L1

Diagnosis and treatment of monogenic diabetes in children

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Historically, sub-classification of diabetes in children has been on clinical grounds but recently classification has become etiological. Monogenic diabetes provides strong support to the shift to etiological classification.

A major advance in the last decade has been the defining of the molecular genetics of monogenic diabetes. This has lead to the recognition of novel genetic diabetes syndromes e.g. Hepatic Nuclear Factor (HNF)-1b mutations causing renal cysts and diabetes (RCAD) (1). The previously clinically defined maturity-onset diabetes of the young (MODY) is now defined by etiological mutations in the glycolytic enzyme glucokinase and the transcription factors HNF-1a, HNF-1b, HNF-4a, Insulin Promoter Factor-1 and Neuro-D1 (2). Glucokinase patients have mild stable hyperglycemia and rarely require treatment or develop complications while patients with transcription factor mutations show progressive hyperglycemia and may have severe complications if not appropriately treated. The most striking evidence of pharmacogenetics is in HNF-1alpha MODY where, in a randomised trial, we showed a 4× greater fall in fasting glucose than in BMI matched type 2 patients (3). HNF-1a patients control can be improved by taking them off insulin injections after 10–30 years continual treatment and putting them on sulphonylureas.

There have been considerable recent advances in the genetics of neonatal diabetes. Patients with the resolving transient neonatal diabetes mellitus (TNDM) have abnormalities in the imprinted region on 6q and recently with the help of many ISPAD members mutations in Kir6.2 were found to be the major cause of permanent neonatal diabetes mellitus (PNDM) (4). There is a striking genotype phenotype relationship with specific Kir6.2 mutations being associated with neurological features (DEND syndrome – developmental delay, epilepsy and neonatal diabetes) by altering function in the K_{ATP} channels in muscle, nerve and brain. All mutations showed reduced channel closure in response to ATP but this response was more severe in mutations associated with neurological features. Despite being insulin dependent these patients can discontinue insulin injections and show improved glycaemic control on high dose sulphonylureas which act to close the K_{ATP} channel by a non ATP dependent route (5). Our data on 25 patients show improved control with less hypoglycaemia episodes.

The ability to make a molecular genetic diagnosis which helps explain the clinical features, predicts prognosis and can improve treatment has lead to the widespread introduction of diagnostic genetic testing in diabetes (see www.diabetesgenes.org). Making the correct diagnosis is probably even more important in children than in adults and I would favor all patients having autoantibodies at diagnosis to help identify patients who possibly have non-Type 1 diabetes. All patients diagnosed in the first 6 months of life should be tested for Kir6.2 mutations as they can achieve much better control off insulin.

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L2

Patient empowerment: optimising dietary outcomes

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The care of children and young people with type 1 diabetes can present a variety of particular problems compared with adults. Many aspects of care and decision-making are taken by parents/carers until the child is mature enough to demonstrate increasing independence in the management of their diabetes. Dietary management is complex because it has many aims: to optimise growth, development and glycaemic control; reduce hypoglycaemia and macrovascular risk and maintain ideal body weight. These are difficult targets when the dietary intake of young people with diabetes is influenced by many factors e.g. age, peer group, indigenous diet, cost, family eating patterns, food availability, trends/fads.

Over the last two decades the emphasis has changed in the theoretical basis of nutritional interventions, moving towards not simply imparting knowledge but to changing behavior and influencing factors such as self-care and readiness to change. This is a radical change from the old acute prescriptive medical model to a newer chronic disease approach that involves a collaborative self-management plan. The new approach now focuses on the process of change. This includes a trusting, equal, non-judgemental and supportive relationship between the health professional and child/family. The basis of this relationship promotes the patient as the decision-maker and self-carer. Past evidence suggested that children and young people found it difficult to achieve dietary targets with traditional teaching methods. The results from studies that have used a behavioral approach have been more successful. The findings from the adolescent group in the Diabetes Control and Complications Trial (DCCT) produced more favorable results showing that the development of a good relationship between the dietitian and the patient was necessary to negotiate treatment goals and achieve HbA_{1c} targets. Pediatric diabetes centers have also demonstrated that improved HbA_{1c} levels can be achieved, without an increase in levels of hypoglycaemia, through close attention to a patient-centered relationship with the health professional and a problem solving approach.

Dietary interventions are always more successful when they have a degree of personalisation with goals tailored to the individual's dietary intake. It is important that dietary goals are not perceived as imposed by the heath professional but were self-initiated and personally endorsed by the child or young person. The benefit of this approach is that autonomous motivation is expected to yield the long-term adherence required in dietary change. A behavioral approach to goal setting has been found useful in pediatric diabetes to achieve successful dietetic outcomes. A useful technique is described by the acronym 'SMART': Specific, measurable, achievable, relevant to the goal of treatment, and time specific. This technique is useful as setting unrealistic goals would set the child up for failure.

Structured patient education programs have been positively evaluated in adults in many countries. They focus on knowledge and skill development to adjust rapid acting insulin around food intake. These programs improve self-management skills, clinical outcomes and quality of life measures. Although many pediatric centers have educational programs, few are structured, use formal curricula, provide training for educators or are quality assured. Preliminary data from a structured education program specifically designed for young people with diabetes in the UK called 'Kids in control of food' (KICK-OFF) is showing positive results in quality of life. Children and young people comment that they like the flexibility of meal times and the freedom of eating a wider variety of foods. Other pediatric centers in the UK also have preliminary data that suggest that there may be other beneficial outcomes to this approach such as decreased insulin doses and weight control. The results of these structured programs will be extremely important to dietary educational approaches in the future.

New technology has given children and young people more control over the management of their diabetes. Current blood glucose monitoring machines are small, more attractive and require minute amounts of blood. These new machines facilitate blood testing around specific foods and provide greater understanding about the glycemic effect of different foods. Children are then more likely to use rapid acting insulin, especially in pens, to prevent or treat hyperglycemia. Teaching the skills of insulin adjustment increases the food choices for young people but at the same time limits hyperglycemia. It is important that this type of education includes healthy eating messages to reduce cardiovascular risk. The increasing use of delivering insulin through continuous subcutaneous insulin infusion (pump therapy) has also improved eating patterns and the ability to control for the glycemic effect of different foods.

Diet is a difficult aspect of diabetes care and one that children find particularly hard to manage. To empower our young people to improve dietary outcomes we need several approaches that include a patient-centered relationship, evaluated skill-based education programs, behavioral approaches including goal setting and continued development of new technology. This combination hopefully will have positive effects on clinical outcomes but also improve quality of life for children and young people with type 1 diabetes.

L3
The accelerator hypothesis

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It has been customary to view those with type 1 and type 2 diabetes mellitus as two different populations, but the distinction is artificial. People who develop type 1 inhabit the same obesogenic environment as those who develop type 2, and they reap the same consequences. One consequence of obesity, fundamental to the development of type 2, is the emergence of insulin resistance and the metabolic up-regulation of the beta cells that ultimately accelerates their loss. Metabolic up-regulation also renders the beta cells more antigenic. Those who carry 'jumpy' genes react to the increase in antigenicity with an intense immune response, which further accelerates beta cell loss.

The Accelerator Hypothesis asserts that *Type 1 and Type 2 diabetes are the same disorder of insulin resistance, set against different genetic backgrounds.* Insulin resistance accelerates beta cell death, which is further modulated by immune response genes. Any distinction between them is merely one of tempo.

The implications are wide-ranging, most particularly for prevention. If insulin resistance drives the beta cell loss and immune response of type 1, it may be more effective, safer and cheaper to treat those at risk of type 1 with lifestyle change or insulin sensitisers, rather than with immuno-modulatory drugs (the much talked of type 1 'vaccines') or islet transplantation. Immune interventions are potentially harmful – and they may not be necessary.

A theory attempting to associate type 1 with insulin resistance would have to demonstrate a rise in obesity concurrent with that of type 1. The relationship is not in dispute, but association is not proof of causation. Causation would have to demonstrate that children who develop type 1 are heavier before onset than their peers who do not – the rule of temporality – and there are several reports that confirm the link.

More importantly still for causation, the hypothesis would need to show, in an unselected group of children with established type 1 diabetes, that the heavier children developed it younger – true evidence of acceleration. There are now two full-length reports of independent cohorts where age at onset was inversely related to BMI.

The probability of developing a complex condition such as type 1 has two components – genetic susceptibility and environmental risk. As both are proportions, a rise in one will result in a fall of the other, and there is evidence that the genetic contribution to type 1 is falling as the environmental risk – obesity/insulin resistance – is rising. There is also direct evidence that those who develop type 1 are more insulin resistant than those who do not, and some intriguing data from identical twin studies that the co-twin of an affected pair who goes on to develop type 1 is more insulin resistant, with poorer beta cell function, than the co-twin who does not. The hypothesis predicts that the clinical phenotypes of type 1 and type 2 will ultimately converge as the contribution made by the genetic difference between them progressively diminishes. Difficulty with the clinical classification of children with diabetes is already being reported from pediatric diabetic clinics.

The Autoimmunity Hypothesis of defective immune regulation has not resolved the cause of type 1 in thirty years, and offers no mechanism to explain its rising incidence. It has been calculated that the curve describing the rising incidence of type 1 regresses to around the middle of the 20th century – arguably the point when our modern, obesity-promoting way of life began. The Accelerator Hypothesis offers compelling evidence that a physically active lifestyle and a healthy BMI might prevent type 1 as it clearly does type 2. Lifestyle change should be the next intervention for those at risk of type 1 diabetes – less newsworthy than islet transplantation or immunotherapy, but more logical.

L4
Parenting the child with diabetes: coping challenges and lessons from research

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Over the last two decades behavioral science research on children with type 1 diabetes has documented that diabetes has a profound impact on parents and families. At the same time, parents have a critical impact on the psychological functioning and metabolic control of the child. First, I will discuss the critical parenting tasks which diabetes uniquely thrusts upon parents. Secondly, I will review parent behaviors that have been shown in empirical studies to relate to positive outcomes in children with diabetes from infancy through school-age. These parent behaviors fall into two categories – parent involvement in diabetes management tasks; and parent–child conflict about diabetes. Finally, based on an integration of unique diabetes coping challenges and previous studies on parenting and diabetes outcomes, I will suggest new directions for research and for optimizing the multidisciplinary care of families with children with diabetes.

In addition to the many challenges all parents face, diabetes confronts parents with a number of unique tasks. Some of the most challenging include: 1.) learning to live with uncertainty; 2.) mastering complex clinical decision-making; 3.) advocating and educating about diabetes management; 4.) fostering autonomy in overall development, including diabetes management, while maintaining involvement in diabetes care; 5.) integrating the child's temperament and normal developmental tasks into disease management expectations; 6.) maintaining and modeling hope, courage, and optimism.

Parent involvement in diabetes management tasks: Research has consistently documented that parents of very young children with diabetes experience higher levels of family stress than do parents of older children.

The expanding skills and increased cognitive abilities of the elementary school child make it seem reasonable to transfer more and more daily diabetes care responsibilities to the child. However, there is growing consensus among recent empirical studies that older children who are given greater responsibility for their diabetes management make more mistakes in their self-care, are less adherent, and are in poorer metabolic control than those whose parents are more involved. The key seems to be finding a developmentally-appropriate balance between parent and child involvement, or 'family teamwork' in diabetes management. Moreover because studies suggest that participation with peers, positive self-image, and regimen flexibility are critical and interrelated goals for the school-aged child, parents must avoid unrealistic demands for adherence to a regimen schedule that restricts the child from active participation in age-appropriate school and peer activities.

Parent–child diabetes conflict: In the school-aged child, studies have consistently documented that lower levels of diabetes conflict and higher levels of 'authoritative parenting' are related to better adherence and glycemic control. 'Authoritative parenting', which describes a parenting style in which conflict is minimized as parents set consistent, realistic limits on children's behavior while displaying warmth and sensitivity to their child's needs and feelings has been linked to improved behavioral outcomes in the general child development research literature as well as in empirical studies in school-aged children with diabetes.

Future research and clinical strategies: Because of the unique parenting challenges parents face when their child is diagnosed, new diabetes education strategies are needed in pediatric centers that include 'parenting classes' focused on these complex, continuous challenges such as fostering autonomy while maintaining involvement in the tasks of diabetes management or setting limits realistically and constructively. Because the many important technological advances (CGMS, pumps) in the management of diabetes increase the burden of care on the parent and the child, empirical studies are needed about how diabetes care teams can help to build the involved, optimistic parents needed to socialize the next generation of resilient and healthy children who can take full advantage of these tools for a lifetime of improved glycemic control.

L5
Insulin-like growth factor 1 and type 1 diabetes

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Insulin like growth factor I (IGF-I), as its name suggests, has insulin like properties but it is also an important mitogen and a mediator of the growth promoting effects of growth hormone. The type 1 IGF receptor also shares remarkable structural and sequence homology with the insulin receptor, with similar pathways of post receptor signaling and as yet it is unclear how the actions of insulin and IGF-I are separated at the post receptor level.

The majority of circulating IGF-I is derived from the liver where the growth hormone (GH) receptor is partially insulin dependent. Thus in newly diagnosed subjects with type 1 diabetes mellitus (TIDM), who are insulinopaenic, circulating levels of IGF-I, IGFBP3 and GHBP (the soluble extracellular domain of the GH receptor) are reduced. Although these levels are partially restored with the introduction of insulin therapy they remain subnormal largely because peripheral administration of insulin fails to achieve normal portal levels of insulin. The persisting low circulating levels of IGF-I in subjects with TIDM has been linked to the insulin resistance of puberty either directly or indirectly through the resultant GH hyper-secretion.

Low IGF-I levels from the diagnosis of TIDM may have other important implications as IGF-I is a potent regulator of lymphocyte function and IGF-I replacement in animal models protects pancreatic islet cells from cytokine mediated inhibition of insulin secretion, stimulation of nitric oxide formation and cell death from apoptosis. Our first speaker will discuss the importance of IGF-I and its binding proteins in the regeneration and apoptosis of normal and diabetic human β cells.

The low IGF-I levels in subjects with TIDM have also been linked to the pathogenesis of diabetic complications. The development of retinopathy, nephropathy, neuropathy have been linked, in epidemiological studies, to low circulating IGF-I levels. These associations may be explained by the lack of direct effects of IGF-I to inhibit apoptosis but alternatively they may relate to the growth hormone hyper-secretion which results from low IGF-I levels. Extra-hepatic GH receptors are not dependent on insulin levels and the increased GH secretion may lead to enhanced paracrine production of IGF-I in tissues such as the retina and kidney. The potential role of IGF-I and other growth factors in the pathogenesis of diabetic complications will be covered by our second speaker.

L6
Regeneration and apoptosis of normal and diabetic human β cells by growth hormone, IGF-1 and IGF-1 binding protein-1

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Long-term alterations in pancreatic islet β cell mass constitute an important means to accommodate an increased demand for insulin. Since previous studies have established a defective insulin secretory response to glucose as well as a decreased β cell volume in diabetic patients, further elucidation of factors governing insulin production and β cell proliferation is clearly warranted. In contrast to other tissues (*e.g.* the liver) that readily regenerate, the adult insulin-producing pancreatic β cell is characterized by a limited proliferative potential. Additionally, its capacity to divide diminishes by increasing age, when glucose intolerance becomes more prevalent. Conversely, expansion of the pancreatic β cell mass by recruitment of β cell to proliferate may constitute a means by which the organism can compensate for the loss or dysfunction of β cells occurring in diabetes. Thus, if β cells could be induced to replicate at a higher rate, this may prove beneficial in maintaining normoglycemia. Importantly, when the adult β cell population is expanded *in vivo* by "cellophane wrapping" of the pancreas, this not only induces islet hyperplasia resembling nesidioblastosis, but also ameliorates experimental diabetes in hamsters.

Regenerative stimulation by growth promotion and apoptosis inhibition may be useful in improving *ex vivo* the growth and function of islets intended for transplantation, which is of crucial significance in obtaining a viable and functional islet graft. Among the environmental factors, growth factors, such as growth hormone (GH), insulin-like growth factors (IGF) and their binding proteins

(IGFBP) have effects on metabolism, cell function and apoptosis of the β cell. Elevation in cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) is a common mechanism in signaling events to initiate or alter cellular processes. On the other hand, abnormal elevation of $[Ca^{2+}]_i$ directly affect apoptosis of the β cell. Therefore, understanding of Ca^{2+} handling in the β cell is an important aspect in protecting the β cell from dysfunction and cell death.

Our studies show that GH-stimulated mitogenesis of fetal rat islet β cells was associated with increased $[Ca^{2+}]_i$ and diacylglycerol production via a phosphatidylcholine-specific phospholipase C (Sjöholm et al., J. Biol. Chem. 2000). IGFs are secreted from almost all types of cells, acting in autocrine, paracrine and endocrine manners, and stimulating β cell proliferation. The functions of IGFs are modulated by IGFBPs, among which IGFBP-1 is elevated in diabetes and was shown to be involved in glucose homeostasis. Whether the elevated IGFBP-1 in diabetics has any direct effect on pancreatic β cell function is unknown.

The inhibition of glucose-sensitive insulin release exerted by IGFBPs suggests an important regulatory role of local IGF in β cell function. This may be of significance in both physiologic and pathologic aspects of insulin exocytosis. Since serum levels of IGFBP-1 are elevated in diabetes, a state caused by impaired glucose-sensitive insulin secretion, locally produced IGF and IGFBPs may exert a critical regulatory in the pathogenesis of diabetes.

The studies on GH signaling will help to understand intracellular Ca^{2+} handling regulated by GH in pancreatic β cells, which is important for GH signaling and is involved in GH actions. In their entirety, the GH studies may be useful in improving *ex vivo* the growth and function of islets intended for transplantation into diabetic patients, which is of crucial significance in obtaining a viable and functional islet graft.

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L7

Guardian® RT Continuous Glucose Monitoring System with real time glucose values and alarm functions: a new tool for improving metabolic control in patients with T1DM. Results of the Guardcontrol trial

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Background: The Guardian® RT (Medtronic MiniMed) is a telemetered glucose monitoring system, with continuous real-time display of glucose values and low/high alerts at preset glucose levels. The efficacy of the device was evaluated for the first time in a clinical study by measuring the impact on glycaemic control and patients diabetes self-management.

Methods and results: The Guardcontrol Trial was a 3-arm randomised controlled trial in 8 European and Israeli centers. 162 adult (mean age: 38.68 ± 11.25 yrs) and pediatric patients (mean age: 14.46 ± 2.78 yrs) with T1DM for at least 1 year and on intensive insulin therapy (78 CSII, 84 MDI) for at least 3 months, characterised with inadequate glycaemic control (mean starting HbA1c 9.6 ± 1.2%) were assigned in equal numbers to either continuous use of the Guardian® RT, bi-weekly use, or control group (standard self-monitoring of capillary blood glucose). Metabolic control was assessed by HbA1c determined centrally (DCCT-standard) at the start, at 30 days and after 90 days of study. Average 24 h glucose levels, frequency and severity of hypo- and hyperglycemic excursions were assessed through downloads of the Guardian® RT data or blinded CGMS (standard continuous glucose monitoring system, Medtronic MiniMed) for the control group. Quality of life changes, patients' own use of the real time values and alerts, and treatment changes were evaluated. Preliminary data on metabolic outcome in a pediatric patient group will be presented at the ISPAD 2005 congress.

Conclusion: Results of this trial provide strong evidence that increased patient awareness of glucose fluctuations through alerts and real time glucose values allows patients to achieve significantly better glycaemic control.

L8

Effect of postprandial hyperglycemia and hyperlipemia on beta-cells – do we have to worry?

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In the natural history of type 2 diabetes (T2DM), individuals progress from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) to overt T2DM and this progression has been demonstrated in populations of diverse ethnic background. It is widely recognised that both insulin resistance and beta-cell dysfunction are important in the pathogenesis of glucose intolerance. In populations with a high prevalence of T2DM, insulin resistance is well established long before the development of any impairment in glucose homeostasis but as long as the beta-cell is able to secrete sufficient amounts of insulin to offset the severity of insulin resistance, glucose tolerance remains normal. This dynamic interaction between insulin secretion and insulin resistance is essential to the maintenance of NGT and interruption of this cross-talk between the beta-cell and peripheral tissues results in the progressive deterioration of glucose homeostasis. Early in the development of T2DM, the initial burst of insulin release in response to food intake is compromised, allowing postprandial hyperglycemia to develop. Meal-associated hyperglycemia further contributes to increase insulin resistance and decrease insulin production.

Why is glucose control so important? In the fasting state, the suppression of insulin and stimulation of glucagon production control the concentration of blood glucose. These processes allow the liver to mobilize glucose from its glycogen stores and synthesize glucose from amino acids and pyruvate (gluconeogenesis). In addition, when insulin levels are low, the uptake of glucose by muscle is minimized, and adipocytes release free fatty acids (FFA).

In the fed state, insulin is released in two phases. The first phase, a short, small burst released on food intake or an increase in plasma glucose concentration, preempts and decreases the postprandial glucose elevation. Later, a more sustained, second-phase insulin release directly proportional to the plasma glucose elevation occurs. In response to this biphasic release of insulin, the liver and muscle take up glucose, converting it to glycogen, and adipose tissues also take up glucose, storing it as triglycerides. Furthermore, the production of FFA in adipocytes is suppressed. The loss of first-phase insulin release has adverse metabolic and physiologic consequences, i.e. hyperglycemia on insulin-producing beta-cells and insulin-sensitive tissues (glucotoxicity), even if the second-phase release is adequate or even excessive.

Does altered FFA metabolism play a role? Insulin exerts its action not only toward glucose but also as a potent inhibitor of lipolysis since even small increments in the plasma insulin concentration exert a potent antilipolytic effect, leading to a marked reduction in plasma FFA. The decline in plasma FFA results in increased glucose uptake in muscle and contributes to the inhibition of hepatic glucose production. Thus, changes in the plasma FFA in response to increased plasma levels of insulin and glucose play an important role in the maintenance of normal glucose homeostasis. Increased FFA have *per se* a direct effect on insulin secretion and beta-cell function. Increased plasma FFA levels after 2–4 h and 24 h of lipid infusion have been shown to enhance both basal and glucose-stimulated insulin secretion. On the other hand, an overnight reduction of plasma FFA results in improvement of glucose tolerance despite reduction in circulating insulin levels. Thus, excess release of FFA into the circulation, as occurs in obesity or abdominal fat accumulation, has the effect to promote insulin secretion.

In summary, both insulin resistance and beta-cell dysfunction play a role in the transition from normal glucose tolerance to hyperglycemia. Excess FFA release and lack of inhibition of lipolysis (hyperlipemia) worsen this state.

L9
Function of adrenal cortex in type 1 diabetes

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Treatment of type 1 diabetes is far from physiology, what evokes many compensatory reactions in organism, including change in the hormonal balance of hypothalamo-pituitary-adrenal axis. On the other hand, glucocorticosteroids play an important role in homeostasis – stimulate gluconeogensis, act in counterregulatory repertoire saving from hypoglycemia, can evoke insulin resistance, modify adipose tissue endocrine function and influence on other points in metabolism, catecholamine secretion and action, blood pressure and electrolytes, immune system etc. All of these may interact with diabetes, so the problem seems worth to be evaluated.

The set of results from few studies performed in type 1 diabetic children and in their control coevals was presented. Basal blood cortisol (F) was elevated permanently in diabetes but parallel higher blood concentrations of steroid intermediate products: 17αOH-progesterone, 11-deoxycortisol as well as dehydroepiandrosterone and androstenedione) indicated on steroidogenesis hyperactivity in its early steps. It was confirmed in results from studies with $ACTH_{1-24}$ (Synacthen) stimulation test. An increased effect was observed in rapid (30 min) response to very small dose (1 µg) as well as after 2-h Synacthen (1 µg + 50 µg) iv. infusion. Serum F was analyzed as total and added AUC, and in each 30 minute's interval up to 150 min of observation. There was evidence for diabetes dependent enhanced steroidogenic responsiveness of adrenals. Gender differences were observed at basal blood F as well as after stimulation (higher reactivity in girls) but not in patients without diabetes.

7-h urine collections started together with Synacthen test in investigated patients. It was found by RP-HPLC analysis of excreted corticosteroids that F and cortisone (E) are depending on increased blood F levels in diabetes. The excreted E amount has extremely positive correlation with urine amounts (it depends on renal 11OHSD2 activity). No differences were found among diabetic and control groups in amounts of excreted F and E tetrahydro-derivatives (alloTHF, THF, alloTHE, THE). However, they had frequent positive correlations of insulin (alone or with BMI) to F 5α-reduction and E 5β-reduction. Other correlations suggested limitation in F and E metabolism toward to tetrahydro-metabolites with age and hyperinsulinism in type 1 diabetic children. So, apart from adrenal cortex increased reactivity to ACTH (a form of *diabetic adrenocorticopathy*?), there is a possible change in peripheral liver metabolism of cortisol and probably there is no effect of diabetes on kidney cortisol conversion to inactive cortisone.

In another study, there was checked pituitary gland reactivity in diabetic vs. control children to hCRH test. Blood ACTH and cortisol were estimated. Results indicated on partial suppression of pituitary gland in patients with type 1 diabetes. It was the next confirmation of permanently elevated blood cortisol, which is not related to low glycemia episodes, for example. An increased ACTH secretion in diabetic patients was not found.

In the course of diabetes therapy, hyperinsulinemia could be discussed as being responsible for exaggerated steroidogenesis. The role of CBG (corticosteroid binding globulin) must also be taken into consideration, when bound vs. free cortisol is analyzed.

Type 1 diabetes changes the cortisol balance toward hypercortisolism in young patients, which appears before other known diabetic complications, and thus cannot be their consequence. However, enhanced cortisol action has some implications for understanding of therapeutic difficulties and/or possibility of increased risk of complications in type 1 diabetic children and adolescents.

L10
Carbohydrate counting during transition from childhood to adolescence – an evolving skill

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Historically, carbohydrate restriction as a method of controlling blood glucose levels, led to poor growth, delayed puberty and an increased consumption of fat. Liberalisation of unrefined carbohydrate intake, together with traditional insulin regimens helped to improve the quality of diets, and led to the introduction of systems such as the 'plate model'. Glycaemic control was variable, but the requirement for regular meals and snacks whilst eating a 'free diet' frequently resulted in excessive weight gain, and led to a fixed eating pattern which could not adapt to changing requirements during adolescence.

Since the recognition that intensive insulin and dietary management can result in reduction in complications, and more recently the introduction of insulin analogues, we now have the incentive and the opportunity to adapt insulin and diet to address these changing requirements.

Carbohydrate counting as the basis for insulin adjustment, has been shown to be an integral part of intensification of insulin therapy in various studies including the Diabetes Control and Complications Trial (DCCT) and the Dose Adjustment for Normal Eating (DAFNE), and is an essential component of continuous subcutaneous insulin infusion therapy. There are various methods of carbohydrate counting, and their use will depend on individual and cultural factors. These include; the standard meal, where patients give a set dose of insulin for a particular meal type and

size; carbohydrate portions, which will vary from 10–15 g carbohydrate, where patients give a specific quantity of insulin per portion; and the use of a unit of insulin to number of grams of carbohydrate ratio. An individual or family may use any or all of these methods on a day-to-day basis, alongside regular pre- and post-prandial blood glucose monitoring.

Adolescents with diabetes in the UK frequently have poor glycaemic control. There is excessive weight gain together with disordered eating, particularly in the girls, and fear of hypoglycemia may result in both excessive food intake, high blood glucose levels and avoidance of tight control. Dietary regulation and lack of dietary freedom are often reported by adolescents to be the worst aspect of living with diabetes, and so dietary advice is often ignored.

We do not believe that children and adolescents should be denied the right to choose how they manage their diabetes, but we need to give them the tools to do this effectively. Carbohydrate counting is just one of those tools.

In Oxford UK carbohydrate counting as part of a healthy diet is taught to all children and families, regardless of regimen, with the aim of developing this skill as children grow up. We have audited the short-term outcomes in our clinic in teenagers using this with multiple daily injection regimens, and the data will be presented. The effect of this method of education on diet composition has also been examined.

In summary, as we gain more experience with this approach, we have seen that carbohydrate counting can be used as one of a range of tools to empower children and young people to mange their own diabetes more effectively within their own lifestyle.

L11
Diet-insulin relationship in the treatment of diabetic children and adolescents: experience acquired in Brussels

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Diet has traditionally played an important role in diabetic therapy. Over the years, various diets have been proposed often without scientific evidence. One of the main errors was (is!) to speculate that there exists a direct linear correlation between the injection of x units of insulin and the utilization of y grams of glucose. If this were true, one should give more insulin to practice physical activity! In reality, it is the reverse!

Dietary recommendations issued over the last few years are the same for diabetic and non-diabetic individuals in order to avoid degenerative diseases. In many countries, the intake of fat is too high, and of complex carbohydrate too low. The so-called 'Mediterranean diet', in combination with appropriate insulin therapy, may be optimal. It consists mainly of fiber-rich complex carbohydrates (grains), vegetables, fruits, fish, and olive oil. Explanations regarding this diet should focus on quality rather than quantity of foodstuffs and should be provided by a multidisciplinary team. Prescription of a highly rigid diet has proved ineffective in producing adequate metabolic control, and increases the risk of deviations from the diet.

In our experience, the proper use of the 2-injection regimen, in countries where the meal schedule allows correct allocation of diet, may lead to 'intensive conventional therapy' and good metabolic control. It is inadequate to systematically assimilate the multiple-insulin injection regimen to intensified insulin therapy, and the 'conventional' 2-injection regimen to a non-intensified insulin therapy.

The proper use of the basal-bolus regimen, with increased flexibility in daily life and dietary freedom, cannot be always applied successfully before adolescence. The adjustment of insulin dosage is more complicated than in the twice-daily injection regimen because dose alteration cannot be done only according to sliding scales based on the glycemia measured immediately before the insulin injection. The simplistic use of these unphysiological sliding scales is the main error in the multiple daily insulin injection regimen. The use of fast-acting insulin analogs in the basal-prandial regimen improves post-prandial glycemia at the expense of an increase in pre-prandial glucose levels, if the time-period between 2 meals, and therefore 2 injections, exceeds 3 to 4 hours because of the short duration of action. If there are 4 to 6 or 7 hours between 2 meals, it is better to use a rapid-acting insulin.

No dogmatism! Only the objective results (good glycated hemoglobin and lipid levels, as well as good quality of life) are important.

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L12
Do diabetic children really need dietary rules?

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It is old knowledge that carbohydrates increase blood glucose, and therefore fat, proteins and alcohol were prescribed to diabetic patients before the discovery of insulin. Gradually, after 1922 dietary advice changed. As late complications to diabetes were unknown the trend swung to the other extreme, 'free diet', to lessen the burden of children with diabetes. However, poor metabolic control and very high incidence of serious complications led to a return to different dietary rules.

All children need dietary rules. Lack of, or wrong, rules with too much sugar and fat is probably one important reason for the global epidemic of obesity among children with perfect insulin substitution, namely, non-diabetic children. Some of them get insulin resistance and develop type 2 diabetes. There is no reason to believe that type 1 diabetes protects against obesity or type 2 diabetes, and therefore diabetic children need the same restrictions regarding intake of sugar, fat, calories as non-diabetic children. As non-diabetic children they also need the same amount of energy, proteins, vitamins and minerals, but do they need more rules?

The aim of our treatment is normal growth and development, no acute or late complications and good quality of life. In our area we have been able to reduce complications dramatically (Bojestig NEJM 1994, Nordfeldt Diab Care 1997, Nordwall Diabetologia 2004) with a yearly average HbA1c of 6.5–6.7% (= 7.5–7.7 DCCT HbA1c) of all patients beyond partial remission. The insulin treatment is crucial. With extremely good insulin substitution no special dietary rules would be needed, but such good insulin substitution is rare if at all existent. Multiple Insulin Therapy or CSII with multiple meal boluses is no guarantee. The first requisite is that insulin doses are given. Most important is then honest but optimistic information from diagnosis of the disease and onwards, that the patient and his/her family learns to take the treatment seriously, without being fed up by tests, rules, adjustments. Too heavy burden by using complicated strict dietary rules may cause rebellious behavior, while total freedom is to leave, to give up responsibility.

The golden mean may vary from culture to culture, but we suggest: too much saturated fat may give macro-vascular complication, and too much protein may be negative for kidney function. Regular meals, reasonably regular amounts of carbohydrates at the meals, reduction of fat and increase of fibers, adaptation of meal

bolus to major changes of meal size, restricted consumption of sweets/sugar ('Saturday-sweets' for most children) are usually enough dietary rules to reach acceptable metabolic control in most patients who get adequate insulin treatment and learn how to use blood-glucose profiles to adjust their insulin regimen. Clever, interested patients may learn more about exchange units of carbohydrates (related to, but less laborious than, carbohydrate counting), which may increase their freedom without deterioration of metabolic control.

Conclusion: Limited clear dietary rules and recommendations are important for diabetic children, but adjusted to their capacity to avoid either overload of rules or a false freedom they cannot handle.

L13

Prevalence of long term complications in a group of type 1 diabetes patients supplied, from the diagnosis, with nutritional education regarding international RDA

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Introduction: A balanced nutritional programme is fundamental in the prevention of many chronic degenerative conditions. Our vision is to educate DMT1 patients and their families to a dietary approach according to recommended daily allowances (RDA). Our nutritional education includes: 5 to 6 servings of fruits and vegetables; hunger and satiation management; 3 main meals and 2 snacks correct composition of meals in respect of low GI carbs, fibre, fat and protein (mostly vegetable) consideration of all nutrients, not specifically carbs as appears to be the current fashion, consultation with a specialist dietician and progress monitoring 5 times a year. We term this: THE PREVENTIVE NUTRITION PROGRAMME (PNP). This approach (first published in 1998) has yielded positive results compared to other similar programmes. We now have a greater majority of patients adhering to recommended daily allowances.

Aim: To demonstrate the positive influence of our PNP with regards to long term complications.

Methodology: Retrospective cross sectional study in an unselected group of patients. Patients with DMT1 for more than 5 years (251); mean age: 21.2 yrs ± 7.08 (7.0–49.4); diabetes duration 13.29 yrs ± 5.8 (5.6–34.8). HbA1c: Bio-Rad D-10, DCCT/NGSP standardisation (reference range: 3.8–6%). Retinopathy: fundus photography; classified according to a modified Airlie House protocol. Persistent microalbuminuria: albumin/creatinine ratio (ACR) >2.5 mg/mmol in 2 out of 3 consecutive spot urine specimen.

Results: BMI: normal group (BMI <25) = 74.9%; overweight (BMI 25–30) = 21.5%; obese group, BMI > 30 = 3.6% No s.d. between males and females. The mean HbA1c % was: 8.47% (HbA1c % ≤ 7:7.6%; ≤8:36.7%; ≤9:74.9%; ≤10:92%; ≤11:97.2%) Blood pressure: systolic ≤ 130 mm/Hg = 80.5%. Diastolic ≤ 80 mg/HG = 89.6% Blood lipid levels (ADA 2005 recommendations). Results are as follows. Total cholesterol < 200 mg/dl = 86.1%; HDL cholesterol > 40 mg/dl = 95.6% LDL cholesterol < 100 mg/dl = 65.3%; Triglycerides < 150 mg/dl = 96%. The mean ACR was 0.86 ± 0.7 mg/mmol. ACR > 2.5 mg/mmol was present in 5.2% of patients (maximum level 4.6 mg/mmol). The prevalence of retinopathy was: absent in 57%; background 41.3%; preproliferative was 0.6% and proliferative 1.1%. In subjects with a diabetes duration of more than 15 yrs, the prevalence of retinopathy was 39.5%. Proliferative was present in 2 subjects with a duration of diabetes between 10–15 yrs. Correlation exists between: retinopathy and total cholesterol >200 mg/dl (no retinopathy 7%; retinopathy present 25% ($p < 0.001$); LDL cholesterol >100 mg/dl: no retinopathy 26%; retinopathy present 42% ($p = 0.03$). Triglycerides >150 mg/dl no retinopathy: 2%, retinopathy present: 10% ($p = 0.004$) ACR >2.5 mg/mmol and HbA1c expressed in quartiles and retinopathy.

Conclusion: When this programme is adhered to, we achieve the same proportion of carbs in every meal but our formulation also allows vital, protective nutrition from other sources. Our patients complications are less prevalent and overall less severe. As the levels of HbA1c in our group are above the ideal range, we can only assume that preventive nutrition is responsible for these positive results.

L14

The effects of rhIGF-I administration on metabolic control and insulin requirements in children with type 1 diabetes mellitus

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For many young people with Type 1 diabetes mellitus (T1DM) childhood & adolescence is a time of instability in terms of glycaemic control. Changes occurring in the hormonal milieu are partially responsible for this, particularly during puberty. In T1DM the pubertal increase in insulin resistance is exaggerated and is largely due to abnormalities occurring in the growth hormone / insulin-like growth factor-I (GH / IGF-I) axis. Typically, spontaneous GH secretion is greater than normal, whereas circulating bioavailable IGF-I is reduced. Both lead to reduced insulin sensitivity, and may contribute to the risk for the later development of microvascular complications. Insulin deficiency, particularly within the portal circulation, plays a pivotal role in the development of these abnormalities, yet intensification of subcutaneous insulin therapy can only partially restore GH and IGF-I levels to normal, and does so at the risk of increased hypoglycemia and excessive weight gain. Alternative treatment strategies have been proposed including the use of recombinant human IGF-I (rhIGF-I) therapy given as an adjunct to sc insulin. Administration of physiological replacement doses of rhIGF-I (20 to 40 µg/kg/day) subcutaneously restore circulating IGF-I levels back to within the normal range and are effective in suppressing GH hypersecretion and in restoring insulin sensitivity to normal. Short-term randomised, placebo controlled trials of rhIGF-I therapy have demonstrated reductions in insulin requirements and improvements in HbA1c in a dose responsive manner. However, larger, supraphysiological doses of rhIGF-I (>80 µg/kg/day) were associated with an increased incidence of retinopathy, either due to rapid improvements in glycaemic control or direct effects of high levels of free, unbound IGF-I. Recently, pilot studies using a rhIGF-I / rhIGFBP-3 complex have confirmed physiological efficacy of IGF-I therapy. This combined treatment is better tolerated and may result in reduced tissue exposure to high levels of free IGF-I. Longer-term clinical trials to assess the therapeutic efficacy and safety of this novel combination therapy are required.

L15

The signaling pathways regulating the metabolic profile of human skeletal muscle

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On current trends, around one in 10 people alive today will develop type 2 diabetes during their lifetime. Thus diabetes and complications arising from this disease place a considerable burden on the afflicted individual and society. We have explored insulin signaling in skeletal muscle, a principle organ involved in whole body insulin resistance, from type 2 diabetic patients and healthy individuals and

provided evidence for selective defects in insulin action in diabetic muscle at the level of IRS1 tyrosine phosphorylation, with insulin signaling to mitogen-activated protein (MAP) kinase unaffected. This highlights that in people with type 2 diabetes, selective defects in insulin signaling contribute to impaired glucose homeostasis. To further explore insulin action at the cellular level, we have utilised primary cultures of human skeletal muscle. Cultured primary skeletal muscle is the closest available cell system to intact skeletal muscle, and although they do not fully differentiate to mature muscle, the cells maintain a skeletal muscle phenotype and genotype. Thus, primary human muscle cultures are a suitable model system to resolve intrinsic from acquired defects in the pathogenesis of diabetes. This model has been used to explore the regulation of the metabolic phenotype in skeletal muscle.

L16
The mitochondria: another therapeutic target in type 2 diabetes?

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Over the last decades, the incidence of type 2 diabetes mellitus has increased dramatically, affecting over 110 million people world wide in 2000, with estimates of 250 million people in 2020.

At the heart of the problem of type 2 diabetes lies insulin resistance, an early detectable and cardinal feature on the way to full-blown and clinically overt type 2 diabetes. Of the insulin resistant tissues, skeletal muscle is responsible for the majority of post-prandial glucose disposal and therefore plays a critical role in the development of type 2 diabetes.

Frequently reported characteristics of the insulin resistant state include increased plasma free fatty acids, increased muscular storage of fatty acids as intramuscular triglycerides (IMTGs) and a decreased triacylglycerol/fatty acid recycling of the IMTG, all of which may result from a mismatch between delivery of fatty acids to the mitochondria and the failure to divert these fatty acids towards mitochondrial β-oxidation. Interestingly, recent reports have shown abnormalities of mitochondrial morphology and reduced oxidative capacity in type 2 diabetic patients and in their first-degree relatives. These findings nicely match with reports showing that a cluster of genes under control of the mitochondrial transcriptional Cofactor PGC1α is downregulated in type 2 diabetes and that the level of downregulation is associated with insulin resistance. While the reduction in PGC1α helps to understand the decreased mitochondrial biogenesis and hence fat oxidative capacity, the factors underlying abnormal mitochondrial morphology are not yet fully understood.

Next to their essential role in maintaining cellular ATP levels, mitochondria are the major source of reactive oxygen species (ROS), an obligatory side product of the electron transport chain. Production of ROS in the inner mitochondrial membrane may result in peroxidation of the lipids present in the vicinity of the mitochondria and lipid-induced damage to mtDNA, RNA and proteins present (lipotoxicity). Whereas mitochondria are poorly equipped with a repair mechanism to restore this damage, muscle mitochondria do contain a so-called uncoupling protein (UCP3) that may serve to prevent lipotoxicity by facilitating outward translocation of fatty acids or lipid peroxides away from the mitochondrial matrix. Alternatively, UCP3 may directly lower ROS production after activation by lipid peroxides thus resulting in a feed forward loop resulting in modulation of lipid peroxidation. Interestingly, we have shown that UCP3 levels are reduced by ~50% in type 2 diabetes and in pre-diabetic subjects, suggesting that reduced UCP3 content may contribute to diabetes pathogenesis. One of the most potent ways to treat type 2 diabetes, albeit not particularly popular, is increasing physical activity. If type 2 diabetics undergo an exercise intervention they (re)gain insulin sensitivity, and UCP3 levels are restored to control values. Similarly the novel class of insulin sensitizers, thiazolidinediones, restore UCP3 to control levels and improve muscular insulin sensitivity.

The aim of this presentation therefore is to clarify the role of mitochondria in the pathogenesis of type 2 diabetes and to guide you through putative mitochondrial targets for treatment of this disease.

L17
The birth of human beta cell

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There is a slow turnover of pancreatic beta cells throughout life and regulation of the beta cell mass is an important component in the overall glucose homeostasis of the body. During embryonic development, both endocrine and exocrine pancreatic cells develop from common stem cells within the early gut endoderm. The pancreatic islet stem cells have been fairly well characterized in the developing pancreas. A specific cell type, characterized by the expression of two transcription factors, Pdx-1 (human equivalent is Ipf-1) and neurogenin-3 (Ngn-3), represents the precursor of all islet cell types. However, much more controversy remains concerning the possible existence and nature of stem or precursor cells in the adult pancreas. Transgenic cell lineage tracing experiments in mice have suggested that beta cell replication is the major mechanism of expansion of beta cell mass in the adult, while islet neogenesis from precursors would not contribute at all. However, human beta cell replication is very low and there is abundant indirect evidence for the budding of new islets from pancreatic ducts both *in vivo* and *in vitro*. Recent *in vitro* experiments show that the plasticity of differentiated human pancreatic cells is greater than previously thought. A new concept of transient dedifferentiation, proliferation and re-differentiation of beta cells is emerging as an important mechanism for human beta cell expansion. Availability of human embryonic stem cells has led to an active area of research aiming to achieve targeted differentiation of these cells into a safely transplantable beta-like cell. After inititial excitement, it appears that a lot of basic research is still required before this goal could be achieved. The major challenge is to achieve early commitment of these pluripotent cells towards definitive endoderm progenitors instead of the default pathway of neuroectodermal differentiation.

L18
Gene expression regulating-cell dysfunction and death

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Accumulating evidence indicates that beta cells die by apoptosis in early type 1 diabetes mellitus (T1DM). Apoptosis is an active, gene directed process, and recent observations by our group suggest that beta cell apoptosis depends on the parallel and/or sequential up- and down-regulation of hundreds of genes.

We have utilized microarray analysis and detailed promoter studies to clarify the pattern and regulation of gene expression in primary rat β-cells and in human islets exposed for different time points to the pro-apoptotic cytokines interleukin-1β (IL-1β+ interferon-γ (IFN-γ. Nearly 2000 cytokine-induced genes were identified, and the picture emerging from these findings is that β-cells are not passive bystanders of their own destruction. β-Cells respond to cytokine-mediated damage by triggering several genes involved in defense/repair and endoplasmic reticulum stress, by decreasing their

most differentiated functions and their capacity for growth and regeneration, and by inducing expression of diverse cytokines and chemokines. Several of these effects of cytokines depend on the activation of the transcription factors NF-κB and STAT-1, and by blocking NF-κB or STAT-1 activation we prevented both cytokine and dsRNA (double stranded RNA) + cytokine-induced rat β-cell death. Subsequent experiments, combining NF-κB blocking and microarray analysis, suggested that NF-κB functions as a 'master switch' of IL-1β effects on β-cells, controlling diverse networks of transcription factors and effector genes that contribute to β-cell apoptosis. STAT-1 probably plays a similar role for IFN-γ-induced genes. This hypothesis was further investigated by time course and cluster analysis of gene expression in cytokine-treated insulin-producing INS-1 cells, and by 'in silico' and molecular biology analysis of the promoter regions of genes located in different clusters. Based on the data obtained by our different microarray analysis, we are presently constructing a 'Beta Cell Gene Expression Bank', which is already accessible at http://t1dbase.org/cgi-bin/enter_bcgb.cgi. By combining functional studies with microarray analysis, performed with or without targeted perturbations of the system (following the systems biology approach), we hope to eventually understand the complex mechanisms regulating the cytokine-induced β-cell 'decision' to undergo apoptosis. This information may point out new approaches to prevent β-cell death in early T1DM.

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