Epidemiology – Genetics – Immunology

OP 008

Childhood body mass (BMI) and risk of type 1 diabetes: findings from a longitudinal National Birth Cohort

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Introduction: Case-control studies have suggested that high rates of growth and/or obesity in early childhood are related to greater risk of type 1 diabetes. However, other studies have not confirmed this, and the association has not been tested in longitudinal cohort studies. It is also unclear whether this association is confounded by breastfeeding, which may be associated with reduced risk of both obesity and of type 1 diabetes.

Methodology: Analysis of 1970 British Birth Cohort: longitudinal follow-up of 16 567 babies born in 1970, of whom 12 160 had accurate weight and height measured at 10 years. 11 211 were resurveyed in year 2000 and gave data on history of diabetes (type, age at onset). Logistic regression was used to estimate the risk of type 1 diabetes onset ≥ age10 years conferred by BMI z-score at 10 years, birthweight and breastfeeding history.

Results: 8736 subjects had data on childhood BMI and adult diabetes history. 61 (0.7%) reported having insulin-dependent diabetes mellitus, of whom 14 had onset < age 10 and 47 with onset ≥10 years. Odds ratios (OR) for risk of diabetes onset ≥10 years are shown:

Factor	N	OR adjusted for height at 10 yr and sex (95% CI)	p	OR adjusted for all other factors and height at 10 yr (95% CI)	p
BMI z-score 10 years	8722	1.9 (1.3, 2.6)	<0.001	1.9 (1.3, 3.0)	0.003
Birthweight (kg)	8552	1.1 (0.6, 2.1)	0.8	1.0 (0.4, 2.3)	0.9
Breastfeeding	Never	1		1	
(n = 7751)	<3 month	1.4 (0.6, 3.5)	0.4	1.3 (0.5, 3.2)	0.6
	>3 month	1.3 (0.4, 4.4)	0.7	1.1 (0.3, 4.0)	0.9
High social class 10 years	8843	1.5 (0.8, 3.0)	0.2	2.3 (1.0, 5.4)	0.05
Female sex	9119	–	–	0.8 (0.4, 1.9)	0.7

Conclusion: Higher BMI at 10 years strongly predicted risk of later developing type 1 diabetes independently of birthweight and breastfeeding history. Each additional standard deviation of BMI increased risk approximately two-fold. Reduction in childhood obesity may reduce the incidence of type 1 as well as type 2 diabetes.

OP 009

A randomised trial with calcitriol versus nicotinamide in patients with recent onset type 1 diabetes (*IMDIAB XI*)

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Introduction: Several epidemiological, experimental and in vitro studies have indicated that vitamin D (vit D) may be an important regulatory factor in type 1 diabetes (T1D) including: (a) supplementation with vit D at birth reduced the incidence of T1D later in life; (b) epidemiological data from the EURODIAB study showed that vit D supplementation has protective effects on T1D; (c) recent data from our group identified a reduction of 1,25(OH_2) vitamin D_3 (the active form) in patients with recent onset T1D. We designed a clinical trial aimed at evaluating whether supplementation with the active form of vit D (calcitriol) at the time of diagnosis of T1D can favour clinical remission and improve the integrated parameters of metabolic control (HbA1c, insulin requirement and C-peptide secretion).

Materials and methods: In the IMDIAB XI randomised trial, a total of 76 patients with recent onset (<4 weeks) T1D, mean age 12.8 years ± 7.6 SD were enrolled. Patients were randomised either to calcitriol (0.25 µg/day on separate days) or nicotinamide (NA) 25 mg/kg daily. In both groups of patients, intensive insulin therapy was implemented with three administrations of regular insulin daily + NPH insulin at bedtime. Frequent telephone consultations (at least once per week) were arranged with the enrolled patients.

Results: We report now results 1 year after diagnosis. In calcitriol and NA groups HbA1c and insulin requirement dropped significantly after 3 months of therapy. In both groups mean HbA1c levels were below 7% throughout the first year of the disease. Baseline C-peptide values were not different between the two groups at diagnosis and at 1 year, and did not drop at 1 year compared to diagnosis in both groups. A mixed meal test was performed 1 year after diagnosis to evaluate the stimulated C-peptide secretion. The increase of C-peptide and the area under the curve following the test were significantly higher in the calcitriol vs. the NA treated group ($p < 0.03$). Finally, in the calcitriol group treated, calcium levels remained within normal ranges and no adverse effects were noted.

Conclusions: Data reported here are encouraging as they indicate that calcitriol may be beneficial in increasing residual beta-cell function 1 year after diagnosis without inducing hypercalcemia at the dose used in the present trial.

OP 010

Extracellular nucleic acid clearance and altered immune response in juvenile diabetes – new hypothesis

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Introduction: Type 1 diabetes remains the classical diabetes in childhood, well documented as immune-mediated disease. The goal of secondary prevention is to delay further destruction of the remaining β-cells and therefore to stop entry into the final stages of the disease. It was recognized that nucleic acid degradation products are capable of stimulating dramatic Th1 response presumably through the activation of T helper cells, IL-2 synthesis and its receptor expression, IL-5 production. But up to now, there is no literature data about the level, metabolism or possible impact of nucleic acid degradtion pathway and circulating oligonucleotides on development and/or progression of insulin-dependent diabetes.

Methodology: The objective of this study was to test new hypothetic approach that altered nucleic acid metabolism and decreased plasma

oligonucleotide clearance by exctracellular nucleases/oligonucleases may be a missing link between altered innate immunity (INF-induced RNase activity) and enhanced Th1 immunity in juvenile diabetics. Children (40) with type 1 diabetes (age group of 5–12 years, baseline serum glucose 15.46 ± 5.34 mmol/l and HbA1c 10.06 ± 3.27%.), together with age-mached healthy children (25) were included in study.
Results: Significantl decrease in plasma RNase-degrading activity was documented for different supstrates (RNA: 0.76 ± 0.22 vs control 2.03 ± 0.24 U/l $p < 0.001$ polyU: 52.6 ± 6.22 vs control 88.83 ± 9.45 U/l $p < 0.001$ and poly C: 39.66 ± 4.45 vs control 59.23 ± 5.32 U/l $p < 0.001$). As a result, the accumulation of different-sized oligonucleotide/deoxynucleotide acid soluble fragments was obtained (385.33 ± 16.24 vs control 265.44 ± 12.48 µmol/l $p < 0.001$), confirming at first time the dysregulation in extracellular RNA and polynucleotide degrading pathway.
Conclusion: The autoimmune implications of circulating different-sized oligonucleotides were supported by the observations that strain (bacterial or viral) oligonucleotide sequences can mimic the immunostimulatory activity, by triggering the production of reactive oxygen species, the secretion of proinflammatory cytokines and chemokines and strongly activated Th1-mediated immune responses.

OP 011
Insights into the acute cerebral metabolic changes associated with childhood diabetes

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Introduction: Type 1 diabetes is a prevalent chronic disease in childhood with the commonest single cause of death being cerebral edema in the context of diabetic ketoacidosis (DKA). The nature of the alterations in cerebral metabolism that may result in vulnerability to neuronal injury remain unknown. The aim of this study was to analyse the magnetic resonance imaging (MRI) and spectroscopy (MRS) brain data from 8 children with diabetes following acute presentation with hyperglycaemia with or without ketoacidosis, to determine the nature and timing of any alterations in cerebral structure and metabolism.
Methods: This study used MRI and MRS to investigate regional cerebral abnormalities in a small series of diabetic patients with and without DKA. Changes were compared to the clinical and biochemical features of the patients studied.
Results: Our small series of patients all demonstrated abnormal signal changes in the frontal region on fluid attenuated inversion recovery (FLAIR) MR imaging, suggestive of edema, and spectroscopic abnormalities of increased taurine, myoinositol and glucose levels. The MR abnormalities varied in severity but did not correlate with any clinical or biochemical parameters.
Conclusions: These changes indicate that many of the diabetic children, particularly at presentation, may have alterations in cerebral metabolism with implications for the pathogenesis and treatment of the cerebral complications of DKA. In addition, our findings suggest that increased taurine may be one of the important differentiating factors in the response of the brain of diabetic children to DKA that may reflect an increase in their vulnerability to cerebral edema as compared with diabetic adults.

OP 012
Early childhood predictors of islet autoimmunity and diabetes

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The incidence and predictors of persistent islet autoimmunity (IA = autoantibodies to insulin, GAD_{65} or IA-2) and type 1 diabetes (T1D) in early childhood are largely unknown. Between 1993–2003, among 31,075 general population HLA-screened newborns, 2.3% had the high-risk HLA-DR3/4,DQB1*0201/0302 (abbrev. DR3/4) and 6.2% had moderate-risk genotypes. High-risk general population children (N = 1,284, including 366 with DR3/4) and 973 young relatives of T1D patients (350 siblings and 623 offspring, 15% and 8% with DR3/4, respectively) have been followed for up to 10 yrs. 107 children have developed persistent IA and 32 of those have developed T1D. Survival analysis showed that by age 6 yrs, IA developed in 9% of siblings, 5% of offspring and 2.3% of all general population children and, respectively, in 33%, 21%, and 3.3% of those with DR3/4. Among the relatives, DR3/4 increased the risk of IA 5.4-foold ($p < 0.0001$) and progression to diabetes 3.1-fold ($p = 0.02$), compared to other DR3 or DR4,DRB1*0302 genotypes. Among the general population children, DR3/4 increased 7-fold the risk of progression from IA to T1D. Adjusting for HLA, the risk did not differ between siblings and offspring RR = 1.3 (95%CI 0.6–3.1) or between offspring of diabetic fathers and mothers 1.4 (0.6–3.4) nor by ethnicity, gender, or age of T1D onset in a relative. Fewer maternal infections and lower intake of vitamin D during pregnancy as well as introduction of cereal to infant diet before 4 or after 6 months of age increased the risk, while exposure to cow's milk, entero-, rotavirus infections, routine immunization and day care attendance were unrelated to IA. Newborn screening and follow-up of at risk children indicates higher than previously reported incidence of IA in early childhood. Both infectious and dietary factors appear to be involved.

OP 013
First phase insulin response in i.v. glucose tolerance tests in children with HLA-conferred genetic risk for type 1 diabetes and autoantibodies: a superb predictor of T1d risk and time of onset?

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Introduction: The aim of this study was to analyze the relationship between decreased first phase insulin response (FPIR) values in IVGGTs, appearance of autoantibodies and onset of T1D in children carrying HLA-conferred risk for T1D and participating in Type 1 Diabetes Prediction and Prevention Trial (DIPP). All children included in this study had developed ICA alone or together with IAA, GADA or IA-2A before IVGTTs were performed.
Methodology: 380 IVGTTs were performed to 145 index children followed from birth and 174 IVGTTs to 61 older at-risk siblings. In IVGTT the children received 0.5 g/kg glucose (ad 35 g) as 25% solution i.v. as a steady 3 min ± 15 s infusion. Samples were drawn from the catheter at –10, –4, 1, 3, 5, 7, 10, 30 and 60 min. FPIR was calculated as the sum of serum insulin concentrations at 1 and 3 min. The 95% lower limit calculated from 60 healthy 1- to 5-year-old children was 38 mU/L.
Results: Mean FPIR values increased with increasing age in children who did not develop T1D during follow-up (mean ± SD 3.3± 2.0 years). In all age categories mean FPIR-values were lower in children who developed multiple autoantibodies compared to those who were constantly positive for only one autoantibody. A FPIR value under 38 mU/L

strongly predicted development of T1D. Only 1.4% of the children whose all FPIR values were over 38 mU/L developed T1D whereas 32.8% of the children whose FPIR value had at least once been under 38 mU/L developed T1D during follow-up. Of the 22 children who developed T1D, 20 (91%) had had subnormal FPIR-values before onset of the disease.

Conclusion: Measurement of FPIR values seems to improve accuracy of prediction of risk and time of onset of T1D in children genetically at risk and having developed autoantibodies.

OP 014

Exclusive and partial breastfeeding, introduction of cow's milk-based formulas and development of type 1 diabetes in a prospective birth cohort study (DIPP)

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Introduction: Duration of exclusive and partial breastfeeding and age of introduction of cow's milk-based formula to infant feeding may regulate development of humoral islet autoimmunity and type 1 diabetes (T1D). We analyzed the impact of these factors on T1D development using data prospectively collected in Type 1 Diabetes Prediction and Prevention Project (DIPP), by far the largest ongoing trial aiming at T1D prediction and prevention.

Methodology: HLA-conferred genetic risk for T1D (HLA-DQB1*02/*0302, or DQB1*0302/x, x = other than *02, *0301 or *0602) of >80,000 newborn babies has been screened in DIPP since 1994. Over 7100 children carrying genetic risk are being followed-up for development of islet autoimmunity at 3- to 12-mo intervals; children with autoantibodies are seen every third month. For each case child developing T1D, 3 autoantibody-negative controls matched for age, gender, genetic risk and city of birth were selected. Half of the case children participated in a double-blinded trial testing efficacy of nasal insulin vs. placebo in T1D prevention. The trial group was not taken into account in the analysis. Comparisons were performed using logistic regression analysis.

Results: Thus far, 60 study children have progressed to T1D. Logistic regression analysis showed no difference between the cases and controls in the duration of exclusive or partial breastfeeding or introduction of cow's milk-based formulas.

Conclusion: Duration of exclusive or partial breastfeeding and age of introduction of cow's milk-based products do not influence child's progression to T1D.

Diabetes care

OP 015

Differential effect of age of onset of diabetes on psychological, social and employment outcomes in adult life: findings from a National Birth Cohort

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Introduction: Cross-sectional and longitudinal studies in adults differ on the impact of diabetes on social, educational and employment outcomes. The impact of age of onset of diabetes on adult outcomes has been little studied.

Methodology: Analysis of 1970 British Birth Cohort: longitudinal follow-up of 16 567 babies born in 1970, of whom 11 211 were resurveyed in year 2000. Self-report data included history of diabetes (type, age at onset) and social, occupational and health data. Logistic regression was used to estimate the risk conferred by diabetes onset ≤19 yrs or ≥20 yrs compared with the general cohort population. Analyses were controlled for sex, BMI and child and adult social class.

Results: 84 of 11 211 (0.8%) currently had diabetes. 36 (43%) had onset of diabetes ≤19 years (all "insulin-dependent") and 48 (57%) had onset ≥20 years. 9 (11%) were registered disabled and 2 (2%) had abnormal vision in both eyes. Odds ratios (OR) for risk of each adult outcome are shown below:

Adult outcomes	N	Adjusted OR Diabetes onset ≤19 yr	Diabetes onset >19 yr
Professional/managerial social class	9135	1.1 (0.5, 2.1)	0.7 (0.4, 1.2)
Never married	9332	0.6 (0.3, 1.2)	0.8 (0.5, 1.4)
Education: achieved A levels or higher degree	9333	1.0 (0.4, 2.3)	1.2 (0.6, 2.3)
Current depression (Malaise Inventory score ≥7)	9333	1.6 (0.7, 3.7)	2.0 (1.1, 3.7)*
Eating disorder (current or past)	9333	4.7 (1.3, 17)**	2.0 (0.6, 6.5)
Alcohol problems (high score on CAGE screening tool)	9165	0.7 (0.2, 2.0)	1.5 (0.6, 3.4)
Used illicit drugs in past 12 months	9233	0.8 (0.3, 2.0)	1.2 (0.5, 2.8)
Regular smoker (>10 per day)	9325	1.3 (0.5, 3.0)	2.2 (1.1, 4.5)*
Obesity (BMI ≥ 30 kg/m^2)	9333	1.1 (0.4, 3.1)	3.1 (1.5, 6.2)**

*p < 0.05, **p < 0.01; Reference category: Never had Diabetes.

Mean annual net income was lower in adult but not juvenile onset in males than the general population (p = 0.02). Duration or type of diabetes were not associated with outcomes measured.

Conclusion: Distinct patterns of poorer mental and physical health were identified for child / adolescent onset compared with adult onset diabetes which were independent of duration and type of diabetes.

OP 016

Benefits of insulin detemir over NPH insulin in children and adolescents with type 1 diabetes: lower and more predictable fasting plasma glucose and lower risk of nocturnal hypoglycaemia

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Introduction: This 26-week, open-label, randomised, parallel-group trial compared the efficacy and safety of insulin detemir and NPH insulin in children and adolescents with Type 1 diabetes.

Methodology: Subjects received insulin detemir or NPH insulin once or twice daily plus pre-meal insulin aspart. In total, 347 subjects (detemir: 232, NPH: 115) with HbA_{1c} 8.8% ± 1.2% [mean ± SD], age 11.9 ± 2.8 yrs, BMI 19.2 ± 2.8 kg/m^2 and duration of diabetes 5 (range 1–15) yrs received treatment.

Results: Mean HbA_{1c} decreased by ~0.8% with both treatments. Glycaemic control with insulin detemir was non-inferior to NPH insulin (HbA_{1c}: 8.02% versus 7.93%, mean difference: 0.09%, [95% CI: –0.12; 0.29]). Mean self-measured fasting plasma glucose (FPG) (8.44 versus 9.58 mmol/L, p = 0.022) and within-subject variation in FPG (SD = 3.32 versus 4.29 mmol/L, p < 0.001) were lower with insulin detemir. Risk of nocturnal hypoglycaemia was also lower with insulin detemir (p = 0.011) as was baseline-adjusted BMI (19.3 versus 19.8 kg/m^2,

$p < 0.001$). The general safety profile of insulin detemir was similar to that of NPH insulin.

Conclusion: Basal-bolus therapy with insulin detemir or NPH insulin, plus insulin aspart, for 26 weeks in children and adolescents with Type 1 diabetes was safe and improved HbA_{1c} to a similar degree. The lower and more predictable FPG, lower risk of nocturnal hypoglycaemia and lower BMI observed with insulin detemir are clinically significant advantages compared to NPH insulin.

OP 017

Differences between parent and adolescent perceptions of the psychological impact of diabetes

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Introduction: Recent research shows that adolescents with diabetes do not have increased levels of psychopathology. However, problems with adjustment to diabetes are commonly reported by parents and seen in clinical practice. This may reflect differing perceptions of the impact of diabetes between young people and parents, however little data has addressed this question.

Methodology: As part of a wider study, we collected questionnaire data from 64 adolescents with type 1 diabetes (duration ≥12 months) aged 10–17 years and their parents, drawn from metropolitan specialist paediatric diabetes clinics. Mean age 13.0 years; 58% female. Mean duration of diabetes 5.9 years. Adolescent psychological distress was assessing using the Strengths and Difficulties Questionnaire (SDQ) in young person self-report and parent report versions. These are reliable and valid screening measures with extensive UK normative data. Subscales include Conduct problems, Peer problems, Emotional problems and Hyperactivity, which are summed to produce a Total problems score. The distribution of Normal, Borderline and High scorers in the general UK population is 80%, 10% and 10% respectively in each subscale. Differences between groups were assessed by Chi Square.

Results: Distribution of Parent Total problem scores were similar to normative data; 75% normal, 11% Borderline and 14% High scorers. However, parents reported twice the expected rate of High Emotional problems (20%, $p < 0.001$) and Conduct problems (19%, $p < 0.04$) in their adolescent. In contrast, adolescents themselves reported normal distributions of Total problems, Emotional and Conduct problems, but significantly fewer Peer problems than expected (6%, $p < 0.03$).

Conclusion: Parents and young people differ in their perceptions of psychological problems in adolescents with diabetes. This may explain differences between research findings and clinical reports of the impact of diabetes in young people. Understanding these differing perceptions may have important therapeutic implications in working with families to improve poor diabetic control in adolescence.

OP 018

Continuous glucose monitoring correlates poor glycaemic control with an increased rate of externalizing behaviours

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Introduction: Anecdotally parents report that their children's behaviour fluctuates with glycaemic control. The aim of this study was to ascertain whether glycaemic indices calculated from repeated continuous glucose monitoring were associated with behavioral outcomes calculated from a parent report questionnaire

Methods: Forty-two children (15 male, 27 female) attending the Royal Children's Hospital, Melbourne undertook continuous glucose monitoring on two occasions six months apart. On each occasion the parent completed a Behavioral Assessment System for Children (BASC) questionnaire which assessed the child's behaviour over the previous six months. Glycaemic outcomes calculated from continuous glucose monitoring included mean blood glucose (MBG), mean of daily differences (MODD), percentage time spent in hypoglycaemia (CGMS < 4.0 mmol/L), normoglycaemia (CGMS 4.0–12.0 mmol/L) and hyperglycaemia (>12.0 mmol/L) and CONGA (continuous overall net glycaemic action). The BASC was summarized with t scores standardized for gender and age. Pearson's calculation correlated glycaemic and behavioral variables on each occasion. Identification of relationships between glycaemia and behaviour over time were analyzed individually (linear regression) and for the group (t-test).

Results: Mean blood glucose and percentage time in hyperglycaemia both positively correlated with externalizing behaviour over time, $p = 0.01$ (95% C.I. 0.41–3.45) and $p = 0.02$ (95% C.I. 0.03 to 0.39). Both parameters negatively correlated with adaptive ability, $p = 0.003$ (C.I. –0.37 to –0.77) and $p = 0.003$ (95% C.I. –0.43 to –0.09). An improvement in percentage time in normoglycaemia over the six month period was reflected in an improvement in adaptive scores, $p = 0.005$ (95% C.I. 0.09 to 0.47). Percentage time in hypoglycaemia and glycaemic variation did not significantly change over the six month period and were not found to be associated with behavioral indices.

Conclusion: Poor glycaemic control evidenced by high mean blood glucose and a high proportion of time in hyperglycaemia on a CGMS trace is predictive of a high level of externalizing behaviour and poor adaptive skills.

OP 019

The pharmacokinetic profile of the new rapid acting analogue insulin glulisine (GLU) in pediatric patients with type 1 diabetes

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Introduction: The pharmacokinetic (PK) and pharmacodynamic properties of GLU compared with regular human insulin (RHI) in pediatric patients with type 1 diabetes were investigated.

Methodology: Ten children aged 7–11 y (mean 10 y), and 10 adolescents aged 12–16 y (mean 14 y), were enrolled in a single-dose, double-blind, randomized, crossover study. Blood glucose (BG) levels of fasted patients were maintained at (5.6–8.9 mmol/L) with variable iv insulin infusions. GLU or RHI was injected sc (0.15 IU/kg) 20 min after cessation of insulin infusion; 2 min later a weight adjusted standardized liquid meal was served.

Results: Maximum serum concentration (INS-C_{max}) and area under the initial insulin concentration–time curve (INS-AUC_{0-2h}) were higher, mean residence time (MRT) was shorter, and baseline corrected blood glucose excursions (BG-AUC_{0-6h}) were lower for GLU than for RHI (Table).

	Geometric mean				Overall point estimate for the ratio GLU to RHI (95% CI)
	GLU		RHI		
	children	adolesc.	children	adolesc.	
Pharmacokinetic results					
INS-C_{max} (μU/mL)	55	61	25	44	171 (127; 229)
INS-t_{max} (min)†	54†	52†	59†	76†	–8 (–24; 7)
MRT (min)	87	90	132	144	64 (59; 70)
INS-AUC_{0-2h} (μU.min/mL)	4948	5534	2383	3860	169 (127; 224)
INS-AUC_{0-6h} (μU.min/mL)	7934	8811	5581	9145	116 (90; 150)
Pharmacodynamic results					
BG-AUC_{0-6h} (mg.h/dL)‡	492	790	729	872	80 (67; 95)

*$p < 0.05$ (ANOVA); †median; ‡arithmetic mean

Children and adolescents showed an almost equal PK profile for GLU with a trend towards higher exposure in adolescents. In contrast, the comparison between age classes for RHI revealed ~60% higher exposure in adolescents. GLU was well tolerated, and incurred less postprandial glucose excursion compared with RHI.
Conclusions: The PK properties of GLU in pediatrics are comparable to those of adults, classifying GLU as a rapid-acting insulin analogue also in pediatric patients. The age-effects for RHI require further investigation.

OP 020
Kinetics of Glargine interaction with a soluble IGF1R and lack of evidence for IGF1R interactions in vivo

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Introduction: Insulin analogs may have increased mitogenic effects due to slow dissociation from the insulin receptor. This has not been demonstrated for Insulin Glargine. Despite of increased IGF1 receptor (IGF1R) affinity, Glargine stimulates thymidine incorporation equipotent with insulin in human muscle cell cultures. Whether this may be related to the kinetics of their interactions with the IGF1R has not previously been investigated. Furthermore, the significance of Glargine interaction with the IGF1R in humans in vivo has not been fully explored.
Methodology: We have examined the kinetics of Glargine interactions with a soluble IGF1R by surface plasmon resonance using Biacore. We have also examined the changes in serum IGF-I concentrations during 12 weeks of Glargine treatment in adolescent T1DM with the assumption that significant Glargine effects via the IGF1R should cause a primary feedback inhibition of GH and suppression of IGF-I.
Results: Glargine had approximately 8-fold higher affinity for the soluble IGF1R than insulin but a 10-fold lower affinity than IGF-I. Glargine had 14-fold faster association and almost 2-fold faster dissociation than insulin. In the clinical study, mean IGF-I increased almost 50% already after 2 weeks of treatment. The mean IGF-I level was markedly subnormal (−1.8 ± 0.4 SDS) before Glargine, increased to −0.7 ± 0.3 SDS after 4 weeks and remained so throughout the study.
Conclusion: Glargine has higher IGF1R affinity, with markedly faster association and faster dissociation than insulin. Such kinetics may explain that Glargine appears to predominantly induce metabolic signals since slow ligand dissociation from the IR is associated with mitogenic actions. Furthermore, in adolescents with T1DM Glargine normalizes IGF-I concentrations but do not fully restore normal levels. This argues against significant actions of Glargine via the IGF1R in vivo at least at the pituitary/hypothalamic level.

OP 021
Glycemic control unaffected by mixing glargine with short acting insulin analogues

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Insulin Glargine is a "long acting and peakless insulin" analogue. The major drawback to its use is that it has to be given as a separate injection, and cannot be mixed with other insulins. This results in: 1). Additional insulin injections; 2). Complicates treatment plan; 3). Decreases compliance. We hypothesized that mixing insulin Glargine with short acting insulin would not adversely affect glycemic control.
Method: 13 adolescents with type 1 diabetes (7M/6F, 13.5 ± 1.6 y, 22.4 ± 1 kg/m^2, A1C of 7.7 ± 0.7% and duration of diabetes of 22.4 ± 1 mo) were studied on 3 occasions. All subjects were on Glargine (as a separate injection at bedtime) and short acting insulin (Aspart or Lispro) with meals. Using the Minimed CGMS®, subjects were studied at baseline on their current insulin regimen (study B), and then 7 days following splitting the dose of Glargine into two halves and administering one half before breakfast and one half before dinner, either by mixing Glargine and short acting insulin in one syringe (study M), or by administering them as separate injections (study S). Studies M and S were randomized. 24 h glucose excursions were analyzed using repeated measure ANOVA.
Results: The 24 h average glucose concentrations (Mean ± SE) were similar in the three studies (167 ± 4 mg/dl in studies B and S, and 150 ± 6 mg/dl in study M) ($P < 0.6$). There were no reported side effects.
Conclusion: Mixing insulin Glargine with short acting insulin (Aspart or Lispro) in children with type 1 diabetes does not negatively impact the glycemic control. Mixing Glargine with short acting insulin may provide predictable insulin action, improve compliance and increase the use of Glargine insulin in the pediatric population.

Diabetic complications

OP 022
Effects of irbesartan on intracellular antioxidant enzyme activity in adolescents with early diabetic nephropathy

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Introduction: Defective intracellular antioxidant enzyme production (IAP) has been demonstrated in adults with diabetic nephropathy. Angiotensin II receptor antagonists have renoprotective effects in early nephropathy in both type 1 and type 2 diabetes.
Methodology: To evaluate the effects on IAP of irbesartan in young patients with type 1 diabetes and early signs of nephropathy, 11 adolescents (aged 12–18 years, diabetes duration 11–16) were studied. All had persistent microalbuminuria, defined as an albumin excretion rate > 20 µg/min in 2 out of 3 overnight urinary collections in 6 months. Skin fibroblasts were obtained by forearm skin biopsies and cultured in Dulbecco's modified Eagle's medium. CuZnSuperoxide-dismutase (SOD), MnSOD, catalase (CAT), and glutathione-peroxidase (GPX) activity and mRNA expression were measured before and after 6 months of irbesartan (150 mg/day); in both occasions IAP was evaluated at different *ex-vivo* glucose concentrations (5 and 22 mmol/L). Twelve adolescents (aged 12–20 years) without angiopathy and 10 healthy volunteers (aged 13–22 years) participated as controls.
Results: In normal *ex-vivo* glucose concentration, CuZnSOD, MnSOD, CAT, and GPX activity and mRNA expression were not different among the three groups. In high glucose, CuZnSOD activity and mRNA increased similarly in all groups (in angiopathics: 0.94 ± 0.31 U/mg protein; 10.1 ± 3.1 mRNA/GAPDH). CAT and GPX activity and mRNA did not increase in high glucose only in adolescents with angiopathy (0.36 ± 0.10; 4.3 ± 0.2 and 0.55 ± 0.16; 2.6 ± 0.8, respec-

tively). MnSOD did not change in any group. Irbesartan in microalbuminuric adolescents normalized all the enzymatic activity and mRNA in both normal and hyperglycemic conditions.

Conclusion: Adolescents with early diabetic nephropathy have defective IAP and activity; 6-month treatment with irbesartan is effective in normalizing skin fibroblasts antioxidant activity.

OP 023

Analysis of MTHFR 677c → T and 1298A → C polymorphism as risk factors for the microvascular complications in adolescents with type 1 diabetes

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Introduction: The thermolabile variant (677C → T) in the methylenetetrahydrofolate reductase (MTHFR) gene with reduced enzyme activity & subsequent hyperhomocysteinemia[1] has been reported to be associated with diabetic nephropathy & diabetic retinopathy in type 2 diabetic patients.[2,3] A second polymorphism in MTHRF, 1298A → C has been reported to be associated with lower incidence of diabetic nephropathy in the homozygous state in a small number of patients with type 2 diabetes.[4] These polymorphisms are in strong linkage disequilibrium & we therefore investigated the role of both MTHRF polymorphisms in the risk of retinopathy & nephropathy in adolescents with type 1 diabetes.

Methodology: We compared genotype distribution & allele frequency for the 2 MTHFR polymorphisms in 205 adolescents without retinopathy with 270 with retinopathy & 390 adolescents without microalbuminuria were compared with 36 with microalbuminuria (median age 16.2 [14.2–18.0] years and duration 7.9 [5.8–11.4] years). We also compared proportions of subjects who were compound heterozygotes for both polymorphisms between the same groups. Retinopathy was diagnosed by 7-field stereoscopic fundal photography. Albumin excretion rate (AER) was estimated from 3 consecutive timed overnight urine collections using a polyclonal radioimmunoassay (Pharmacia AB, Uppsala, Sweden). Microalbuminuria was defined as 2 of the 3 urine collections with AER > 20 μg/min. The MTHFR polymorphisms were detected by gene specific PCR followed by allele specific oligonucleotide &/or restriction fragment length polymorphism analysis. Chi-square test was used to assess association between MTHFR genotypes and complications.

Result:

	No Retinopathy	Retinopathy	P-value
MTHFR 677			
TT	33 (16%)	52 (19%)	
TC	92 (45%)	114 (43%)	0.65
CC	78 (38%)	101 (38%)	
Total	203	267	
MTHFR 1298			
AA	64 (45%)	84 (44%)	
AC	62 (44%)	85 (44%)	0.91
CC	15 (11%)	23 (12%)	
Total	141	192	
677TC & 1298AC	34/173 (20%)	41/244 (17%)	0.46
	AER < 20 μg/min	Microalbuminuria	P-value
MTHFR 677			
TT	67 (17%)	5 (14%)	
TC	167 (43%)	18 (50%)	0.72
CC	151 (39%)	13 (36%)	
Total	385	36	
MTHFR 1298			
AA	120 (44%)	9 (35%)	
AC	120 (44%)	15 (58%)	0.37
CC	35 (13%)	2 (8%)	
Total	275	26	
677TC & 1298AC	61/341 (18%)	8/33 (24%)	0.37

There was no difference in either allele or genotype frequency for either polymorphism when comparing subjects with or without complications. No difference was observed in frequency of compound heterozygotes between the same groups.

OP 024

Are microvascular complications declining in adolescents with type 1 diabetes mellitus?

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Introduction: Since the results of DCCT showed that intensive therapy delays the onset & slows the progression of complications in adolescents, diabetes management goals have changed (1994). The objective of our study was to compare the frequency of microvascular complications between the study period of 1990–94 & 1995–2002.

Methodology: We screened for complications in 1083 adolescents with type1 diabetes mellitus with duration over 5 years. Retinopathy was assessed by 7-field stereoscopic fundal photography using Topcon Fundus camera & graded according to modified Airlie House classification. Albumin excretion rate (AER) was estimated from 3 consecutive timed overnight urine collections (Pharmacia Albumin RIA till March 2000 & then the IMMAGE analyzer). Microalbuminuria was defined as 2 of the 3 AER > 20 μg/min. Peripheral nerve function was tested by thermal & vibration thresholds. Cardiovascular reflexes & pupillometry were used for measuring autonomic nerve function. HbA1c was measured by the colorimetric method till February 1994, then by Diamat HPLC. Comparison was made between study period of 1990–94 & 1995–2002. Subjects were grouped into 12–15 years of age & 15–18 years of age. Chi-square test and Wilcoxon rank-sum test were used to compare categorical and continuous variables respectively between the two groups.

Results:

Age 12–15 yrs	90–94 group (N = 173)	95–02 group (N = 319)	p-value
Retinopathy	72/164 (44%)	63/306 (21%)	<0.0001
Retinopathy 31/21	11/163 (7%)	5/280 (2%)	0.0069
AER ≥ 7.5 μg/min	38/138 (28%)	60/284 (21%)	0.14
Microalbuminuria	3/138 (2%)	5/284 (2%)	0.72
Any autonomic nerve abnormality	31/171 (18%)	39/223 (17%)	0.87
Any peripheral nerve abnormality	16/173 (9%)	75/294 (26%)	<0.0001
Any pupillary abnormality	24/104 (23%)	75/115 (65%)	<0.0001
Median HbA1c (%)	8.6 [8.0–9.3]	8.6 [8.0–9.3]	0.96
BMI SDS	0.31 [–0.11 to 0.77]	0.64 [0.07–1.16]	<0.0001
Age 15–18 yrs	90–94 group (N = 196)	95–02 (N = 395)	p-value
Retinopathy	96/187 (51%)	110/373 (29%)	<0.0001
Retinopathy 31/21	31/186 (17%)	14/348 (4%)	<0.0001
AER ≥ 7.5 μg/min	59/113 (52%)	106/333 (32%)	0.0001
Microalbuminuria	17/113 (15%)	20/333 (6%)	0.0026
Any autonomic nerve abnormality	36/193 (19%)	51/298 (17%)	0.66
Any peripheral nerve abnormality	26/194 (13%)	90/372 (24%)	0.0025
Any pupillary abnormality	32/102 (31%)	89/155 (57%)	<0.0001
Median HbA1c (%)	8.5 [7.8–9.3]	8.7 [8.0–9.4]	0.16
BMI SDS	0.60 [0.15–1.02]	0.78 [0.26–1.22]	0.0067

No significant change was found in HbA1c value between former & later study period. However insulin dose/kg, number taking more than twice daily injections & Body mass index has increased. There was a significant fall in the rate of retinopathy in both age groups and microalbuminuria in the older age group, but an increase in pupillary & peripheral nerve function abnormalities.

Conclusion: Except for nerve function abnormality, there appears to be a declining rate of micro vascular complication over time in this nonpopulation-based cohort. Surprisingly glycaemic control was

not significantly different in the 2 groups. Subtle change in detection methods & more intensified management may account for the difference.

OP 025

The association of prorenin with the progress to micro-angiopathy and/or macro-angiopathy in type 1 diabetes

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Introduction: Prorenin in circulation has been known as one of the indictors of diabetic nephropathy. Recently in transgenic mice, prorenin is taken up by tissues where it can contribute to the local synthesis of angiotensin peptides. Therefore, we tried to clarify the association of prorenin with *arteriosclerosis, which includes* both mico-angipathy and macro-angiopathy, in type 1 diabetes patients by clinical aspect.
Methodology: Prorenin was measured (by the novel antibody-activating direct enzyme kinetic assay of human prorenin) in 102 child-onset type 1 diabetes patients (age of onset; 7.1 ± 0.13 y.o. age; 15.8 ± 5.23 y.) and 30 non-diabetic patients. At the same time, HbA1c, Total-cholesterol (TC) HDL-C, LDL-C, and high sensitive CRP were measured. The association between prorenin and the following clinical data, were investigated; gender, age, duration, stature, weight, diabetic nephropathy, diabetic retinopathy and macro-angiopathy (any abnormality of blood pressure, ratio of blood pressure upper limbs to lower limbs, or ultrasonography of carotid artery).
Results: Prorenin was significantly higher in type 1 diabetes patients (119.6 ± 76.1 pg/ml) than non-diabetic patients (62.2 ± 26.4 pg/ml, $p < 0.001$), and was also significantly higher in male (F; 101.2 ± 53.1 pg/ml M; 149.2 ± 96.5 pg/ml, $p = 0.0016$). Furthermore, prorenin was significantly higher in the patients with any complication than those without complication ($p < 0.0001$). A significant positive correlation was seen between the prorenin and age ($p = 0.0034$), duration ($p = 0.004$), HbA1c ($p = 0.04$), high sensitive CRP ($p = 0.011$). Prorenin shows no relation to TC or LDL-C, but negative relation to HDL-C ($p = 0.015$) and positive relation to arteriosclerotic index (AI = (TC-HDL-C)/HDL-C, $p = 0.046$). By step-wise regression analysis, any micro-angiopathy (diabetic nephropathy and diabetic retinopathy), any macro-angiopathy and high sensitive CRP were selected as independent variables ($r^2 = 70\%$).
Conclusion: We have demonstrated that prorenin may become an important marker of not only micro-angiopathy but also macro-angiopathy, while the patho-physiologic role of prorenin remains to be clarified.

OP 026

The presence of limited joint mobility in adolescents and young adults with type 1 diabetes is predictive of the development of microalbuminuria: the oxford regional prospective study

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Introduction: To determine risk factors for the development of microalbuminuria (MA^+) in relation to detection of limited joint mobility (LJM^+) of the interphalangeal joints in a longitudinal cohort of young type 1 diabetic (T1DM) subjects.
Methodology: 479 T1DM subjects diagnosed <16 years were followed from diagnosis to receive annual assessments consisting of: clinical examination, measurement of $HbA1_c$ and insulin-like-growth-factor-I (IGF-I) and 3 urine samples for albumin: creatinine ratio (ACR).
Results: After a median follow-up of 10.9 years, 162 subjects (35.1%) developed LJM at median age 13.0 years and duration of diabetes 5.2 years. More subjects developed LJM after compared to before onset of puberty (67.6 v 32.4%). In LJM^+ compared to LJM^- subjects; HbA1c (10.1 v 9.6%, $p < 0.001$) and ACR levels (1.1 v 0.9 mg/mmoL, $p = 0.004$) were higher whilst height SDS (0.0 v 0.2, $p = 0.001$) and IGF-I levels (182.6 v 199.8 ng/mL, $p = 0.04$) were lower. ACR levels were higher after detection of LJM compared to before (1.2 v 0.8 mg/mmoL, $p = 0.003$). In a Cox model probability of developing LJM was related to puberty and $HbA1_c$. In MA^+ subjects ($n = 95$) compared to normoalbuminuric subjects; HbA1c levels were higher (10.7 v 9.6%, $p < 0.001$), prevalence of females greater (58.1 v 42.2%, $p = 0.006$) and height SDS and IGF-I levels were lower. The probability of developing MA was related to pubertal onset (a 6.6 fold increased risk with pubertal onset), higher HbA1c (a 20% increased risk for a 1% rise in HbA1c), female sex (a 2 fold increased risk for females) and presence of LJM (a 1.9 fold increased risk if LJM was present).
Conclusion: The presence of LJM is associated with an increased risk of microalbuminuria, independent of glycaemic control. Associations with height SDS and IGF-I levels may relate to underlying pathogenic mechanisms predisposing to this risk.

OP 027

The influence of long-term glycemic control on the fibrinolytic system in type-1 diabetic patients

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Disturbances in the haemostatic system are well-known risk factors for atherothrombosis. Diabetic individuals are known to be prone to atherothrombosis and coronary heart disease (CDH). In the present study we have evaluated the relation between long-term glycemic control in type 1 diabetes and some fibrinolytic and inflammatory variables. The population consists of 38 type-1 diabetes patients (35–56 years, 40% women) who participated in the Oslo study from 1982. Mean age at diagnosis of diabetes was 12 years. They were all regularly followed during 18 years with HbA1c measurements. Mean HbA1c levels during 18 years were 8.2% and correlated significantly with PAI-1 activity ($r = 0.497$, $p = 0.002$) and tPAag ($r = 0.450$, $p = 0.005$) and inversely with tPA activity ($r = -0.497$, $p = 0.004$) and serum D-dimer (global test of fibrinolysis) ($r = -0.371$, $p = 0.022$), all remaining statistically significant after adjustments for BMI and serum lipids which also correlated significantly with HbA1c (BMI $p = 0.002$; triglycerides $p < 0.0001$; HDL-C (inversely) $p = 0.004$). HbA1c was also significantly correlated with TNFα ($r = 0.430$, $p = 0.007$), however not after adjustments for the highly intercorrelated variables serum lipids and BMI. No correlation with high sensitivity CRP was found. When analyzing the variables according to the tertiles of the HbA1c, the group with the highest tertile (≥8.4 %) had, compared with the two lower, significantly higher levels of PAI-1 activity (10.0 vs 6.3 U/mL), tPAag (6.7 vs 5.3 ng/mL) and TNFα (2.45 vs 1.79 pg/mL) and significantly lower levels of tPA activity (0.82 vs 1.60 IU/mL) and serum D-dimer (0.56 vs 1.53 µg/mL). Conclusion: Long-term glycemic control (assessed as mean HbA1c over time) was highly associated with atherothrombotic markers, especially reduced fibrinolysis.

OP 028

Use of the bedside neuropathy disability score compared to vibration and thermal perception thresholds for the detection of diabetic neuropathy in youth with type 1 DM

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Objective: To validate the bedside neuropathy disability score (NDS) against vibration and thermal perception thresholds in youth with type 1 DM.
Methods: One hundred sixty-six patients with type 1 diabetes, of median age 21 years and median disease duration 10 years, were evaluated for diabetic neuropathy (DPN) by the NDS and by vibration and thermal perception thresholds (using the VSA-3000 and TSA-2001, respectively, Medoc, Israel). Sensory testing was also done in 43 healthy matched controls. DPN grade by both methods was correlated with glycemic control and other disease-related variables.
Results: DPN was detected by the NDS in 23 patients (14%). The diabetic group had significantly higher mean scores for vibration ($p < 0.05$) and warm sensation ($p < 0.001$) than controls, and lower scores for cold sensation ($p < 0.001$); however, there was a great degree of overlap. There was significant correlation between the NDS and mean thresholds for vibration ($p < 0.001$), warm sensation ($p < 0.05$) and cold sensation ($p < 0.05$). The NDS was highly correlated with age at testing, diabetes duration, and long-term and current HbA1c levels, and significantly associated with the presence of microalbuminuria and diabetic retinopathy ($p < 0.001$ for all). Significant correlations were found for vibration ($p < 0.001$) and warm sensation ($p < 0.05$) with age at testing and disease duration, but not for cold sensation. None of the quantitative tests was significantly correlated with long-term or current glycemic control. Analysis of other microvascular complications yielded a weak correlation between vibration threshold and retinopathy ($p = 0.05$) and a nonsignificant correlation between thermal thresholds and retinopathy and all thresholds and microalbuminuria.
Conclusions: The significant correlation of the NDS with vibration and thermal perception thresholds in youth with type 1 diabetes combined with its stronger association with glycemic control and other microvascular complications compared to perception thresholds testing indicates that the NDS may serve as the preferred method for detecting DPN in this population.

Diabetic complications – Obesity

OP 029
The UK prospective study of cerebral oedema complicating DKA

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Introduction: A prospective case control study to determine risk factors for the development of cerebral oedema complicating diabetic ketoacidosis.
Methodology: 62 suspected cases of cerebral oedema were notified through the British Paediatric Surveillance Unit over a three year period. 43 cases were confirmed after scrutiny of notes, radiological and post mortem records. Control episodes of DKA were identified through parallel monthly reports from 243 consultants in 231 UK hospitals. Cases and controls (1 : 3 ratio) were matched for sex, age, and new and old cases. Biochemical and treatment data were extracted from clinical notes. Risk factors were identified initially in univariate and then in multivariate models using STATA.
Results: There were no significant differences in age, gender or new/old between cases (n 43) and controls (n 169), but these variables were allowed for in all subsequent analyses. The risk for cerebral oedema was strongly related to pH or HCO_3 levels at presentation (p for trend across quartiles of pH or HCO_3, 0.001). A high K^+ (OR 5.0, p 0.009), and sodium (OR 0.36, p 0.09) at presentation conferred risk and these were taken forwards in subsequent models. Higher volume of fluid administered in the first hour (OR 4.76, p 0.06), 0–2 hours (OR 6.79, $p < 0.02$), 0–3 hours (OR 9.49, p 0.005), 0–4 hours (OR 7.3, $p < 0.02$) also independently contributed to the risk of cerebral oedema. Preliminary analysis also identified insulin dose in the first two hours (p trend 0.045) and the change from normal saline to more dilute fluids in the second and third hours (OR 5.6, p 0.13, and OR 7.9, p 0.03 respectively) as additional risk factors.
Conclusion: This study highlights that volume and type of fluid administered may be important determinants for risk of cerebral oedema in the severely acidotic child with DKA.

OP 030
TNFA and PPARG2 polymorphisms and body weight in children with type 1 diabetes

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Introduction: Metabolic disorders at the onset of type 1 diabetes and subsequent insulin therapy may affect adipose tissue metabolism including body mass index in children. Thus, this study aimed to evaluate variability of body mass index (BMI) during the first years of diabetes duration and to determine an association between BMI and polymorphisms in genes encoding for TNFα and PPARγ.
Methodology: The study group comprised of 201 children with type 1 diabetes and minimum three years of the disease duration (M/F: 123/78, age at onset 9. ± 4.0 years). BMI, which was standardized for age and gender (Zscore), was measured at the onset and at follow-ups: 6, 12, 24 and 36 months of disease duration. Functional polymorphisms: –308 G/A of *TNFA* and Pro12Ala of *PPARG2* were chosen for genetic analysis.
Results: We found linear increase in Zscore for BMI during subsequent follow-ups from –0.04 ± 0.72 in 6th month to 0.36 ± 0.79 ($r = 0.83$, $p = 0.01$). However, average values do not excided 0.5 of standard deviation for healthy individuals. Genetic analysis revealed that the polymorphism in *TNFA* gene was associated with constitutive differences in BMI (6^{th} month: 0.18 ± 0.60 *vs.* –0.19 ± 0.81 ($p = 0.03$); 12^{th} month: 0.31 ± 0.81 *vs.* 0.00 ± 1.06 ($p = 0.04$); 24^{th} month: 0.35 ± 0.79 *vs.* –0.07 ± 0.58 ($p = 0.03$); 36^{th} month: 0.50 ± 0.85 *vs.* 0.03 ± 0.65 ($p = 0.04$) for GG and A+ (GA + AA) carriers, respectively. Further analysis showed that the Ala allele of *PPARG2* polymorphism was associated with increase in BMI in subsequent follow-ups which became significant in 24^{th} and 36^{th} months of the disease duration (–0.01 ± 0.65 *vs.* 0.61 ± 0.77; $p = 0.0007$ and 0.12 ± 0.64 *vs.* 0.80 ± 0.88; $p = 0.001$; respectively). Moreover, an interaction between studied polymorphisms was found in these two time points in ANOVA analysis. In the 24^{th} Zscores for BMI were as follows: 0.70 ± 0.87; 0.48 ± 0.43; 0.06 ± 0.67; –0.20 ± 0.53 and in the 36^{th} month of diabetes duration 1.0 ±

0.80; 0.44 ± 0.32; 0.11 ± 0.58; –0.015 ± 0.43 for GG/Ala$^+$, A$^+$/Ala$^+$, GG/ProPro and A$^+$/ProPro, respectively.
Conclusions: Our results suggest that increase in body mass in children with type 1 diabetes may be genetically determined.
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OP 031

Chronic hyperglycemia and TNF-α secretion by lymphocytes isolated from type 1 diabetic children

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Introduction: Chronic hyperglycemia preceding the onset of clinically apparent type 1 diabetes may exert glucotoxic and immunosuppressive effects. An influence of hyperglycemia on secretion of TNF-α, a cytokine involved in the pancreatic β-cells destruction, is suggested. The aim of the study was to evaluate the secretion of TNF-α by lymphocytes isolated from children at the moment of type 1 diabetes diagnosis and at the time when near-normoglycemia was achieved.
Methodology: 10 type 1 diabetic children (aged 11.4 ± 3.3 years), in whom humoral markers of an autoimmune β-cell destruction (ICA and anti-GAD) were detected, constituted the study population. Blood samples were collected at the moment of diabetes diagnosis ('0') and after 6 months of the disease ('6'), when the HbA_{1c} level was below 7.5%. Glycemic levels at the diagnosis of type 1 diabetes were from 373 to 1250 mg/dl, base reserve (BE) was from –25.5 to 1.1 and HbA_{1c} levels: from 8.1 to 16.4%. Lymphocytes had been isolated from the peripheral blood, then cultured and specifically, with glutamic acid decarboxylase (GAD) antigen, and non specifically, with PMA/IONO, stimulated. The control culture (NS) did not contain any activator. TNF-α secretion was measured by '*intracellular staining*' with TNF-α-FITC/CD69-PE/CD4-PerCp-Cyt5,5 antibodies. Cell analysis consisted of the estimation of the percentage of activated and TNF-α secreting cells.
Results: The percentage of activated and TNF-α secreting cells in GAD stimulated cultures was similar to non stimulated cultures (respectively: '0' Me = 12.3% vs 6.8% of activated cells and Me = 0.5% vs 0.48% of TNF-α secreting cells; '6' Me = 12.6% vs 12.3% and Me = 0.5% vs 0.6%). The percentage of activated and TNF-α secreting cells after PMA/IONO stimulation increased compared to GAD containing cultures and controls. Higher percentage of activated cells was observed in the 6th month compared to the moment of diabetes diagnosis in non stimulated (Me = 12.3% for '6' vs Me = 6.8% for '0'; $p < 0.03$) as well as in PMA/IONO stimulated cultures (Me = 63.6% for '6' vs Me = 43.9% for '0'; $p < 0.04$). The percentage of TNF-α secreting cells in the 6th month was higher, too (Me = 7.6% for '6' vs Me = 5.1% for '0'; $p < 0.03$).
Conclusions: Chronic *in vivo* hyperglycemia decreases the TNF-α expression in CD4 lymphocytes, and this effect is reversible when good glycemic control and near normoglycemia is achieved.
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OP 032

Children are more frequently affected by the metabolic syndrome (Ms) than deterioration of glucose tolerance

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First cases of type 2 diabetes mellitus (T2D) in children have been reported in France, where we know that obesity increases in this age group. The objective was to evaluate glucose tolerance, insulin resistance (IR) and the MS in obese children. 310 children and adolescents (168 girls and 142 boys; age = 7 to 17 years; BMI = 28.8 ± 5.5 kg/m^2 or 4.9 ± 1.5 DS for age and sex), 143 (46.4%) of whom were prepubertal. 40 (12.9%) had a family history of T2D. 52 (6.9%) were Sub-Saharian, 170 (55.2%) were European Caucasian, 28 (9.1%) were African, 44 (14.3%) were Caribbean, 3 (1%) were Asiatic and 11 (3.6%) were from an other origin. Body fat was assessed by absorptiometry (DEXA) and distribution of adiposity on abdominal MRI section. Glucose tolerance was read on OGTT according to 1997 ADA criteria; IR was defined as HOMA > 75th perc. of the distribution for age and gender. SM was defined according to the ATP III guidelines. There were 11 cases (3.5%) with deterioration of glucose tolerance (1 T2D, 8 IGT, 2 HFG). Other metabolic features were as follow:

	IR$^+$	IR$^-$
MS$^+$	41 (13.2%)	10 (3.2%)
MS$^-$	181 (58.4%)	78 (25.2%)

Glucose tolerance was significantly affected by puberty ($p = 0.007$); male gender ($p = 0.0003$) and family history of T2D ($p = 0.01$). MS and IR were significantly associated with higher BMI ($p = 0.04$ and $p = 0.006$); visceral fat mass ($p = 0.03$ and $p < 0.0001$) and the ratio of visceral/subcutaneous abdominal fat mass ($p = 0.02$ for each). IR was significantly associated with male gender ($p = 0.0005$) and pubertal development ($p = 0.01$). In contrast to what observed in some countries like USA, disorders of glucose tolerance do not seem highly frequent in European obese children and are less frequent then MS which in turn is less frequent than IR. However MS is influenced by the same determinants as previously described in obese children.

OP 033

Somatometric development of children of mothers with gestational diabetes

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Introduction: In pregnancies with diabetes, an adverse intrauterine environment resulting from maternal metabolic disturbances may imprint a life-long increased risk of metabolic syndrome in these children. Our study investigated (1) the somatic development of children from pregnancies with gestational diabetes (GDM) and (2) the association of intrauterine growth pattern and the quality of maternal glycemic control with anthropometric parameters at birth and in early childhood.
Methodology: Weight and height were measured at follow-up (FU) in 324 offspring of women with GDM selected from an ongoing database. Somatometric data from routine examinations at 6, 12 and 24 months were retrospectively obtained, while data from birth, maternal glycemic values and measurements of the fetal abdominal circumference (AC) were obtained from the database. The standard deviation score (SDS) was calculated for weight and body-mass-index (BMI) based on age-correspondent data.
Results: Median age at FU was 5.5 years (range 2.5–8.5). BMI at birth was related to BMI at FU ($r = 0.26$, $p < 0.001$) with a rate of BMI > 90th percentile of 31.8% and 28.2%, respectively. BMI-SDS was 0.90 at birth, 0.56 at 6, 0.35 at 12, 0.32 at 24 months, and 0.66 at FU, being significantly different ($p < 0.001$) from normal population at each time point. AC of the 3rd trimester was significantly ($p < 0.001$) related to

BMI at birth (r = 0.39) and at FU (r = 0.22). There was no association of oGTT values with BMI at birth or FU. BMI/mother, BMI/father and BMI at birth (alternatively AC) were independently correlated with BMI at FU (r = 0.40, p < 0.001).

Conclusion: In children of mothers with GDM, neonatal obesity leads to early childhood obesity with the highest increase at birth and at age 2 to 8 years. The risk of childhood obesity seemed to be influenced mainly by intrauterine growth pattern, postnatal domestic environment and/or genetic predisposition.

OP 034

Insulin resistance and impaired glucose tolerance in obese children and adolescents referred to a tertiary care center in Israel

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Objective: To establish the prevalence of insulin resistance and impaired glucose tolerance (IGT) and their determinants in obese children and adolescents.

Methods: The study included 256 obese patients aged 5 to 22 years referred to a tertiary-care center in Israel. Estimates of insulin resistance [homeostatic model assessment (HOMA-IR)]; insulin sensitivity [ratio of fasting glucose (GF) to fasting insulin (IF) (GF/IF), the quantitative insulin sensitivity check index (QUICKI)], and pancreatic β-cell function [HOMA – derived β-cell function (HOMA %B)] were derived from fasting measurements. An oral glucose tolerance test (OGTT) was performed in 192 patients to determine the presence of IGT.

Results: Insulin resistance was detected in 81.2% of the patients, IGT in 13.5%, and silent diabetes in one adolescent girl. GF/IF and QUICKI decreased significantly during puberty (p < 0.005). Insulin resistance and insulin sensitivity indexes were not associated with ethnicity, presence of acanthosis nigricans or type 2 diabetes in family. Only 2 patients with IGT also had impaired fasting glucose. Compared with subjects with normal glucose tolerance, patients with IGT had significantly higher fasting blood glucose (p < 0.05), higher 2-hour post-OGGT insulin levels (p < 0.001), and a lower QUICKI (p < 0.05). There was no difference between these groups in fasting insulin, HOMA-IR, HOMA %B or the male-to-female ratio, age, BMI-SDS, presence of acanthosis nigricans, and family history of type 2 diabetes.

Conclusions: Insulin resistance is highly prevalent in obese children and adolescents. The prevalence of IGT in the Israeli young population is higher than in Europe but lower than in the USA. As there are no predictive cut-point values of insulin resistance or insulin sensitivity indexes for IGT, an OGTT is required in all subjects at high risk. Longitudinal studies are needed to identify the metabolic precursors of the development of type 2 diabetes in these patients.

OP 035

Effects of feeding and RHGH on gluconeogenesis in humans

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A number of factors are thought to increase the rate of gluconeogenesis (GNG), e.g. growth hormone, insulin deficiency and glucocorticoids. Recent data from our laboratory demonstrate that GNG is not suppressed during feeding when compared to short term fasting. To determine the effect of short term rhGH on GNG, 6 healthy volunteers (23.5 ± 6 yr, 61.3 ± 7.6 kg, 161.6 ± 7.3 cm and 23.6 ± 2.5 kg/m^2) were studied on two separate occasions. Subjects received, in random order, either of normal saline (NS) or recombinant human growth hormone (rhGH), (0.05 mg/kg · day) for 7 days. Rate of glucose appearance (Ra) and GNG were measured using [U-^{13}C]glucose MIDA following 14 h fasting, and following 8h of feeding with Boost® High Protein (34 g every 15 minutes).

Results: GNG was significantly higher during feeding than fasting in the presence or absence of rhGH, despite significant and prolonged hyperinsulinemia. No significant differences were observed in the GNG following rhGH injection when compared to saline in either the feeding or fasting conditions (see table 1). In summary, GNG increases during feeding, and rhGH does not acutely affect GNG.

Conclusion: Using [U-^{13}C]glucose MIDA in human: 1) GNG is not acutely under the control of either insulin or rhGH. 2) Feeding a mixed macronutrient diet increases GNG, thus 3) The only mechanism to decrease glucose production is via decreased glycogenolysis.

Table 1.

	Fasting/NS	Feeding/NS	Fasting/rhGH	Feeding/rhGH
Glucose mmol/l	4.87 ± 0.08	6.01 ± 0.21	5.31 ± 0.28	5.94 ± 0.23
Insulin u/ml	10 ± 2*§	69 ± 14*§	22 ± 5*§	101 ± 29§*
Ra mol/kg · min	10.89 ± 0.40*	29.17 ± 2.04*	12.84 ± 0.9*	31.25 ± 1.28*
GNG · mol/kg · min	5.02 ± 0.33*	8.84 ± 1.53*	5.44 ± 0.73*	9.26 ± 1.11*

Feeding vs. Fasting * = P < 0.05, NS vs. rhGH § = P < 0.05