory infertility (PCOS), so we examined its impact on the composition of trios (child, mother and father) which are increasingly used in the search for insulin resistance/diabetes genes.

Methods: Insulin resistance (HOMA-IR) was measured fasting in 137 EarlyBird girls (mean age 4.9 y), 177 EarlyBird mothers (mean age 34.0 y), and 90 women with polycystic ovarian syndrome (PCOS, 29.5 y) attending a hospital clinic for anovulatory infertility. Both groups spanned a BMI range 20-40.

Results: The girls at 5 y had already gained 0.5 BMI SD scores since birth, and there was a clear correlation between their BMI and insulin resistance ($r=0.33$, $p<0.0001$). The highest insulin resistance recorded in their (ovulatory) mothers was 5.4 units, and the greatest waist circumference 110 cm. Of the anovulatory women, 23% and 11% respectively lay above these levels. BMI did not separate individuals in the two groups. The gradient of the regression of insulin resistance on waist circumference (3.6 v 2.7×10^{-2} , $p=0.02$) was greater in PCOS women than mothers ($p<0.01$). The key correlate with IR was not BMI but visceral fat mass. Once waist was known in the PCOS women, BMI was excluded from a predictive model of their insulin resistance. Furthermore, once IR was known, both BMI and girth were excluded from the prediction of anovulation.

Conclusions: There appears to be a threshold of IR, above which women are unable to reproduce. This has implications for insulin resistance research, which turns increasingly to parent-child trios to distinguish genetic from gestational inheritance. The rising adiposity of contemporary girls will mean an increasing proportion of women in the future who are not represented in such studies - women who, by implication, have the highest insulin resistance of all.

**Clinical picture and diagnosis of non-insulin-dependent diabetes
in Silesian children in chosen cases**

Maria Kalina, Przemyslaw Jarosz-Chobot, Malgorzata Muchacka-Bianga,
Grazyna Deja, Mariola Minkina-Pedras, Ewa Malecka-Tendera
*Department of Paediatric Endocrinology and Diabetes,
Medical University of Silesia, Katowice, Poland*

Background: Recently the recognition of non-insulin-dependent diabetes in childhood has been shown to occur with increased frequency. Since 1999 the diagnosis of diabetes in children has been checked in the Outpatient Clinic of the Medical University of Silesia. We studied the clinical and laboratory features of children and adolescents with untypical course of diabetes.

Methods: 18 patients (8 boys, 10 girls) aged 10.8-17.8 years (mean 15.6, SD 2.0) were analyzed. Either their daily insulin requirement was low (0.1-0.2 U/kg/24h) after at least two years duration of the disease with satisfactory metabolic control, or they had been treated with diet and/or oral hypoglycemic agents since the diagnosis of carbohydrate disturbances. The following parameters were considered: age at manifestation, reason for the primary diagnosis, BMI, C-peptide, HbA1c, presence of antibodies (a-GAD, IAA, IA2), lipid profile, clinical course of the disease, family history of diabetes.

Results: The duration of the disease was 1.6-6.5 years (mean 2.3 years) and HbA1c during this period ranged from 6.2 to 7.8%. Age at manifestation was 8-15.8 years (mean 13.01, SD 2.29); the beginning of the disease was always benign. C-peptide was within the normal range in all cases except one (0.5-3.5 ng/ml, mean 1.62, SD 0.82). If present, antibodies were detected in only one class. On the grounds of clinical picture the patients were separated into three subgroups: 1. Features as in type 2 diabetes: BMI 90-97 pc or above, at the beginning on insulin, presently on diet or oral medication, tendency towards dyslipidemia, positive family history of diabetes or metabolic syndrome (6 patients). 2. Features as in MODY: low BMI, positive family history, from the beginning on diet and/or oral medication (7 patients). 3. Unclassified group: variable parameters (5 patients).

Conclusions: Careful clinical follow-up of diabetic patients during the first 3 years after diagnosis often leads to changes in the initial classification of diabetes, which may modify therapy.

**Increasing incidence of non-type 1/type 2 diabetes
in a pediatric diabetes center in Paris, France**

Nadia Tubiana-Rufi, Lydia Bubuteishvili, Marie-Aline Guitteny,
Christine Bellané-Chantelot, Claire Lévy-Marchal, Paul Czernichow
Robert Debré Hospital, St-Antoine Hospital, Paris, France

Background: Incidence of type 2 diabetes in children is increasing worldwide, but few data are available in Europe. Our aim was to evaluate the frequency of non-type 1 diabetes during a 1-year period (2001), and to compare to previous published data in the same reference pediatric center.

Methods: All new cases of diabetes in children referred to our center in 2001 were classified for the type of diabetes (1997 ADA criteria) and the distribution was compared to the period 1993-98. Clinical data, familial anamnesis, routine biological, and markers of autoimmunity (ICA, GAD-65, IA2) were recorded. Genetic study was performed when appropriate.

Results: 90 new cases of diabetes (age<16 yr) were diagnosed in 2001. Type 1 was diagnosed in 65 patients (72.2%), among them type 1A (autoimmune) n=61 and type 1B (idiopathic) n=4; autoantibodies were negative in 28 patients; features of type 2 were found in 5 cases of type 1. Diagnosis in non-type 1 patients was as follow: type 2 n=5 (5.5%), MODY2+3+X n=6 (6.6%), other specific types n=8, non-classified n=1, transitory hyperglycemia n=5. Among 7 patients with type 2 phenotype (obesity, ethnicity, familial anamnesis of T2), two were genetically identified as MODY 3. Patients with type 2 diabetes (4F/1M) had the following characteristics: mean age at diagnosis 13.5±1.1 yr, mean BMI at diagnosis 35.2±8.5; 3 were Caucasians, 1 of Asian and 1 of African origin; mean HbA1c=8±1.9%, one of them was initially treated by insulin therapy. One year later, under diet (n=5) and oral therapy (n=1), mean BMI was 39.7±7.7 and mean HbA1c 6.4±0.8%. Incidence of type 2 was higher than reported before in the same pediatric clinic: 5.5% (n=5/90) in 2001 vs 2.2% (n=8/370) in 1993-98.

Conclusions: Although this study is not population-based, the incidence of type 2 seems to be increasing in France. French pediatricians must be aware of the frequency of non-type 1 diabetes, especially type 2 and MODY, in children and adolescents.

**Molecular analysis of glucokinase and HNF-1 α genes
in 80 Spanish MODY families**

Itziar Estalella, Luis Castaño, Jose Antonio Vazquez, Pedro Martul,
Jose Ramón Bilbao, Spanish Group of MODY (GEM)
Hospital de Cruces, Baracaldo-Bizkaia, Spain

Background: Maturity Onset Diabetes of the Young (MODY) is a genetically and clinically heterogeneous sub-type of type 2 diabetes mellitus characterised by early onset, autosomal dominant inheritance and absence of autoimmunity. Mutations in six genes (MODY1-MODY6) have been shown to cause most of the MODY cases, and among them, glucokinase (MODY2) and HNF-1 α (MODY3) genes are the most frequently affected, representing around 70% of all MODY cases.

Methods: Molecular analysis of glucokinase and HNF-1 α genes was performed in 80 families with clinical diagnosis of MODY. Screening for sequence variants in both genes was performed by single-strand conformational polymorphism (SSCP) analysis. Samples that were seen to migrate abnormally in SSCP were sequenced to identify the mutation.

Results: Alterations of the glucokinase gene sequence were detected in 38 out of the 80 families studied (47.5%). DNA sequencing identified 30 different mutations, 22 of them had not been described previously. To date, the HNF-1 α gene has been analysed in 30 out of the 42 families without changes in the glucokinase gene. Six of these families (10.5%) presented mutations in this gene, one of them being a novel mutation. We have also noticed that most exons of HNF-1 α are very polymorphic and thus, direct sequencing is the method of choice rather than SSCP analysis in this gene.

Conclusions: 1. 58% of MODY cases in our population are due to mutations in glucokinase (MODY2) or HNF-1 α (MODY3) genes.

2. We describe a total of 22 novel mutations in the glucokinase gene and one in the HNF-1 α gene.

3. SSCP technique is not an efficient method for HNF-1 α screening.

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A novel mutation of MODY type 1. Three cases in one family

Christer Gundewall, Gunnar Stenström, Kevin Colclough,
Kate Thomson, Andrew T Hattersley
*Kungsbacka Hospital, Kungsbacka, Sweden,
Peninsular Medical School, Exeter, UK*

Background: Type 2 diabetes is more frequently recognized in young age groups, some of these patients being diagnosed as MODY 1-5 with characteristic gene mutations. The association between a novel mutation in the HNF-4 α gene and age at diagnosis together with results of treatment in a three generation family is reported.

Methods: HNF-4 α on chromosome 20 was analyzed using PCR/direct sequencing (ABI377). Islet autoantibodies (ICA, GADA, IA-2A), C-peptide and HbA1c (MonoS method) were analyzed on a commercial basis.

Results: All three family members had a novel insertion mutation, L332insCTG, in exon 8 of the HNF-4 α gene. The age when diabetes appeared for the first time (7, 15 and 39 years of age) was lower than in the previous generation. The hitherto obtained results showed normal C-peptide levels and no islet autoantibodies; data not yet available from the grandfather. The proband immediately received insulin treatment, 2 years later switched to glipizide and repaglinide; however, only with temporary decrements in HbA1c levels. Her mother and her grandfather, being reluctant to antidiabetic treatment, had high HbA1c; in the mother even on insulin treatment. In the last years, however, on small sulfonylurea dosages their HbA1c has stabilized at 7%. Retinopathy developed in the grandfather (laser treated) and in the mother 24 and 18 years after diabetes diagnosis, respectively. The grandfather also had hyperlipidemia, arterial hypertension and a minor stroke at age 65.

Conclusions: This MODY family with the new insertion mutation, L332insCTG, in the HNF-4 α gene presented at a lower age for each successor generation. In this family female diabetes patients seem to be more difficult to treat.

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Impaired glucose tolerance (IGT) and diabetes mellitus in cystic fibrosis: A multicenter screening approach

Reinhard W Holl, Christine Müller-Brandes, Magnus Nastoll,
Manfred Ballmann

University of Ulm and Hannover Medical School

Background: The prevalence of secondary diabetes in adolescents and adults with CF is high. However, there is still controversy on the clinical consequence of IGT and the requirement for general screening.

Methods: A total of 914 OGT tests in 745 CF patients from 35 participating centers was available for analysis.

Results: In the first test, 508 patients displayed NGT, 50 IFG, 113 IGT and 68 were classified as diabetes. NGT patients were significantly younger than patients with abnormal glucose (17.8 versus 21.7 yrs, $p < 0.0001$). 41% of CF patients with a diabetic OGT test were younger than 18 yrs. Abnormal glucose metabolism was associated with lower SD scores for height (-0.93 versus -0.74, $p = 0.06$), weight (-1.22 versus -0.91, $p < 0.005$) and body mass index (-0.85 versus -0.65, $p = 0.05$). 2-hour blood glucose in the OGT test was significantly and negatively correlated to SD score for weight ($p < 0.03$). Multiple regression analysis revealed that age, gender and 2-hour glucose were significant predictors of weight SD score (p-values of 0.07, 0.06 and 0.05, respectively), while fasting glucose had no additional effect. In 141 patients, repeat OGT tests were performed due to pathologic results in the first test or clinical judgement, the mean interval was 0.7 years. Out of 46 CF patients with a first diabetic OGT test, diabetes was confirmed in 23 (50%), while 10 (22%) displayed IGT and 13 (28%) NGT. None of the 10 patients with IFG on the first test displayed a diabetic repeat test (5 NGT, 2 IFG, 3 IGT). In contrast, out of 37 patients with IGT, 9 had a diabetic repeat test (14 IGT, 14 NGT).

Conclusion: In summary, this large multicenter study from Germany and Austria confirms the high prevalence of abnormal glucose tolerance in CF. Patients with pathologic glucose metabolism are shorter and more underweight compared to CF patients with NGT. In individual CF patients, the OGT test displays a high variability.

Diabetes mellitus after abdominal radiation therapy:

A case report in an adolescent

Anne-Marie Bertrand, Brigitte Mignot,

Emmanuel Plouvier, Alfred Penfornis

Centre Hospitalier Universitaire Besançon, France

Background: Abdominal radiation may induce exocrine and endocrine pancreatic insufficiency with diabetes mellitus (DM). The prevalence of DM is higher than expected in young adults: 6.6% of 121 patients irradiated for nephroblastoma in a French study based on systematic glycaemic screening. The risk seems to increase with irradiation at young age and with left tumoral localization because of pancreatic tail injury. The delay between the radiation and the onset of DM may be long, 10 years or more.

Patient report: We report a case of a 4 year-old girl treated for neuroblastoma by chemotherapy, surgery, bone marrow graft with total body irradiation and complementary abdominal irradiation (25 Gy) leading to remission. She developed several long-term complications: primary ovarian insufficiency, scoliosis, hepatic nodules with cytolysis, 2 papillary carcinomas requiring total thyroidectomy and iodine-131 therapy. DM was diagnosed 13 years after irradiation. She was symptom-free, but fasting and post-prandial plasma glucose were high, 132 and 231 mg/dl respectively with insulinemia at 34 μ UI/ml and 99 μ UI/ml respectively. HbA1c was 7.5% (N < 6%). B-cell antibodies (GAD and IA2) were negative. She had HLA-DR3 allele. There was no family history of diabetes. Body mass index was in the upper normal range. She also had secondary left renal atrophy. The irradiation therapy was considered as the etiological factor of this pancreatic diabetes.

Conclusion: This case emphasizes the need for long-term follow-up of survivors of childhood cancer. Screening glucose tolerance is strongly recommended in patients with a history of abdominal radiation therapy.

**HES-1 expression in primary human pancreatic tissue
and pancreatic endocrine cell lines**

Carla Demeterco, Chris Padilla, Björn Tyrberg, Fred Levine
University California San Diego, Cancer Center, San Diego, USA

Background: Our laboratory studies the mechanisms that control growth and differentiation of primary cells and cell lines from the human pancreas with the goal of increasing the supply of β -cells for transplantation. One attractive source of β -cell precursors is duct cells, but efforts to induce endocrine differentiation from those cells has had limited success. Another possible source is human β -cell lines. While our human pancreatic cell lines retain many features of endocrine cells, including β -cell restricted transcription factors such as MafA and Nkx6.1, they lose insulin expression. Thus, a problem with the use of cultured cells is difficulty in inducing β -cell differentiation. To address that problem, we have been studying positive and negative factors that control β -cell differentiation. Previously, we found that c-myc is induced in proliferating pancreatic cells and inhibits hormone expression. Here, we present evidence that the Notch pathway, a negative regulator of endocrine differentiation, is induced in cultured primary pancreatic cells and cell lines.

Methods and Results: Using RT-PCR and immunofluorescence, we found that human islet cell lines express high levels of the repressive bHLH transcription factor Hes-1, a downstream effector in the Notch signalling pathway that plays an important role in the control of pancreatic growth and differentiation. We also investigated the expression of Hes-1 in human primary pancreatic tissue and demonstrated that it is strongly expressed in a subpopulation of cells in the fetal pancreas. Although expressed at low levels in the adult pancreas, Hes-1-positive cells were found in isolated adult ductal cells.

Conclusion: We propose that the induction in cultured cells of factors that negatively affect hormone expression, such as Hes-1, may be an important consideration in the use of those cells for transplantation. An improved understanding of those pathways, e.g. Notch, in the human endocrine pancreas may allow us to better facilitate the differentiation of cells and cell lines along the endocrine, and particularly the β -cell, lineage.

**The microbiological safety of pig islet
xenotransplantation in humans**

Robert B Elliott, Olga Garkavenko
Diatranz Ltd, Auckland, New Zealand

Methods: Neonatal pigs have been used to transplant 18 patients with islets, 16 of these in the paediatric age group. The source 'SPF' pig herd is monitored for clinical infectious disease and receives immunization for Leptospirosis and Parvovirus. Sows are isolated before and during farrowing. 7-10 day-old piglets are used for pancreas supply. They are checked clinically and microbiologically for infections which might transfer to humans. The islets subsequently isolated from these pancreata are checked for microbiology and ability to infect human cells co-cultured with them. PERV is present in all pigs, and remains non-infective *in vitro* and *in vivo* with islets from our colony. Mice, rabbits, primates and humans injected with these islets fail to develop any evidence of infection despite evidence of blood microchimerism for PERV in several recipients. Either alginate micro-encapsulation or Sertoli cell co-transplantation was used to avoid rejection. There was evidence of long-term function of these islets in many of these animals and humans. Other pig viruses present in New Zealand which may putatively cause infection in humans - including hepatitis E, cytomegalovirus, lymphotropic herpes virus and circovirus 2 - can be avoided by the use of neonatal donors or herd selection and isolation. Circovirus 2 is vertically transmitted and is endemic in most herds. A unique herd of feral pigs from a remote sub-Antarctic island is free of all of these.

Conclusion: Islet xenotransplantation using islets from neonatal animals from a highly selected herd of pigs is a safe procedure, and shows therapeutic promise.