ISPAD + APEG 2015

ORAL ABSTRACT SESSIONS

APEG Young Investigators Session

O1

Accumulation of skin advanced glycation end-products is a significant indicator of early retinopathy in adolescents with type 1 diabetes

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Objectives: Advanced glycation end-products (AGE) are strongly linked to the pathogenesis of vascular complications. Skin autofluorescence (AF) provides a non-invasive measure of AGE accumulation in skin collagen, which has been correlated with systemic vascular dysfunction. We examined the relationship between diabetic retinopathy (DR) and skin AF, cumulative HbA1c and duration in youth with type 1 diabetes (T1D).

Methods: Forearm skin AF was measured non-invasively (Diagnoptics AGE-Reader; mean of 6 readings), in 132 participants with T1D (median age 15.7 years, duration 7.9 years) and 31 age-matched controls. DR was assessed using 7-field stereoscopic fundal photography, defined as ≥21 in any eye on Modified Airlie House Criteria. Mean HbA1c for the past 1–5 years was calculated from HbA1c levels at each clinic visit.

Results: Skin AF was higher in T1D vs. controls (median 1.17 vs. 1.01 AU; $p < 0.001$). Adolescents with DR, compared to those retinopathy-free, had higher skin AF (1.37 vs. 1.15 AU; $p = 0.001$), were older (16.3 years vs. 15.6 years; $p = 0.03$), had longer T1D duration (11.0 years vs. 7.6 years; $p < 0.001$), higher concurrent HbA1c (9.9 vs. 8.4%; $p = 0.02$) and mean HbA1c over 1, 2, 3 and 4 years ($p < 0.05$). There was no significant difference in cholesterol levels, SBP and DBP SDS and BMI SDS. In binary logistic regression, DR was associated with skin AF ($R^2 = 0.17$), older age ($R^2 = 0.08$), longer duration ($R^2 = 0.18$), higher concurrent HbA1c ($R^2 = 0.11$) and mean HbA1c over the past 1, 2, 3, 4 and 5 years ($R^2 = 0.13, 0.13, 0.20, 0.17, 0.11$). In multivariable analysis, Model 1 ($R^2 = 0.28$): DR was associated with skin AF ($\beta = 21.2$, 95% CI 2.5–180.6; $p = 0.005$) and duration (1.3, 1.1–1.5; $p = 0.006$); or Model 2 ($R^2 = 0.29$): skin AF (37.4, 2.7–526.1; $p = 0.007$) and mean HbA1c over past 4 years (1.6, 1.0–2.6; $p = 0.04$), but not age.

Conclusions: Skin AF is associated with DR, which remained significant adjusted for HbA1c or duration. This supports the independent role of AGE accumulation in the pathogenesis of DR in adolescents.

O2

Prevalence of maturity onset diabetes of the young in a Western Australian paediatric diabetes clinic using targeted massively parallel sequencing

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Objectives: Maturity Onset Diabetes of the Young (MODY) is an autosomal dominant diabetes with 13 known genes. To date, no prevalence study has assessed for variants in all MODY genes. We aimed to assess MODY prevalence in a paediatric diabetic population, using massively parallel sequencing (MPS).

Methods: All West Australian children diagnosed with type 1 diabetes (T1DM) and many with type 2 diabetes (T2DM) are seen at The Princess Margaret Hospital (total clinic = 1052 cases). 13 cases had been previously diagnosed with MODY. Of the remainder, 60 of 104 antibody negative (ab-ve) T1DM and 18 of 62 T2DM samples were available for MPS, assessing all exons of known MODY genes (268 amplicons). Primers were designed using Illumina Design Studio. DNA libraries were constructed using Illumina TruSeqDNA sample preparation kit, then multiplexed and sequenced using Illumina MiSeq. Data were demultiplexed using CASAVA, aligned to the human genome (hg19) using the Novoalign alignment tool and converted using SAMtools and Picard tools. Single nucleotide polymorphisms and indels were called using GATK and annotated using ANNOVAR. After QC, data were filtered for coding variants with minor allele frequency <1% predicted to be deleterious/damaging by SIFT and/or Polyphen.

Results: Damaging variants in MODY genes were seen in 6/60 cases with ab-ve T1DM (10%) and 3/18 cases with T2DM (17%): two with *HNF1A* and *ABCC8*, one each with *HNF4A, GCK, HNF1B, KCNJ11* and one with both *PDX1* and *PAX4* mutations. The 13 previously identified cases had variants in *GCK* (9), *HNF1B* (1), *PDX1* (1) and one unknown.

The prevalence of MODY in the entire clinic was 2.1%; however, this is an underestimate due to lack of testing in missing samples.

Conclusions: The prevalence of MODY is at least 2.1% in a paediatric diabetic population but is 10% in presumed ab-ve T1DM and even higher in presumed T2DM. MODY is under diagnosed; however, MPS is an efficient and cost-effective means of screening patients at risk.

O3

Pupillometry changes over time in type 1 diabetes

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Introduction: Diabetic autonomic neuropathy (AN) is a frequently undiagnosed complication of type 1 diabetes (T1D) and is associated with increased mortality. Early detection of AN would be desirable for better individual risk stratification. Pupillometry is a sensitive tool to detect subclinical AN in patients with diabetes. We examined the prevalence and predictors of pupillometry in an adolescent cohort with T1D.

Research designs and methods: 171 patients with T1D assessed between 1990 and 2015 with 2 or more pupillometry assessments over a minimum of 10 years. Pupillary autonomic function was assessed using an infrared computerized pupillometer. Longitudinal analysis was performed using generalised estimating equations.

Results: At baseline assessment median age was 13.9 years [12.5–15.8] and median diabetes duration was 6.2 years [4.2–9.4]. Median follow-up was 13.3 years [8.6–17.6]. All three pupillary function tests declined over time.

	Baseline	Last follow-up	p-value
Resting pupil diameter (mm)	6.2 ± 0.9	5.6 ± 0.7	$p < 0.0005$
Maximum constriction velocity (mm/s)	6.3 ± 1.5	5.2 ± 1.2	$p < 0.0005$
Reflex amplitude (mm)	2.1 ± 0.5	1.6 ± 0.3	$p < 0.0005$

[Pupillary function tests over time]

Resting pupillary diameter was inversely associated with weight SDS (beta: −0.15, 95% CI −0.26 to −0.05, $p < 0.05$) and diabetes duration (beta: −0.04, 95% CI −0.05 to −0.03, $p < 0.05$). Reflex amplitude was inversely associated with BMI SDS (beta: −0.11, 95% CI −0.18 to −0.05, $p < 0.05$) and diabetes duration (beta: −0.25, 95% CI −0.03 to −0.19, $p < 0.05$). Maximum constriction velocity was also associated with BMI SDS (beta: −0.24, 95% CI −0.41 to −0.07, $p < 0.05$) and diabetes duration (beta: −0.57, 95% CI −0.08 to −0.36, $p < 0.05$). Mean HbA1c was not associated with measures of pupillometry function.

Conclusion: AN function assessed using pupillometry declines over time in adolescents with T1D followed into adulthood. Body mass index is a modifiable risk factor for AN in this population.

O4

Antidiuretic hormone as a mediator of the glycaemia-increasing increasing effect of high intensity exercise in individuals with type 1 diabetes

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Adrenaline ans noradrenaline are generally acknowledged to be the primary mediators of the rise in glycaemia associated with high intensity exercise in individuals with type 1 diabetes (T1D). However, since high intensity exercise causes a fall in plasma volume, and the levels of antidiuretic hormone (ADH), a potent activator of hepatic glucose production, increase in response to dehydration, the aim of this study was to test the hypothesis that ADH contributes to the glycaemia-increasing effect of high intensity exercise in individuals with T1D. Nine individuals with TID [21.5 ± 4.0 years, HbA1c 7.9 ± 0.8% (60 mmol/mol), BMI 25.4 ± 5.5 kg/m^2, VO_2peak 34.8 ± 5.1 ml/kg/min, mean ± SD] underwent a morning euglycaemic euinsulinaemic clamp during which euglycaemia and basal plasma insulin levels were maintained and deuterated glucose was infused to determine the rates of endogenous glucose production and utilisation. Then, the participants exercised at 80% VO_2peak for up to 40 min. Before, during and after exercise, blood samples were collected for analyses of glucoregulatory hormone levels and plasma volume change. In response to exercise, mean blood glucose levels increased from 5.7 ± 0.2 mmol/l before exercise reaching peak levels of 6.3 ± 0.2 mmol/l at 5 min post-exercise ($p < 0.05$). This rise in blood glucose level was accompanied by significant increases in endogenous glucose production and glucose utilisation rates and a fall in plasma volume. Exercise resulted in a significant increase in the levels of plasma adrenaline (0.36 ± 0.07 nmol/l and 2.38 ± 0.44 nmol/l, before and at the end of exercise, respectively; mean ± SEM), noradrenaline (2.6 ± 0.25 and 17 ± 1.9 nmol/l), growth hormone (5.7 ± 1.7 and 26.1 ± 7.7 μg/l) and ADH (1.8 ± 0.2 to 10.5 ± 2.1 pmol/l). Our results suggest that in addition to adrenaline and noradrenaline, the rise in ADH levels may contribute to the glycaemia-increasing effect of high intensity exercise, but the importance of ADH in this process remains to be determined.

O5

Higher dehydroepiandrosterone levels within the normal range relate to better vascular endothelial function in girls with type 1 diabetes

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Objectives: The association between androgen levels and vascular function in females is complex. Androgens may have a protective effect on vascular function by increasing nitric oxide production and reducing oxidative stress. The effect of serum androgen levels within the normal range on endothelial function in girls with T1D is unknown. We therefore aimed to evaluate the association between vascular function and androgen levels in T1D, obese and normal weight girls.

Methods: 164 girls with T1D (from RCT ANZCTR 126111000148976), obesity or normal weight, without hyperandrogenism, were evaluated with vascular function (Flow mediated dilatation, Glyceryl trinitrate induced dilatation), serum androgen levels (free testosterone, androstenedione, dehydroepiandrosterone [DHEA] measured by LC/MS) and biochemical variables.

Results: Girls with T1D had higher androgens levels (Table 1), mean diabetes duration of 5.6 (3.6) years and their pubertal status (19/19/54 pre/early-mid/late puberty respectively) did not differ from controls. Linear regression showed an independent association

Table 1. Characteristics of female participants

	T1D (n = 92)	Obese (n = 29)	Non-obese T1D controls (n = 43)	p (ANOVA)
Age (years)	13.8 (2.77)	13.1 (2.5)	14.1 (3.12)	0.25
BMI Z score	0.75 (0.70)	2.26 (0.26)	0.39 (0.85)	<0.001
FMD (%)	5.91 (4.52)	4.39 (3.49)	7.10 (4.87)	0.04
GTN (%)	25.64 (6.70)	22.93 (5.12)	28.6 (9.1)	0.04
Free testosterone (ng/ml)	0.26 (0.15)	0.17 (0.10)	0.20 (0.10)	<0.01
Androstenedione (nmol/l)	0.96 (0.56)	0.76 (0.27)	0.86 (0.48)	0.19
DHEA (nmol/l)	2.72 (2.12)	1.24 (0.81)	1.85 (1.66)	<0.001
SHBG	81.40 (36.08)	42.09 (25.68)	83.86 (31.65)	<0.001

Mean (SD)

between DHEA and FMD (β = 0.45, p = 0.049 and a borderline association between testosterone and FMD (β = 6.18, p = 0.059). There were no associations in the obese and normal weight groups.
Conclusion: Higher DHEA levels within the normal range related to better endothelial function and may have a protective role in early vascular changes in adolescent girls with type 1 diabetes.

O6
Insulin resistance remains a feature of children born preterm

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Background and objective: Fifteen years ago we reported that children born very preterm (<32 weeks of gestation) were insulin resistant. Since, there have been changes in neonatal intensive care (notably nutrition). Thus we aimed to assess whether pre-pubertal preterm children still had reduced insulin sensitivity compared to term children.
Methods: Participants were pre-pubertal children aged 4–11 years born very preterm (<32 weeks of gestation; n = 25; 68% boys) or at term (37–41 weeks; n = 56; 70% boys). Frequently sampled intravenous glucose tolerance tests with insulin were performed. Insulin sensitivity was calculated using Bergman's minimal model. DXA-derived body composition data were obtained. Data were analysed using linear mixed models, adjusting for important confounders such as age, sex, and adiposity (% total body fat).
Results: Participants in both groups were of similar age (p = 0.11) and sex ratio (p = 0.88). Preterm children were lighter (weight SDS 0.06 vs. 0.67; p = 0.002), shorter (height SDS 0.01 vs. 0.39; p = 0.047), and had lower BMI SDS (0.06 vs. 0.70; p = 0.002) than children born at term. Importantly, after adjustment for adiposity, children born preterm had lower insulin sensitivity than term controls (9.0 vs. 11.1 $\times$ 10^{-4}/min (mU/l); p = 0.041). There were no differences in fasting insulin between groups (4.4 mU/l vs. 4.9 mU/l; p = 0.40).
Conclusions: Despite changes in neonatal intensive care of the past 15 years, adverse metabolic programming (i.e. lower insulin sensitivity) remains a feature of pre-pubertal children born very preterm. However, it seems that the magnitude of the reduction in insulin sensitivity has been attenuated (~19% compared to the previously observed difference of ~34%), which may be a result of improvements in neonatal care.

Diabetes Oral I Diabetes Genetics, Immunology and the Environment

O7

Prediabetes, short-term therapy with alagberium chloride improves glycaemic control and reduces pancreatic infiltration in experimental type 1 diabetes

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Objectives: Advanced glycation end products (AGE) are considered independent predictors of type 1 diabetes (T1D) progression in islet autoantibody positive children. Alagebrium chloride (ALT-711) is a therapeutic agent which decreases the formation of AGEs. Using a murine model of T1D, we aimed to study the effects on glucose homeostasis and immune cells of ALT-711 therapy prediabetes.

Methods: Randomised, female NODShiLt (n = 10/group) received

1) no treatment or
2) ALT-711 (1 mg/kg/day s.c.) prediabetes from day 50–100 of life and were followed until diabetes diagnosis or day 200.

Results: Fifty days of ALT therapy, conferred protection from T1D until day 200 of life compared to controls (20% vs. 80% incidence, p = 0.005). Toward the end of the treatment period, ALT treated mice had lower glycated haemoglobin (7.4% vs. 11.1%, p = 0.0005), improved insulin secretion during OGTT (11.8 nmol/l/min vs. 10.2 nmol/l/min, p = 0.02) and less pancreatic islet infiltration compared to controls (p = 0.0005). After 30 days of ALT treatment, pancreas digests showed a modest reduction in $CD45.1^+$ cell number (1.17×10^5 vs. 3.87×10^5 cells, p = 0.07) and reduced $F4/80^+$ macrophages (4.7×10^3 vs. 20.5×10^3 cells vs., p = 0.03), compared to controls. No differences in $CD4^+$, $CD8^+$, $CD4^+CD25^+$Foxp3^+ T cells, $CD19^+B220^+$ B cells or $CD11b^+CD11c^+$ dendritic cell numbers or proportions were observed in the pancreatic lymph node or spleen. Splenocyte function at this time was not different between groups after immune stimulation by ovalbumin (163.8 vs. 213.63 interferon-producing cells). Adoptive transfer of diabetes by splenocytes to NODScid recipients from ALT treated or control mice, did not differ between groups (69% vs. 62%). *In vitro*, AGE modified albumin enhanced antigenic peptide activity by the enzyme ERAP-1, part of the MHC Class I pathway.

Conclusion: Short-term, ALT-711 therapy prediabetes improves islet function, and reduces immune infiltrate. This may be via effects on antigen presentation by pancreatic islets.

O8

Sitagliptin-anti CD4 mab conjugated T-cell targeting therapy for the effective treatment of type I diabetes

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Antibody drug conjugate (ADC's) concept is a less explored and more trustable for the treatment of Type 1 diabetes (T1D). T lymphocytes, include specialized subsets of regulatory T cells (Tregs) that are crucial for the maintenance of immunological tolerance. Their major role is to shut down: T cell-mediated immunity towards the end of an immune reaction and suppress auto-reactive, T cells that escaped the process of negative selection in the thymus. Destruction of beta cells via autoimmune processes occur, when immune-regulatory mechanism fails, allowing auto reactive, and T cell clones to infiltrate the pancreas, thereby selectively destroying the beta cells in the islets of Langerhans which initiated the use of biological agents that selectively interfere with the activation of lymphocyte population, namely anti-CD4 T cell antibodies, which can induce/restore self tolerance to well defined beta cell antigens. In this concept we are using sitagliptin nano-particle conjugated through Sulfo-MBS cross linkers with Anti-CD4 antibodies to target auto reactive $CD4^+$ T cells which are present in Pancreas. Sitagliptin which is a DPP-IV inhibitor drug, which also stimulates β-cells proliferation as the half life of GLP-1 hormone is less due to rapid degradation by DPP-IV enzyme which conquers the replacement and reserve β-cells mass. Thus in the present study CD4 mAbs could arrest the autoimmunity in T1D and sitagliptin loaded polymeric nanoparticles could regenerate pancreatic beta cells. In future prospects antibody-drug conjugates could substitute subcutaneous insulin therapy.

O9

Increased C peptide with longer diabetes duration in type 1 diabetes

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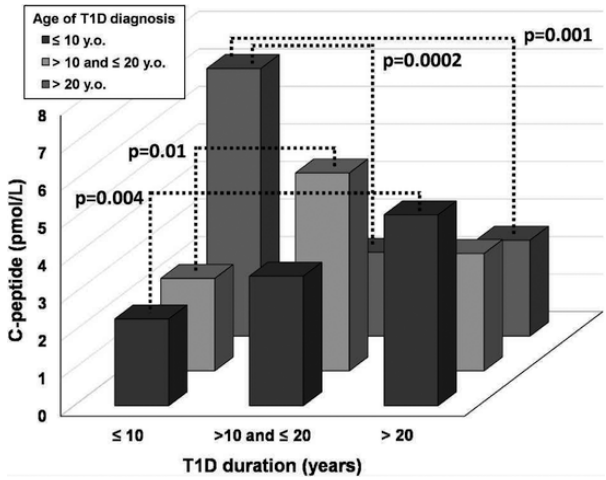
Objectives: People with long duration of type 1 diabetes (T1D) may demonstrate residual β-cell function. This study aims to compare c-peptide levels in children and adults with T1D at varying age of diagnosis and duration, and in children, its relationship to persisting diabetes autoantibodies.

Methods: 358 T1D patients between ages 10–80 years (mean 31 ± 16 years) and 249 controls were recruited. T1D patients were stratified by age at diagnosis (≤10, 10–20, >20 years) and duration (≤10, >10–20, >20 years). Plasma c-peptide was quantified by ultrasensitive ELISA (Mercodia, Sweden; detection limit 1.16 pmol/l). In children, IA2 and GAD were also quantified.

Results: C-peptide levels were lower in T1D vs. controls (3.4 ± 1.1 vs. 572.7 ± 1.0 pmol/l; $p < 0.001$). Lower rates of detectable c-peptide were found in T1D patients diagnosed at age ≤10 years vs. >10 years ($p < 0.001$). In those diagnosed at age ≤10 years, c-peptide was detected in 44% after >20 years duration; 30% with 10–20 years duration; and 19% in those with ≤10 years duration.



[Figure]

In the paediatric T1D cohort (median age 16.0 years, duration 8.0 years), 39% had detectable c-peptide (51/130) and 74% had persistent IA2 ± GAD (67/91). C-peptide was not associated with positive antibodies.

Conclusions: T1D adults diagnosed in early childhood were more likely to have detectable c-peptide compared to adolescents diagnosed at the same age group, in keeping with islet regeneration over time. Higher c-peptide was not associated with persisting diabetes autoimmunity.

O10

Broad blood transcriptional abnormalities and complexity of pathophysiology in patients with type I diabetes

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Although Type I diabetes is a well-studied disease, the events leading to initiation of the disease are still poorly understood, which hinders not only our understanding of the disease, but also the development of effective treatments. In this study, using genome-wide gene expression arrays, we analyzed blood RNA isolated from young female patients with Type 1 diabetes (T1D) and matched healthy controls. Our data showed significant alterations (≥2-fold, FDR < 10%) in 270 genes in the blood of TID patients, including 258 genes that are known to play roles in diverse biological processes. Specifically, the T1D blood transcript abnormalities involve pathways of RNA binding/transcription/splicing, protein synthesis/post-translational modification, metabolism, immune response, cell migration/trafficking/transport, apoptosis, cytoskeleton and extracellular matrix, DNA binding and synthesis, oxidative stress, and erythrocytes development and function. Importantly, numerous dysregulated genes in T1D blood have been linked to various disease conditions, including, metabolic disorders and diabetes, inflammation, neurodegeneration and hematological disorders. Noteworthy, we observed alteration of numerous tissue-specific genes in T1D blood, involving brain, retina, skin, immune system, heart, bone marrow/erythrocyte, skeletal muscle and kidney. While the mechanisms are unclear, we demonstrate here that blood transcriptional profiles reflect the complex pathology in T1D, and major long-term T1D complications, such as neuropathy, retinopathy and cardiovascular disorders, emerge early during the disease progression. Importantly, some of the observed transcriptional abnormalities have not been previously linked to T1D. Therefore, genome-wide blood transcriptional profiling may offer an invaluable tool to reveal early pathophysiological changes in T1D and identify new therapeutic avenues.

O11

A high perinatal blood iron level increases the risk of type 1 diabetes

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Objectives: Immunologic events during fetal life may play a part in the pathogenesis of type 1 diabetes (T1D). Iron is a key element in immune function, and excessive iron can lead to inducement of oxidative stress, which is involved in the destruction of the pancreatic β cells.

Through a population-based case-control study, we examined whether a high perinatal iron level was associated with increased risk and earlier age of onset of T1D.

Methods: 199 cases, born between 1991 and 1998, who developed T1D before the age of ≤15 were randomly selected from the Danish T1D patient registry (DanDiabKids). Neonatal dried blood spot samples (NDSB) from the cases and 199 age-matched controls were acquired from the Danish Newborn Screening Biobank.

Iron was analyzed via laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS).

Linear logistic regression was used as the main analysis, with *HLA-DQB1* alleles, birth data, and mother's age and diabetes status as possible confounders. The association between iron status and onset age of T1D was analyzed via linear regression.

Results: Mean iron level in cases and controls were significantly different. Each doubling in iron level increased the relative risk of developing type 1 diabetes with 2.74 ($p = 0.02$). The relative risk increased to 3.28 ($p = 0.009$) when adjusting for possible confounders. In contrast, iron level was not found associated with age of onset of T1D.

Furthermore, in agreement with earlier studies, both *HLA-DQB1* alleles and gestational age were also found to affect the risk of developing T1D significantly.

Conclusions: The risk of developing T1D in Danish children was positively associated with perinatal iron level. However there was no significant association between iron level and age of onset. This is to our knowledge the first study, on the influence of iron levels on the risk of T1D. If future research confirms our findings, guidelines for iron/mineral supplementation may need to be revised.

Variables	RR	95% CI	p value
Iron status (log2 transformed)	3.28	1.34–8.06	0.009
HLA-DQB1 allele's (n/% of total)			
Protective or low risk	1		–
Moderate or high risk	7.39	4.30–12.27	<0.0001
Gestational Age (mean of weeks)	0.80	0.66–0.95	0.01

[Adjusted logistic regression model with case-control]

O12

Islet autoantibody measurements from dried blood spots can be used to screen for type 1 diabetes risk

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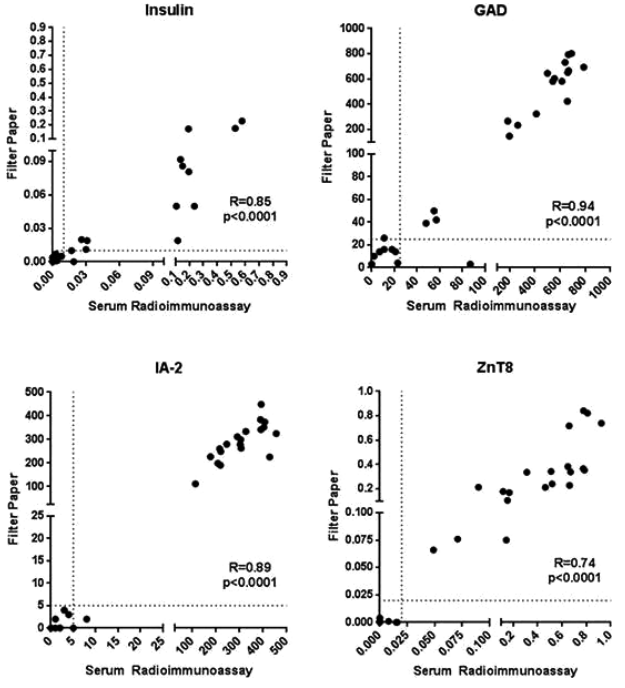
Type 1 Diabetes (T1D) risk can be identified before disease onset by measuring islet autoantibodies (IA) to insulin, glutamic decarboxylase (GAD), islet antigen 2 (IA-2), and zinc transporter 8 (ZnT8) in first-degree relatives (FDR) and individuals with high genetic risk. With ≥2 IA, the risk of developing T1D is nearly 100% over time. FDRs are offered IA screening through research trials; however, more than 85% of T1D patients have no family history. Barriers to screening the general population include cost, venipuncture, sample transport and difficulty measuring IA. Insulin autoantibodies are historically difficult to measure and often the first IA to develop in young children. We hypothesize that screening the general population for IA using dried blood spots (DBS) will help address these barriers. After establishing methodology to measure IA from DBS, we enrolled new onset T1D (n = 27) and controls (n = 26) to validate DBS to measure IA. For each subject, a 6 mm DBS was transferred to one well of a nonbinding 96 well plate in elution buffer and agitated. IAs were measured from eluate and serum by sensitive radioimmunoassays (RIA). Serum RIA levels strongly correlate with DBS levels for each IA in new onset T1D patients (Figure). The false negative rate using DBS was 4% (GAD, insulin, IA-2) and 0% (ZnT8). There were no false positive DBS results for insulin, IA-2 or ZnT8 in controls. We conclude that measuring IA from DBS may allow screening the general population for T1D risk.



[Autoantibody Measurements with Dried Blood Spots]

O13

High affinity pan-isotype transglutaminase and islet autoantibodies improve early diagnosis of celiac disease and pre-type 1 diabetes

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Objectives: Screening for celiac disease uses the presence of IgA transglutaminase autoantibodies (IgA TGA). However, they are rarely detectable <18 month of age and not as sensitive as a combined IgA/IgG TGA test. Multiple IgG autoantibodies to insulin (IAA), GAD, IA-2 or ZnT8 identify pre-type 1 diabetes (pre-T1D); presence of only one of these (usually low affinity) is not diagnostic. We developed novel assays for TGA, IAA, GADA, and IA-2A, based on electrochemiluminescence (ECL) that detects only high-affinity autoantibodies of any isotype (IgG, IgA, IgM, IgD and IgE). We evaluated the ability of ECL-based assays to accurately detect the onset of celiac autoimmunity or pre-T1D.
Methods: The Diabetes Autoimmunity Study in the Young has followed from birth, for up to 20 years, 2562 children at high genetic risk for celiac disease and T1D. TGA and islet autoantibodies were tested starting from 9 month of age. ECL-IAA and ECL-GADA were measured in all children who developed T1D (n = 68) or multiple autoantibodies without yet progressing to diabetes (n = 26). Seroconversion to ECL-TGA was studied in an additional 70 TGA+ study participants.
Results: ECL-IAA or ECL-GADA detected pre-T1D earlier than radioassays in 30% of children who progressed to T1D or multiple autoantibodies. The median lag between ECL and radioassay positivity was 1.9 year. The positive predictive value of ECL-IAA or ECL-GADA as a single autoantibody was superior ($p < 0.01$) to that of single IAA or GADA by radioassay. ECL-TGA detected seroconversion to TGA earlier than IgA TGA in 54% of the children. The median lag between ECL and radioassay TGA positivity was 2.5 year. ECL-TGA correlated more strongly ($p = 0.03$) with positive intestinal biopsy than radioassay TGA levels.
Conclusions: The novel ECL-based assays often antedate the onset of autoimmunity and should be used in population screening for CD and/or pre-T1D and in time-to-event analyses evaluating candidate triggers of autoimmunity.

O14

Characterization of rapid progressors to type 1 diabetes among children with HLA-conferred disease susceptibility

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Objective: To assess the characteristics of rapid progressors to type 1 diabetes (T1D) in children with HLA-conferred disease risk.
Methods: We followed from birth 7410 children (52.6% males) with HLA-conferred disease risk for development of islet autoimmunity

and progression to T1D over a median follow-up time of 13.2 years (range 0.9–18.2). Islet cell autoantibodies (ICA), autoantibodies to insulin (IAA), glutamic acid decarboxylase (GADA), and islet antigen 2 (IA-2A) were used for screening for β-cell autoimmunity. Rapid progression was defined as progression to T1D within 1.5 years after prediabetic seroconversion. We analyzed the association between rapid progression and 25 non-HLA single nucleotide polymorphisms (SNPs) predisposing to T1D.

Results: Among the 7410 children, 1563 (21.1%) developed islet autoantibodies and 221 (14.1%) progressed to T1D by the end of 2012. The median time from seroconversion to diagnosis was 0.35 (0.02–1.46) years in rapid (n = 46, 20.8%) and 4.3 (1.5–15.8) years in slower progressors. Compared to slower progressors, rapid progressors were more often multipositive (76% vs. 54%; $P < 0.01$), had higher titers of ICA (10.0 vs. 5.0 JDFU; $P < 0.01$) and IAA (9.1 vs. 6.6 RU; $P = 0.02$) at seroconversion, and higher prevalence of a SNP in the FUT2 gene (major allele G homozygotes, 74% vs. 25%; $P < 0.001$). Compared to seroconverted non-progressors, rapid progressors were younger (1.6 years vs. 5.5 years; $p < 0.001$) and had higher titers of ICA, IAA, GADA, and IA-2A (10.0 vs. 4.0 JDFU; 9.1 vs. 0.3 RU; 6.9 vs. 0.1 RU; 0.11 vs. 0.08 RU, respectively; $p < 0.001$) at seroconversion, and carried more often the high risk HLA-DQB1*02/*0302 genotype (48% vs. 26%; $p = 0.001$).

Conclusion: At seroconversion, rapid progressors to T1D are characterized by young age, high-risk HLA genotype, higher autoantibody titers, multipositivity, and higher prevalence of the predisposing SNP in the FUT2 gene. Such children might benefit from careful monitoring and intensive intervention.

Diabetes Oral II Diabetes Treatments and Technology

O15

MiniMed 640G with SmartGuard: a predictive low glucose management system that prevents hypoglycemia and is acceptable in children

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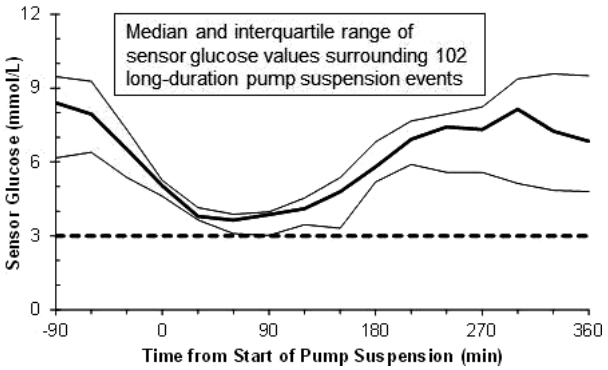
Objectives: To evaluate a commercially-available sensor-augmented insulin pump system that suspends insulin delivery in response to predicted hypoglycemia.

Methods: The MiniMed 640G system (Medtronic) uses second-generation Enlite glucose sensors and includes SmartGuard technology that can stop insulin delivery 30 min before a predicted low sensor glucose (SG) value occurs and automatically restart the pump upon recovery. Questionnaires were completed at the end of the 4-week study.

Results: Sixteen pediatric subjects age 9–17 (mean ± SD, 13.4 ± 2.53) years, 9 male, A1C 7.4 ± 1.10%, were enrolled. All completed the study. SmartGuard Suspend before Low events were activated 1010 times (2.1 times per subject-day). At the time of activations, the median (interquartile range) SG value was 5.0 (4.8–5.4) mmol/l and was falling at 0.08 ± 0.02 mmol/l per min. The figure shows SG values surrounding 102 suspensions lasting 2 h. Four hours after the start of these long pump suspensions, the median SG value was 7.4 (5.6–7.9) mmol/l. On questionnaires, subjects agreed with the following: fit into my lifestyle, helped me avoid hypoglycemia, want to continue using it, and prefer it.

Conclusions: Pediatric subjects responded favorably to the the MiniMed 640G with its SmartGuard feature. SmartGuard activation prevented 83.5% of predicted hypoglycemic episodes.



[Sensor Glucose Values (Median, IQR)]

O16

Pharmacokinetics (PK) of a new suspension of glibenclamide for use in young patients and infants with neonatal diabetes

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Background: Sulfonylurea therapy allows a better metabolic control than insulin in patients with neonatal diabetes secondary to mutation in potassium channel. Its galenic form (tablets) is not suitable for children, as the dosage can't be easily modulated and as it induces large PK variations when administer to young children.

Objective: To measure relative biodisponibility of a new galenic form of glibenclamide and to assess its safety and tolerability.

Method: Open-label, cross over randomised phase 1 study in 18 healthy male subjects. Single oral administration, in fasted conditions of two new oral glibenclamide suspensions (0.83 ml of a 6 mg/ml suspension (S6), 8.33 ml of a 0.6 mg/ml suspension (S0.6)) and of 5 mg of Daonilâ crushed tablet (DCT).

Results: When suspensions were administered, glibenclamide plasma concentrations peaked 0.5 h earlier than observed with a DCT (median value of 2.5 h post-dose versus 3.00 h post-dose). Mean plasma peak Cmax values were similar for the two suspensions (S6: 201.71 ± 71.43 ng/ml S0.6: 206.93 ± 67.33 ng/ml, approximately 40% higher than the DCT one (148.34 ± 46.74 ng/ml). Exposures were similar for the two suspension dosages (AUC0-¥ values: S6: 1120.9 ± 400.5 ng.h/ml, S0.6: 1172.3 ± 422.0 ng.h/ml), and superior to that observed after DCT administration. Relative bioavailability was 121.6% for the 0.6 mg/ml and 114.1% for the 6 mg/ml formulations when compared to the DCT. Elimination half-lives were similar for the two suspensions (close to 8 h) and a little shorter than that observed with DCT (10.45 h). No adverse events were reported.

Conclusion: Suspension of glibenclamide appears to be more suitable for use in pediatric patients as its dosage can be adjusted to patients needs with great precision more easily. PK studies reported it to be better absorbed than glibenclamide tablets. Tolerance and acceptability are being evaluated in patients with neonatal diabetes (ClinicalTrials.gov Identifier: NCT02375828).

O17

The "Clay Pot Olympics" – testing the efficacy of insulin cooling devices

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Aim: Insulin loses potency when stored at high temperatures. However refrigeration is not available in many settings in lower-resourced countries. Various clay pots part-filled with water, and other evaporative cooling devices are used across the world. This study examined the cooling efficacy of such devices.
Methods: 14 devices from 7 countries (Sudan, Ethiopia, Tanzania, Mali, India, Pakistan and Haiti) were studied. 11 were clay pots of various designs, also studied were a goat skin, vegetable gourd, and bucket filled with wet sand. Two commercially manufactured "Frio" brand cooling bags (suspended and lying flat) were also studied. Internal and ambient temperature, and ambient humidity were measured simultaneously every 5 min by electronic loggers for two 44-h periods in a well-ventilated internal room in Khartoum, and a similar room in Mali (the two devices from Mali only, as pots had broken twice during air shipment). Cooling efficacy was assessed by the average absolute difference in temperature (internal vs. ambient), and also by % of maximal possible evaporative cooling (adjusting for humidity). Cluster analysis was used to find devices showing similar cooling efficiency.
Results: In Khartoum, mean temperature was 31.0°C (range 29.1–33.4°C) and humidity 32.0% (23.6–48.6%). All devices reduced temperature ($p < 0.001$ vs. ambient) with a mean temperature reduction of 2.2–7.8°C depending on the device. When expressed as % maximal cooling, the efficacy ranged from 20.4–71.2%. Cluster analysis separated the devices into 4 groups. The most effective group consisted of the goat skin, 2 of the clay pots, and the suspended Frio bag. Results from Mali are pending analysis.
Conclusion: Low-cost devices are effective in reducing insulin storage temperatures. Devices are more effective when humidity is lower. Use of more effective designs should be encouraged. Further studies are needed on insulin stability to determine when these devices are necessary.

O18
To study the efficacy of bromocriptine mesylate as an add-on therapy to oral anti-diabetic agents in type 2 diabetes mellitus

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Introduction: There is a need for new oral anti diabetic agents with different modes of action from existing anti-diabetic agents. Most important player implicated in the pathogenesis of type 2 diabetes is the brain. Bromocriptine Mesylate is one such drug which acts on brain up-regulating dopaminergic tone thereby reducing insulin resistance and improving glucose tolerance.
Objective: To study the efficacy of Bromocriptine Mesylate as an add-on therapy to oral anti-diabetic agents in type 2 diabetes mellitus.
Material and methods: 50 patients according to inclusion and exclusion criteria formed the subject matter of this prospective, non-randomized study. Bromocriptine Mesylate was added in weekly 0.8 mg increments to achieve a target dose between 1.6 and 4.8 mg depending upon the patient tolerance. Baseline measurements of fasting, post prandial blood sugar and HbA1c were followed up at 6 and 12 weeks. BMI was measured at 0 and 12 weeks. Appropriate history, examination and lab tests were done at each visit to identify any adverse effects. Paired student '*t*'-test was used for analysis using SPSS 17 statistical software.
Results: Patients showed significant reduction in fasting, post prandial blood sugar and HbA1c levels both at 6 and 12 weeks without any significant adverse effects. Reduction in HbA1c was more in diabetics with poor baseline glycemic control compared to those having fair and good control. Overweight and obese diabetics showed significant reduction in BMI at 12 weeks.
Conclusion: Bromocriptine Mesylate is an effective anti-diabetic drug which when added on to existing oral anti-diabetic therapy in uncontrolled diabetes helps achieve optimal glycemic control.

O19
Glycemic variability: a peril of modern insulin therapy among youths with type 1 diabetes?

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Objective: Glycemic variability (GV) predicts severe hypoglycemia and may predict risk for vascular complications in T1D. We tested whether GV increases as a function of coefficient of variation in both mealtime insulin bolus score (BolusCV) and in carbohydrate content of meals/snacks (CarbCV) in a sample of youths with T1D.
Methods: We collected 30-day carbohydrate (Carb), bolus, and blood glucose (BG) data in 298 youths and regressed the two outcome variables: standard deviation (SDBG) and average daily risk range (ADRRBG) of BG, on BolusCV, CarbCV, diabetes duration, and age in two linear models in SAS 9.4.
Results: Mean age and T1D duration were 12.0 ± 3.5 and 4.5 ± 3.4 years, respectively. Means for youths' mealtime bolus score, meal/snack carb content, ADRRBG, and SDBG were 2.2 ± 0.49, 40.8 ± 19.2 g, 44.4 ± 15.5, and 5.8 ± 1.5 mmol/l respectively. All 4 explanatory variables significantly associated with ADRRBG and SDBG after Bonferroni correction (Table 1; $p < 0.004$). A 1-unit increase in BolusCV yielded an estimated average increase of 44.7 (95% CI: 31.7–57.8) in ADRRBG and 4.94 mmol/l (95% CI: 3.68–6.22) in SDBG. A 1-unit increase in CarbCV yielded an estimated average increase of 11.8 (95% CI: 4.2–

Table 1. Multiple regression results

Outcome	Predictor	Beta	SE	DF	*t*	2-sided p	1-sided p	Lower95	Upper95
ADRRBG	BolusCV	44.73	6.63	280	6.74	<0.0001	<0.001	31.67	57.80
	CarbCV	11.80	3.88	280	3.04	0.0026	0.0013	4.16	19.44
	Duration	1.18	0.26	280	4.58	<0.0001	<0.001	0.67	1.68
	Age	−1.27	0.27	280	−4.61	<0.0001	<0.001	−1.81	−0.73
SDBG	BolusCV	89.0	11.60	280	7.67	<0.0001	<0.001	66.19	111.87
	CarbCV	12.06	6.79	280	1.78	0.0768	0.0384	−1.30	25.43
	Duration	2.28	0.45	280	5.08	<0.0001	<0.001	1.40	3.17
	Age	−2.07	0.48	280	−4.31	<0.0001	<0.001	−3.020	−1.12

19.4) in ADRRBG and 0.67 mmol/l (95% CI: −0.07 to 1.41) in SDBG.

Conclusion: Variation in adherence to meal boluses and in carbohydrate content of meals/snacks associates with increased GV. Whether interventions targeting these factors can reduce GV should be studied.

O20

Risk of severe hypoglycemia is associated with treatment regimen not HbA1c in youth with type 1 diabetes – analysis of three contemporary pediatric diabetes registry databases

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Objective: To examine the relationship between treatment regimen and severe hypoglycemia (SH) in a contemporary cohort of patients with Type 1 diabetes (T1D) aged <18 years, using data from the US T1D Exchange(T1DX), German/Austrian DPV and Australian WACDD registries.

Methods: Patients seen in the last 12 months were identified from a collated dataset comprising of 7,102 T1DX, 21,724 DPV and 943 WACDD patients. After determining the number of SH (coma/convulsion) events, SH rates were calculated per 100 patient years and analyzed by registry, treatment regimen and median HbA1c.

Results: Sixty percent of patients were treated with CSII in T1DX, 40% in DPV and 35% in WACDD. Overall, the SH rate per 100 patient years was 7.1, 3.2, and 7.6 in T1DX, DPV and WACDD patients, respectively. After adjusting for sex, age, duration of diabetes and HbA1c%, the SH rate was 26% ($p = 0.005$) higher in T1DX and 35% ($p < 0.0001$) higher in DPV patients on injections compared to CSII (Table), but no significant difference was observed in the smaller WACDD cohort. No significant correlation was found between SH rate and HbA1c by treatment regimen in all 3 registries.

	Injections	CSII	p-value* (adjusted for sex, age, duration and HbA1c%)
T1DX			
Mean HbA1c%	7.8	7.7	
SH rate/100 pt years	3.7	2.5	<0.0001
DPV			
Mean HbA1c%	8.7	8.1	
SH rate/100 pt years	8.3	6.3	0.005
WACDD			
Mean HbA1c%	8.3	7.8	
SH rate/100 pt years	7.6	7.7	NS

Conclusion: The SH rate is higher in patients treated with injections compared to CSII, and does not continuously increase with lower HbA1c% in either regimen. These findings, based on data from large diabetes registries in countries with advanced diabetes care options, support the use of CSII in minimizing SH in pediatric patients with T1D.

O21

Optimal pump settings differ according to age and insulin dose

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Objectives: Knowledge is scarce about optimal bolus guide settings, but may be important in increasing adherence in young persons with diabetes. Our aim was to estimate the carbohydrate factor (CF) and insulin sensitivity factor (ISF) in well-controlled patients.

Methods and material: Medtronic pumps were uploaded at clinical visits and bolus guide settings were linked to HbA1c. Multiple regression analysis was used to explore data. The CF and ISF was calculated using insulin dose*carbohydrate ratio and *sensitivity ratio respectively.

Optimal control was defined as HbA1c <59 mmol/mol and no severe hypoglycemic events.

Results: A number of 108 children (58 males) with HbA1c below 59 mmol/mol, mean age 11.6 (±0.5), diabetes duration 5.1 (±0.4) were included. Insulin dose/kg varied from 0.5 (±0.1) to 0.8 (±0.1). CF varied from 268 in the youngest to 436 in the oldest and the ISF from 100 to 121, the highest level in 6–12 years old. Using backward elimination age, insulin dose and number of boluses per day were significantly associated with CF. Age, pump duration, insulin/kg and percentage of insulin as boluses were associated with ISF.

Conclusion: In children with an optimal metabolic control age, pump duration, use of pump and insulin dose all influence the bolus-guide settings. Calculation factors vary with age and total insulin dose, and therefore age and insulin dose dependent calculation factors are needed. There is a lack of intervention studies aiming at optimizing bolus guide settings during child growth.

O22

Toddlers and preschool children with type 1 diabetes: a snap shot of CSII settings in a contemporary population based service in Western Australia

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Introduction: Achieving optimal glycaemic control in toddlers and preschool children with T1D is challenging. Despite increasing insulin pump use in this age group there is a paucity of evidence to guide pump settings when starting pump therapy in this population.

Objective: To describe the contemporary insulin regimens and characterise CSII settings of children aged less than 5 years with T1D in Western Australia (WA).

Methods: All children in WA, with T1D, aged <5 years were included in the study. Data was extracted from the Western Australia Children's Diabetes Database.

Results: Our cohort included 40 children with T1D (19F, 21M), mean age 3.7 years (range 1.1–4.9 years) and mean duration of T1D of 1.43 years (range 0.1–3.8 years). Mean total daily dose (TDD) of insulin was 0.6 U/kg/day (range 0.2–1.4 U/kg/day). 18/40 (45%) children were on CSII. Median HbA1c for CSII, MDI and BD regimens were 7.35%, 7.45% and 7.6% respectively.

For CSII cohort, mean TDD of insulin increased from 0.44 U/kg/day at pump start, to a current mean TDD of 0.56 U/kg/day. Basal insulin dose as % of the TDD decreased from 48% at pump start to 41%. Mean Insulin to Carbohydrate Ratio (ICR) × TDD

was 485 at pump start (ICR's calculated by dividing 500 by the TDD, known as "500 rule"), compared to 290 using present settings.

Conclusion: In our <5 year-old cohort patients on CSII had a lower median HbA1c than patients on injection regimens. This study provides guidance on rates and ICR for commencing children <5 years on CSII. We recommend use of a "300" rule instead of a "500" rule when calculating ICRs at pump initiation. Further research will assist in making recommendations specific to CSII use in toddlers and preschool children with T1D. This is paramount to ensure that these very young children are launched on a trajectory of optimal long-term glycaemic control.

Diabetes Oral III Diabetes Education, Exercise and Nutrition

O23

The impact of the quantity of protein in a meal on postprandial blood glucose levels in individuals with type 1 diabetes mellitus using intensive insulin therapy

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Objectives: Dietary protein increases the postprandial glycaemic response and insulin requirements in individuals with type 1 diabetes mellitus (T1DM). Previously, we demonstrated that ≥75 g of protein alone produced a late glycaemic response comparable to 20 g of carbohydrate (CHO). The aim of this study was to determine the effects of varying quantities of protein when consumed with 30 g of CHO on blood glucose levels (BGL's) in individuals with T1DM using intensive insulin therapy (IIT).

Methods: 14 subjects (7 male) with T1DM (HbA1c <8.1%/65 mmol/mol) aged 7–40 years were recruited. Over 5 days subjects were fed 5 test meals containing 30 g pure glucose with 0, 12.5, 25, 50 and 75 g of protein (whey protein isolate) in randomised order. Insulin was given for 30 g CHO 15 min prior to consumption. Postprandial glycaemia was measured using 5 h of continuous glucose monitoring.

Results: There was a dose dependent effect of the protein on postprandial glycaemia. This effect was statistically significant at all-time points beyond 135 min. When protein meals were examined individually and compared with CHO only (0 g protein) meals, the glycaemic excursion from 75 g of protein at 300 min was significantly higher than 0 g protein (5.35 mmol/l vs. −0.02 mmol/l; $p < 0.001$). Importantly, 75 g protein resulted in a delayed and sustained glycaemic excursion, commencing approximately 2 h postprandially and continuing for at least 5 h. The postprandial glycaemic excursion of 50 g of protein was statistically significantly higher than 0 g of protein at 225 min.

Conclusions: 75 g of protein when consumed with 30 g of CHO significantly increases postprandial BGL's in individuals with T1DM using IIT. This study demonstrates that the postprandial glycaemic impact of protein is higher when consumed with CHO than when the same amount is consumed alone. These findings have significant clinical implications for insulin dosing for protein and further studies are needed to determine this.

O24

Evaluation of a social media site to improve diabetes glycaemic control, knowledge and self-efficacy: the ASSIST study

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Objectives: To assess the use of a social network site as a resource-light intervention medium to increase diabetes knowledge, self-efficacy and glycaemic management of adolescents with T1DM.

Methods: 73 adolescents (44 male, mean age 14.3 ± 1.0 years, T1DM >12 months) were randomised into 2 arms (42 intervention, 31 control). The intervention arm was engaged in closed Facebook groups allowing them direct contact with both their diabetes team and peers with diabetes. They were engaged in semi-structured educational and behaviour-change conversation threads based around diabetes related topics over a 3 month period. HbA1c, diabetes knowledge (assessed by the ADKnowl across 8 domains) and diabetes self-efficacy (assessed by the Self-efficacy for Diabetes scale) were measured at baseline (T1), 4 (T2), 8 (T3), and 12 months (T4).

Results:

ADKnowl Domain	Group	T1 Score	T2 Score	T3 Score	T4 Score
Diabetes Treatment/ Testing	Intervention	84.9 (19.9)	95.6 (10.8)	90.5 (17.5)	93.7 (13.2)
Diabetes Treatment/ Testing	Control	89.1 (13)	86.3 (14.5)	83.7 (16.2)	86.8 (15.7)
Diabetes Complications	Intervention	75.5 (17.1)	87.1 (9.9)	85.1 (12.7)	87.1 (14.2)
Diabetes Complications	Control	81.9 (15.9)	86.2 (8.8)	85.2 (12)	86.6 (12.3)

ADKnowl scores in both the (a) diabetes treatment/testing and (b) reducing risk of complications domains were significantly increased in the intervention group relative to the control group at 4 months ($p = 0.001$; $p = 0.028$). Intervention group score increases were also seen in both the (c) diet/food and (d) insulin management/use domains but not to a statistically significant level ($p = 0.06$; $p = 0.073$). Differences in knowledge scores were maintained at 8 months and 12 months for the diabetes treatment/testing domain but not for the diabetes complications domain. There were no statistically significant differences in HbA1c or self-efficacy for diabetes between the groups at 4, 8 or 12 months.

Conclusions: Social network sites provide a valuable and resource-light intervention medium for increasing and maintaining diabetes-specific knowledge in adolescents with T1D.

O25

Dietary proteins contribute to the insulin dose required to maintain post-prandial euglycaemia in type 1 diabetes (T1D)

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Objectives: Meals high in protein, independent of fat content, have been shown to prolong postprandial hyperglycaemia in individuals with T1D. The quantity and distribution pattern of the additional insulin required is unclear. Usual clinical practice is to determine insulin dose only on the carbohydrate content of the meal. The aim of this study was to use a novel variation of the insulin clamp technique to determine the insulin requirement for a high protein meal (HP) compared to a of low protein meal (LP), controlling for carbohydrate and fat.

Methods: Three subjects with T1D >1 year, aged 12–21 years have been studied in this collaborative RCT. Subjects attend for 2 days and are randomised to HP meal (60 g) or LP meal (5 g), each with 30 g CHO and 8 g fat. On both days IV insulin infusion is titrated to stabilise BGL in euglycamic range for at least two hrs prior to test meal. Five to fifteen minute blood sampling allows frequent adjustments to the insulin infusion to maintain BGL between 4 and 8 mmol/l for the five hour postprandial period.
Results: To date three of the proposed fifteen subjects have completed the study. Results have demonstrated that in the five hours following the HP meal insulin requirements were 136, 91 and 128% of the total insulin required for the LP meal.
Conclusions: Study findings indicate that the novel clamp technique using rapid insulin rate adjustments used in this study will be successful in determining the pattern of increased insulin requirements on consumption of a HP meal when compared to a LP meal.

O26
Diabetes ambulatory service: lessons learned from health care professionals' point of view

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Objectives: A RCT was conducted to compare inpatient (n = 25) with ambulatory care and education (n = 25) for children newly diagnosed with Type 1 Diabetes (T1D). The average stay for the inpatient and ambulatory group was 5 and 2 days. Ambulatory participants received Hospital in the Home (HiTH) visits for 2 days post-discharge for support with injections. They were visited at home 3 times by a diabetes nurse and either a dietitian or social worker. The aim was to identify lessons learned and utilise these in future ambulatory programs.
Method: At the end of the trial, an anonymous questionnaire was circulated to 26 Healthcare professionals (HCPs) involved in the home visiting program. The survey included questions on duration of education, staff safety, staff knowledge and confidence, patient safety, and the pros and cons of early hospital discharge and delivering education in the home. Anecdotal learnings were documented weekly.
Results: HCPs (90%) felt they gained better insight into participants' learning ability, diet and activities. HCPs (73%) found that parents appeared to be more comfortable asking questions related to their child's diet and activity, and 81% felt participants were more receptive in the home environment. Combined HCP visits created an opportunity to problem solve the complexities of diabetes management. HiTH nurses (88%) felt patients were safe in diabetes management at home.

Challenges included staff lacking confidence in explaining the program to parents, inconsistent messages from HCPs, scheduling of visits, availability of both parents, distractions at home, not having a suitable place for education at home and distance of travel. Communication and collaboration between departments was important.
Conclusion: All HCPs involved found the home visits to be beneficial for the participants. The lessons learned and recommendations will be utilised in developing an ambulatory diabetes program and can be applied to other ambulatory models of care.

O27
A new pediatric diabetes knowledge test: instrument development and factor analysis

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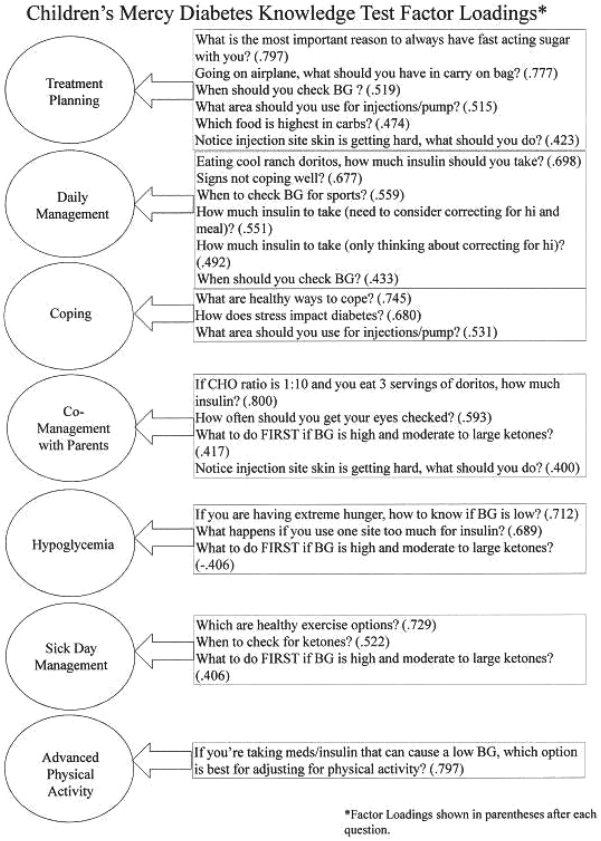
Objectives: Youths with type 1 diabetes (T1D) must acquire significant knowledge to effectively manage their T1D. Previously developed knowledge tests for youths have not incorporated modern changes in therapy. We are developing a measure of T1D knowledge that is aimed at youths and is based on contemporary management standards.
Methods: An initial 88-item test was derived from the American Association of Diabetes Educators 7 Self-Care Behaviors. Next, a multidisciplinary team selected the best 49 items which were piloted in a sample of 107 youths (59 males, ages 12–18, having a mean HbA1c of 85.5 mmol/mol). Items were all four-response multiple choice.



[CMH Diabetes Knowledge Test Factor Loadings]

Results: Point-biserial correlation coefficients for items revealed the 21 most discriminating items. Factor analysis of these items yielded 7 factors that explain 61% of question variances. Factors were associated with the latent variables: treatment planning, co-management with parents, problem solving, managing hypoglycemia, advanced physical activity, complication reduction, and healthy lifestyle.
Conclusion: A new T1D knowledge test for youths was refined from 88 to 21 questions based on expert opinion, empirical test construction, and factor analysis. Our next goals are to validate this 7 factor

model with another cohort and confirm concurrent validity based on youths' HbA1c and adherence behaviors. However, our new T1D knowledge measure appears to be initially valid and promising as a new clinical and research tool.

O28

Strengths, risk factors, and resilient outcomes in adolescents with type 1 diabetes (T1D): results from diabetes MILES Youth – Australia

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Objective: Despite the challenges of living with T1D, many adolescents achieve 'resilient outcomes': good adherence and quality of life (QOL), and in-range glycemic control (A1c). T1D strength behaviors (e.g. seeking support, communicating calmly, expressing confidence) are associated with resilient outcomes, yet the combination of risks and strengths in relation to resilient outcomes is unclear. We aimed to investigate relations among T1D strengths and resilient outcomes across psychological and family risk levels.

Method: 514 adolescents with T1D (age 13–19 years, M = 15.7 ± 1.9; T1D duration 6.9 ± 4.2 years; 38% male; 53% insulin pump) completed a national cross-sectional survey about their T1D strengths, risks (depressive/anxiety symptoms, family conflict), and resilient outcomes (blood glucose monitoring [BGM] frequency, QOL, A1c).

Results: More T1D strengths were related significantly (all $p < 0.001$) to the 3 resilient outcomes: more frequent BGM ($r = 0.39$), higher QOL ($r = 0.49$), and lower A1c ($r = -0.33$), and to lower risks: fewer depressive [$F(4,468) = 29.0$] and anxiety [$F(3,469) = 29.6$] symptoms and less conflict ($r = -0.29$). In multivariate regressions, T1D strengths consistently related to all 3 resilient outcomes, even with significant risk factors included in the models. Strength-risk interactions did not relate significantly to resilient outcomes.

Conclusions: In a large sample of Australian adolescents, T1D strengths were strongly related to key resilient T1D outcomes, even in the presence of well-documented psychological and family conflict risk factors. Null interactions suggest behavioral strengths may be too closely related (inversely) to behavioral/psychological risks to effectively buffer their impact. More research is needed to determine whether strengths reduce or buffer other types of risks. Nevertheless, given associations with adherence, QOL, and A1c, monitoring and enhancing T1D strengths may support resilience promotion during a vulnerable developmental period.

O29

Exercise is associated with glycemic control and QoL in young persons with type 1 diabetes (T1D): the Global TEENs study

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Objectives: TEENs is the largest worldwide (20 countries), contemporary, cross-sectional observational T1D study ($N = 5887$) in 8–25 year old (y/o) patients. The influence of exercise on A1c and Quality of Life (QoL) is reported.

Methods: 219 centers collected data by interview, record review and survey from 3 age groups: 8–12 y/o, 13–18 y/o and 19–25 y/o. A1c was measured uniformly using A1cNow™ (Bayer); A1c targets were defined as <7.5% (58 mmol/mol) for ≤18 y/o (ISPAD) and <7% (53) for 19–25 y/o (ADA). QoL was measured by PedsQL™ 3.0 Diabetes. Factors associated with A1c and QoL were identified by multivariate linear regression, controlling for age group and region.

Results: Mean A1c was 8.5 ± 1.8% (69 ± 20 mmol/mol); A1c target was met in only 32% of 8–12, 29% of 13–18 and 18% of 19–25 y/o. Regular exercise was performed more often in the younger age groups (Table). In a multivariate analysis, exercising at least 30 min, 3–7 days/week (vs. 0–2 days/week) was significantly associated with lower A1c by 0.28% (adjusted means: 8.9% vs. 9.2%, $p < 0.01$), and with higher QoL(adjusted QoL means: 66.7 vs. 64.5, $p < 0.01$).

Conclusions: One potentially modifiable lifestyle factor (exercise) was significantly associated with lower A1c and higher QoL. Encouraging exercise as a routine part of diabetes management could affect both A1c and QoL positively and can potentially be implemented globally, even in resource restricted areas.

Study sponsored by Sanofi

	8–12 y/o (*N* = 1715)	13–18 y/o (*N* = 2846)	19–25 y/o (*N* = 1326)	Overall (*N* = 5887)
Number of days per week spent exercising*, *n* (%)				
N	1687	2812	1301	5800
0–2	554 (32.8)	1062 (37.8)	576 (44.3)	2192 (37.8)
3–4	518 (30.7)	811 (28.8)	337 (25.9)	1666 (28.7)
5–7	615 (36.5)	939 (33.4)	388 (29.8)	1942 (33.5)

*At least 30 min doing any physical activity or exercise.
[Exercise frequency according to age]

O30

Diabetes Community Care Ambassador Program: a feasibility report

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Objectives: To assess the feasibility of the Diabetes Community Care Ambassador (DCCA) Program, a pilot study designed to improve the health of children with poorly controlled diabetes by extending the clinical care model into the home, school, and community.

Methods: Both participant and caregiver were required to consent and engage in the intervention for 9 months. DCCAs were non-medical staff hired from within the local community and trained extensively in diabetes self-management. DCCAs worked closely with the participant's primary diabetes team. Each DCCA assessed and coached families and school caregivers on self-management skills through home visits, school visits, and community support groups. Caregiver experience with the program was evaluated using an adapted CAHPS® survey.

Results: Of 342 eligible participants (T1D patients ages 3–19, A1C ≥8.5% and/or ≥1 episodes of DKA in last year, duration of diabetes ≥1 year, English or Spanish-speaking), 109 enrolled (32%) and are reflective of our patient population (1/3 public insurance, 2/3 white race). Retention for families that had an initial home visit is currently 85% (n = 76/89). The 28 caregivers that have completed the program rated their DCCA highly on a 0–10 scale (9.71 ± 0.46 SD). On a 4-point composite scale, caregivers felt DCCAs communicated effectively with patients (3.91 ± 0.17 SD). 100% of caregivers felt that the DCCA was caring, inspired trust, and would recommend DCCA to other families.

Conclusions: The enrollment rate suggests that the intervention is feasible, though the lower than anticipated number may reflect the intensity of the intervention. The retention rate indicates high engagement. This, along with the overwhelmingly positive caregiver experience, suggests that DCCAs working in the community can be effective members of the diabetes care team. At the study's conclusion, the impact on A1C, quality of life, cost-effectiveness and medical-legal issues will also be evaluated.

Diabetes Oral IV Epidemiology and Other Diabetes

O31
Genetic risk for co-occurrence of type 1 diabetes and celiac disease is modified by HLA-C and killer-immunoglobulin like receptors

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Objective: The prevalence of celiac disease (CD) in type 1 diabetes (T1D) has been reported to be 5–7 times higher than in the general population. Risk factors for co-occurrence of both diseases have not been entirely established. The human killer cell immunoglobulin-like receptors (KIRs) are expressed on NK cells. HLA class I molecules are ligands for most inhibitory and some activating KIRs and are in this way key regulators of NK cell activity. The aim of our study was to analyze possible impact of HLA class I and KIRs on the co-occurrence of T1D and CD.

Methods: We analyzed 67 patients with T1D, 68 patients with CD, 69 patients with both diseases and 130 controls. PCR-SSO and PCR-SSP molecular typing was used for the HLA and KIR gene determination. Statistical analysis was based on two tailed Fischer's exact test with corrections for multiple testing.

Results: In the group of patients with coexisting diseases the presence of HLA-C*07 ($p = 8.65 \times 10^{-3}$) and HLA-B*08 ($p = 0.03$) but not HLA-A*01 increased the susceptibility. There was increased frequency of C1 KIR ligand (OR = 21.23, $p_c = 3.67 \times 10^{-5}$). Analysis of the combinations of KIRs with ligands revealed the positive association of the weak inhibitory combination KIR2DL3-C1 ($p_c = 1.97 \times 10^{-4}$) with the co-occurrence of both diseases. When the C1 was analysed in combination with its stronger inhibitory receptor KIR2DL2 no association was observed.

Conclusions: The influence of class I alleles was observed only in patients with coexisting diseases. C*07, contributing C1 ligand could have an impact on the innate immunity rout of this susceptibility. The significantly higher frequency of the weak inhibitory combination, namely C1-KIR2DL3 observed in patients with coexisting diseases supported this hypothesis. Predominantly weak inhibition in patients with coexisting diseases could lead to a NK cell response, making them vulnerable for developing more than one autoimmune disease.

O32
Serum miRNA profiles as biomarkers of HNF1B-MODY

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Transcriptional regulation of miRNAs coded by non-intronic genomic sequences is unclear and we hypothesized that transcription factors may be implicated in this process. This may result in altered profiles of serum miRNAs in forms of MODY caused by mutations of transcription factor genes.

The study was performed on two groups of subjects. The Polish cohort ($N = 60$) consisted of 11 patients with *HNF1B*-MODY, 17 with *HNF1A*-MODY, 11 with *GCK*-MODY, an HbA1c-matched type 1 diabetes (T1DM) group ($n = 9$) and 10 healthy controls. As a validation group, 61 clinically matched British patients were used mirroring the groups in the Polish one. The Polish group underwent miRNA serum levels profiling with qPCR arrays (Exiqon, Denmark) to identify differentially-expressed miRNAs. Validation was performed using qPCR. To determine whether serum expression reflects alterations at cellular level, we quantified miRNA levels in HepG2 and HEK293 cells with si-RNA knockdowns of *HNF1A* or *HNF1B*.

Significant differences (adjusted $p < 0.05$) were noted for 11 miRNA. Six of them differed between HNF1A-MODY and HNF1B-MODY, and amongst those four (miR-24, miR-27b, miR-223, and miR-199a) were confirmed to show HNF1B-MODY specific expression levels in the validation group. In all four cases the miRNA expression level was lower in HNF1B-MODY than all other tested groups. Areas under the ROC curves for all four differentially expressed miRNAs were between 0.79 and 0.86 with sensitivity and specificity reaching 91.7% (miR-24) and 82.1% (miR-199a) respectively. Cellular expression pattern of miRNA expression was consistent with their serum levels as they all were significantly higher in *HNF1A*- than in *HNF1B*-deficient HepG2 cells and in mock transfection controls than in *HNF1B*-knockdown HEK293 cells alike.

In summary, we identified four miRNAs that seem to be specifically dependent on *HNF1B*-transcriptional control. Serum expression levels of those miRNAs show promise as sensitive and specific biomarkers.

O33

HbA1c associated to increased mortality in a Danish cohort of young patients with type 1 diabetes followed for 25 years: the Danish cohort of pediatric diabetes 1987 (DCPD1987)

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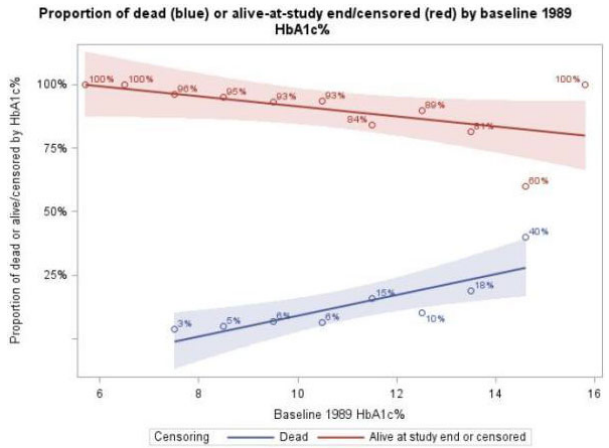
Objective: Type 1 Diabetes (T1D) has been associated with a higher mortality compared to the general population. The aims of the present study are to determine the mortality rate in a Danish cohort of patients diagnosed with diabetes in childhood and compare these to the general population.

Methods: In 1987 and 1989 we included 884 and 1020 children and adolescents aged 19 years or less, respectively, attending the outpatient clinics at pediatric departments in Denmark. This corresponded to 75% of all Danish children and adolescents with T1D. Those who had participated in both investigations were followed clinically in 1995 and 2011. Patients were followed until January 1, 2015 using the nationwide Danish CPR-registry on death certificates and emigration data. Survival analysis was performed using Cox proportional hazard. Covariates were selected after forward and backwards stepwise regression ($p = 0.05$).

Results: Of the 720 patients who participated in both 1987 and 1989, HbA1c was obtained in 718 patients (53.3% men). In 1989 mean age was 14.0 years (range 4.4–20.3 years) and mean diabetes duration 6.4 years (range 1.9–17.6 years). Median HbA1c was 9.2% (range 5.0–15.9%), 9.6% (range 5.7–15.8%) and 9.6% (range 5.7–15.8%) in 1987, 1989 and 1995, respectively. Mean observation time was 25.0 years (range 0.2–26 years). During the 25 years of follow-up 49 (6.8%) out of 718 patients died. Baseline HbA1c % (1989) was the only significant covariate to predict death resulting in a hazard ratio of 1.4 (95% CI 1.2–1.6, $p < 0.0001$). Assuming a linear effect, this suggests a 40% higher mortality rate per 1% increase in HbA1c.

Conclusion: The best predictor for increased risk of death up to 25 years after inclusion in the study was the HbA1c level in 1989. This illustrates the importance of an excellent glycemic control among young T1D patients and should encourage clinicians to stress the importance of a tight diabetes control to their patients.

[Proportion of dead by HbA1c%]



1989 HbA1c %	6	7	8	9	10	11	12	13	14	15	16	Total
Dead (number)	0	0	4	8	11	8	10	3	3	2	0	49
Alive/Censored (number)	8	49	99	152	149	116	53	26	13	3	1	669
	8	49	103	160	160	124	63	29	16	5	1	718

O34

Measuring changes in glucose rate of appearance in patients with diabetes

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Objectives: To understand the effects of diabetes therapies, it is critical to be able to measure the contributions of inadequate insulin secretion and insulin resistance (IR) to hyperglycemia. Hepatic IR can be assessed by the glucose rate of appearance (Ra) during a multi-stage hyperinsulinemic euglycemic clamp with isotopic tracers, and expressed as

(1) percent suppression of basal Ra or
(2) insulin concentration needed to suppress 50% of basal Ra.

However, these measures require accurate measures of basal Ra, which is problematic in subjects requiring overnight exogenous insulin. We present two methods for analyzing changes in Ra in insulin-requiring patients.

Methods: Twelve youth 12–18 years of age (4 control, 4 T1D, 4 T2D) were recruited from pediatric clinics at Children's Hospital Colorado and the Barbara Davis Center for Childhood Diabetes for a 3-stage hyperinsulinemic euglycemic clamp with stable glucose isotopes. Glucose Ra was calculated using Steele equations and the relationship between Ra and insulin was modeled using two statistical methods:

(1) the standard two-stage (STS) algorithm and
(2) mixed-effects models (MEM).

Results: Glucose Ra at baseline and stage 1 was similar across groups. In stage 2, glucose Ra decreased in controls, with minimal change in T1D and T2D. During stage 3, glucose Ra in T1D and T2D decreased but was still elevated relative to controls. For STS and MEM, quadratic regression models were the best fit. STS parameter estimates were more variable than MEM. Using MEM, T1D and T2D were significantly different from controls ($p = 0.0044$, $p = 0.0037$). Using STS, there were no differences between groups.

Conclusions: The STS and MEM methods can be used to describe the relationship of Ra and insulin in individuals with diabetes, and gave discrepant results when applied to our dataset. Advantages and disadvantages of each will be discussed.

O35

Clinical risk factors for psychiatric disorders in young adults with childhood onset T1DM: insights from 20 years of follow up

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Objective: To examine the association of clinical risk factors with incidence of psychiatric disorders in a population-based setting.
Methods: The Western Australian (WA) paediatric diabetes database was established in 1987 and serves a population of 2.4 ml. Patient data is collected at 3 monthly clinic visits. All patients in the database with T1DM, aged ≥18 years at Dec 2011, were included. The WA Mental Health Information System (MHIS) contains coded records for WA inpatient hospitalisations and community-based psychiatric services. MHIS records from 01/1992-01/2012 were linked to the T1DM cohort. Cox regression models were used to generate hazard ratios HR (95% CIs) for clinical risk factors.
Results: Of 1316 eligible patients (50.4% males, 34,547 person-years (PY) of follow-up), 366 had at least one record in the MHIS. Overall incidence rates were 2.72/1000 PY for broad affective disorders (including depression) (n = 94), 3.85/1000 PY for anxiety disorders (n = 133), 0.41/1000 PY for eating disorders (n = 14), 0.49/1000 PY for adult personality and behaviour disorders (n = 17) and 0.35/1000 PY for substance dependencies (n = 12).

Glycaemic control (mean paediatric HbA1c) was not associated with eating disorders or substance dependencies. The HR for an increase in mean HbA1c of 1% was 1.38 (1.20, 1.59) for anxiety disorders, 1.49 (1.28, 1.74) for broad affective disorders and 1.61 (1.10, 2.34) for adult personality and behaviour disorders. Age at T1D diagnoses was associated with a reduced risk, HR 0.94 (0.90, 0.98) per year older at diagnosis, of anxiety disorders; the direction of effect was consistent for all other psychiatric disorders but significance was not reached. No association was observed with history of severe hypoglycaemia.
Conclusions: Psychiatric disorders affect a large proportion of people with childhood onset T1DM. Poor glycaemic control and female sex may be important risk factors associated with the onset of psychiatric disorders.

O36

Reduced incidence of severe hypoglycemia and changing trends in treatment of type 1 diabetes (T1D) in children the last 12 years in Norway – a nationwide study

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Objectives: To assess the rate of severe hypoglycemia (SH) defined as having seizures and/or unconsciousness, and the relationship to HbA1c and changing mode of treatment.
Methods: Standardized examinations from 96% of all Norwegian with T1D age 0–14 years from 2001 to 2013 were reported annually to the Norwegian Childhood Diabetes Registry (NCDR).
Results: The study population included 3299 individuals (51% boys) with T1D, representing 14,041 person-years (PY). Mean age at diabetes onset was 6.3 years (range 0.2–13.9), mean diabetes duration was 4.7 years (1.0–14.5). 1054 events of SH were reported. Annual grand mean HbA1c varied between 8.0–8.6%. The incidence of SH decreased significantly from 2001 with 14.4 pr 100 PY to 4.2 pr 100 PY in 2013 ($p < 0.001$). There was no significant reduction in mean HbA1c. The use of insulin pens decreased from 53.1% to 26.2%, and use of long acting insulin analogues increased from 1.5% to 78.5%. Continuous subcutaneous insulin infusion (CSII) increased from 6.7% to 73.2% ($p < 0.001$) and use of premixed insulin declined from 36.7% to 0.1% ($p < 0.001$). Mean self-monitoring of blood glucose (SMBG) increased from 3.6 to 6.6 a day ($p < 0.001$). Overall the group using CSII had 6.5 SH pr 100 PY, whereas the MDI group had 7.7 pr 100 PY ($p = 0.01$). The group using premixed insulin had 14.1 SH pr 100 PY ($p < 0.001$). Those performing SMBG >5 times a day had 7.0 SH pr 100 PY compared to 9.0 in the group performing <5 SMBG a day ($p < 0.001$).

Stratifying the population in good metabolic control (HbA1c <7.5%), moderate control (7.5–9.0) poor control (>9.5) significantly more patients had good control in 2013 compared to 2001 ($p < 0.001$). There was no significant difference in SH within these categories.
Conclusion: In Norway during 2001–2013 there has been a shift in diabetes treatment towards CSII, long-acting insulin analogues and closer glucose monitoring. In the same period events of SH have been reduced by 70%.

O37

1,5-Anhydroglucitol, a discriminatory gas chromatography-mass spectrometry (GC-MS) metabolomic marker in adolescents with type 1 diabetes- quantified using the plasma Glycomark® assay

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Objective: Assess plasma 1,5-Anhydroglucitol (1,5-AG) by the quantitative GlycoMark® assay in adolescents with type 1 diabetes (T1D) and controls after identifying this discriminative metabolomic marker using semi-quantitative GC-MS profiling.
Methods: We reported a case control study in a tertiary paediatric hospital: 27 (14F) adolescents with T1D (age (median, interquartile range) 15.5, 14.7–16.4 years; duration 7.7; 6.0–11.8 years; HbA1c 9.1, 8.1–10.1%); glucose 13.35 (7.60–17.85) and 27 (14F) controls (age 15.1, 14.4–16.8 years). BMI was <95th percentile.

Fasting plasma and urine metabolomes were profiled by GC-MS and compared between cohorts (1). The molecule most influential in separating the groups was 1,5-AG (identified by an open profiling approach, without any bias or specific targeting).

1,5-AG is a short-term marker of glycaemic control (7–14 days) that competes with glucose for renal reabsorption. Otherwise stable levels of 1,5-AG are depleted as blood glucose exceeds the renal threshold.

Plasma 1,5-AG was subsequently quantified using a two-step enzymatic assay (GlycoMark®).

Statistics Univariate: Mann–Whitney *U*-test, Spearman correlation. Multivariate: PCA, OPLS-DA, OPLS.

Results: Plasma 1,5-AG measured by GlycoMark® (μg/ml) was 0.48, 0.10–1.66 and 24.50, 21.60–31.15 (median, interquartile range) for T1D ($n = 26$) and control ($n = 27$) participants respectively. Levels were significantly lower in the T1D group ($p < 0.0001$). T1D group values are overestimated (8 values below the assay limit of 0.1 μg/ml). Plasma 1,5-AG measured by GlycoMark® was also more influential on group classification than fasting glucose or HbA1c.

Conclusion: Plasma 1,5-AG measured by the quantitative GlycoMark® assay, like the semi-quantitative GC-MS result, was more influential on group classification than fasting glucose or HbA1c. The metabolic profile of adolescents with diabetes appears to be influenced by short term (7–14 days) hyperglycaemia.

(1) Conwell LS et al. APPES/APEG 2014

O38
The Australasian diabetes data network (ADDN): first national audit data

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Objectives: Other national registries have found many children with type 1 diabetes (T1D) are not meeting the ISPAD target of <7.5%. In addition, frequency of continuous subcutaneous insulin infusion (CSII) use varies by age-group internationally. We report the first national data on HbA1c, treatment regimen and BMI SDS in Australian young people with T1D.

Methods: ADDN, funded by the Juvenile Diabetes Research Foundation Australia, is a nationwide longitudinal diabetes registry of data pooled every 3 months from large and geographically diverse paediatric diabetes centres in Australasia.

Results: The sample includes $n = 2570$ participants, 51% male, mean age 12.7 years and mean duration of diabetes 5.6 years from 4 centres in NSW, WA, QLD and VIC. The use of CSII, multiple daily injections (MDI) or twice daily injections (BD), HbA1c, % meeting target HbA1c <7.5% and mean BMI SDS are reported for the group and by age-group.

		Age Group (*n*)			
	Sample *n* = 2570	<6 years (152)	6–10 years (474)	10–14 years (831)	14–18 years (1113)
%CSII	39	32	34	41	39
%MDI	38	28	20	34	49
%BD	21	38	43	20	10
Mean HbA1c	8.1	7.8	7.6	8.1	8.4
Median HbA1c	7.9	7.8	7.6	7.9	8.1
% HbA1c <7.5%	34	39	46	35	29
Mean BMI-SDS	0.9	1.3	0.9	0.7	0.9

[Treatment Regimen, HbA1c and BMI-SDS by Age Group]

Conclusions: This audit of national data demonstrates that more than half of young people are not meeting glycaemic targets, particularly adolescents. Only ~1/3 of young people are using CSII; this is likely to be due to access impeded by the financial cost of insulin pumps in Australia. Young people with T1D are overweight, in keeping with national rates of overweight/obesity of ~25%. The addition of additional sites in Australian and New Zealand will enable exploration of factors associated with variation in glycemic targets and therapeutic regimens.

Endocrine Oral Session

O39
Understanding the role of introns in growth

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Objectives: Meta-analyses of genome-wide association studies (GWAS) have highlighted genetic variation in many disorders affecting adult height, infant length, or pubertal growth. 3-M Syndrome is a disease of development, marked by severe prenatal and postnatal growth retardation, resulting in very short adult height. For example, analyses of 3M Syndrome have identified changes in CUL7 as causing the disease.
Methods: We performed a novel analysis of 164 study cohorts comprising 893 GWAS variants that were significantly linked to adult height, infant length, and/or pubertal growth. These variants were analysed for:

1) 3D spatial (physical) connections (GWAS3D, as captured by proximity ligation);
2) accessibility to transcription factors (DNAse Hypersensitivity Sites; UCSC database); and
3) linked gene expression (eQTL analysis; Genevar 3.3.0).

Results: Despite the importance of CUL7 in 3-M syndrome, no GWAS variants that are significantly associated with growth and development have been published in or within 1 Mb of the gene. By contrast, variants have been found in intronic regions of FBXW11. 3D spatial genomic analysis reveals that there is a spatial connection between the FBXW11 and CUL7 genes on chromosomes 5 and 6, respectively. Functionally, the FBXW11 variants all reside within DNase sensitive sites. eQTL analysis reveals that FBXW11 variants have no significant effect on FBXW11 expression, but instead one FBXW11 variant significantly effects expression of CUL7 in a Kenyan population.
Conclusions: Our novel results provide evidence that GWAS variants in FBXW11 are functionally and spatially associated with the CUL7 locus. Furthermore, this supports the hypothesis that GWAS findings outside of gene coding regions (intronic) play important roles in gene regulation through 3D genomics, thus helping to shift the paradigm for understanding the underlying genetics influencing development and adult height.

O40
[18F]-DOPA PET/CT imaging in congenital hyperinsulinism – first 5 years of the Australian experience

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In the southern hemisphere, [18F]-DOPA PET/CT (Positron Emission Tomography/Computed Tomography) of the pancreas became available in Brisbane, Australia in early 2010. This may enable pre-operative distinction of focal and diffuse forms of Congenital Hyperinsulinism (CH) without the challenges of travel to an overseas centre.

Objectives: Determine the clinical utility of [18F]-DOPA PET/CT in distinguishing focal from diffuse disease in patients with CH.
Methods: Case records were reviewed for clinical details, metabolic and genetic investigations, PET/CT result, histology (if surgery performed) and clinical outcome.
Results: Fifteen PET/CT scans had been performed. Patients were 3 weeks to 4 years of age, with 10 patients ≤6 months of age.

Eight patients had genetics suggestive of focal disease (5 with paternal *ABCC8* and 3 with paternal *KCNJ11* mutations), of which PET/CT indicated focal disease in 5 (2 in head, 2 in head / uncinate process, 1 in neck). Focal lesions were identified in all four who had surgery (3 paternal *ABCC8*, 1 paternal *KCNJ11* mutations). Medical support was able to be withdrawn for these patients.

Seven patients had genetics suggestive of diffuse disease (2 homozygous *ABCC8*, 1 heterozygous *GLUD1*, 1 mosaic whole genome uniparental disomy) or non-informative genetics (1 heterozygous de novo *ABCC8*, 1 negative for *ABCC8*, *KCNJ11*, *GLUD1*, *GK* and *HNF4α*). Diffuse histology was confirmed in the 2 infants who had surgery (near-total pancreatectomy).
Conclusions: In the 6 patients who proceeded to surgery, PET/CT scanning demonstrated 100% accuracy in discriminating focal from diffuse disease. The accuracy of PET/CT cannot be confirmed by histology in the remaining 9 patients who are medically managed.

PET/CT scanning was instrumental, in combination with genetic background, in facilitating targeted resection of focal lesions and enhancing confidence in performing a near-total resection in those with diffuse disease.

O41
Increased levels of placenta-derived exosomes within maternal circulation is associated with a higher susceptibility in developing gestational diabetes mellitus in obese women

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Objectives: We recently established that release of placental exosomes increase across normal gestation and placental cells cultured under diabetic conditions altered the release and bioactivity of exosomes. The aim of this study was to test the hypothesis that placenta-derived exosome concentration is higher in obese women who develop GDM compared to normal pregnancies.
Methods: Women were recruited with informed written consent from the Ochsner Baptist Medical Center (New Orleans, USA). Groups were classified according to the body max index (BMI) in two categories: lean (n = 6, BMI 18.5–24.9 kg/m^2) and obese (n = 6, BMI >30 kg/m^2) who develop GDM. Exosomes were isolated from the plasma by differential and buoyant density centrifugation. The total number of exosome vesicles and specific placenta-derived exosomes were determined by quantifying immunoreactive exosomal CD63 and placental PLAP markers using the ELISA kit and nanoparticle tracking analysis NanoSight NS500).
Results: Exosome concentration was 2-fold greater in obese-GDM than lean-GDM women ($p < 0.05$). In obese-GDM women, the

number of exosomes present in the maternal plasma ($1.2.34 \times 10^9 \pm 3.0 \times 10^8$ particles per ml plasma) was significantly higher compared those in lean-GDM women ($7.14 \times 10^8 \pm 2.3 \times 10^8$ particles per ml plasma). The concentration of placenta-derived exosomes in maternal plasma (as indicated by exosomal PLAP concentration) is higher in obese-GDM compared to lean-GDM women (1320 ± 75 and 734 ± 56 pg/ml, respectively).

Conclusions: While the role of placental exosomes on the maternal metabolic adaptation during pregnancy remains to be elucidated, placental exosomes could be use as potential biomarker for the determination of placental metabolic status during pregnancy.

O42
Subclinical atherosclerosis and glucose homeostasis in Indian obese children

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Objective: To evaluate the role of Carotid intimo-medial thickness (CIMT), as a marker of subclinical atherosclerosis in Indian obese children and detect derangements in glucose and lipid homeostasis.

Methods: Eighty children of constitutional obesity were recruited and compared with age and gender matched controls. Body mass index and waist hip ratio were calculated. Total body fat was estimated with DEXA. The CIMT was measured with B-mode ultrasonography in both cases and controls. The laboratory testing was performed only on obese subjects.

Results: The mean age of cases was 12.8 ± 3 years with a male: female ratio of 1.29. The mean BMI of cases was 29.2 ± 4.8 (range −21.7 to 46.4) kg/sqM. Twelve (15%) cases were hypertensive and 31 (39%) patients had abnormalities in glucose metabolism. Both HOMA-IR and Matsuda index correlated significantly to blood pressure. Hypertriglyceridemia was commonest dysplipidemia seen in 44%. The mean CIMT was significantly higher in cases (0.534 ± 0.13) than controls (0.402 ± 0.08); $p < 0.001$. CIMT was significantly higher in hypertensive subjects than those with normal blood pressure ($p = 0.04$). CIMT showed a positive correlation with total percentage body fat ($r = 0.28$, $p = 0.011$) but not by WHR. Significant positive correlation was also observed between CIMT and blood glucose at 1 h ($r = 0.22$; $p = 0.04$), serum insulin at 60 min ($r = 0.28$; $p = 0.01$), total AUC for insulin ($r = 0.29$; $p = 0.01$). No significant correlation was noticed with lipid profile or post-meal blood glucose. Whole body insulin sensitivity as measured by Matsuda index was inversely correlated with CIMT ($r = -0.27$; $p = 0.01$) while no significant correlation was seen between CIMT and HOMA-IR.

Conclusion: Indian obese children were detected to have dyslipidemia and deranged glucose metabolism. CIMT correlated significantly to known cardiac risk factors and metabolic parameters and can serve as a screening tool for predicting cardio-vascular risk in obese Indian children.

O43
Reduced birth weight is associated with partial growth hormone resistance

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Objective: We aimed to assess whether term children with idiopathic short stature (ISS) showed different growth hormone (GH) sensitivity to an insulin-like growth factor I (IGF-I) generation test in comparison to children of normal stature.

Methods: Forty-six prepubertal children (aged 7.1 ± 2.1 years) born at term were studied, and categorized into two groups: ISS ($n = 23$; 74% boys) and normal stature ($n = 23$; 57% boys). All children underwent a modified IGF-I generation test via a daily dose of recombinant human growth hormone (rhGH; 0.05 mg/kg/day) over four consecutive days. Hormonal concentrations were measured at baseline and day 5, with anthropometry assessed at first clinic.

Results: When the cohort was assessed as a whole, decreasing birth weight SDS across the normal range (−1.9 to 1.5 SDS) was associated with reduced IGF-I response to rhGH stimulation ($p = 0.008$).

ISS children were lighter ($p < 0.001$) and shorter ($p < 0.0001$), but were of similar BMI SDS than children of normal stature. At baseline, ISS children had lower IGF-I (−27%; $p = 0.024$) and GHBP (−32%; $p = 0.026$) concentrations, but higher leptin concentrations (+53%; $p = 0.031$). Following rhGH stimulation, ISS children showed a greater percentage increase in IGF-I concentrations (104% vs. 66%; $p = 0.018$). Further, while children of normal stature displayed no change in GHBP concentrations, these were suppressed in ISS children (−19%; $p = 0.009$).

Conclusions: Progressive decrease in birth weight is associated with decreased GH sensitivity in both normal and short children. We speculate that partial GH resistance may contribute to the shorter stature seen in lower birth weight children.

O44
Thyroid function in children with Prader-Willi syndrome (PWS) treated with growth hormone (GH)

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Objective: Children with PWS, may be at risk of central hypothyroidism after commencing GH treatment. FT4 (Free Thyroxine) and TSH (Thyroid Stimulating Hormone), recorded in Australian GH database, OZGROW, were used to assess thyroid function in PWS patients before and after GH treatment.

Methods: Between 2003 and 2014, 30 PWS patients were identified who had at least one recorded FT4 test and 31 with at least one TSH test. FT4 and TSH results were standardized by expressing them as a % of the reference range (RR) – where RR was available. x % = 100x − LU − L, where x is the test result and U and L are the upper and lower values of the RR. Mean test results were calculated in the year before GH treatment and from 4 weeks post-GH in the 1st year of treatment. These were compared to an expected mean of 50%. For individuals who had two such before and after tests for both FT4 and TSH, their change in test % was recorded and these differences tested against 0 (paired *t*-test).

Results: In the year prior to GH, for 19 patients who were tested for both FT4 and TSH, mean(SD) FT4 = 18.8(16.9)%, $p = 2 \times 10^{-7}$ with 3 below RR and TSH = 37.7(27.1)%, $p = 0.06$, all within RR. Similarly for 13 patients in the 1st year of treatment all were within RR and FT4 = 19.9(10.5)%, $p = 3 \times 10^{-7}$ and TSH=20.8(14.0)%, $p = 7 \times 10^{-6}$. 8 patients had both FT4 and TSH tests before and after (1st year) GH commencement thus enabling us to test mean change in test% (Δ%). For FT4 Δ% mean(SD) = +1.4(11.6), $p = 0.7$ and for TSH Δ% mean(SD) = −12.3(8.2), $p = 0.004$.

Conclusions: Most PWS patients are in the low normal RR for both TSH and FT4 prior to GH although mean FT4 is lower with some patients falling below the RR. Following GH, TSH decreases further

while FT4 remains at a similar low level. GH, through increased somatostatin release, decreases TSH but also increases deiodination of T4 to T3. Although no patients were reported as receiving thyroxine, this possibility needs exclusion.

O45

Effects of a motivational lifestyle intervention (the Healthy Eating and Lifestyle Programme (HELP)) on metabolic outcomes in obese adolescents: findings from a randomized controlled trial

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Aims: To assess whether a motivational multi-component lifestyle intervention delivered in the community was effective in improving cardiometabolic health outcomes in obese adolescents.

Methods: 174 obese adolescents (13–17 years; 109 females) from a UK community setting were randomised into intervention or control arms. Intervention participants received 12 sessions across 6 months, addressing lifestyle behaviours and focusing on motivation to change and self-esteem. The intervention was delivered by trained Graduate Health Workers. Control participants received a single 2 h session providing didactic weight management advice. Primary outcome was BMI change at 6 months. Secondary outcomes included body fat and fasting glucose, insulin and lipids and BP. Analysis was by random-effects linear regression. The primary analyses used the intention to treat sample.

Results: Cardiometabolic data were available on 145 (83.3%) adolescents who completed the intervention. Mean BMI across the whole group was 32.3 kg/m^2 (SD 4.4) at baseline. There were no significant baseline differences between arms in BMI or any cardiometabolic outcomes. We found no significant difference in the primary outcome (BMI) at 6 months: effect estimate −0.06 (95% CI: −0.57 to 0.45) p = 0.8). No significant differences were observed for changes in any secondary cardiometabolic outcomes (all p > 0.4) between intervention and control groups at 6 months. Fidelity monitoring showed moderately strong fidelity to treatment.

Discussion: A motivational multi-component lifestyle modification intervention delivered in the community was not effective in reducing BMI or improving cardiometabolic risk factors in obese adolescents. Our findings suggest that obesity interventions with a strong theoretical basis and evidence of effectiveness when delivered by psychologists may not be effective when delivered at lower intensity in the community by entry-level health workers.

O46

A novel TSHβ gene mutation with impaired immunoreactivity but normal bioactivity

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Background: TSH deficiency due to mutations in the *TSH*β gene is a rare cause of congenital central hypothyroidism.

Clinical case: The proband was a 4-year-old male, the youngest of a consanguineous Pakistani family. Fatigue led to thyroid function testing revealing undetectable serum TSH despite normal thyroid hormone levels and no clinical signs of hypothyroidism. His 10-year-old brother had the same thyroid function profile, whereas their older siblings and their mother were biochemically and phenotypically normal. These findings prompted sequencing of *TSH*β gene; both brothers were found to be homozygous for a single nucleotide substitution in exon 3 (c.223A >G) predicting the replacement of normal arginine-55 with a glycine in the mature TSHβ protein. Their brother and mother were heterozygous and their sister homozygous for the normal allele. Serum TSH levels of the two brothers were measured by five different automated platforms (Roche Elecsys, Siemens Immulite 2000, Siemens Centaur, Beckman Coulter DXI and Abbott Architect) and were undetectable only in two produced by Siemens. Interestingly, in the two Siemens assays, the TSH levels of the heterozygote brother and mother were half compared to the TSH measurements using other platforms. To mimic the effect of the mutation, serum with normal TSH levels was exposed to phenylglyoxal, which leads to arginine modification and loss of the amino acid properties. When measured with the Siemens platforms, TSH levels were reduced from 4.1 to 0.16 mU/l. This further supports the conclusion that replacement of arginine-55 is responsible for the loss of immunoreactivity through the undisclosed (proprietary) monoclonal antibody used to detect the TSHβ molecule. The consequence is falsely low TSH measurements.

Conclusion: To our knowledge, this is the first report of a TSHβ gene mutation that impairs the immunoreactivity of the protein without affecting its biological activity.

Diabetes Oral V Diabetes Complications

O47

Evaluation of serum bicarbonate and anion gap to define resolution of diabetic ketoacidosis

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Objective: To investigate the utility of serum bicarbonate (HCO3) levels and anion gap (AG) to define resolution of diabetic ketoacidosis (DKA) in children with new onset diabetes (NODM).

Methods: Retrospective study of all patients with NODM presenting with DKA to Boston Children's Hospital from 10/1/07 to 7/1/13. DKA was defined as blood glucose ≥200 mg/dl and venous pH (vpH) <7.3; severity as mild <7.3, moderate <7.2, or severe <7.1, and resolution of DKA as vpH ≥7.3. We used Cox regression to determine time to resolution, and compared different HCO3 and AG cut-offs to vpH ≥7.3.

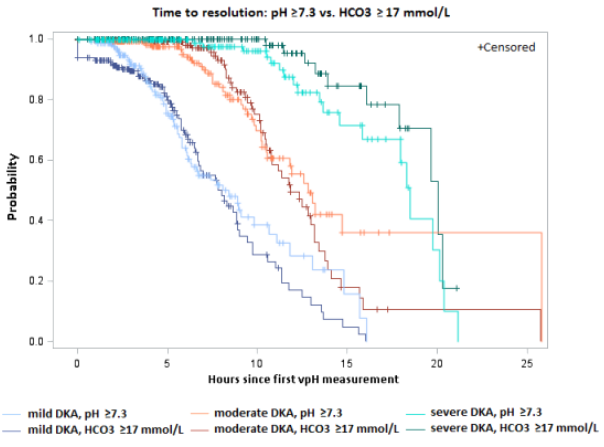
Results: 249 patients (125F, mean age 9.9 ± 4.4 years, 75% White) met inclusion criteria. DKA was mild in 132 (53%), moderate in 69 (28%) and severe in 48 (19%). HCO3 ≥ 17 mmol/l and AG ≤14 corresponded best to vpH ≥7.3. Based on these values for vpH, HCO3 and AG, median times to DKA resolution were 13.6 (95% CI 12.2–15.8), 12.9 (95% CI 11.8–13.8) and 9.0 h (95% CI 8.4–9.7), respectively. Time to resolution increased with DKA severity. In moderate and severe DKA, AG normalized before vpH reached 7.3 (Figure).

Resolution of DKA by severity

Median time (hours) to resolution

DKA Severity	vpH ≥7.3	HCO3 ≥17	AG ≤14
Mild	8.3 (95%CI 6.2-10.6)	7.8 (95%CI 6.7-8.9)	7.7 (95%CI 6.4-8.4)
Moderate	12.7 (95% CI 10.5-25.8)	11.8 (95%CI 10.5-13.2)	8.4 (95%CI 7.6-9.4)
Severe	18.4 (95%CI 15.8-20.1)	20.1 (95%CI 17.9- .*)	11.4 (95%CI 10.4-12.5)

*largest value censored



[Resolution of DKA by Severity]

Conclusions: In the management of pediatric DKA, HCO3 ≥ 17 mmol/L, as compared to normal AG, is a better predictor of vpH ≥7.3. HCO3 and AG are good surrogates for vpH in mild DKA; however, in moderate and severe DKA, AG normalizes before vpH. Future studies should compare these indirect parameters of metabolic acidosis with serum ß-hydroxybutyrate levels to define resolution of DKA.

O48

Elastargene 3C helps to improve glycated haemoglobin in children and adolescents with type 1 diabetes using insulin pump therapy

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Objective: Elastargene 3C (E3C) is a cream designed to improve lipoatrophy in patients with diabetes. It is made by many ingredients, among whom are elastin, arnica, collagen, caffeine, and L-carnitine. We started a 6-month, double-blind, randomized trial to test the efficacy of E3C in children with type 1 diabetes (T1D) using insulin pump (CSII), in whom infusion set usually left little withe scars.

Method: Forty children and adolescents using CSII, were randomized into 2 arms:

a) *n* = 20 E3C once a day on the skin of abdomen or other sites where infusion sets have been placed;
b) *n* = 20 placebo once a day on the skin of abdomen or other sites where infusion sets have been placed. BMI, HbA1c, insulin requirement, were determined in each child before at baseline and after 6 months.

Results: At the end of the study, 5 patients dropped using the E3C or placebo and were excluded from the analysis. In E3C group, 18 patients with T1D were evaluated: age 15.2 ± 4.8 years, diabetes duration 8.0 ± 5.3, time using a pump 4.1 ± 3.0; in the placebo group, 17 patients with T1D were evaluated: age 15.1 ± 5.7 years, diabetes duration 8.3 ± 5.8, time using a pump 4.7 ± 3.0. No significant difference has been observed for age, disease duration and time since insulin pump started. HbA1c significantly improved in E3C group (baseline 8.08 ± 0.80%, after 6 month 7.51 ± 0.53%, p = 0.005, delta −0.53%), but not in placebo group (baseline 7.98 ± 0.74%, after 6 month 7.76 ± 0.79%, p = 0.19, delta −0.22%). No difference has been observed regarding BMI or insulin requirement. In the E3C group, withe scars completely disappeared in 8 patients and improved in 10; in the placebo group withe scars did not change in any of the patients.

Conclusions: This is the first time a direct effect of E3C have been shown to improve little withe scars that appear on the skin after infusion set removal in children with T1D using CSII. HbA1c significantly improved only in the E3C group, probably because improved insulin absorption.

O49

The impact of altered glycaemic states on working memory in adolescents with type 1 diabetes

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Objective: To assess acute changes in working memory during hyperglycaemia and hypoglycaemia.

Methods: Functional MRI (fMRI) & a simultaneous working memory task (WMT), the "n-back", were used to assess changes in brain function between euglycaemia, hyperglycaemia or hypoglycaemia in youth (N = 18, aged 12–18 years) with T1D. Exclusion criteria were HbA1c >9.0%, IQ <70, prior history of DKA, seizure, CNS disease or substance abuse. Standard insulin clamp techniques were used to study participants in baseline euglycaemia (5.0 ± 0.5 mmol/l), a challenge state of either HYPER (18–20 ± 0.5 mmol/l n = 9 or HYP0 (2.6 ± 0.5 mmol/l, n = 9) & again in euglycaemia (recovery). The "n-back" is a well-established working memory paradigm with two conditions (a) 0-back -a response is made if a pre-specified letter "X" appears on the screen and (b) 2-back condition-a response is made when the letter presented on the screen is the same as the letter presented 2 back i.e. one before the preceding letter.

Results: FMRI findings are reported elsewhere. Performance on 0-back did not vary with glycaemia. Compared to baseline, false alarms were greater (p = 0.04) in the 2-back condition and there were trends for a lower hit rate (p = 0.07) and more missed targets (p = 0.07) during HYPER. The HYPO group made more errors than HYPER at baseline (p = 0.04), but hits, misses and false alarms did not change during the challenge state. There were trends for reaction times to be faster during recovery compared to baseline and challenge, significantly so after HYPO (p = 0.04), but no difference between baseline and challenge.

Conclusions: Dysglycaemia is unavoidable in individuals with TID. We have shown increased impulsive responding and trends for increased errors on a WMT during hyperglycaemia, consistent with pathophysiological changes in left frontal cortex and basal ganglia demonstrated on FMRI. Interestingly, performance on WMT was preserved, suggesting some neuroadaptive capacity, during hypoglycaemia in this T1D cohort.

O50
Incidence of DKA hospitalisations in adolescents with type 1 diabetes: results from diabetes MILES Youth – Australia

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Objectives: We assessed frequency of self-reported DKA hospitalisations in the past 12 months in adolescents with established T1D and the associations with demographic and clinical factors, insulin omission and disordered eating (DE).

Methods: In a national online survey, adolescents (13–19 years) with T1D for ≥12 months, reported frequency of hospital admission for DKA (very high blood glucose, very high ketones, vomiting, drowsy). Frequency was assessed on a scale from 0 to ≥5 admissions in the past 12 months and recoded into ≥2 vs. no or one admission(s). Insulin omission in the past 14 days was recoded into ≤3 and ≥4 days. DE was measured with the Diabetes Eating Problem Survey-Revised (DEPS-R). Participants also reported age, gender, insulin delivery mode and HbA1c. In a stepwise logistic regression we compared adolescents with ≥2 DKA hospitalisations with those with no or one event.

Results: DKA hospitalisation data were provided by 494 (96%) adolescents (62% girls; mean age 16 ± 2 years; diabetes duration 7 ± 4 years; 52% insulin pump; HbA1c 66 ± 17 mmol/mol). Overall 104 (21%) had been hospitalised for DKA in the past year. Adolescents with ≥2 DKA events (41; 8%) had a higher HbA1c and were more likely injecting insulin (vs. pump) than those with no or one event (both p < 0.001). Age, gender and diabetes duration were not associated with recurrent DKA. In a stepwise logistic regression insulin injections, forgetting to inject insulin (≥4 days) and DE were significant factors for hospital admission for recurrent DKA (p < 0.001). HbA1c became insignificant after adding unintentional insulin omission and DE.

Conclusions: In this cross-sectional study, one in five adolescents with established T1D reported being hospitalised for DKA in the past year. Although self-reported incidence of recurrent DKA was higher compared to clinical samples, unintentional insulin omission and DE were confirmed risk factors. Whether insulin delivery mode affects DKA rates remains unclear.

O51
Mechanisms of central nervous system dysfunction in hypoglycaemia and hyperglycaemia in youth with type 1 diabetes

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Objectives: While the impacts of hypoglycaemia and hyperglycaemia on cognition have been well described, the underlying processes remain unclear. Our objective was to define the pathophysiological events within the brain during hypoglycaemia and hyperglycaemia in youth with Type 1 Diabetes (T1D).

Methods: This prospective study used functional MRI (fMRI) & a working memory task (WMT) to assess changes in brain function between euglycaemia (5.0 ± 0.5 mmol/l) & hypoglycaemia (2.6 ± 0.5 mmol/l) or hyperglycaemia(18–20 ± 0.5 mmol/l) in youth aged 12–18 years with T1D. Exclusion criteria were HbA1c >9.0%, IQ <70, prior history of DKA, seizure, neurological disease or substance abuse. Standard insulin clamp techniques were used to study 19 participants in euglycaemia (baseline), then hypoglycaemia (n = 9) or hyperglycaemia (n = 10) & again in euglycaemia (recovery). fMRI was performed at rest (fixation) & during WMT in each state. Blood oxygen level dependent (BOLD) signaling & arterial spin labeling (ASL) assessed neuronal activity & perfusion respectively. Group level t-tests identified regions of altered activation for hypoglycaemic & hyperglycaemic WMT & fixation blocks compared to euglycaemia.

Results: Compared to baseline, hyperglycaemia was associated with significantly decreased cerebral perfusion (ASL) most marked in the basal ganglia & subcortical frontal regions. Hyperglycaemia was also associated with decreased neuronal activity (BOLD) in the frontoparietal areas during WMT. In contrast, hypoglycaemia was associated with increased perfusion in the left prefrontal cortex and thalami, areas associated with working memory and higher executive function (p < 0.001 for all).

Conclusions: This study is one of the first to describe mechanisms of acute brain dysfunction in T1D youth during glycaemic extremes. We have shown differential mechanistic and regional effects of hypoglycaemia & hyperglycaemia. This experimental paradigm will allow neuroprotective therapeutic options to be explored.

O52
Early identification of cardiac autonomic neuropathy using complexity analysis

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Objectives: Abnormal heart rate variability (HRV), a marker of cardiac autonomic neuropathy (CAN), contributes to mortality in adults with diabetes. Traditional HRV measures do not consistently detect abnormalities in youth with type 1 diabetes (T1D). We hypothesised that complexity analysis of HRV would identify CAN earlier than traditional methods in youth with T1D.
Methods: We studied 17 youth with T1D [age 12.8 ± 1.8 years, duration 4.5 ± 2.6 years, HbA1c 7.9 ± 1%] and 15 controls (age 12.9 ± 1.6), with 12 lead ECGs (sampling frequency 1000 Hz) recorded continuously for 10 min. Recordings were analysed using Labview software and an algorithm for complexity analysis (sample entropy and detrended fluctuation analysis), with standard methods for time-domain and spectral analysis. Clinical and laboratory data, as well as heart rate (HR) and BP responses to standing were obtained. Data were analysed using chi-squared, student's *t* or Mann–Whitney *U*-tests and Pearson's or Spearman's correlation.
Results: Youth with T1D had significantly higher sample entropy (0.149 ± 0.011 vs. 0.136 ± 0.014, p = 0.015) than controls suggesting increased complexity in HRV, but similar detrended fluctuation analysis (0.67 ± 0.12, 0.65 ± 0.13, p = 0.68). They had increased % high frequency (62 ± 14% vs. 46 ± 16%, p = 0.017) and reduced mid-frequency (18 ± 7.5% vs. 26 ± 1.5%, p = 0.019) power on spectral analysis, but no differences in HR or BP responses to standing or time-domain analysis of HRV. In T1D, sample entropy correlated strongly with triglycerides (TGs) ($r = 0.76$, p = 0.001) and detrended fluctuation analysis correlated strongly with serum potassium (K^+) ($r = -0.86$, $p < 0.001$).
Conclusion: Complexity analysis of HRV may detect CAN earlier than traditional measures. Our data suggest serum potassium and TGs may contribute to abnormal HRV. It is conceivable an interaction between abnormal HRV and serum K^+ contributes to the "dead-in-bed" syndrome in youth with T1D, which would be worth further investigation.

O53
Incidence of severe hypoglycemia in children with type 1 diabetes in the Nordic countries in the period 2008–2012. Influence of HbA_{1c} and treatment modality

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Objectives: Treatment of type 1 diabetes (T1D) has been intensified aiming at normalizing blood glucose, which may increase the risk of severe hypoglycemia (SH). The objectives of the study were to compare incidence of SH events between the four Nordic countries – Denmark, Iceland, Norway and Sweden – with comparable access to health care, and explore the influence of HbA_{1c} and treatment modalities on the frequency of SH.
Methods: Data from children less than 15 years registered in the national childhood diabetes databases in the four Nordic countries from 2008 to 2012 were compiled. All 89 Nordic centers treating T1D in children registered data in the national databases, and data completeness was nearly 100%.
Results: There were 8806 (48% females) patients with T1D duration >1 year enrolled in the study, giving 29,715 person years with 1,775 SH events. Mean (SD) age was 11 (3.0) years and mean diabetes duration was 4.8 (2.9) years. Totally the SH incidence did not change over the five years period, but the Swedish population constantly had the lowest SH incidence while it decreased significantly in the Danish population. HbA_{1c} decreased significantly over time ($p < 0.01$) while the number of pump users increased ($p < 0.01$). Stratifying for HbA_{1c} levels showed significant higher incidence of SH in patients with HbA_{1c} >70 mmol/mol (8.6%) ($p < 0.01$). There was a tendency to lower SH incidence in pump users, but it was only statistically significant in the Danish population.
Conclusion: The total incidence of SH in the Nordic countries remained stable in spite of a significant decrease in HbA_{1c} in the period 2008–2012. Although all patients had an equal access to health care, the incidence of SH differed between the countries and only in Denmark pumps significantly decreased the risk of SH. A target of HbA_{1c} below 50 mmol/mol (6.7%) seems possible without increasing the risk of SH. On the contrary, those with high HbA_{1c} had the highest risk of SH.

O54
HLA genotype and EV infection at diagnosis of type 1 diabetes predicts vascular complications in type 1 diabetes

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Background: Identifying early factors predictive of microvascular complications risk may allow targeted risk stratification and intervention.
Objective: To determine if features at T1D diagnosis (adiposity, enterovirus (EV) infection, vitamin D deficiency, HLA genotype) predict risk of microvascular complications.
Methods: Study population: incident cohort of youth with T1D ($n = 206$) diagnosed 1997–1999 at The Children's Hospital at Westmead, Australia; 156 (76%) followed longitudinally to 2014 and assessed for retinopathy (DR), microalbuminuria (MA) and autonomic neuropathy at min. 2 years diabetes duration. The effect of factors at diagnosis on development of complications was examined using generalised estimating equations, predictors of time to onset of complications using Cox hazard regression and time to development of complications using Kaplan-Meier survival statistics.
Results: At final visit ($n = 156$, 40% male), median age was 17.1 years [interquartile range 15.7–18.8], duration 10.2 years [6.3–14.1] and HbA1c 8.8% [7.9–9.8]. DR was present in 35%, MA in 4%, abnormal pupillometry in 77% At diagnosis, 30% had EV detected by PCR; 85% had high risk HLA genotypes.

A lower odds of DR was associated with EV infection at diagnosis (OR 0.47, 95% CI 0.25–0.88, p = 0.02) after adjusting for gender, age and HbA1c. MA was associated with higher BMI (OR 1.1 (1.0–1.2), p = 0.03). Abnormal pupillometry was associated with younger age (OR 0.9 (0.86–0.98), p = 0.007) and DRB1*04-DQB1*0302/x haplotype (OR 0.5 (0.3–0.8), p = 0.007). Vitamin D deficiency was not associated with the risk of any complication. Patients with DRB1*03 or DRB1*04 vs. all other alleles had a longer time to onset of MA (median time to onset 15.7 years (15.2–16.3), p = 0.02).

Conclusion: T1D heterogeneity is associated with variable complication risk after accounting for known risk factors. Those with a viral trigger are at lower risk of retinopathy while high risk HLA genotypes are protective for development of albuminuria.