Diabetes Care, Education, Psychosocial Issues

O/WED/1/01

The clinical trial of metformin in children and adolescents with type 2 diabetes mellitus, T2DM, in Japan

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Objectives: In Japan, metformin is not officially approved in pediatric patients, while even in adults metformin has been restricted by maximum dose of 750 mg/d. The Pediatric Clinical Trial supported by the Health and Labor Research Grants examines whether 750 mg/d and its double dose of metformin can be applied to Japanese pediatric patients with T2DM.
Methods: This study was an open, not-randomized single arm trial. The main outcome was set by 80% efficacy in terms of HbA1c changes by metformin between 12th and 24th week in 50 patients (20 > ages > 10 y/o), enrolled either if any anti-diabetic medication had not been given at least for 28 days before study (group A) or only metformin without any other anti-diabetic medication had been taken at dose of 750 mg/day at least for 28 days before study (group B). At entry HbA1c should be > 5.8% (the reference upper limit < 5.8%) and SDS-BMI should be > 0 for age and sex. Several other outcome measures including fasting plasma glucose (FPG) and adverse events including lactic acidosis were observed. In both groups metformin was given at 750 mg/d for the first 12 weeks. For the second 12 weeks, metformin dosed up at 1500 mg/d, if HbA1c exceeded > 6.5% at 12 weeks, whereas metformin dose remained at 750 mg/d, if HbA1c was < 6.4% at 12 weeks.
Results: Finally 47 patients (24 in group A and 23 in group B) were enrolled and 38 patients completed the clinical study. Both HbA1c and FPG levels between 0 week and 24 weeks improved statistically significantly in the above 38 patients. The efficacy of metformin was 0.79 (0.65–0.89 of 95% CI) which did not reach a statistical significance, since the efficacy was set by > 0.8 of the lowest point before trial. Any serious adverse event was not observed.
Conclusion: This fourth clinical trial of metformin for pediatric use in the world proved again metformin to be effective and safe in children and adolescents with T2DM. Further trial of a long-term use of metformin is warranted in Japan.

O/WED/1/02

Age, insulin regimen and HbA1C: The Search for Diabetes in Youth Study

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Objective: To examine the relationship between age, insulin regimen, and age-specific HbA1C goals as recommended by the American Diabetes Association (ADA) for pediatric age groups with T1D.
Methods: Search for Diabetes in Youth is a population-based study of diabetes diagnosed before age 20 years. This report includes 2600 participants with T1D, mean age 13.1 years, mean duration 5.1 years. Demographic and clinical data including current insulin therapy were collected at an in-person visit. Insulin regimens were grouped as: 1) insulin pump; 2) glargine + rapid acting insulin; 3) glargine with multiple insulins; 4) 3 or more injections/day without glargine; 5) 2 or less injections/day.We examined the distribution of insulin regimens, their associations with HbA1C, and frequency of attaining ADA HbA1c target by age group.
Result: Insulin regimens use varied by age, with fewer young children on insulin pump therapy. For the youngest age group, insulin regimen was not significantly associated with HbA1C results, but there were significant differences in the two older age groups ($P < 0.001$). Whereas the youngest age group was most likely to have an HbA1C in the target range, youth over age 12 were unlikely to have an HbA1C in target range regardless of insulin regimen ($P < 0.001$).

	1	2	3	4	5
Age Group in years (*n*)	mean HbA1C/ % in target	mean HbA1C/ % in target	mean HbA1C/ % in target	mean HbA1C/ % in target	mean HbA1C/ % in target
< 6 (159)	7.8/95	8.3/65	8.6/59	8.1/74	8.0/72
6–12 (1053)	7.7/69	8.2/47	8.5/29	8.5/41	8.4/46
> 12 (1388)	8.2/27	8.8/21	9.4/14	8.7/24	9.1/26

Conclusion: In youth with T1D, the use of insulin pump is associated with better glycemic control. Multiple factors affect selection of insulin regimen, as well as success with the prescribed treatment regimens, including sociodemographic variables and frequency of blood glucose monitoring. Younger children are more likely to achieve the HbA1C target range. Much improvement is needed in glycemic control, particularly adolescents.

O/WED/1/03

A comparative study of an experimental 4 mm needle and Novofine® 6 mm needle in relation to anatomical deposition of sterile air in lean diabetic children

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Background: Correct insulin deposition, in the subcutaneous tissue, is of major importance since 1) a superficial deposition of insulin might cause leak out of insulin and 2) too deep deposition of insulin might cause intramuscular insulin deposition which might cause irregular absorption and thus increase the risk of hypoglycaemia. Consequently, both needle length and injection technique are of clinical relevance, particularly when injecting insulin in lean children.
Objectives: To detect tissue deposition of a simulated insulin bolus of 300 µl of sterile air injected with a 4 or 6 mm needle in lean children with diabetes mellitus.
Patients and Methods: A total of 28 children (19 males) with an average age of 10.3 years (range: 6–17.3), a median body mass index of 16.6 kg/m^2 (range: 12.7–19.5), and a diabetes duration of a median 4.2 years (range: 0.6–10.7). All participants received an injection of 300 µl of sterile air using a NovoPen® 3. The air was injected perpendicular to the cutis without skin fold at the thigh and abdomen with an experimental 4 mm needle and 6 mm NovoFine® needles, respectively. Tissue deposition of the

air was detected by ultrasound (transducer model 8802/8811, 5–12 MHz.

Results:

Table 1 shows ratio of intramuscular injections with 4 and 6 mm needles at the thigh and the abdomen when injecting without a skinfold.

Needle length	4 mm	6 mm
Thigh	2/28	8/28
Abdomen	14/28	17/28

Conclusion: The study demonstrated that injecting with a 4 or a 6 mm needle without a lifted skin fold resulted in a substantial amount of intramuscular injections. Therefore, we propose still to inject with an elevated skin fold and a 45°angle when using 6 mm needles in very lean patients as this injection technique and needle length previous have been reported to cause a very low number of IM injections.

O/WED/1/04

Insulin omission and glycaemic control in adolescents with type 1 diabetes from 21 international centres

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Objective: To examine the extent of self-reported insulin omission due to concerns about weight, and its relationship to glycaemic control, in a large international cohort of adolescents with type 1 diabetes.

Methods: At sequential visits to their local centre, adolescents and their parents/carers completed questionnaires investigating age, gender, diabetes duration, insulin-regimens and dose adjustments and glycaemic targets. HbA1c (DCCT adjusted) was measured centrally.

Result: Questionnaires were completed by 2062 adolescents (aged 14.5 ± 2.0 years, male 50.6%, diabetes duration 6.1 ± 3.5 years). Different insulin regimens were used within and between the 21 centres. Mean HbA1c was 8.2% SD 1.4, significantly influenced by age ($r = 0.1$; $P < 0.001$), gender (females 8.3% ± 1.5, males 8.1% ± 1.3; $t = 3.0$; $P < 0.005$) and diabetes duration ($r = 0.29$; $P < 0.001$). 5.1% and 4.2 of females and males stated they never missed insulin to control weight, 91.7% and 93% stated they missed insulin to control weight once a month, 2.5% and 1.9% omitted insulin once a week and 0.7% and 0.9% missed insulin every day. Multiple regression analysis showed that omission of insulin to control weight was significantly associated with poor HbA1c after controlling for age and gender effects (ß = 0.103, $t = 2.558$, $P = 0.011$). Missing insulin more frequently was also significantly associated with worse well-being ($P = 0.013$).

Conclusion: Self-reported omission of insulin to control weight among both female and male adolescents with diabetes was very frequent in a large international cohort. The association between omission of insulin and poor glycaemic control warrants further research into the possible negative clinical impact of patient concerns over insulin-related weight gain and related omission of insulin. Our findings confirm the clinical relevance of addressing concerns about insulin-related weight gain in adolescents with diabetes.

O/WED/1/05

Assessing the needs of children with diabetes in the school

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Objective: To identify children with special diabetes needs in school settings.

Methods: Children aged 6–16, with type 1 diabetes, their parents (P) and teachers (T) were eligible. Those who agreed to participate completed a questionnaire to assess the needs and wishes related to children's integration, disease control, insulin administration, meals, sports, trips and attitudes of teachers and school colleagues to their disease. The study was performed January–April 2006 with the collaboration of nine paediatric hospitals in Castilla-La Mancha (Spain).

Result: A total of 589 (children: 226; parents: 232; teachers: 131) questionnaires were completed and validated. Median age of children was 11.9 years. Median diabetes evolution was 5 years. Children's results showed that when they are at school, 9% need to inject insulin (P:10%, T:11%); 56% check their blood glucose (P:61%, T:48%); 51% have a main meal at school (P:26%, T:4%); 97% do physical education (P:98%), 85% went on day trips with their colleagues (P:84%, T:89%); 27% experienced hypoglycaemias before or during an exam (P:20%, T:7%); 46% experienced hypoglycaemias as a consequence of physical exercise (P:37%); severe hypoglycaemia accounted for 19% (P:10%, T: 5%). In order to overcome the burden of diabetes at school children, asked for: improved teachers knowledge on diabetes 67% (P:74%, T:96%); written information on diabetic emergencies 85% (P:95%, T:95%); diabetes to be explained to all students 57% (P:69%, T:72%); readily available glucagon 52% (P:62%); nurse among staff 42% (P:46%).

Conclusion: The low percentage of children injecting insulin and performing blood sugar controls suggests sub-optimal diabetes treatment due to lack of support at schools. Training sessions on diabetes and emergencies management for teachers and physical trainers, talks on diabetes for students, glucagon availability and a nurse among staff were identified as key factors in providing a safer environment for diabetic children at school.

O/WED/1/06

Monitoring health-related quality of life in adolescents with type 1 diabetes improves psychosocial health and satisfaction with care

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Objectives: Systematic monitoring of psychosocial functioning in adolescents may help to improve physical and psychosocial well-being and glycemic control. In an ongoing RCT, we investigate the effects of monitoring health related quality of life (HRQoL).

Methods: A total of 91 adolescents with type 1 diabetes (age 14.9 ± 1.1, HbA1c 8.8 ± 1.7, duration 6.4 ± 4.2 years) were recruited from four outpatient clinics in the Netherlands. Clinics were randomized over control (care as usual) or monitoring condition. In the monitoring condition, patients completed a three monthly computerized HRQoL assessment (PedsQL) and discussed the outcomes with the pediatrician or nurse. At

baseline and follow-up (12 months) we assessed physical and psychosocial well-being by questionnaires (Child Health Questionnaire, Center for Epidemiological Studies scale for Depression, Diabetes Family Conflict Scale), satisfaction with care and medical outcomes (HbA1c, BMI, treatment regimen). MANCOVA analyses, correcting for baseline values, were used to test the effect of the intervention. Multiple linear regressions was used to identify factors predicting HbA1c.

Results: A significant improvement was found in the psychosocial summary scale of the CHQ for the monitoring group compared to the control group ($P < 0.001$, R2 = 0.37), especially in reduction of behavioural problems and improvement of self-esteem. We found no change in HbA1c, depression and diabetes specific family conflict in either of the groups. Patients' satisfaction with care significantly improved in the monitoring group compared to the control group. For the whole group, HbA1c levels at follow up were predicted by glycemic control and number of diabetes specific family conflicts at baseline ($P < 0.001$, R2 = 0.42).

Conclusion: This is the first study to demonstrate monitoring HRQoL improves psychosocial well-being and satisfaction with care in adolescents with diabetes but not glycemic control. Additional interventions are needed to decrease HbA1c levels.

O/WED/1/07

Quality of life in children with type 1 diabetes and psychological burden in parents during the first year after diabetes onset: A prospective multicentre study

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Aim: In a prospective multicentre study metabolic control, quality of life and emotional well-being of children with diabetes and their families during the first year after onset were assessed.

Methods: All eligible parents (81 families cared for in 10 German pediatric diabetes units) of newly diagnosed children (age: 4–14 years; mean 8.1 ± 2.9 years.) received a personalized education. Outcome parameters: time needed for education, children's glycaemic control, children's health related quality of life (HrQoL) (KINDL-R), parents' well-being (WHO-5) at onset (t0), 6 (t1) and 12 (t2) months later.

Results: On average 30.6 ± 10.1 education lessons (45 min) were required. Mean HbA1c: 10.8% ± 2.7% (t0); 6.8% ± 1.0% (t1); 7.2% ± 1.2% (t2); severe hypoglycaemia (t0–t2): 9.1/100 patients/years. Children's HrQoL at onset in all but one of Kindl-R subscales (physical well-being) corresponded to standard values of healthy controls. At (t1) and (t2) parents assessed their children's HrQoL significantly better than before and than the standard values of healthy controls (physical well-being, psychological well-being, self-esteem, kindergarden/school and total HrQoL; each $P < 0.001$). Compared to standard values of WHO-5 mothers' psychological well-being was poor (raw scores: (t0) 11.9 ± 6.9; (t1) 12.8 ± 5.6; (t2) 14.5 ± 5.0. Scores < 13 (indicating depression) were seen at 50% (t0), 41% (t1) and 29% (t2) of the mothers. There was a systematic association between children's HrQoL and their mothers' well-being (t0: $r = 0.47$; t1: $r = 0.48$; t2: $r = 0.35$; each $P < 0.001$).

Conclusion: Twelve month after diabetes onset, the target of good metabolic control (HbA1c < 7.5%) was met by 71% of the children. HrQoL of children was unexpectedly good and paradoxically better than in healthy controls. The poor well-being of mothers indicates their need for specialized care. Thus, early support interventions for mothers concerned should be developed and evaluated to sustainable improve their family's emotional and physical health.

O/WED/1/08

Parent well-being and support are associated with better metabolic control and quality of life in adolescents with type 1 diabetes

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Objectives: To assess and determine the roles of parent well-being and support in glycemic control and quality of life of adolescents (QOL) with type 1 diabetes.

Methods: Clinical data and centrally analysed HbA1c were collected on 2062 adolescents, aged 11–18 years and from 1994 parents from 21 centers in 19 countries in Europe, Japan, North America and Australia. Adolescents completed QOL, well-being and Life Ladder questionnaires. Parents completed WHO-5 well-being and Family Burden questionnaires.

Results: Mean HbA1c was 8.2 % (± 1.4%), higher in girls 8.3 (± 1.5%) than boys 8.1(± 1.3%, $P < 0.0001$). Good parent self rated well-being scores were associated with lower HbA1c ($P < 0.005$), greater adolescent well-being ($P = 0.001$), less impact of diabetes ($P < 0.001$), less worries ($P < 0.001$), less adolescent perception of parent over involvement ($P < 0.01$), greater health perception ($P < 0.001$) and life ladder ($P < 0.001$), less physical and psychological symptoms ($P < 0.001$). Greater family burden relating to long term health concerns was associated with higher HbA1c values ($P = 0.000$), poorer adolescent well-being ($P = 0.000$), greater impact of diabetes ($P = 0.000$), more worries ($P = 0.000$), more parent over involvement ($P = 0.000$), poorer health perception ($P = 0.000$) and life ladder ($P = 0.000$), more physical and psychological symptoms ($P = 0.000$). Adolescents accompanied to clinic by parent had lower HbA1c 8.1% vs. 8.6% ($P < 0.007$), greater well-being ($P = 0.000$), less worries (P 0.000), greater health perception ($P < 0.003$) and life ladder ($P = 0.000$).

Conclusion: Better reported parent well-being, lower parent perceived family burden relating to diabetes and parent attending diabetes clinic are associated with better glycemic control and QOL in the adolescent. Parent QOL and family burden assessment form an important part of diabetes care.

O/WED/1/09

Evaluation of a transition to adult diabetes care program for youth with type 1 diabetes (T1D) and their parents

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Background: After a 1989 survey of our adolescent population showed a 24% dropout rate after graduating from our pediatric

diabetes clinic, we established a transition program targeting teens, their parents, and health care professionals. The program includes strategies to promote awareness of transition issues and to provide guidance and support for teens and parents during the transition process.

Objectives: We aimed to evaluate our transition program by determining 1) the rate of F/U for those with T1D graduating from pediatric care 2–4 years ago, 2) factors associated with F/U status and 3) level of satisfaction with the transition process.

Methods: We identified and attempted to contact by telephone and by mail 213 youth with T1D who left our clinic from 1997–1999 at 16–18 years of age. Sociodemographic and health information and F/U status were obtained through health record review and telephone survey.

Results: 80 individuals (40M/40F) mean age 21.4 ± 1.2 years were evaluated; 75 (38M 37F) were engaged in F/U; 5 (2M 3F) were not. Compared to those without F/U, those in F/U were in better metabolic control prior to discharge from pediatric care (A1c 8.8 + 1.5 vs. 10.9 + 1.6, $P < 0.002$). After transiton they reported better control ($P < 0.005$) and well-being ($P < 0.002$) and were fairly satisfied with the transition process and with their current care. Most (95%) were seeing an endocrinologist; 60% had > 3 assessments/year; 59% were connected with a diabetes education center; 99% had an eye assessment in the past 24 months.

Conclusion: Our data suggest that the introduction of a transition program is associated with a significant improvement in the rate of health care F/U after discharge from our pediatric clinic (94% F/U vs. 76% prior to program implementation). Teens with very poor metabolic control in the transitional year remain at highest risk for no F/U. We are currently evaluating a program targeting this high-risk group in our pediatric clinic.

Immunology and Genetics of Diabetes

O/WED/2/01

Specific immune response to GAD65 in type 1 diabetic children treated with GAD65 (Diamyd™)

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Background: In a Phase II study in type 1 diabetic (T1D) patients, 10–18 years, we have found that GAD65 vaccination gives remarkably good preservation of residual β-cell function. Our hypothesis is that GAD65 vaccination can induce a specific immune response towards a protective immune profile in T1D children.

Methods: A phase II, randomised, double-blind, placebo-controlled, multi-centre trial including 70 T1D children (42 female, 28 male, 10–18 years), < 18 months duration, fasting C-peptide > 0.1 nmol/L, pos for GADA. 35 patients got 20 mikrog GAD65 (Diamyd™) and 35 placebo on day 1 and 30. PBMC, collected before and 15 months after the primary vaccination, and from a reference group (12 healthy children; 8 female, 4 male, 11–15 years), were stimulated with GAD65 (Diamyd™) and PHA for 72 hours. Secretion of cytokines (IL-5, IL-6, IL-10, IL-12, IL-13, IL-17), IFN-g, TNF-α and chemokines (IP-10, MCP-1, MIP-1α, MIP-1β and RANTES) were detected in cell supernatants by Luminex. Expression of FOXP3 mRNA was detected together with endogenous rRNA by multiplex real-time RT-PCR.

Results: Stimulation *in vitro* with GAD65 induced higher secretion of IFN-g, IL-5, IL-13, IL-10 and IL-17 ($P < 0.0001$), IL-6, TNF- α, IP-10, MIP-1a and MIP-1b in the diabetic children 15 months after treatment with GAD65 compared to placebo. Also FOXP3 mRNA increased after GAD65 stimulation in those treated with GAD65 compared to the placebo ($P < 0.0001$). GAD65-induced secretion of IL-5, IL-13, IL-17 TNF-α, and expression of FOXP3mRNA were higher in children treated with GAD65 than in healthy children. Spontaneous expression/ secretion of markers did not differ between children treated with GAD65 or placebo either before or 15 months after injection. PHA induced prominent response in all children regardless of treatment.

Conclusion: Treatment with GAD65 seems to induce a specific T-cell population, with a subsequent deviation of a GAD65 specific immune response, towards a protective immune profile.

O/WED/2/02

GAD65-vaccination preserves residual insulin secretion in children and adolescents with recent onset type 1 diabetes: Results of a randomized controlled phase II trial

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Background: Residual insulin secretion is crucial to make type 1 diabetes milder, and prevent complications, but so far interventions to preserve β-cell function have been either ineffective or unsafe. The current trial assessed the safety and efficacy of GAD-alum vaccination to reverse T1D in 10–18 years old recent-onset patients.

Methods: A total of 70 T1D patients within 18 months from diagnosis, with fasting C-peptide > 0.10 pmol/ml and positive for GAD autoantibodies were recruited. Participants were randomly assigned to receive either 20 μg GAD-alum ($n = 35$) or placebo ($n = 35$) administered subcutaneously at day 1 and one month later. At day 1 and at month 3, 9 and 15, a mixed meal tolerance test (Sustacal) was performed to evaluate the impact of treatment on residual β-cell function (measured as C-peptide).

Results: Both groups lost insulin secretion up to 15 months. Change in fasting serum C-peptide was not significantly different between treatment groups, but the rate of decline in stimulated C-peptide secretion (measured as area under the curve), was approximately one-half as much in the GAD-alum as in the placebo group ($P = 0.01$). Maximum stimulated C-peptide at 15 months also decreased less in the GAD-alum group compared to the placebo group ($P = 0.04$). The protective effect was most pronounced in patients treated within 3 months of diagnosis. These patients preserved their endogenous insulin secretion over 15 months, while the placebo patients lost a considerable portion of their stimulated C-peptide response. The incidence of clinical adverse events did not differ between GAD-alum and placebo groups.

Conclusion: GAD-alum vaccination has a statistically and clinically significant protective effect on residual insulin secretion. GAD-alum intervention represents a safe and easily administered treatment in patients with newly diagnosed T1D.

O/WED/2/03

Age is the most important factor for the decline in β-cell function during the first year after diagnosis of childhood type 1 diabetes

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Background and Aim: Type 1 diabetes is characterized by immune mediated progressive destruction of the pancreatic β-cell. Young children are found to have lower residual β-cell function 12 months after disease onset. The aim of the present study was to investigate if the loss of residual β-cell function occurs at a higher rate compared to older children during the first year after onset.

Methods: A total of 275 children and adolescents were followed for 12 months after diagnosis of T1D. At 1, 6, and 12 months after diagnosis, the residual β-cell function was evaluated by stimulated C-peptide levels 90 min after the ingestion of a liquid mixed meal (Boost test) and blood glucose levels were measured. HbA1c was recorded 1, 3, 6, 9, and 12 months after diagnosis. C-peptide is considered on logarithmic scale. Interaction between visit and age was analysed using a repeated measurements analysis with unstructured variance matrix.

Results: Meal stimulated C-peptide declined by 50% from the first visit at one month after diagnosis to the 12 month visit ($P < 0.0001$). Throughout the study period the older children had higher residual β-cell function and overall 10% higher C-peptide levels per year of age ($P < 0.0001$). During the 11 month follow-up, the decline in β-cell function occurred at a faster rate in young children [by a factor of −0.04 ($P = 0.02$) per year of age] compared to the older children. For a 5 year old child, this corresponded to a decrease in residual β-cell function of 61% between the one and 12 month visit, while for a 15 year old child a decrease of only 40% occurred.

Conclusion: In the present study we show that the progressive loss of β-cell function during the first year after onset of childhood type 1 diabetes is highly dependent on age. This explains why younger children have either no or a very short remission phase.

O/WED/2/04

Genetic variation within the PPARγ2 gene associates with residual beta cell function and glycaemic control in children and adolescents with newly diagnosed type 1 diabetes during the first year after disease onset

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Objective: The Pro12Ala single nucleotide polymorphism (SNP) of the type 2 diabetes susceptibility gene PPARG2 has also been found to confer a risk for type 1 diabetes (T1D). Therefore, we tested the hypothesis that variation in the PPARG2 locus has an impact on residual β-cell function and glycaemic control in newly diagnosed T1D.

Methods: Totally, 257 children and adolescents were followed for 12 months after diagnosis of T1D. The residual β-cell function was determined as 90 min meal-stimulated C-peptide (Boost test) 1, 6, and 12 months after diagnosis. HbA1c and the daily insulin dose (IU/kg/day) were recorded 1, 3, 6, 9, and 12 months after diagnosis. Genotyping of four SNPs within the coding and promoter region resulted in 5 haplotypes (h1, 364 alleles; h2, 73 alleles; h3, 43 alleles; h4, 17 alleles; and hx, 15 alleles) within PPARG2, generated by MALDI-TOF. Statistical analyses were performed by multiple regression and compound symmetric models on log transformed variables C-peptide and HbA1c with PPARG haplotypes, age and sex as covariates. The most common haplotype h1 was used as reference.

Results: Of the five haplotypes the combination of the Ala allele from the Pro12Ala SNP together with the T (Ala-T, h3) or the C allele (Ala-C, h4) of the C1431T SNP associated with residual β-cell function during the first year after diagnosis: the h3 haplotype with a 27% lower C-peptide ($P = 0.02$) and the h4 haplotype with a 39 % lower C-peptide ($P = 0.01$). h4 also associated with a 0.42% (absolute) higher HbA1c ($P = 0.05$) during the study period.

Conclusion: Variation in the PPARG2 locus influences disease progression during the first year after onset of T1D. The effect of the 12Ala allele appears to be stronger in the C1431 haplotype (h4) than in the T1431 haplotype (h3) as the h4 associated with lower residual β-cell function and a higher HbA1c than the h3 haplotype. Our study confirms that the PPARG locus play a role in T1D.

O/WED/2/05

The risk of celiac disease among children with diabetes has increased three-fold over the last ten years

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Objectives: Childhood type 1 diabetes (T1D) is frequently accompanied by celiac disease (CD). Clinical experience suggests that the risk of a diabetic child to develop CD is increasing. The aim of our study was to assess the trends in risk of CD in a large and well defined retrospective cohort of children with T1D.

Methods: Seven large central European pediatric diabetes centres participated in the study. Data of all newly diagnosed T1D patients of European Caucasian descent, aged 0–14.9 years at onset, were collected over the years 1996–2006. The patients were regularly assessed for CD using endomysium and/or transglutaminase antibody tests at least once a year. The date of CD was defined as the date of the first out of two or more independently positive antibody tests, regardless of biopsy results. Celiac disease risk was

calculated by the proportional hazards model, adjusted for age, time since T1D onset, calendar year, centre and sex.
Results: A total of 1544 patients with T1D contributed 4805 person-years of observation; 108 of the patients developed CD. The risk of CD increased by 14% yearly (95%CI 2.0% to 28%; $P = 0.02$) over the period 1996–2005, and a further sharp peak was noted in the first months of 2006. Annual CD incidence rates for children younger than 5 years diagnosed with T1D in 1996–2000 were 3.4% compared to 6.9% for children diagnosed in 2001–2004. Among children older than 5 years, annual CD incidence rates were 1.4% if T1D diagnosis was made in 1996–2000 compared to 4.1% for children diagnosed in 2001–2004.
Conclusion: We bring the first documented evidence of a significant increase in CD risk among children with T1D. The increase in risk over the ten years of observation cannot be attributed to an effect of the age at T1D onset, as the distribution of age at onset remained stable over the whole study period. We may therefore witness an analogy to the increase in T1D incidence over time, whose causes are yet to be disclosed.

O/WED/2/06

GADA positive children and adolescents with Type 1 diabetes (T1D) have an increased risk of autoimmune thyroiditis (AIT)

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Objective: This study aimed to evaluate whether the presence of diabetes-specific autoantibodies at T1D onset may be predictive for the development of AIT which presents the most common second autoimmune disorder in patients with T1D.
Methods: Diabetes-specific autoantibodies GADA, IA2A and IAA were determined at T1D onset in 341 children and adolescents (160 girls; mean age 8.9 ± 4.2 years, range 1–17 years). Thyroid antibodies (anti-TG, anti-TPO), TSH, T3 and T4 were measured at T1D onset in 335 of these patients and, thereafter, annually with a follow-up time of 1–15 years. In case of thyroid antibody positivity and/or TSH elevation, sonographic evaluation of the thyroid gland was performed and treatment with L-thyroxine (100 $\mu g/m^2$) was started if persistent elevation of TSH (> 4.5 μU/ml) and/or thyroid volume (> 97th percentile) was present.
Results: The majority of patients (314 of 341, 92.1%) had at least one diabetes-specific antibody at T1D onset (71.6% GADA, 73.0% IA2A and 44.9% IAA). GADA positive patients were older than those without GADA ($P < 0.001$). Thyroid autoimmunity was found in 15 of 335 patients (4.5%) at T1D onset with female pre-ponderance ($P = 0.013$). At the end of follow-up, a total of 70 patients (20.9%) had developed thyroid autoantibodies (cumulative incidence [CI] 0.38 ± 0.06 at 10 years of T1D). In 30 patients (9.0%), AIT was diagnosed up to 9.4 years after T1D onset (CI 0.24 ± 0.03 at 10 years). The incidence of AIT was not influenced by IAA or IA2A positivity at onset. However, by multivariate analysis, GADA positive patients were estimated to have a 3.5-fold increased risk of AIT (CI 0.31 ± 0.11 at 10 years) compared to those without GADA ($P = 0.024$).
Conclusion: According to current ISPAD recommendations, AIT screening should be performed in children at T1D onset and in regular intervals thereafter. Based on our results, a special focus should be given to patients positive for GADA at T1D onset since they are at increased risk to develop autoimmune thyroiditis.

O/WED/2/07

Two families with a novel H241q mutation in NEUROD1 causing MODY6 diabetes

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Objectives: The aim of this study was screening for mutations of the NEUROD1 and IPF-1 genes in patients with clinical characteristics of maturity-onset diabetes of the young (MODY) who carried no mutations in the HNF-4A (MODY 1), GCK (MODY 2) and TCF1 (MODY 3) genes.
Methods: We studied 30 unrelated probands of Czech origin (14 males, 16 females) with a clinical diagnosis of MODY. The median age of probands was 18 years (interquartile range 17–35.5) and the median age at the first recognition of hyperglycaemia was 16 years (interquartile range 14–22). The promotor, exons and exon/intron boundaries of the NEUROD1 and IPF-1 genes were examined by PCR-dHPLC followed by direct sequencing.
Results: While no mutations were found in the IPF-1 gene, a novel substitution in the NEUROD1 gene was identified in two unrelated probands. This H241Q substitution is located in the transactivation domain of the protein. The H241 residue is evolutionary remarkably conserved. In the first proband, the H241Q mutation lead to early-onset (20 years) hyperglycaemia followed by serious diabetic microvascular complications by the age of 32 years. The second proband suffers from slowly progressing hyperglycaemia first detected at 30 years. He developed no diabetic complications by his current age of 39 years. We identified several symptomatic as well as pre-symptomatic mutation carriers in both families.
Conclusions: The Q allele of the H241Q NEUROD1 variant co-segregated with diabetes mellitus in affected families suggesting that it represents a new disease causing mutation that is responsible for autosomal dominant transmission of diabetes mellitus.

O/WED/2/08

Protection against diabetes: Application of coppering lowering effect of tetrathiomolybdate

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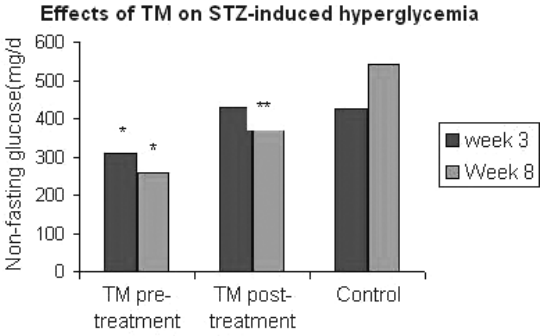
Objectives: Tetrathiomolybdate (TM) is an anticopper drug developed for the initial treatment of Wilson disease. We have showed that TM can inhibit levels of the inflammatory cytokines, and delay the onset of diabetes in NOD mice . In this study, we evaluated the protective effects of therapy with TM against streptozotocin (STZ)-induced diabetes in mice.
Methods: To induce diabetes (DB), STZ was administrated by a single intraperitoneal injection in C57BL/6 mice, which were separated into four groups: blank control, TM pre-treatment, STZ only, TM post-treatment after DB developed. TM treated mice received TM by oral gavage (0.2 mg daily). Non-fasting blood glucose was measured weekly. Plasma ceruloplasmin (CP) was followed as a measure of body copper status.
Results: After 3 weeks with STZ, we saw marked lower blood glucose in TM pre-treatment group than STZ only group ($P < 0.01$). While in TM post-treatment group, the blood glucose was significantly lower than STZ controls at week 8



Effects of TM on STZ-induced hyperglycemia after 3 weeks and 8 weeks of STZ injection

* P<0.01 TM pre-treatment vs Control in both Week 3 and week 8

** P<0.05 TM post-treatment vs Control in week 8

($P < 0.05$). CP levels were maintained between 20 and 60% of baseline in TM group.[Effects of TM on Diabetes].

Conclusion: TM has a significant effect on lowering the blood glucose. By composing a stable TM-copper-albumin tripartite complex, TM can quickly and effectively decrease free copper level. This probably leads to the inhibition of some of the immune respond pathways in STZ induced-pancreas damage.

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O/WED/2/09

Genetic protection from metabolic syndrome in young girls: APM1 -11,391G>A polymorphism

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APM1/adiponectin promoter -11,391G > A polymorphism has been found associated with enhanced APM1 transcription and higher serum adiponectin levels. Our aims was to confirm the association of APM1 -11,391G > A with higher serum adiponectin in young girls and to estimate the clinical relevance of this polymorphism as regards not only insulin sensitivity but also HDL levels. Of 278 young girls (age: 10.86 ± 1.52), we calculated the Z-score of BMI according to local charts; we measured plasma glucose, insulin, and HDL, and serum adiponectin. We genotyped -11,391G > A by Light Cycler. We used SPSS.15 to perform linear regression, ANOVA and Pearson Chi Square tests needed for the analysis.

Serum adiponectin is inversely related to HOMA-IR independently of age and Z-BMI ($\beta = -0.26$, $P = 0.001$), while it is directly related to HDL independently of age, Z-BMI and HOMA-IR ($\beta = 0.31$, $P = 0.000$). -11,391G > A is not associated with any difference in Z-BMI, while it is associated with higher adiponectin concentrations, lower HOMA-IR and higher HDL; it is also associated with lower risk of HDL < 60 mg/dl.These associations disappear when HDL and HOMA-IR are adjusted for adiponectin concentration.Our results shows for the first time that APM1 -11,391G > A polymorphism plays a protective role not only against insulin resistance but also against sub-optimal levels of HDL, by means of modulation of adiponectin levels. For this reason we think this polymorphism should be considered as a genetic protection from metabolic syndrome in young girls.

	G/G	G/A	A/A	*P* value
Number	235	40	3	
Age (y)	10.85 ± 1.52	10.89 ± 1.53	11.13 ± 1.15	0.940
Z-BMI	-0.21 ± 0.92	-0.16 ± 0.96	-0.06 ± 1.25	0.920
Adiponectin (μg/mL)	13.88 ± 4.10	16.54 ± 5.36	17.43 ± 6.93	0.004
HOMA-IR	2.85 ± 1.43	3.02 ± 1.47	1.87 ± 0.096	0.401*
HDL (mg/dl)	59.58 ± 9.84	59.71 ± 11.42	70.00 ± 5.56	0.200*
Prevalence of HDL < 60 mg/dl	136/235 (0.57)	17/40 (0.42)	0/3 (0)	0.042

P = 0.000 for A/A versus A/G + G/G (recessive model)

Diabetes Acute and Chronic Complications

O/FRI/1/01

Further insights into the mechanisms and effects of brain injury in diabetic ketoacidosis

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Objectives: DKA can result in brain injury in children and adolescents. Pilot data has indicated that taurine may be of significance in the pathogenesis of cerebral edema (CE) in the context of type 1 diabetes mellitus (T1DM) and DKA. Our aim was to provide insight into the pathophysiological changes in the cerebral structure and biochemistry on a new series of patients with newly diagnosed T1DM presenting with and without DKA.

Methods: Magnetic resonance imaging, magnetic resonance spectroscopy (MRS), electroencephalogram (EEG) and clinical parameters were performed prospectively on days 1528 and 6 months from initial presentation. Neuropsychological data will be presented separately.

Results: Data is presented on a sub-group of four patients with newly diagnosed diabetes: one patient without (pH = 7.35), one with moderate (pH = 7.10), one patient with severe DKA (pH = 6.70) and one with severe DKA and CE (pH = 7.00). Focal neuronal swelling was found in the fronto-medial, hippocampal and parietal subcortical neurons with associated neurochemical changes of taurine and myo-inositol on spectroscopy particularly for the most severe DKA (pH = 6.70) presentation. Encephalopathic EEG changes were noted with the DKA patients. These were most pronounced in the first 24 hours after presentation and resolved gradually over 5–28 days. In the most severe DKA presentation (pH = 6.70), an unidentified spectroscopic peak was observed on day 1 that disappeared by day 5. The patient with CE was treated with i.v. mannitol prior to imaging and the taurine level on spectroscopy was significantly elevated (22 Institutional Units) that returned towards normal by day 28. The EEG showed Aochi grade 4 encephalopathic changes that settled completely by day 5.

Conclusion: In the examined regions of the brain in healthy individuals taurine is not readily detected. In our patients taurine

was detected implying that taurine may be a significant osmolyte contributing to the pathophysiology of CE.

O/FRI/1/02

Comparison of 2 protocols for treatment of diabetic ketoacidosis in children with T1D

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Objectives: Diabetic ketoacidosis is the most severe acute complication of T1D. We worked out an improved method of DKA treatment that differs from the standard one. Aim of this work is to compare two protocols for treatment of DKA children.
Methods: Two groups were involved. 1st group included 25 patients (middle age - 10.3 ± 2.7 years, entering pH - 7.1 ± 0.9, glycemia - 23.3 ± 3.7 mmol/l). The 2nd included also 25 patients (middle age - 11.6 ± 2.3 years, entering pH - 7.07 ± 0.6, glycemia - 24.2 ± 2.8 mmol/l). Clinically, all these patients detect degree 2 of DKA. Standard protocol of DKA treatment was used in the 1st group. That is IV infusion (100 ml/h) 0.9% NaCl to maintain glycemia < 17 mmol/l and short effect insulin 0.1–0.12 IU/kg*h. When glycemia achieved the value < 17 mmol/l infusion with 5–10% (depends on glycemia) glucose solution was starting. Improved protocol was used in the 2nd group. That is IV infusion of 2.5%–5% (depends on glycemia) of glucose solution with the same speed. Other components of the solution were the same. Efficiency of DKA treatment was estimated by pH increasing speed, infusion therapy complications, glycemia and decreasing glycemia speed. Optimal rate of glycemia decreasing is 2–5 mmol/l*h and optimal glycemia is > 12 mmol/l. Data processing was carried out with STATISTICA by StatSoft.
Results: Speed of pH increasing in 1st group was 0.018 ± 0.007 per hour, in the 2nd – 0.034 ± 0.009/hour. Speed of glycemia decreasing in the 1st group was 3 ± 1, 4 mmol/l*h, 1.67 ± 1.2 mmol/l*h in the 2nd. Duration of acidosis in the 1st group was 10.3 ± 4, 7 h, 4.8 ± 3.3 h in the 2nd, in all calculations $P < 0.05$. Increasing pH speed in treatment with improved protocol was significantly more than with standard.
Conclusions: The effectiveness of both protocols is similar. But improved protocol is more physiological: acidosis lasts for a shorter time, decreasing glycemia levels are safer for patient.

O/FRI/1/03

Diabetic nephropathy in 27,643 children, adolescents and adults with type 1 diabetes: Effect of diabetes duration, Hba1c, hypertension, dyslipidemia, diabetes onset and gender. Analysis from the prospective German diabetes documentation and quality management system (DPV)

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Objective: To give an up to date profile of nephropathy and involvement of potential or known risk factors in a large, prospective cohort of patients with type 1 diabetes and largely pediatric and adolescent onset of disease.
Methods: From the DPV-Initiative, patients with at least two documented urine analysis and given consent for data evaluation were included in the present analysis. Cohort characteristics of the 27,643 patients included were: mean age at diagnosis 12.9 years, mean age at last follow up 21.4 years, and mean follow up time 3.3 years. Influence of the covariates diabetes duration, age at diagnosis, gender, hypertension, HbA1c, dyslipidemia, and smoking was tested by Kaplan-Meier analysis and logistic regression.
Results: Kaplan-Meier analysis included 26,644 patients with normal urine albumin, 921 patients with microalbuminuria and 78 patients with macroalbuminuria. After calculated diabetes duration of 40 years, 25.4% (CI 22.1–28.3%) of patients had microalbuminuria but only 4.9% (CI 3.8–7.1%) macroalbuminuria. Logistic regression identified diabetes duration (OR1.033, $P < 0.0001$), HbA1c (OR1.13, $P < 0.0001$) and dyslipidemia (OR1.727, $P < 0.0061$) as common risk factors for any nephropathy, while systolic and diastolic blood pressure (OR1.008, $P < 0.0074$) increased and young diabetes onset (OR1.011, $P < 0.0001$) decreased risk for microalbuminuria. Moreover, male gender and smoking were not associated with nephropathy.
Conclusion: Cumulative incidence of micro and macroalbuminuria was lower in this recent, large cohort from Germany and Austria than reported earlier. Young age of our patients and lower rates of risk factors might account for this phenomenon. Diabetes duration, HbA1c, dyslipidemia and blood pressure have been identified as independent risk factors for development of nephropathy. Therefore, diabetes care must focus first on long term metabolic control, but also on reduction of other risk factors, like dyslipidemia and hypertension.

O/FRI/1/04

Arterial hypertension and pre-hypertension in children and adolescents with type 1 diabetes

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Objectives: The aim of the study was to evaluate the prevalence of hypertension and pre-hypertension, as well as daily blood pressure (BP) profile disturbances and also to analyze several cardio-vascular risk factors in children and adolescents with diabetes type 1.
Methods: The group consisted of 100 young patients (51 girls and 49 boys) in mean age 15.4 years (8–19 years) and mean diabetes duration 6.89 years (0.5–17 years). The 24-hour blood pressure monitoring was being performed in all patients.
Results: A total of 30 (30%) patients during the day period and 26 (26%) during the night period had their mean systolic BP (SBP) elevated beyond the 95th percentile (for sex, age and height) in more than 40% of measurements. Corresponding diastolic BP (DBP) elevation occurred during the day in 3 (3%) and during the night in 2 (2%) patients. Pre-hypertension was revealed in 39 (39%) patients. Lack of physiological BP decrease during the night (non-dipper) appeared in 48 (48%) subjects. The analysis of relationship between the existence of hypertension, pre-hypertension and several risk factors revealed a negative correlation between HDL level and mean SBP during the hole BP measurement period ($r = -0,412$, $P < 0.05$) in the hypertension group. Similar negative correlation occurred with mean SBP during the night period alone ($r = -0.52$, $P < 0.01$) in these children. There was also a positive correlation between triglycerides (TG) level and mean DBP during the hole measurement period ($r = 0.443$, $P < 0.05$) as well as mean SBP during the night period alone ($r = 0.467$, $P < 0.05$) in the hypertension group. Furthermore the comparison of non-dipper group with dipper group showed significantly higher BMI ($P < 0.05$), higher TG level ($P < 0.05$) and lower HDL level ($P < 0.05$) within the Non-Dipper group.

Conclusion: 1) Both hypertension and pre–hypertension are common disorders in children and adolescents with diabetes type 1. 2) Non-dipping seems to be connected with some of the cardio-vascular risk fact

O/FRI/1/05

Insulin binding to antibodies is a risk factor for inexplicable severe hypoglycaemia in children with type 1 diabetes mellitus

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Background: Type 1 diabetic patients differ with regard to both the formation of circulating insulin antibodies, and the incidence of severe hypoglycaemia (SH).
Aim of the study: To assess the association of insulin binding to antibodies with the incidence of SH.
Patients and methods: In a cross sectional study, 73 children with type 1 diabetes mellitus (median age 14 years, duration of diabetes 6 years) were investigated, 22 of whom had experienced SH during the preceding 18 months and 51 never had experienced SH. Of the patients with SH, 16 had experienced SH deemed inexplicable, and 6 had experienced SH which deemed explicable (by missed meals, unplanned physical exercise etc.). Insulin binding was measured by radioimmunoassay, and expressed as ratio bound/unbound insulin; a binding >15% was considered relevant insulin binding (RIB).
Results: A total of 38 patients displayed RIB (17 of whom had experienced SH), and 35 patients did not display RIB (5 of whom had experienced SH; $P = 0.0055$, Fisher's exact test). Patients with RIB were younger (13 vs. 15 years, $P = 0.006$) than patients without RIB. Of the 16 patients with inexplicable SH, 15 displayed RIB, compared to 2 of the 6 patients with explicable SH ($P = 0.009$). The association of any SH, and of inexplicable SH, with RIB was significant (odds ratio 4.8 (95% CI 1.5–15.2), and 22.1 (95% CI 2.7–179.6), $P < 0.006$). Patients with/without RIB, or with/without SH, were comparable regarding sex, duration of diabetes, number of insulin injections per day, HbA1c and C-peptide levels (ANOVA).
Conclusion: Insulin binding to antibodies > 15% appears to be a strong risk factor for inexplicable severe hypoglycaemias in type 1 diabetic children. This study was supported by: Die Stiftung 'Das Zuckerkranke Kind'

O/FRI/1/06

The glucagon response to hypoglycaemia is lost early in adolescents with type 1 diabetes mellitus and not preserved by strict glycaemic control initiated at diagnosis

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Objectives: A major contributing factor to the vulnerability of patients with T1DM to hypoglycaemia is a defective counter-regulatory hormone response. We have previously shown that the glucagon response to hypoglycaemia is lost early in T1DM in adolescents. To investigate whether tight glycaemic control initiated at diagnosis can preserve the glucagon response to hypoglycaemia, we enrolled adolescents prospectively and studied the natural history of their glucagon response to hypoglycaemia.
Methods: Hypoglycaemic hyperinsulinaemic clamp studies were performed at 6 weeks, 9 months and 18 months after diagnosis. At each time point we assessed Glucagon response following 40 min of hypoglycaemia, Glucagon response to Arginine stimulation and C-peptide response to a meal challenge. HbA1c was measured monthly thrice.
Results: To date, 11 adolescents (13.2 years, 4F) have completed all 3 clamp studies. A further 26 have been enrolled and are yet to complete the 18 months. Good glycaemic control was achieved and maintained: HbA1c 12.2 ± 0.4% at diagnosis, 6.9 ± 0.4% at 9 months and 7.2 ± 0.4% at 18 months ($P < 0.05$). Glucagon response to hypoglycaemia was lost by the first 6 weeks in 10 of the 11 subjects and did not improve over the 18 months.Glucagon levels (mean ± SE in pg/mL) at the 3 time points, at euglycaemia and in response to hypoglycaemia were: 6 weeks: 49.5 ± 5.1; 49.2 ± 5.8, 9 months 48.1 ± 5.1; 43.9 ± 5.8 and 18 months 41.9 ± 4.7; 40.9 ± 5.3, respectively. In contrast, glucagon responses to Arginine were preserved throughout the study period at all time points in all patients (peak 130.9 ± 13.9 after Arginine stimulation, $P < 0.05$ vs. baseline). Fasting C-peptide (mean ± SE in nmol/L) was low and could not be stimulated after a meal challenge: 0.3 ± 0.05 at baseline, peak 0.4 ± 0.04 after meal challenge.
Conclusions: Glucagon response to hypoglycaemia was lost in adolescents early after diagnosis of T1DM and was not preserved by tight glycaemic control.

O/FRI/1/07

Screening for diabetic retinopathy by non-mydriatic retinal imaging

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Objectives: To determine the sensitivity, the specificity and the clinical impact of a digital non-mydriatic digital stereoscopic retinal imaging as a screening tool in detecting diabetic retinopathy in a group of patients with type 1 diabetes mellitus.
Methods: We reviewed the records of 87 patients with type 1 diabetes mellitus who had a dilated funduscopic examination and a contemporary non-mydriatic digital retinal imaging (obtained by a Topcon Fundus Camera TRC-NW200). Patients were 54 males (aged 16.33 ± 5.91 years) and 33 females (aged 15.99 ± 5.8 years); the duration of diabetes was 78 ± 64 months and their HbA1c was 7.8 ± 1.15%.
Results: 78 patients (89%) had no retinopathy, 1 (1.1%) patient had severe non-proliferative diabetic retinopathy, 1 (1.1%) moderate non-proliferative retinopathy and 7 (8%) mild non-proliferative retinopathy. No signs of retinopathy were found by both techniques before 152 months of diabetes duration and before 18 years of age. We found full agreement between retinopathy gradation made from dilated funduscopic examination and the gradation made by non-mydriatic digital retinal imaging (both sensitivity and specificity were 100% for all 174 eyes). We also found a significant relationship between retinopathy and duration of diabetes ($P = 0.0001$); between 10 and 15 years of diabetes duration 7/19 (36%) patients had retinopathy and after 15 years 2/4 (50%) had retinopathy. We didn't found any significant correlation between retinopathy and gender, HbA1c, blood pressure, microalbuminuria, antibodies, asymptomatic hypoglycemic values, BMI.
Conclusions: As reported in a recent article by Ahemd J et al. (Diabetes Care, 2006) our results show good agreement between dilated fundus oculi examination and non-mydriatic digital retinal images and suggest that this later technique is suitable and recommendable for ophthalmologic screening for diabetic patients.

O/FRI/1/08

Transcutaneous electrical nerve stimulation in paediatric patients with painful diabetic neuropathy

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Introduction: Management of pain is a difficult task in children with painful diabetic neuropathy. The drugs available are limited in efficacy and tolerability. There is a need, therefore, to study non-pharmacological methods of pain relief in this population. This paper studies the effect of transcutaneous electrical nerve stimulation (TENS) on painful neuropathy in pediatric patients of diabetes mellitus.

Methods: 15 pediatric diabetic patients receiving five sittings of TENS on daily or alternate day basis were compared with 15 age-matched, disease-matched patients who were given daily oxcarbamazepine and five sittings with sham electrodes. Glycemic control was maintained with insulin as per protocol.

Results: Pain scores reduced significantly in both groups, but much more so in the TENS group (from 4.60 ± 0.54 to 2.40 ± 0.54) than the sham electrodes + oxcarbamazepine group (from 4.40 ± 0.54 to 3.60 ± 0.54). A significant change was seen in health distress and disease intrusion scores in the TENS group.

Discussion and conclusions: This study demonstrates the beneficial effect of low dose TENS in pediatric patients with painful neuropathy due to diabetes mellitus.

O/FRI/1/09

Association between Leu54Met polymorphism at the paraoxonase gene (pon1) and plantar fascia thickness in young patients with type 1 diabetes

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Objective: Abnormal plantar fascia thickness (PFT) has been found in diabetic patients compared to controls. PFT may be a novel measure of tissue advanced glycation and predictor of diabetes complications[1]. Paraoxonase is an HDL-bound anti-oxidant enzyme and polymorphisms at the paraoxonase gene (PON1) have been implicated in microvascular disease[2]. We investigated the relationship between abnormal PFT and PON1 polymorphisms.

Methods: Cross-sectional study of 331 patients with childhood onset T1DM (162 male; 169 female) attending annual diabetes complications assessment. PFT was assessed by ultrasound (normal < 1.6 mm[1]). PON1 genotyping was performed by PCR followed by RFLPT. Serum PON1 activity was determined by rates of hydrolysis of paraoxon and phenylacetate. Predictors of abnormal PFT (gender, total cholesterol, HbA1c, duration of diabetes, retinopathy by 7-fundal photography, albumin excretion rate, SBP and BMI centiles and PON1 genotype) were assessed by multiple logistic regressions (SPSS 13.0).

Results: The median (IQR) age was 15.4 years (13.5–17.3) and duration 7.6 years (4.9–10.6). Abnormal PFT (≥ 1.6 mm) was present in 159 (48%). Leu54Met polymorphisms of PON1 were LL 135 (40.8%); ML 149 (45%) and MM 47 (14.2%). PON1 activity (paraoxon substrate) was significantly different across the 3 groups (mean ± SD; MM 28.8 ± 12.9, ML 53.0 ± 45.1 and LL 85.4 ± 46.9; Kruskal–Wallis test, $P < 0.001$). Significant predictors of abnormal PFT were:

Predictors	Odds Ratio	95% CI	P value
Gender (male)	2.98	1.70–5.23	<0.001
SBP centile	1.01	0.99–1.02	0.07
BMI centile	1.02	1.01–1.03	0.005
Leu54Met PON1 (MM vs ML/LL)	0.28	0.12–0.64	0.002

Conclusion: In young patients with T1DM, homozygosity for the M allele of the PON1 Leu54Met polymorphism is associated with reduced risk of abnormal PFT.

References:

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Beta Cell and Adipocyte Function, New Insulins

O/FRI/2/01

Can we build a beta cell? Induction of beta cell genes in transcription-factor targeted cells

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Objectives: Developing sustainable insulin delivery responsive to all nutrients is a major challenge for long-term diabetes care. Engineering tissues from a patient's own cells should confer control superior to mechanical devices, without immunological responses potentially provoked by embryonic stem cells. The transcription factor (TF) network emerges as a key element of cellular engineering. Hepatocyte nuclear factors HNF4α, HNF1α and HNF1β are central to network regulation. They are expressed early in the β-cell lineage and exhibit self-regulating and self-sustaining features. Disruptions in their genes impair glucose and lipid homeostasis in humans and mice. HNF4α and HNF1α activate each other's expression and both are dependent on HNF1β. HNF4α transcription from two promoters and alternative splicing lead to 9 potential variants that differ in transcriptional activation activity.

Methods: To test the feasibility of utilizing endogenous transcription factor genes to reproduce β-cell behaviors, we tested HNF4α variant induction of Hnf1α and HNF1α induction of specific HNF4α variants in Cos7 monkey kidney cells and (in combination with coregulator CREBBP) in NIH3T3 mouse fibroblasts.

Results: In NIH3T3 cells, transient expression of HNF4α3 but not HNF4α1 or HNF4α7 (in combination with coregulator p300) induced the endogenous HNF1α gene. Conversely, transient HNF1α expression induced some HNF4α variants (HNF4α1, HNF4α7). In Cos7 cells transiently expressing HNF1α, only HNF4α1 was induced.

Conclusion: Non-β cells can sustain expression of HNF4α and HNF1α if appropriately stimulated by transcription factor variants. Ultimately, activation of β-cell target genes could move us closer to engineering full β-cell functionality to cure diabetes by creating regulated insulin secretion in non-β cells.

O/FRI/2/02

Effect of A20 gene on animal pancreas islet xenotransplant

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Objectives: Transplantation of islets of langerhans represents a potential cure for T1DM, but the success of it is hampered by destruction of the islets and loss of β-cell to apoptosis. A20 has a dual anti-apoptosis and anti-inflammatory function in primary endothelial cells. Our aim was to evaluate the protective effect of A20 gene on pancreas islet xenotransplant.

Methods: Mice islets were isolated, purified and cultured. Then the islets were transplanted under the left kidney capsule of the diabetes rats, and the diabetes rats were made by injecting STZ into the abdominal cavity. The recipients were divided into two groups randomly, experiment group and control group. In the experiment group, the graft islets were infected with Lentivirus vector expressing A20; in the control group, the islets weren't infected. After transplantation the blood glucose were measured to evaluate the effect. The statistical analysis was conducted using SPSS 10.0.

Results: On the first day after transplant the blood glucose began decreasing obviously in both groups. In the experiment group, the effective existing time of islets was 3 to 7 days; the average was 5.1 ± 1.87 days. Compare with the experiment group, the time of the control group was 2 to 4 days; the average was 2.9 ± 1.12 days.

Conclusion: The data showed that A20 gene could prolong the existing time of xenograft islets. A20 might be a relevant gene for protection of β-cells against the autoimmunity in islets xenotransplant.

O/FRI/2/03

In vitro (re)programming of human bone marrow stromal cells towards insulin producing phenotypes

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Adult stem cells are investigated as an alternative source for β-cell replacement therapy. So far, no consistent differentiation capacity for insulin producing cells has been shown in human bone marrow derived mesenchymal stem cells (MSC). Here we investigated *in vitro* the ability of human bone marrow derived MSC-line hMSC-TERT to differentiate into insulin producing cells under the regulation of Neurogenin 3 (Ngn3) and Pdx-1, master regulator genes in development of endocrine pancreatic lineages and/or β-cell function. hMSC-TERT cells stably over-expressing hNgn3 and/or hPDX-1 were generated (hMSC-TN, hMSC-TP and hMSC-TN/P). Islet-cell gene regulation and protein synthesis were analyzed by RT-PCR, Western blotting, reporter gene assays and immunocytochemistry. Insulin content and secretion were evaluated by ELISA. Our results indicate that introduction of key endocrine pancreatic transcription factors into human bone marrow derived mesenchymal stem cells is able to induce differentiation programs towards insulin producing phenotype. Overexpression of hNgn3 alone is enough to trigger pancreatic endocrine differentiation cascade through activation of endogenous Pdx-1. Similar to its role in endocrine pancreatic development Ngn3 seems to lie upstream of Pdx-1 transcription factor. Finally, in a human system of MSCs, insulin was expressed, produced and stored under the regulation of hNgn3 and/or Pdx-1. Insulin secretion though was not regulated in a glucose dependent manner in these cells. We conclude that human bone marrow derived hMSC-TERT cells have the potential to differentiate into β-cell-like phenotypes. However, higher maturity level must be achieved in these cells in order to obtain a functional source of insulin producing phenotypes for the cell-based therapy of type 1 diabetes.

O/FRI/2/04

Improvements in cognition with insulin pump therapy in children with type 1 diabetes mellitus (T1DM)

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Objectives: There is some evidence that high, low or widely fluctuating blood glucose levels are related to cognitive difficulties in children with T1DM. Insulin pump therapy (continuous subcutaneous insulin infusion; CSII) has been associated with improved metabolic control and reduced glucose fluctuations. We investigated changes in cognition following commencement of CSII in children with T1DM.

Methods: A total of 13 children with T1DM aged 6–16 years were recruited once accepted into the CSII program at Children's Hospital Westmead, Sydney (n = 15) and Royal Children's Hospital, Melbourne (n = 15). A comprehensive test battery was administered, comprising measures of intelligence, attention, processing speed and executive skills. Participants were assessed one week before, and 6–8 weeks after, commencing CSII. Alternative test forms were used where possible to minimize practise effects. HbA1c was used to assess glycemic control at each time point.

Results: Paired sample t-tests revealed no significant improvement in performance on simple tasks dependent on basic cognitive skills, including focused, sustained and selective attention. In contrast, significant improvement following commencement of CSII was observed on cognitively demanding, complex tasks such as divided attention (P < 005, n^2 = 0.29), working memory (P < 0.05, n^2 = 0.19), processing speed (P < 0.05, n^2 = 0.20), and self-monitoring (P < 0.05, n^2 = 0.27). All effect sizes were large (n^2 > 0.14) as indicated by Cohen (1988). Mean blood glucose level (BGL) at time of testing did not differ across assessments, indicating that cognitive changes did not reflect intercurrent BGL. Mean HbA1c improved from 8.21 to 7.47% (P < 0.001, n^2 = 0.58).

Conclusion: Even mild decrements in ability are relevant for children who are still acquiring new skills and knowledge. Our results suggest that, with CSII, specific cognitive skills improve and may enhance learning efficiency in children with T1DM.

O/FRI/2/05

Novel adipokines retinol binding protein-4 and lipocalin-2 in childhood obesity: Differences from adult obesity

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Recently, much evidence has emerged regarding the roles of newly described adipokines, such as retinol binding protein-4 (RBP4) and lipocalin-2, in systemic insulin resistance. Both RBP4 and lipocalin-2 are elevated in adult obesity but sparse data exist on childhood obesity. Aim of the study was to investigate the impact of BMI on the circulating concentrations of RBP4 and lipocalin-2 in obese children and adolescents, in comparison to markers of inflammation, such as high sensitivity CRP (hsCRP) and well-established adipokines, such as leptin and adiponectin.

Patients and methods: We studied 80 girls aged 9–15 years divided in 4 groups according to their BMI–SDS: 20 overweight (mean BMI–SDS 1.8 ± 0.4), 20 obese (mean BMI–SDS 2.2 ± 0.4) and 20 morbidly obese (mean BMI–SDS > 3.0) patients and 20 lean subjects serving as controls (mean BMI–SDS < 1.4). We measured plasma soluble RBP4, lipocalin-2, leptin and adiponectin levels by immunoenzymatic techniques and hsCRP by immunonephelometry. We calculated HOMA values from the fasting glucose and insulin concentrations using the formula G0xI0/22.5 as a marker of insulin resistance.

Results: a) Plasma RBP4 and lipocalin-2 levels were higher in lean than in obese children ($P < 0.01$) and correlated negatively with BMI-SDS values ($P < 0.0018$ and $P < 0.05$, respectively); b) similarly, adiponectin levels correlated negatively with BMI-SDS values ($P = 0.0017$); c) hsCRP and leptin concentrations correlated positively with BMI-SDS values ($P < 0.0001$).

Discussion: While leptin, adiponectin and hsCRP levels in children correlated with BMI similarly to adults, the concentrations of RBP4 and lipocalin-2 in children correlated with BMI inversely compared to adults. Although systemic inflammation and insulin resistance are present in childhood obesity, protective mechanisms of the organism in children might lead to decreases of both RBP4 and lipocalin-2 levels in an attempt to counteract the detrimental effects of insulin resistance.

O/FRI/2/06

Serum adiponectin and expression of AdipoR1, PPAR-γ and CB1 in primary adipocyte cultures from abdominal adipose tissue of lean pre-pubertal children

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Objectives: Factors associated with insulin sensitivity and lipogenesis are: 1) adiponectin, an insulin sensitizer, and its receptor AdipoR1 2) PPAR-γ, a nuclear receptor associated with lipogenesis and insulin sensitivity and 3) CB1, an endocannabinoid receptor associated with food intake and lipogenesis. It has been reported that older normal pre-pubertal children undergo a decrease in insulin sensitivity. We investigated the serum levels of adiponectin and the expression of AdipoR1, PPAR-γ and CB1 in pre and mature adipocytes of lean healthy pre-pubertal children to assess the normal physiology of these factors.

Methods: Primary cultures of pre and mature adipocytes were developed from routine surgical abdominal biopsies of adipose tissue from 36 lean healthy pre-pubertal children (BMI < 85%) separated into 2 age groups: group A: 2 months–7 years and group B: 8–12 years. AdipoR1, PPAR-γ and CB1 expression was studied at the mRNA level (mR) with RT-PCR and at the protein level (Pr) with Western immunoblotting. Serum adiponectin was measured by ELISA.

Results: There was a significant (S) increase at the Pr of AdipoR1 [55.6%, $P < 0.001$(*)] and CB1 (37.8%*) in the mature adipocytes of the older lean children in comparison to the younger lean. Also an S increase in Pr of AdipoR1 and CB1 was observed during the differentiation of the pre-adipocytes to mature adipocytes of the older lean children (by 11.85%* and 37.8%* respectively). Serum adiponectin was S decreased (50%, $P = 0.012$) in the older lean vs. younger lean children.

Conclusion: The reduced serum adiponectin and increased Pr of CB1 in the older lean pre-pubertal children may possibly lead to decreased insulin sensitivity. The increased Pr of AdipoR1 though, in this group, could be an attempt to increase AdipoR1's availability to the circulating serum adiponectin, as a homeostatic mechanism to possibly restrict the decreased insulin observed in normal pre-pubertal children.

O/FRI/2/07

Differences in waist circumference and expression of AdipoR1, PPAR-γ and CB1 in primary adipocyte cultures from abdominal adipose tissue of obese and lean pre-pubertal children

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Objectives: Childhood central obesity is associated with insulin resistance. Adiponectin, an insulin sensitizer, shows decreased AdipoR1 expression in obesity leading to insulin resistance. PPAR-γ is a nuclear receptor associated with lipogenesis and insulin sensitivity and CB1 is an endocannabinoid receptor associated with food intake and lipogenesis. We studied the expression levels of AdipoR1, PPAR-γ and CB1 in pre (p) and mature (m) adipocytes from obese and lean children in association with their waist circumference.

Methods: Primary cultures of pre and mature adipocytes were developed from routine surgical biopsies of subcutaneous abdominal adipose tissue from 17 healthy obese (BMI ≥ 90%) and 36 healthy lean pre-pubertal children (BMI < 85%) in 2 groups (group A: 2 months–7 years and group B: 8–12years). AdipoR1, PPAR-γ and CB1 expression was studied at the mRNA level (mR) with RT-PCR and at the protein level (Pr) with western immunoblotting. Waist circumference (WC) was measured on the day of surgery.

Results: Group A: AdipoR1 and PPAR-γ showed no significant difference (NS) at mR, whereas CB1 was significantly (S) decreased

in the p (31.8%) and m (66.8%) of obese vs. lean. At the Pr, AdipoR1 and PPAR-γ showed NS, although CB1 was S decreased in the p (64.5%) and m (36.5%) of obese vs. lean. WC showed NS between lean and obese. Group B: AdipoR1 showed NS at the mR whereas, PPAR-γ and CB1 showed an S decrease (29.32% and 64% respectively) in the m of obese vs. lean. At the Pr level NS was observed in PPAR-γ, but AdipoR1 and CB1 were S decreased (60% and 48% respectively) in the m of obese vs. lean. WC was S increased by 22.9% in obese vs. lean.

Conclusion: The increased WC and the reduced Pr of AdipoR1 and mR of PPAR-γ in the mature adipocytes of the obese older children may play a role in the development of insulin resistance in this group. The reduced CB1 at the Pr and mR in these children may be an attempt to protect by reducing lipogenesis.

O/FRI/2/08

Long-acting insulin analogues have mitogenic and antiapoptotic activities

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Introduction: To improve the control of diabetes long acting insulin (INS) analogues have been developed. These analogues have modifications in the C-terminal regions of the α and β chains of human INS, which do not mediate INS binding to its receptor. However, these regions determine ligand affinity towards the IGF-I receptor (IGF-IR), known to play important roles in tumor biology.

Objective: We tested whether Glargine (Gl, Lantus®, Sanofi Aventis) and Detemir (Dt, Levemir®, Novo Nordisk), two new long-acting INS analogues exhibit IGF-I-like enhanced mitogenic and antiapoptotic effects.

Methods: Colon (HCT116), prostate (PC3) and breast (MCF7) cancer-derived cell lines were incubated with IGF-I, regular INS (rINS), Gl and Dt for different time intervals and then harvested and counted with a hemocytometer. In addition, the potential anti-apoptotic activities of the analogues were evaluated using an Annexin V/FITC kit (Bender Med System) and the activated signaling cascades were identified by Western immunoblotting.

Results: In all cell lines, both Gl and Dt significantly ($P < 0.05$) stimulated cell proliferation while rINS did not have any major effect (Table). Apoptosis measurements after 12 hour in HCT cells demonstrated that Gl and Dt exhibit a reduced anti-apoptotic effect similar to that elicited by IGF-I. Specifically, 14.9% apoptosis under Gl treatment, 18.1% (Dt), 16.9% (IGF-I), 24.5% with rINS and 23.2% in control cells. Western blot analysis revealed that Gl activates both the MAPK and PI3K pathways, the major signaling cascades of both the INS and IGF-I receptors. Interestingly, the effect of Gl on the phosphorylation of AKT was stronger than that of IGF-I.

Cell line/ treatment	Control	IGF-I	Reg. Insulin	Glargine	Detemir
HCT116 (4d)	100%	135.8	101.5	122.2	118.2
PC3 (3d)	100%	118.4	101.2	110.0	113.7
MCF7 (2d)	100%	156.2	126.9	146.1	148/6

Conclusion: Both long-acting INS analogues, Gl and Dt exhibit potent mitogenic and anti-apoptotic activities similar to IGF-I. These activities significantly exceed the extent of the effects elicited by rINS. The clinical importance of these findings remains to be established.

O/FRI/2/09

Changes in the use of analogue insulins in 33944 children and adolescents with type 1 diabetes in 254 German centers in the last ten years

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Objectives: We want to describe changes in insulin treatment regarding short acting (SA) and long acting (LA) insulin analogues in different age groups over the last ten years.

Methods: A total of 33,944 children and adolescents with the age of 0–20 years from 254 German centers that were registered in the DPV-database (Dec. 2006) were included into the analysis. The group was subdivided into 4 age groups (A: ≤5 years; B: 5–≤10 years; C: 10–≤15 years, D: 15–≤20 years). We further analysed the use of analogues from onset of diabetes.

Results: A significantly increasing rate of pediatric patients in all age groups with type 1 diabetes use analogue insulins. In 2006, 44.9% used SA, 39.2% LA. 87.8% of pumps are running with short actin analogue. Age group analysis: A: 2000: 9.7% SA, 0.8% LA vs. 2006: 44.7% SA, 10.3% LA; B: 2000:6.3%SA 1.7% LA vs 2006: 31.0% SA, 22.8% LA; C: 2000:15.7% SA,3.8%LA vs. 2006: 44.6% SA, 44.4% LA; D: 2000:27.3% SA,3.0% LA vs. 2006: 58.0% SA, 55.2% LA. This increase in usage of analogues was also found at onset of diabetes. Corrected for age, center and diabetes duration HbA1c was significantly lower in the group with nomal insulin (8.07 ± 0.055%) than with SA (8.20 ± 0.057%) ($P < 0.0001$) as BMI-SDS was significantly but only marginal lower in the group with nomal insulin (0.47 ± 0.01) than with SA (0.50 ± 0.01) ($P = 0.036$). The same differences in HbA1c (8.15 ± 0.060% vs. 8.36 ± 0.062%) and BMI-SDS where seen when NPH was compared with LA respectively. After change to SA and LA, the reduction of severe hypoglycemia with (0.99 patients/100 years) and without coma (2.1 patients/100 years) did not reach significance.

Conclusions: Long term data for the use of new drugs are sparse. In our analysis patients are followed not under study conditions. Still the higher BMI and HbA1c with either SA or LA usage have to be discussed carefully in the context of increasing use of both, long acting and short acting analogues and possible problems with reimbursement.