

EFFECT OF GLUCOSE CONCENTRATION AND CALPHOSTIN C ON ACTIVATION OF NUCLEAR FACTOR- κ B IN CULTURED ENDOTHELIAL CELLS

M. Fazia, F. Chiarelli*, F. Cipollone, A. Iezzi, D. De Cesare,
A. Mohn, F. Cuccurullo, A. Mezzetti

Department of Pediatrics and Medicine, University of Chieti, Italy

Background: Nuclear factor- κ B (NF- κ B) is a transcription factor to many cell types, including endothelial cells. NF- κ B plays a pivotal role in early gene responses by promoting messenger RNA (mRNA) synthesis for various cell-adhesion molecules and inducible nitric oxide synthase.

Aim: To examine whether increases in glucose concentration enhance NF- κ B expression in nuclear fractions of endothelial cells and whether this process is mediated via a protein kinase C (PKC)-dependent pathway.

Methods: Bovine aortic endothelial cells (BAECs) were incubated in media containing 5.5-35 mM glucose. In some BAECs the glucose induced NF- κ B activation was blocked by inhibition of nuclear translocation by using 30 μ g/ml of the peptide SN-50. Other BAECs were incubated with 100 nM staurosporin (a non selective PKC inhibitor) or 50-200 nM calphostin C (a highly selective PKC inhibitor) for 30 min before incubation with 35mM glucose for 2 hours.

Results: NF- κ B activity was increased as early as 1 h (peak activation at 2-4 h) after incubation with 35 mM glucose compared with 5.5 mM. Similar increases at 2 h of incubation were observed by using 25 but not 15 mM glucose.

Glucose-induced NF- κ B activation was blocked by inhibiting nuclear translocation by using a peptide (SN-50) containing the nuclear-localization sequence of NF- κ B p50 linked to a membrane-permeable motif of the sequence for Kaposi fibroblast growth factor. Co-incubation with a selective protein kinase C (PKC) inhibitor, calphostin C, produced a concentration-dependent inhibition of glucose-induced NF- κ B activation.

Conclusion: NF- κ B activation is an early event in response to elevations in glucose, which may elicit multiple pathways contributing to the origin of hyperglycemia- or diabetes-induced endothelial cell injury and consequent diabetic angiopathy.

INCREASED CORTISOL SECRETION IN CHILDREN AND YOUTH SUFFERED FROM TYPE 1 DIABETES MELLITUS

P.Fichna

*Department of Pediatric Endocrinology and Diabetes, Institute of Pediatrics,
University School of Medical Sciences, Poznań, Poland*

Background: Physiologically imperfect insulin therapy in type1 diabetes evokes some adverse reactions. An increased adrenal steroid secretion is thought to be one of them. **Aim:** This study was to answer if type1 diabetes changes cortisol responsiveness to low dose ACTH stimulation test in young diabetic patients. **Methods:** 24 diabetic girls (**dg**) (age: 8.7-17.3yrs, diabetes: 1-11yrs, HbA1c 5.9-12.8%), 25 control girls (**cg**) (age: 8.3-17.3yrs), 29 diabetic boys (**db**) (age:8.3-17.8yrs; diabetes:1-12yrs, HbA1c 6.1-10.7%) and 20 control boys (**cb**) (age:8.3-17.1yrs) underwent to iv stimulation test with Synacthen 1 μ g dose injected at 0 min. and followed with 50 μ g dose in continuous infusion from 30 min up to 120 min. Control patients were without diabetes, obesity nor adrenal disorders. Serum cortisol was measured (RIA) at 0, 30, 60, 90, 120 and 150 min. The results were evaluated in time intervals as area under curve (AUC) in relation to: group, sex, age, diabetes duration, HbA1c, daily insulin dose, number of its injections/day, blood glucose and insulin concentrations during the test. ANOVA and Pearson correlation were applied for statistical analyses. **Results:** Diabetic patients revealed basal ($p<0.05$) and time depended ($p<0.0001$) cortisol (AUC mean \pm SD ng \cdot min/ml) higher than in controls: to-tal 0-150min area (**dg** 51959.4 \pm 12812.4; **cg** 41326.8 \pm 7984.3; **db**48995.7 \pm 11425.2; **cb** 39480.0 \pm 7926.9) depends on diabetes ($p<0.0001$). Total cortisol net-increase (**db** 18090.5 \pm 9350.5; **cb** 13650.0 \pm 5723.5; **dg** 23871.9 \pm 8026.7; **cg**17218.8 \pm 6684.7) depends on diabetes ($p<0.001$) and on sex ($p<0.004$) separately. Cortisol net-incre-ments in time intervals (the highest were found in 0-30min: **dg** 1646.9 \pm 859.5; **cg** 1185.6 \pm 527.4; **db** 1116.7 \pm 782.7; **cb** 1094.3 \pm 480.2) are depending on diabetes ($p<0.0001$) and sex ($p<0.003$) as well. Significant positive correlations were found between basal or total cortisol levels and HbA1c, glycemia, weight or BMI, when net-cortisol secretion was correlated to insulin levels negatively in diabetic girls. Diabetic boys revealed negative correlation between basal or total cortisol levels and HbA1c. **Conclusion:** Diabetes induces elevation of basal and quasi-physiologi-cally stimulated blood cortisol, what should be considered in relations to insulin resistance, down phenomenon,

SEVERE CLINICAL ONSET OF DIABETES AND INCREASED PREVALENCE OF OTHER AUTOIMMUNE DISEASES IN CHILDREN WITH COELIAC DISEASE DIAGNOSED BEFORE DIABETES MELLITUS.

A Franzese*, G Valerio, L Maiuri, R Troncone, P Buono, E. Mozzillo, M Spagnuolo

Department of Paediatrics, University Federico II, Naples, Italy; Faculty of Scienze Motorie, University Parthenope, Naples, Italy

Background: The pathogenetic mechanism underlying the simultaneous occurrence of celiac disease (CD) and type 1 diabetes mellitus (T1DM) has not been elucidated, even though many evidences indicate that a common genetic determinant may play a role.

Aim: To analyse whether the time of diagnosis of CD with respect to the clinical onset of T1DM could differentiate a subgroup of patients with both diseases and different severity.

Methods: Thirty-two T1DM subjects with CD were studied. Gender distribution, age at diagnosis of T1DM, prevalence of ketoacidosis at the onset of T1DM and prevalence of other autoimmune diseases were compared in patients divided according on whether CD was diagnosed before (Group A) or after (Group B) T1DM onset and whether they had presented symptoms of CD. T1DM patients (n= 351) without CD were the control group (Group C).

Results: Patients with CD and T1DM presented higher prevalence of females ($p= 0.003$), but similar age and prevalence of ketoacidosis compared with Group C; 18.7% had a third autoimmune disorder. The increased prevalence of females was confirmed in Groups A and B with respect to Group C ($p = 0.013$), while higher prevalence of both ketoacidosis ($p = 0.009$) and other autoimmune diseases ($p = 0.001$) was found only in Group A. Compared with symptomatic patients, asymptomatic subjects in Group B presented lesser female prevalence, older age at diabetes onset, lower prevalence of ketoacidosis and no other associated autoimmune disease.

Conclusion: A wide clinical spectrum characterises the association of CD and T1DM, with a more severe clinical presentation when CD is diagnosed before T1DM. Distinct phenotypes might imply the contribution of a peculiar genetic background.

EXPERIENCES WITH AN EDUCATIONAL AND PSYCHOSOCIAL INTERVENTION PROGRAM DESIGNED FOR ADOLESCENTS WITH TYPE 1 DIABETES AND THEIR PARENTS.

M.Graue*, T.Wentzel-Larsen, B.R. Hanestad, O.Søvik, Inst.of Social Sciences and Social Medicine, University of Bergen, Centre for Clinical Research, and Dept. of Pediatrics, Haukeland University Hospital, Bergen, Norway.

Background: Abnormalities in aspects of psychosocial and family functioning in adolescents with diabetes are well documented. These facts, as well as a generally poor metabolic control in this age group, highlight the need for novel intervention strategies.

Aim: The objective was to examine the effect of an educational and psychosocial intervention program, as an alternative to traditional diabetes team involvement, in the follow-up of diabetic adolescents and their families.

Methods: All diabetic patients 14-17 yrs old (n=77) and their parents, attending a population-based pediatric outpatient department, were included. The sex ratio (F/M) was 39/38, and mean diabetes duration 7,4 yrs. The patients were randomized to an intervention (n=41) or control group (n=36). The intervention consisted of a 15 months program with computer-assisted consultations and group visits involving physician, nurse, psychologist and social worker. Patients and parents participated in separate groups. The effect of the intervention was assessed by HbA1c, number of hypoglycemic episodes, and the adolescents' assessment of generic (CHQ CF-87) and diabetes-specific (DQOL) quality of life, at 0, 9 and 15 months. The overall acceptance was evaluated by a patient satisfaction-questionnaire.

Results: 10 patients participated in the whole program, 21 participated in parts, whereas 10 declined participation. The negative effect of age on impact of diabetes (DQOL) was reduced in the intervention group as compared with controls (p=0.018), whereas the family functioning (CHQ CF-87) was improved during intervention (p=0,006). HbA1c was not significantly improved. 98% of the parents and 69% of the adolescents reported high degree of satisfaction with the group consultations.

Conclusions: A specific therapeutic educational intervention improved family functioning and reduced general impact of disease. Parents were more easily motivated than adolescents for participation in structured group discussions.

INSULIN REGIME WAS CHANGED BUT HBA1C DID NOT IMPROVE AFTER SUMMER CAMPS FOR CHILDREN

M.Hallman*, B.Lindblad

The Queen Silvia Children's Hospital, Göteborg, Sweden

Background: Camps for diabetics are worldwide and increase the acceptance of diabetes. Improvement in knowledge and self-management is also noted. An assumption of this is that it would also improve the metabolic control which has seldom been evaluated.

Aim: To evaluate 1) if summer diabetes camps made it possible to introduce meal related insulin regimes in those children taking 2-3 injections per day and 2) if HbA1c was improved the year after they participated in their first camp.

Methods: During 1994-2000 sixty children from our diabetic clinic participated in their first 6-day summer diabetes camp together with children from other clinics. The children were 8-13 (mean 11) years old and mean duration of diabetes was 3,8 years (range 0,4 - 11). Among all children in this age range those who for various reasons were judged to benefit more had priority to participate.

Results: Children who before the camp had 2-3 injections per day had during the 6-month-period following the camp increased daily injections from a mean of 2,7 to 3,3 injections per day ($p < 0,001$). During the period 6-12 months after the camp the injection frequency was still equally high. During the 6-month-period following the camp the percentage of soluble or direct reacting insulin increased from 33 % before the camp to 41 % ($p < 0,05$). However, there was no significant change in HbA1c (before the camp 7,5; after 7,8; $p = ns$). Among the participant were children who had difficulties in coping with their diabetes. Contrary to our expectations we found no decrease in HbA1c for those who had HbA1c above 8,0 % (ref. 3,6-5,0) before the camp when comparing the 6-month period preceding the camp with the 6-month period following the camp. In fact in no age group was there a significant change in HbA1c.

Conclusion: Our diabetes children accepted meal related insulin regimes significantly more often after having participated in their first camp. However, the diabetic control was not improved during the following year. This is in agreement with a few earlier studies. A randomised trial seems necessary in order to evaluate which activities during camps that may lead to improved metabolic control.

DOES THE ADDITION OF METFORMIN TO CONVENTIONAL INSULIN THERAPY IMPROVE SHORT-TERM OUTCOME IN TEENS WITH TYPE 1 DIABETES?

J Hamilton, E Cummings, V Zdravkovic, D Finegood, D Daneman*
*Hospital for Sick Children, University of Toronto,
Dalhousie and Simon Fraser Universities, Canada*

Background: HbA1c (HbA1c) is often higher in adolescents with type 1 diabetes when compared with younger children and adults. Insulin resistance (IR) peaks during puberty and is more pronounced in teens with type 1 diabetes compared to nondiabetics. The triad of high HbA1c, high insulin dose requirements and increased weight gain suggests that IR may be an important contributor to deterioration of metabolic control in some teens with type 1 diabetes.

Aims: The objective of our study was to evaluate whether the oral agent, metformin, used in combination with standard diabetes care, would (i) improve insulin sensitivity (Si) and (ii) lower HbA1c and insulin dose requirement.

Methods: We performed a randomized, double-blind, placebo-controlled 3 month trial of metformin therapy in 27 adolescents with type 1 diabetes, high insulin dose (>1 u/kg/day) and high HbA1c (>8%). Insulin sensitivity (Si) was measured at 0 and 3 mo. using: (1) frequently sampled IV glucose tolerance test (FSIGT); and (2) HbA1c, insulin dose (U/kg) and body mass index (BMI).

Results: At the beginning of the study, mean HbA1c was $9.2 \pm 0.9\%$, insulin dose 1.2 ± 0.2 U/kg/day, BMI 24.2 ± 3.9 kg/m² with no difference between metformin (M) and placebo (P) groups. At 3 mo., the mean change in HbA1c was 0.6% lower in the M group compared to P ($p < 0.05$). This was achieved with lower insulin doses (M -0.14 ± 0.1 vs P $+ 0.02 \pm 0.2$ U/kg/day; $p < 0.05$) and no significant change in BMI. Si, measured by FSIGT, did not change significantly between the two groups at study end; however, after adjustment for fasting glucose this approached significance ($p = 0.07$). One subject in the M group withdrew due to vomiting.

Conclusions: Metformin treatment lowered HbA1c, decreased insulin dose with no weight gain in teens with type 1 diabetes in poor metabolic control and clinical evidence of IR. It may act via improving Si, through direct (tissue level) and indirect effects (decreased hepatic gluconeogenesis as well as possible appetite suppression with weight loss). Changes in Si were not documented in this study using the FSIGT. Unanticipated variability in glucose and insulin excursions complicated Minimal Model analysis and make FSIGT an unreliable tool in type 1 diabetes. We conclude that metformin is safe and well tolerated and may represent a useful adjunct to standard type 1 diabetes management in adolescents with poor metabolic control. Long-term studies are needed to determine whether these improvements are sustained.

LONG-TERM OUTCOME OF MULTIPLE DAILY INJECTIONS FROM THE ONSET OF DIABETES

R. Hanas*, P. Adolfsson, C. Andreasson, E. Johansson, A. Kernell.

Dept. of Pediatrics, Uddevalla Hospital, Uddevalla, Sweden,

Background: Results from the DCCT study suggest that persons using intensive insulin therapy have a better long-term outcome even when compared to persons with the same HbA1c using conventional therapy (Diabetes 1995;44:968-83). We began using multiple daily injections (MDI) in children in 1985. From 1987 and onwards we have routinely used it from the onset of diabetes in all age groups, even the youngest children

Aim: To investigate the risk of short- and long-term complications in patients with at least 2 years diabetes duration.

Methods: 109 consecutive patients have been followed (103 began with MDI at diagnosis and 6 within 1 year of diagnosis). One has died (non-diabetes related death). Their average age is 17.2 ± 6.5 years (\pm SD, range 4.5-30.6) with a diabetes duration of 8.8 ± 4.4 years (range 2.1-17.3). 36 (33.3%) use pumps and 9 (8.3%) use indwelling catheters (Insuflo). Fundus photography and urinalysis was performed in patients aged >8 years with >1 year of diabetes and in patients ≤ 8 years after 5 years of diabetes.

Results: 14/96 (13.0% of total study population) had retinopathy (10 simplex, 3 pre-proliferative and 1 proliferative). 5/96 (4.6% of total study population) had persistent microalbuminuria, none macroalbuminuria. 43/97 were negative in fasting C-peptide, 30/97 had 0.3 nM/L or above. 20 patients experienced severe hypoglycemia in 2000, and 12 in 2001. There was no difference in HbA1c between patients with (7.4%) and without (7.1%) severe hypoglycemia. Mean of last HbA1c was 8.5% (DCCT-equivalent numbers) and mean HbA1c over the entire diabetes duration was 8.1%. Patients with retinopathy had a higher mean yearly HbA1c (8.9 ± 1.2 vs. $7.9 \pm 0.9\%$, $p=0.001$) and higher HbA1c load (added yearly mean HbA1c), 114.2 ± 30.3 vs. 70.0 ± 32.7 HbA1c-years, $p<0.001$. Patients with nephropathy also had a higher HbA1c (10.0 ± 1.0 vs. $8.0 \pm 0.9\%$, $p<0.001$) and higher HbA1c load, 133.2 ± 29.5 vs. 75.0 ± 33.1 HbA1c-years, $p<0.001$.

Conclusion: A longer follow-up is needed but so far this non-selected patient group using MDI from the onset of diabetes has a low risk of long-term complications with an acceptable rate of severe hypoglycemia.

THE EDUCATION OF CHILDREN WITH DIABETES IN THE COURSES OF THE DIABETES CENTRE IN FINLAND

S.Härmä-Rodriguez*, E. Autio, E. Haapa, K. Heinonen, P. Holopainen, J. Kauppila, L. Kokkonen, M. Ojala

The Diabetes Centre of The Finnish Diabetes Association , Tampere, Finland

Background : The Finnish Diabetes Association maintains a national Diabetes Centre in Tampere where courses for children under 12 years and their families are arranged. During the course the sessions of education as well as the leisure time of children have certain targets based on the needs of the families. The education of children is realized in three groups according to their age (5-6 years, 7-9 years and 10-12 years).

Aims: The aims of the study are to evaluate , does the course reinforce the skills of self-care (injections, evaluation of the carbohydrates, hypoglycaemia etc.) and do the attitudes of children towards diabetes change after the course.

Methods: 1. A questionnaire and an interview of the family in the beginning of the course

2.. Observation and an interview of children during the course

3. A questionnaire three months after the course

Results: 68 children with their families participated the study during the summer 2000. For the youngest group of children the most useful part of the course was the meeting of other children with diabetes. The two other groups of older children learnt new skills and they became more interested in their self-care as well. Learning in a group helped especially those children who had bigger problems or fears. The attitudes towards diabetes changed in positive way after the course.

Conclusions: The education of children in a group has many advantages and possibilities. The children support and courage each other with their own example. On the other hand, a group is not suitable for everyone, some children need more individual education. In the future it will be a challenge to develop new material for the education of children.

**INSULIN PUMP THERAPY IN PEDIATRIC PATIENTS
WITH TYPE-1-DIABETES: MULTICENTER ANALYSIS
BASED ON THE DPV-SCIENCE INITIATIVE**

B. Heidtmann*, E. Lang, C. Klinkert, R. Lepler, M. Grabert, R.W. Holl
*Catholic Childrens Hospital, Hamburg, HDC NRW, Bad Oeynhausen,
Childrens Hospital Coesfeld, University of Ulm.*

Background: During recent years, insulin pumps have gained new interest in pediatric diabetology, based on reports from the United States. However, few multicenter studies on large numbers of pediatric patients have been published.

Aim: To describe the use of pump therapy in pediatric centers from Germany and Austria participating in a multicenter initiative on quality control.

Methods: The DPV system allows standardized prospective documentation of patients with diabetes, anonymized prospective data are available for centralized descriptive analysis. Pediatric patients (age < 20 years) on pump therapy were documented at 86 institutions. New German reference data (Kromeyer-Hauschild et al) were used to calculate SD-scores for height, weight and BMI. HbA1c was standardized based on the normal range at each center.

Results: A total of 4686 records from 956 patients were available (444 boys, 512 girls, mean age: 15.0 ± 3.6 years, mean diabetes duration 6.9 ± 3.9 years). 12 patients were younger than 5 years, 25 in the age-range 5-10 and 51 between 10 and 15 years, while 868 patients were older than 15 years. On average, patients used pump therapy for 0.8 years [range 0-10 years], the average insulin dose was 0.77 Units/kg. 46.7 % of patients used rapid-acting insulin, a steep increase during the last 5 years (1996: 8 %, 1998: 21 % 2001: 57 %). In the whole group, HbA1c averaged 9.0 ± 2.6 %, the rate of severe hypoglycemia 13,6 per 100 patient-years. While height in pump-patients was nearly identical to the reference group (SD-score $+0.01 \pm 1.04$), weight and body-mass-index were considerably increased (weight-SD $+0.47 \pm 0.97$; BMI-SD $+0.56 \pm 0.88$).

Conclusion: These multicenter data demonstrate considerably recent interest in pump therapy among pediatric centers and their patients. Compared to all patients in the DPV database, patients on CSII tend to have less severe hypoglycemia, while metabolic control is often insufficient. In addition, weight and BMI should be monitored closely. Prospective intervention trials are needed to define the outcome of pump therapy in pediatric patients.

**ASSOCIATION BETWEEN PON 1-POLYMORPHISMS,
PON-ACTIVITY AND AUTONOMIC NEUROPATHY
IN TYPE 1 DIABETES**

S.E.Hofer*, C.Karschimkus§, A.Jenkins§, A.Chan, B.Holloway, B.Bennetts,
J.M.Fairchild, M.Silink and K.C.Donaghue

*Depts. of Endocrinology and Molecular Genetics, The Children's Hospital at
Westmead, Sydney, §Dept. of Medicine, St Vincent's Hospital, Melbourne*

Background: The paraoxonase enzyme is highly associated with HDL and protects lipoproteins from oxidation. Paraoxonase (PON) activity has been found lower in patients with diabetic neuropathy¹. We hypothesise that the paraoxonase enzyme activity mediates the risk of developing autonomic neuropathy and that polymorphisms in PON1 promoter and coding region of the PON1 gene may influence PON activity.

Aim: To investigate the potential association between PON1 polymorphisms, PON activity and neuropathy in adolescents with type 1 diabetes.

Methods: 160 adolescents were genotyped for the two known polymorphisms in the coding region of PON 1 gene (L54M and Q191R) by PCR amplification, followed by polymorphism-specific restriction digestion (PCR-RFLP), using the enzymes Hsp92 II and Dpn II, respectively. Genotyping of the promoter polymorphisms (-107, -126, -162, -824, -907) is currently underway. Paraoxonase activity was measured in lithium heparin plasma, using both paraoxonase and arylesterase as substrates. Computerised infra red pupillometry was used to assess resting pupil diameter and 2 indices of the phasic light reflex, which we have found to be the most sensitive autonomic nerve function tests².

Results: Both the Leu54Met and Gln191Arg polymorphisms significantly influenced PON activity levels using paraoxon as substrates (Leu/Leu >Met/Leu >Met/Met and Arg/Arg >Gln/Arg >Gln/Gln). Using arylesterase as substrate, only Leu54Met influenced activity levels. No significant association was found between PON activity and any form of pupillary abnormality.

Conclusions: As no association between PON activity and autonomic neuropathy was found, we could not confirm an effect of PON activity or PON1 genotypes on the development of diabetic autonomic neuropathy.

¹ Abbott CA et al. *Arterioscler.Thromb.Vasc.Biol* 1995

² Pena MM et al.*Diabetic Med* 1995

Angled 6 mm needles and a pinch technique dramatically reduce intramuscular injections in children

P. L. Hofman*¹, J. M. Peart¹, J. Holt¹, C. A. Jefferies¹, J. M. D. Thompson¹, W. S. Cutfield¹, S. A. Lawton²

¹*University of Auckland*, ²*Diabetes Devices, Novo Nordisk, Copenhagen*.

Background and Aims: Inadvertent intramuscular (IM) injections of insulin in children with type 1 diabetes mellitus (DM) are common and cause variability in glycaemic control. We have previously demonstrated that frequent IM or fat-fascial interface injections occur when using vertical insertion with 31G 6 mm needles or angled insertion with 30G 8 mm needles (40% and 24% respectively). Our aim was to establish whether an angled 31G 6 mm needle could more reliably deliver insulin into subcutaneous (SC) fat.

Materials and Methods: Healthy children with DM were injected with air using an angled NovoFine® 31G 6 mm needle inserted at 45° via either a pinched (lifted) or unpinched abdominal and thigh injection site. This study comprised a 'high risk' group of children most of whom had previously sustained IM, fat/fascia interface, fat/dermal interface or intradermal injections with vertical 6mm needle injections. Four injections of sterile air (200µl) were injected into each subject; 2 in the abdomen and 2 in the anterior thigh. Immediately after each injection an assessment of the injection depth was made by ultrasonography.

Results: 37 children were enrolled (ages 10.8±2.0 years, BMISDS 0.9±1.1, 65% prepubertal, 40% female, mean HbA1c 7.8±1.2). IM injections were detected in 1 child (3%) while air was visualised at the fat/muscle fascia interface in 14%. IM and muscle fascia injections were more frequent with angled unpinched 6 mm (13%) needles than angled pinched 6 mm needles (5%, Chi squared between groups p<0.05). There was no difference in IM or fat/fascia injections between the thigh and abdomen sites (10% versus 8% in the second). Subjects with IM compared to SC fat injections had a lower BMISDS (0.4±1.3 vs 1.4±1.4, p<0.01) and were predominantly male (100% versus 57%, p<0.01). There were no intradermal injections. Compared to their normal injection there was a clear preference for angled 6 mm needles (p<0.01) and an abdominal injection site (p<0.05) with no preference for pinching. Neither IM nor intradermal injections could be identified by pain perception.

Conclusion: Angled 6 mm needles in both the abdomen and thigh reliably administer insulin into SC fat in children.

NUTRIENT INTAKE IN DIABETIC (T1DM) CHILDREN BEFORE AND AFTER NUTRITIONAL COUNSELLING

L. Iughetti^{1*}, B. Predieri¹, M. Gavioli², S. Milioli¹, S. Bernasconi³
*Pediatrics Department^{1,3} and Dietetic Service²,
 Universities of Modena and Reggio Emilia^{1,2} and Parma³, Italy*

For T1DM patients the diet is an important tool to obtain a good metabolic control and prevent the complications. However, few are the studies evaluating the impact of nutritional education on the effective nutrients intake. To understand the subjects compliance to dietetic indications provided at the beginning of the disease, we studied 22 T1DM normal weight patients (12 F, 10 M; age 9.6±3.3 yr.), without complications and other acute and/or chronic diseases. All patients were submitted to careful evaluation of nutrients intake and measurements of HbA1c, cholesterol, HDL, LDL and triglyceride (TG) levels. On the basis of this evaluation we renewed for each patient the individual dietetic schema. After 3 months we repeated the same evaluations.

Time (mo.)	Calories (Kcal)	Protein (%)	Lipids (%)	Cholesterol (mg)	Fibre (gr.)	Carbohydrates (%)
0	1974.4±407.9	13.7±1.8	38.7±5.73 (Sat. 32.1±4.1)	246.8±92.7	14.1±5.3	47.5±5.7 (26.5±11.0)
3	1950.1±310.8	14.1±1.2	37±3.4 (Sat. 33.9±3.9)	238.4±86.3	15.3±4.5	48.7±3.5 (21.5±6.5)

No differences were founded between laboratory values before and after individual nutritional counselling (HbA1c 8.5±1.2 vs. 8.1±1.3%; cholesterol 160.2±29.6 vs. 188.8±32.6 mg/dl; HDL 54.2±14.6 vs. 65.6±8.7 mg/dl; LDL 90.8±24.4 vs. 109.7±28.6; TG 66.6±24.6 vs. 67.1±34.1 mg/dl).

T1DM patients are poorly compliant to the dietetic indications, as demonstrated by the initial data and the lack of significant improvement of diet composition after individual nutritional counselling.

We need a better training for patients and their family to develop an improved understanding of dietary modifications.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY MONITORING OF CML AND CEL IN CHILDREN WITH TYPE 1 DIABETES MELLITUS

V. Jakuš¹, D. Michalková², R. Petrovič³, J. Chandoga³

¹*Dep. of Med. Chemistry and Biochemistry, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic,* ²*Diabetological Center of SR at 1st Pediatric Clinic-DFNsP, Faculty of Medicine, Bratislava, Slovak Republic*

³*Center of Molecular Genetics, Faculty Hospital, Bratislava, Slovak Republic*

Background: It is now thought that advanced glycation endproducts (AGEs) contribute to accelerated micro- and macrovasculopathy observed in diabetes. N-Carboxymethyllysine (CML) and N-carboxyethyllysine (CEL) are products of glycation and glycoxidation reactions and may be biomarkers for glycoxidative stress in diabetes.

Aim: The aim of this work is the monitoring of urinary AGEs - CML and CEL in children with poorly metabolically compensated Type 1 DM.

Methods: We have evaluated 12 h urine in 15 children with type 1 diabetes melitus (age: 8-18 years) and 15 healthy children (age: 9-17 years) because of the control. We have developed a new modified technique for quantifying of CML and CEL in protein to assess levels of glycation and glycoxidation in physiological systems. In this procedure the biological sample (urine) after deproteination and delipidation was derivatized by ethyl chloroformiate in the presence of ethanol and pyridine. For separation and detection of N-ethoxycarbonyl esters of CML and CEL was used gas chromatography-mass spectrometry assay developed in our laboratory. The quantification was performed with using of isotopically labeled internal standards d₄CML and d₈CEL and analyses were carried by SIM (selective ion monitoring).

Results: The values of CML (2.5 ± 0.4 mmol/mol creatinine) and CEL (4.2 ± 0.7 mmol/mol creatinine) in urine of diabetics were significant higher compared with values of CML (1.2 ± 0.2 mmol/mol creatinine) and CEL (1.9 ± 0.3 mmol/mol creatinine) of control group ($p < 0.01$). The significant correlation was found between CML and CEL in diabetics and also in control group ($r = 0.86$; $r = 0.73$, $p < 0.0001$).

Conclusion: The significant increased urinary levels of CML/CEL in children with Type 1 DM indicates that monitoring of these AGE-biomarkers should be used in evaluation of the progression of late diabetic vascular complications

SMOKING AMONG ADOLESCENTS WITH DIABETES

I. Jansson*, K. Berg-Kelly

The Queen Silvia Children's Hospital, Göteborg, Sweden

Background: Although being a health hazard little is known about smoking habits in adolescents with diabetes.

Aim: Do adolescents with diabetes smoke less than their non-diabetic peers? Where do diabetics acquire knowledge about smoking - from the diabetes team or elsewhere?

Methods: All 13-18 years old diabetics in our clinic were invited to answer a written questionnaire about smoking in 1999 (n=127). Results were compared with those from an ongoing life-style and health survey among adolescents, n=3804, in Göteborg (Q90-98). The study was approved by the Ethics Committee of Göteborg University.

Results: Questionnaires from 123 diabetics could be evaluated, i.e. 97 %. Smoking was equally frequent in diabetics (7,3 %) as in non-diabetics (6,6 %), p=n.s. More girls (11,3 %) than boys (4,3 %) with diabetes smoked, p<0,001. This does not differ from that in non-diabetics, girls 9,2 %, and boys 4,5 %. Only 4,5 % of the diabetics thought that they would be smoking as adults. Information about smoking was recognized by 98 %, and had mostly been obtained from school (93 %) but also from media (58 %), less often from the diabetes team (12 %). However, smokers admitted to have had information about smoking from the diabetes team more often (40 %) than non-smokers (8,3 %), p<0,001.

Conclusion: Adolescents with diabetes smoke as much as non-diabetics. Those who do not smoke admit to having heard about smoking less often than those who smoked. An assumption was that they did not pay attention to information that was not regarded as relevant at that time.

**INCREASING TREND OF INCIDENCE OF DIABETES
MELLITUS TYPE 1 IN CHILDREN AGED 0-14 YRS IN UPPER
SILESIA REGION, POLAND, 1989-2000**

P. Jarosz-Chobot* , E. Otto-Buczowska#, B. Koehler

*Dept. of Pediatric Endocrinology & Diabetes, Silesian University of Medicine,
Katowice, Poland, #Center of Diabetes, Gliwice, Poland*

Background: Upper Silesia is one of the most urbanized and industrialized Polish regions where in last decade there have been aggressive economical and life style changes.

The aim of study was to determine both, value and the dynamics of change in the incidence ratio of diabetes mellitus in Silesian children aged 0-14 yrs in period: 1989-2000.

Material and methods: 834 diabetic children with onset of diabetes mellitus type 1 before the age of 15, years 1989-2000 were taken into account. The incidence ratio was evaluated in relation to the whole Silesian children population, according to the EURODIAB criteria.

Results: A dramatic increase in the incidence ratio of diabetes was observed, tripling from 4.71 in 1989 to 13.51/100 000 in 2000. The youngest group of children (0-4) had the most intensive increase of incidence ratio: from 1.67 (1989) to 6.38 (2000). In particular this increasing trend is visible in last 4 years (1996 - 2000). It has been found that the highest incidence ratio was being observed among children aged 10-14, when they reach puberty (incidence ratio: 7.72 in 1989 and 18.46 in 2000). There was no difference in the incidence ratio between girls and boys.

Conclusions: The incidence ratio of diabetes mellitus type 1 in Silesian children is observed to be constantly and steadily increasing. The dynamic changes of life style that took place on the last decade could influence that fact in the post communistic region.

LINEAR GROWTH AND HEIGHT OUTCOMES IN CHILDREN WITH EARLY ONSET TYPE 1 DIABETES

S.Kanumakala^{1*}, P.Dabadghao¹, S.Vidmar², J.B.Carlin², F.J.Cameron¹
*Royal Children's Hospital¹ and Murdoch Childrens Research Institute²,
 Melbourne, Australia*

Background: Growth in diabetic children has been extensively researched in the last 50 years but controversies and considerable debate still exist. Growth failure has historically been associated with diabetes, especially with early or pre-pubertal onset of diabetes.

Aims: To assess the linear growth and the effect of metabolic control on the near final height of children with early onset type 1 diabetes mellitus.

Methods: Retrospective longitudinal evaluation of 99 children with pre-pubertal onset of type 1 diabetes mellitus before 8 years of age, who were regularly assessed, clinically and metabolically for the next 10 years. Near final height was calculated as the average of last 2 measurements after 15 and 16 years of age in girls and boys respectively. Only 92 children who had complete height records from 8 to 18 years were used for statistical analysis.

Results: The mean age of onset of diabetes was 4.87 (SD 1.94) years. The mean height Z score (SD) for each age and sex were

Age group	BOYS (n=43)		GIRLS (n=49)	
	Mean	(SD)	Mean	(SD)
8 years	-0.17	(0.99)*	-0.29	(1.19)
10 years	-0.25	(0.96)	-0.31	(1.20)
12 years	-0.17	(1.01)	-0.27	(1.27)
14 years	-0.38	(1.14)	-0.27	(1.19)
Near final height	-0.39	(0.99)*	-0.13	(1.07)

The mean near final height Z score in boys was 0.22 SD lower than the mean height Z score at 8 years ($p=0.03$)*. There was no evidence of a decline in height in girls ($p=0.13$). There was no significant correlation between metabolic control and linear growth in either males or females.

Conclusions: Linear growth and near final height in children with early onset type 1 diabetes is comparable to the general population. We found some evidence of a small height reduction in boys. In addition, growth appeared to be independent of metabolic control in diabetes. *(Ack: SK is Novo Nordisk Fellow at RCH)*

TWO CASES OF A RARE DISEASE: ROGER'S SYNDROME

E.Khallouf*, C.Saab, R.Medlej, S.Azar, G. Halaby
Chronic Care Center, Beirut, Lebanon

H.K, 13 years old girl had a normal birth. Birth weight and height were normal. She was breastfed for two months. Parents were first degree cousins. At one year and three months of age she was diagnosed with bilateral deafness. At six years old, insulin dependant diabetes mellitus (IDDM) was diagnosed and she was treated by two insulin injections daily. At six years and a half a thiamine responsive megaloblastic anemia (TRMA) was diagnosed and responded progressively to thiamine and folic acid treatment. No neurological signs or cardiological signs were found. Eye fundoscopy was normal. The association of deafness, IDDM, and TRMA lead us to the diagnosis of Roger's syndrome. In January 2002 she had a cochlear implant. At the last medical visit in Feb 2002, she was still treated by thiamine (100mg daily) and following a twice daily insulin regimen: a mixture of regular and lente (1u/kg/day). A genetic study was done (Cohen N and al) and showed a mutation in the slicing site at the end of exon 4 of the SLC19A2 gene. It is a change from G to A in position 1223+1 of the gene at amino acid 408+1.

C. N, 24 years and 5 months old man was born from a normal pregnancy. Birth weight and height were normal. He was not breastfed. His parents were first degree cousins. One brother had the same disease, one brother was in a good health and a third one died early during childhood. Deafness was noticed at school at the age of 4 years and a half and insulin dependant diabetes started almost at the same time. He was treated with a twice daily insulin regimen (1u/kg/day). Anemia was discovered one year later. Treatment with Thiamine was started. No neurological signs, no thyroid anomaly were found. Eye's fundoscopy was normal. Anomaly of cardiac rhythm was diagnosed lately and treated by beta bloquers and anti arhythmic agents. The association of deafness, IDDM, and TRMA confirmed Roger's syndrome. A genetic study was done: there was a deletion of one base© in position 724 of the SLC19A2 gene. The deletion causes a frame shift at aminoacid 242 and subsequent truncation of the protein at aminoacid 259.

DIABETIC KETOACIDOSIS IN A PEDIATRIC INTENSIVE CARE UNIT – A COMPARATIVE STUDY

C..Limbert*², R.Pina², C.Camacho¹, L.Gonçalves¹, G.Cassiano¹, L.Varandas¹,
C.Vale¹, J.Estrada¹, A.Mota², C. Vasconcelos¹

*Pediatric Intensive Care Unit¹ and Pediatric Endocrinology & Diabetes Unit
Children's Hospital D. Estefânia² Lisbon, Portugal*

Background: Diabetic Ketoacidosis (DKA) is a persisting problem in the diabetic children's quality of care.

Aim: To evaluate the efficacy of DKA treatment protocol used during the past 12 years in our intensive care unit (ICU); to look for changes in the characteristics of DKA population; to identify the main triggers of diabetic decompensations in order to evaluate the quality of diabetes care.

Methods: Retrospectively we analyse the records of DKA patients admitted between 1/1/97 and 31/12/01 (n=52) and compared them with a similar study carried out with the same protocol, between 1/1/91 and 31/12/94 (n=44).

Results: The total rate of admissions was 14.7/ year in the first and 10/ year in second study. There were no significant differences in the duration of ICU stay between the two study groups. No DKA complications occurred in the more recent study; in the first one, six case complications were found; cerebral edema was not reported in neither the studies. No significant differences were found in DKA populations concerning age, gender and severity at presentation.

The rate of admissions per year of newly diagnosed patients with DKA decreased 30%; these patients had a longer duration of symptoms prior to admission. The rate of diabetic decompensation remained similar (6 and 4.4 / year). The cases of insulin omission remained identical but infection as a cause of decompensation decreased.

Conclusion: The complete absence of cerebral edema instances in both studies as well as any other kind of complications in the last 5 years in the 52 patients group, is an indicator of the success of our protocol. The long duration of symptoms prior to diagnosis can reveal the need for a better clinician's awareness for symptoms of diabetes in children.

The decrease of infection, as a triggering factor of decompensation, can be related with a more adequate management of intercurrent disease. Nevertheless, as insulin omission number of cases persisted, we conclude that psychological and social support of our diabetic children and families must be improved.

METABOLIC ACIDOSIS AT ONSET OF DIABETES IS EQUALLY FREQUENT IN ALL PEDIATRIC AGES

B.Lindblad*, L.Blom, R.Hanas, B.Lindberg, U.Samuelsson, and the Swedish pediatric diabetes clinics.

The Queen Silvia Children's Hospital, Göteborg, Sweden

Background: The outcome from episodes of ketoacidosis is worse for young children and varies in different studies.

Aim: Prospective evaluation of metabolic acidosis and HbA1c in Swedish children at diagnosis of diabetes. Monitoring the frequency of metabolic acidosis in different age groups on a population basis.

Methods: Data from years 2000-2001 were obtained from 'The National Pediatric Diabetes Registry, age 0 - 18 years', which is run by the Swedish Pediatric Society. HbA1c and the lowest values of pH, standardized bicarbonate, and base excess were registered on day one of insulin treatment.

Results: The registry is estimated to include >96 % (n=1279) of all new pediatric cases in Sweden during these years. Values for acid base status and for HbA1c were obtained from >94 % of these patients. Metabolic acidosis was found in 15 % (pH <7,30), 3,4 % (pH <7,10), and 1,7 % (pH <7,00) of all cases. pH did not correlate with age either for all children or in a separate analysis for those with pH <7,30 ($R^2=0,096$). Standardized bicarbonate and base excess did not correlate significantly with age. Any degree of metabolic acidosis (standardized bicarbonate <21 mmol/L and/or base excess < -3 mmol/L) was present in 31 %. Standardized bicarbonate <15 mmol/L and/or base excess < -13 mmol/L occurred in 13 %. HbA1c correlated weakly with pH ($r= -0,25$, $p<0,001$). In 94 % of those with pH <7,30 HbA1c was >7,3 % (ref. range 3,6-5,0 %). Mean HbA1c ($9,6\pm 2,4$ %, range 4,6-18,0) at diagnosis was lower in children below 2 years of age (8,1%) and increased gradually up to 10 years (10,5 %). Thereafter no increase in mean HbA1c was observed. The frequency distribution of HbA1c was identical during 2000 and 2001.

Conclusion: The frequency of metabolic acidosis in children at onset of diabetes is low in Sweden and is not more frequent in younger children. Mean HbA1c at diagnosis is lower in children below 10 years but cannot be used to evaluate the degree of acute decompensation.

INSULIN SENSITIVITY AND DIABETES IN CHINESE OBESE CHILDREN

L. Liu*, C. J. Huang, X.Y. Zhao, D. Y. Wu, Y. N. Cai

Department of Endocrinology, Guangzhou Children Hospital, China

Background and Aim: Overweight and obese kids are going up in recent years in China. Type 2 diabetes and insulin insensitivity strongly related with obese children in African-American and Japan were reported. The onset of diabetes is considerable varied in different racial and heredity background. The incidence of type 1 diabetes is known low in China. Our study was to evaluate the tendency of insulin insensitivity and type 2 diabetes in Chinese background obese children and their manifestation.

Method: 151 Chinese obese children and 30 age matched non-obese healthy children were studied. In obese group, 44 female, 107 male, aged 9.8 ± 2.8 years, weighed 52.8 ± 17.3 kg, height 139.3 ± 15.4 cm, BMI 26.5 ± 4.3 kg/m². All children were tested serum glucose, insulin and urine glucose in the morning after fasting for more than 10 hours. 59 of these obese children were performed OGTT test for screening diabetes.

	Fast serum glucose (mmol/l)	Fast serum insulin (uiu/ml)	ISI (HOMA)
Control group (n=30)	4.9 ± 0.3	$5.9 \pm 4.2 (2.4 \sim 19.4)$	0.06 ± 0.02
Obese group (n=151)	5.0 ± 0.9	$19.9 \pm 21.1 (2.1 \sim 171)^*$	$0.02 \pm 0.02^*$

Compare with non-obese children, mean fast serum insulin level was significantly higher ($p=0.0005$), insulin sensitivity index was significantly lower in obese group ($p=0.0003 \times 10^{-7}$). But no difference in mean fast serum glucose level ($p=0.66$). The insulin sensitivity index lower more than 2SD was found in 25 obese children. Fast serum glucose level higher more than 7 mmol/l in twice was found in 3 obese children. 2h serum glucose level higher more than 11.1 mmol/l in OGTT was found in 4 obese children. None of these high serum glucose level obese children was tested urine glucose positive or had typical diabetic symptoms, such as polyuria, polydipsia, lost weight and evidence of ketosis et al. **Conclusion:** Chinese obese children also tend to be insulin insensitivity, insulin sensitivity indexes were abnormal in 16.6%(25/151) of them. Just urine glucose or fast serum glucose test for screen diabetes would miss more than half diabetes.

ALTERNATE SITE BLOOD GLUCOSE TESTING AT THE FOREARM OR HAND: IS IT FEASIBLE AND RELIABLE IN CHILDREN WITH TYPE 1 DIABETES?

K. Lüpke, S. Glinda, E. von Walter, W. von Schütz, T. Danne*

Kinderkrankenhaus Auf der Bult, Hannover

Background: Some children complain that frequent capillary blood glucose testing by pricking their fingertips leads to callous fingertips or sore punctures.

Aim: To investigate the feasibility and reliability of alternate sites for capillary blood glucose testing at the ball of the thumb or the forearm in children with diabetes.

Methods: In 204 pediatric patients (age: 12.8 (3.1 – 18.0), diabetes duration: 4 (0.1-16.1) years), HbA1c (DCA 2000): 7.7 (5.0-14.4) %) a random capillary blood glucose determination from the finger tip was assayed with a standard laboratory method (Hexokinase) and compared to point of care testing (OnetouchUltra (Lifescan) with samples from the fingertip, ball of the thumb or forearm. Following the procedure the patients completed a questionnaire which site gave the most and the least discomfort.

Results: The determinations covered a wide blood glucose range 143 (39 – 474) mg/dl (median (range) of the reference method). Values of all sampling sites displayed an excellent correlation with the reference method: (Pearson-correlation-coefficient: forearm: $r=0,97$; ball of the thumb: $r=0,98$; finger tip $r=0,98$; all $p<0.001$). Interestingly the determination at the forearm showed a significantly lower deviation from the reference method compared to the sample from the ball of the thumb ($12,7 \pm 13,2\%$ vs. $15,8 \pm 12,0\%$; $p<0.001$, Wilcoxon-Test) or the finger tip ($14,2 \pm 11,9\%$; $p<0.001$). The clinical relevance of these differences remained minimal judged by Clarke Error grid analysis, as most values fell within zones A and B (forearm: 98,9%, ball of the thumb: 97,9%, fingertip: 97,5%). While 25% of the patients felt no difference between the sites for blood glucose samplings maximum discomfort was felt in 9% at the fingertip, 23% at the forearm and 43% at the ball of the thumb. No effects of age, gender or diabetes duration were observed.

Conclusions: Alternate site testing under routine conditions with a personalized blood glucose meter at the forearm or the ball of the thumb is feasible and reliable. Individual preferences for the different sites varied considerably.

STRONG RELATIONSHIP BETWEEN THE TIME WATCHING TV AND BLOOD GLUCOSE CONTROL IN CHILDREN AND ADOLESCENTS WITH TYPE-1 DIABETES MELLITUS

H.D.Margeirsdottir*, J.R.Larsen, L.Sandvik, K.Lande, K.Dahl-Jørgensen and the Norwegian Study Group for Childhood Diabetes.

Pediatric Dept.and Center for Medical Statistics, Ulleval University Hospital, Clinical Chemistry Dept. Aker University Hospital, Diabetes Research Centre Aker and Ulleval Hospitals, Oslo, Norway.

Background: Physical activity is in general considered to be an important factor in the treatment of children and adolescents with diabetes. Physical activity is difficult to measure in this age group, but physical inactivity may be reflected by the time used watching television.

Aim: We wanted to study whether there was any relationship between blood glucose control and the time used watching TV.

Methods: As part of a national prospective quality study of childhood diabetes in Norway 398 type-1 diabetes patients were included from eight hospitals in the eastern part of the country. The time used watching TV and the time using PC was recorded separately by interview together with clinical data. Mean age was 13.0 years (range 2-20 years), mean diabetes duration 5.5 years (range 1-17 years), mean HbA1c 8.63 (reference range 4.1-6.4%). HbA1c was measured in the same laboratory for all patients.

Results: 34 patients (9 %) watched TV <1 hour daily. Their mean HbA1c level was 8.2 % (SD 0.9). 81 patients (20%) watched TV between 1 and 2 hours daily; Mean HbA1c 8.4% (SD 1.2), 180 (45%) 2-3 hours daily; mean HbA1c 8.5% (SD 1.4), 49 (12%) 3-4 hours; mean HbA1c 8.7% (SD 1.3), and 49 (12%) ≥ 4 hours daily; mean HbA1c 9.2% (SD 1.6). There was a continuous increase in mean HbA1c by every hour of watching TV and test for trend was highly significant ($p < 0.001$). No correlation between HbA1c and the use of PC was observed. 143 patients (36%) used PC ≥ one hour daily, and only 4% ≥ 4 hours daily.

Conclusion: Extensive TV watching is associated with impaired blood glucose control in diabetic children and adolescents.