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Welcome Message from Congress Board

The epidemic increase in the incidence of type 2 diabetes all over the World has intensified the efforts to find ways of treatment and prevention of this disease that is a threat to population health and health care economy. In fact the increase in the incidence of type 1 diabetes is similar but in a lower scale. Both forms may be related to the changes in life style. Type 2 diabetes is closely associated with increased availability of high energy nutrients and decreased physical activity whereas type 1 diabetes seems to be associated with increased hygiene and reduced exposure to infectious agents. In both forms the pancreatic beta cell plays a pivotal role. Type 1 diabetes occurs when the beta cells are destroyed by an autoimmune process whereas type 2 diabetes occurs when the capacity of the beta cells to compensate for the increased demand in obesity is insufficient. Recent genome wide association studies have identified more than 50 susceptibility gene loci of which the majority is expressed in the beta cells. Therefore the beta cells are the focus for much of the current diabetes research and the aim of the conference “Beta Cells in Health and Disease” in Turkey is to bring together some of the leading experts in beta cell biology to present the latest development in the field and outline the avenues for future research that may lead to cure or prevention of this devastating disease.

We are looking forward to welcome you at Kocaeli University-Turkey in May 2014.

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Scientific Program

May 21, 2014, Wednesday

15:00-15:30 Opening Ceremony

15:30-16:30 Session 1: Beta cell biology: development and function
Chair: Sukru Hatun and Tuncay Delibasi

15:30-16:00 Pancreatic alpha and beta cells in diabetes, Ole Madsen
16:00-16:30 Developmental reprogramming of the beta cell, Mulchand Patel

16:30-16:45 Coffee Break

16:45-17:15 Inter-organ control of the functional beta cell mass, Bernard Thorens

17:15-17:45 Signaling mechanisms in insulin secretion, Lena Eliasson

17:45-18:15 Stem Cell Therapy in Iran, Bagher Larijani

18:15-19:15 Brief Presentations
Chair: Jens H. Nielsen

18:15-18:27 Wnt4 regulates canonical Wnt signalling by blocking the increase of active beta catenin into the nucleus: Bowen A, Whatmore J, Kos K and Welters HJ

18:27-18:39 Beta cells and the beneficial effect of gluten-free diet in animal models of type 1 diabetes: Buschard K

18:39-18:51 Soluble TRAIL (TNF-Related Apoptosis-Inducing Ligand) Treatment Induces Proliferation in Both Primary Rat Pancreatic Beta Cells and Mouse Pancreatic Beta Cell Line: Kahraman S, Dirice E, Altunbas HA, Sanlioglu AD

18:51-19:03 Premature weaning exacerbate diabetes resulting from a later injury to pancreatic beta cells: Stolovich-Rain M and Dor Y

19:03-19:15 Comparative analysis of differentiation potential of mouse embryonic stem cells into insulin producing cells by co-culture with pancreatic islets and chemical method: Yilmaz I, Eker Sariboyaci A, Okcu A, Subasi C, Karaog E
May 22, 2014, Thursday

08:30-10:30  Session 2: *Mechanisms involved in beta cell failure in diabetes*
*Chair:* Jens H. Nielsen

08:30-09:00  Insulin resistance, cause or consequence?, Barbara Corkey
09:00-09:30  α/β-Hydrolase Domain 6 accessible monoacylglycerol: implications for beta cell dysfunction, obesity and diabetes, Marc Prentki
09:30-10:00  Molecular mechanisms in beta cell failure and apoptosis, Thomas Mandrup-Poulsen
10:00-10:30  Inflammatory mediators of beta cell apoptosis, Decio Eizirik

10:30-10:45  Coffee Break

10:45-11:15  Studies of rare and low-frequency variants in relation to metabolic phenotypes, Annette M. P. Gjesing

11:15-11:45  Genetics of beta cell dysfunction, Philippe Froguel

11:45-13:15  Session 3: *Beta cell therapy of diabetes*
*Chair:* Ole Madsen

11:45-12:15  Molecular Control of Human Beta Cell Replication for Diabetes, Andrew Stewart
12:15-12:45  Diabetes recovery by age-dependent conversion of pancreatic delta or alpha cells into insulin producers, Simona Chera

12:45-14:00  Lunch

14:00-14:30  Poster Session

14:30-15:15  Visit to Research Labs (Proteomics, Diabetes and Obesity, Medical Genetics, Stem Cell and Gene Therapies)

15:15-16:15  Session 4: *Beta cell replacement therapy*
*Chair:* Erdal Karaöz

15:15-15:45  Fetal derived stem cells: a potential source for islet regeneration, Hamid Reza Aghayan
15:45-16:15  Establishing a cGMP-compliant facility for stem cell and pancreatic islet manufacturing: Our experience in Iran, Babak Arjmand

16:15-16:30  Coffee Break

16:30-18:45  Session 5: *New treatments of type 1 and 2 diabetes*
*Chair:* İlhan Tarkun

16:30-17:00  Incretin therapy, Jens J Holst
17:00-17:30  Restoration of pancreatic beta cell mass and function by GLP-1 gene delivery for diabetes, Salih Sanlioğlu
17:30-18:00  Immunotherapy of Type 1 Diabetes, Anne Cooke
18:00-18:45  Panel discussion: Future prevention and treatment of diabetes

18:45-19:00  Concluding Remarks
Invited Talks

(Abstracts are listed by presentation order)
Compensatory up-regulation of functional beta cell mass is essential to maintain normoglycemia as a consequence of “Western lifestyle”-induced insulin resistance. Diabetes (T2D vs. T1D) is characterized by a relative vs. absolute beta cell deficiency, respectively. Mechanisms and signalling pathways involved appear linked to glucose metabolism and are becoming elucidated to possibly consist of mixtures of both local permissive factors (action via IR/IGF1R and IRS2) as well as liver derived signals such as the recently reported betatropin. Glp-1 has additional profound effects on the functional mass due to its glucose dependent incretin effect on insulin secretion possibly combined with beta cell anti-apoptotic and neogenic activity.

Interestingly, the absence of glucagon signalling (in the liver only?) results in alpha cell neogenesis/proliferation and the gcg-KO has marked hyperplasia of Arx positive, but hormone-negative alpha cells while intestinal L-cells are unchanged. Targeted conditional expression of the single factor, Pax4 (repressor of Arx) reprogram alpha to beta cells, eliminates glucagon signalling and leads to massive islet beta cell hyperplasia. Spontaneous reprogramming (transdifferentiation) from alpha to beta cells can occur in mice with near-total beta cell ablation. Recent data suggests that absence of glucagon signalling minimizes (or eliminates?) the need for insulin to maintain glucose competence (in rodents). Thus the controlled balance of alpha to beta cells may be key to sustain life-long euglycemia. Mice lacking insulin die soon after birth (of diabetes). Mice lacking proglucagon derived peptides (Glu/alpha cells; Glp1, Glp2/L-cells) are viable, normoglycemic and have a normal life span. An ultimate experiment remains to test if the gcg-KO will rescue the lethal ins-KO?

Insulin replacement therapy has remained a successful life-saving treatment for diabetes for >90 years and is unlikely to be replaced by a glucagon-antagonistic principle (potentially leading to a vicious cycle of alpha cell hyperplasia). Combination therapy with long-acting forms of insulin and Glp1 appears highly beneficial in T2D and might find part of the explanation as a unique approach to maintain and stimulate residual beta cell mass. Finally, a third cell type may become relevant to learn more about: the alpha/L cell that remains glucose-responsive, express both converting enzymes PC2 (alpha) and PC1/3 (L) and co secrete all processable forms of proglucagon. The combination of glucagon and Glp1 has been demonstrated to eliminate obesity in rodents and transplantable glucagonomas with “total proglucagon processing” cause severe anorexia.

Altered nutritional experience during critical periods of early development may play a decisive role in metabolic programming of target organs with long-term consequences for the offspring. Maternal malnutrition during gestation and lactation can impact on beta cell development and function in the offspring. Two animal models will be presented. (i) Fetal programming due to maternal obesity: Maternal obesity induced by chronic consumption of a high-fat (HF) diet results in increased weight gains and modified profiles of plasma substrates, hormones and
proinflammatory markers during pregnancy. Term HF fetuses show hyperinsulinemia and islets’ insulin hypersecretory response. These parameters reappear in HF offspring soon after weaning them on lab chow and develop obesogenic plasma profile, glucose intolerance and obesity. (ii) Altered nutritional experience in the suckling period: The HC rat pups fed a high-carbohydrate milk formula develop hyperinsulinemia due to altered islet structure and beta cell functions including glucose-stimulated insulin secretion and increased parasympathetic activity. These changes persist into adulthood predisposing to the development of obesity. Pair-feeding of HC rats (HC/PF) from weaning show normalized body weight gains and serum insulin levels but these parameters are restored to HC levels in the HC/PF/AL rats after ad libitum feeding, indicating that calorie restriction cannot erase the programmed predisposition for hypersecretory capacity of islets and hypothalamic hyperphagic response in obese HC rats. In summary, maternal obese intrauterine environment and feeding practices for babies (early introduction of infant foods high in carbohydrates) may be contributing factors for obesity/diabetic epidemic prevalent in developed and developing countries.

**IT - Signaling Mechanisms in Insulin Secretion**  
**Lena Eliasson**  
*Lund University Diabetes Centre (LUDC), Unit of Islet cell Exocytosis, Dept Clinical Sciences Malmö, Lund University, Clinical Research Centre, Malmö*

Insulin is central in the control of blood glucose levels, and impaired secretion is involved in the development of type 2 diabetes (T2D). Specifically, first phase insulin secretion is absent in patients with the disease. Insulin is released through Ca\(^{2+}\)-dependent exocytosis, and first phase insulin secretion has been linked to the final steps of the beta-cell exocytotic machinery.

We use expression analysis and patch-clamp technology to investigate the importance of exocytotic proteins for functional insulin secretion and to study mechanisms by which these proteins control the release of insulin. We can demonstrate that the expression of obvious exocytotic genes, such as STX1A, SYT4 and SYT7, is reduced in islets from T2D human donors. Moreover, we have recently demonstrated that the chloride channel CFTR, associated with the disease cystic fibrosis, is present in mouse and human beta-cells and is involved in the regulation of beta-cell exocytosis. Finally, the increasing demand to produce and secrete more insulin during the progression of diabetes requires an advanced adaption system. MicroRNAs (miRNAs), small non-coding RNAs regulating the expression of target proteins, have the capacity to participate in the adaptations needed. We could demonstrate that expression of several miRNAs is changed in islets from the diabetic GK-rat and from human T2D donors. Interestingly, several of these miRNAs regulate the expression of target proteins involved in exocytosis.

In conclusion, we suggest that microRNAs have a key function in the adaptation of beta-cell demand during development of T2D through regulation of several pathways including exocytosis. We believe that exocytotic genes have a central role in the signaling mechanism controlling the release of insulin and that their reduced expression contributes to impaired insulin secretion in T2D.
Recently, stem cell research has found great public interest and different cell-based clinical trials have been started in Iran. In 2002, Iran’s supreme leader publicly supported human embryonic stem cell (ESC) research and congratulated the scientists who had produced the ESC lines. In 2005, the Ministry of Health and Tehran University of Medical Sciences jointly developed a set of guidelines regarding research on human gametes and embryos for stem cell research and therapy. Iranian council of stem cell technology was established by Deputy of Research and Technology (Ministry of Health) in 2008. The main goal of this council is promotion of clinical and translational stem cell researches in order to improve public health. This council and Food and Drug Organization have started working on a plan to regulate cell-based therapies in Iran. The objective of this presentation is to provide an overview of clinical cell transplantation researches in Iran, which has assumed a leadership role in the Middle East. By comparison with basic stem cell research, the current status of cell transplantation trials in Iran is not optimal. Joined multicenter research, implementation of national regulations, sharing of facility and staff, international collaborations and bridging the gap between basic and clinical research may improve quality and quantity of clinical cell transplantation research in Iran.
May 22, 2014
Session 2: Mechanisms Involved in Beta Cell Failure in Diabetes  
08:30-10:30

IT - Insulin Resistance: Cause or Consequence?

Barbara E. Corkey  
Boston University School of Medicine  

Many studies have investigated fuel or incretin-stimulated insulin secretion and insulin interaction with target tissues, however, the most striking abnormality in metabolic disease is basal hypersecretion of insulin. It is accepted that obesity leads to hyperinsulinemia and insulin resistance. However, it is generally assumed that hyperinsulinemia follows obesity rather than vice versa. Excess secretion by the β-cell can be the initial and sustaining incident in the development of insulin resistance. This hypothesis is based on a signal transduction cascade in which environmental factors or fuel excess lead to an increased redox state, increased mitochondrial membrane potential, increased ROS and impaired mitochondrial function. Support for this hypothesis includes: 1) Repeated insulin injection causes obesity and insulin resistance; 2) Inhibition of insulin hypersecretion by diazoxide reverses insulin resistance; 3) Rimonabant lowers insulin hypersecretion in islets from obese Zucker rats. We have documented several conditions in vitro that increase insulin secretion in the absence of stimulatory glucose. These include in vitro culture in high glucose (11 mM) plus high lipid (0.1 mM palmitate) or acute exposure to the monoglyceride, 2-monooleoylglycerol (MG) or H2O2. Such glucose-independent stimulation of insulin secretion, if occurring in vivo, would cause insulin resistance and obesity initially, and might ultimately lead to development of diabetes in susceptible individuals. Support for the β-cell-mediated insulin resistance hypothesis would lead to radically different strategies for the treatment of insulin resistance and Type 2 diabetes and would suggest possible early interventions for obesity.

IT - Molecular Mechanisms in Beta Cell Failure and Apoptosis

Thomas Mandrup-Poulsen, MD, PhD

Posttranslational modifications such as methylations, acetylations and phosphorylations are critical regulators of gene expression and protein function. Acetylation and deacetylation of lysine residues control gene expression at the level of modifications of the histone backbone of DNA as well as at the level of transcription factors and many cytosolic signalling proteins. The acetylation status is controlled by lysine acetyl transferases (KATs) and deacetylases (KDACs). We have reported that inhibitors of lysine deacetylases, and in particular of HDAC1 and 3, restore β-cell function and viability in vitro (Larsen L et al Diabetologia 2007; Lundh M et al Diabetologia 2010; Lundh M et al Diabetologia 2012) and in the non-obese diabetic (NOD) mouse model of type 1 diabetes of oxidative and inflammatory stress (Christensen DP et al PNAS USA 2014). The mechanism of action involves hyperacetylation of the master inflammatory transcription factor NFκB p65 subunit, preventing its binding to proinflammatory gene promoters (Christensen DP et al PNAS USA 2014).

Recently we found that HDAC3 inhibition prevents glucolipotoxic apoptosis in vitro and restores glycemia, insulin secretion and β-cell mass in the Zucker Diabetic Fatty (ZDF) rat model of type 2 diabetes, not by affecting p65 transcriptional activity but by intercepting endoplasmic reticulum (ER) and mitochondrial death pathways (Wagner F et al Sci Transl Med, submitted). These studies suggest that HDACs are promising therapeutic targets in both type 1 and type 2 diabetes and warrant clinical trials.
We know that type 2 diabetes is a complex disease with a genetic component. Genome-wide association studies have enabled us to investigate common variants having a minor allele frequency above 5%. At present we have identified approx. 60 common variants associating with type 2 diabetes and related traits. These variants explain a maximum of 30% of the genetic influence on the development of type 2 diabetes. Yet, the effect of each variant is minor. By the use of sequencing, we are now also able investigate the effect of low frequency (between 5 and 1%) and rare variants (below 1%) on the development of type 2 diabetes. The results of studies investigating these less frequent variants are slowly emerging. With the use sequencing of the coding regions, we have investigated the effects of these less frequent variants in 1000 Danish type 2 diabetes patients and in 1000 Danish control subjects and have identified genes which appear to have higher burden of variation in diabetic patients. Yet the effect size of these low frequency variants appears to be less than what we expected.

Another place to look for genetic variants having a significant effect size is in small populations, since deleterious variants have a higher probability of reaching high frequency. Thus, we performed an association study of type 2 diabetes-related quantitative traits in Greenlandic individuals. We discovered a variant with an allele frequency of 17% highly associated with type 2 diabetes-related traits and with an effect size larger than what we have previously seen for common variants.

Finally, very rare variants are also involved in the cause monogenic diabetes and there are several examples where sequencing has been successful in the identification of the causal variants, which may have a large direct impact on the treatment of the individual patient. This approached have been used in relation to the monogenic form of diabetes called MODY. We have identified families and probands having MODY without a known genetic course. Using exome sequencing in 72 individuals, we have identified variants which co-segregate with the disease. These variants are in the process of being further investigated.

**Session 3: Beta Cell Therapy of Diabetes**

**IT - Molecular Control of Human Beta Cell Replication for Diabetes**

*Andrew F. Stewart MD*

*Director, Diabetes Obesity and Metabolism Institute, Icahn School of Medicine at Mount Sinai*

*New York, NY USA*

Human pancreatic beta cells are lost or reduced in numbers in both Type 1 and Type 2 diabetes. This has prompted attempts to induce human beta cells to regenerate, or to replace beta cells from cadaveric donors. In the US, there are 26 million people with diabetes, but only ~2000 pancreas organ donors per year, so that replacement of beta cells from cadaveric sources is not feasible on a large scale. Thus, there is an urgent need to induce human beta cells to replicate or regenerate in vivo in people with diabetes as well as ex vivo to generate sufficient supplies of human beta cells for beta cell replacement therapy. With these thoughts in mind, we have attempted to develop approaches to inducing human beta cells to proliferate and expand. Unfortunately, this has proven difficult, because human beta cells have proven resistant to mitogens, growth factors, small molecules and biologics that induce rodent beta cells to
replicate. Accordingly, we have explored the molecular control at the G1/S checkpoint of human beta cell cycle control, and have shown that human beta cells are amenable to induction of cell cycle entry through manipulation of cyclins and cdks. Moreover, cyclin- and cdk-mediated cell cycle induction leads to enhanced function in human islets transplanted into immunodeficient diabetic rodent models.

Our efforts at expanding human beta cells has now extended to manipulation of upstream signaling pathways and high-throughput small molecule screens, both of which have yielded promising approaches to therapeutic human beta cell expansion. These will be discussed in the meeting.

References.

**IT - Diabetes Recovery by Age-Dependent Conversion of Pancreatic Delta or Alpha Cells into Insulin Producers**

*Simona Chera, G. Gu, F. Reimann, F. Thorel, V. Cigliola, K. Furuyama, L. Ghila & P.L. Herrera*

Restoration of endogenous insulin production in diabetes is a major medical challenge. We recently showed that new insulin-producing cells arise from mature glucagon-producing α-cells in diabetes. Here, using mice in which β-cells can be completely ablated, we studied the influence of age on β-cell reconstitution from non-β-cells. We found that if β-cell loss occurs before puberty, all mice recover from diabetes by the age of 4 months. We will report the mechanism of regeneration in these animals, completely novel, which does not concern α-to-β-cell conversion.
Session 4: Beta Cell Replacement Therapy  

15:15-16:15

IT - Fetal Derived Stem Cells: A Potential Source for Islet Regeneration

Hamid Reza Aghayan*1, Bagher Larijani2, Babak Arjmand3
1- Chronic Diseases Research Center, Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences, Tehran, Iran
2- Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences, Tehran, Iran

During recent years, significant progress has been made in beta-cell replacement therapies with a progressive improvement of short and long term outcomes. Organ shortage, graft rejection, complexity of isolation procedure, lifelong immunosuppression, and high costs are main limitations of islet transplantation. Stem cells represent a promising solution to these limitations, and current research is being aimed at the creation of islet-endocrine tissue from these undifferentiated cells. Stem cells can be found at various stages of development with a declining gradient of potency from embryonic to adult cells. Fetal stem cells appear to represent an intermediate cell type with more safety than embryonic and more potency than adult stem cells. The fetal environment is unique as it is the only time in human development that there is large-scale migration of stem cells into different organs to make up the organism. The suitability of fetal derived stem cells transplantation for treatment of diabetes has been well documented in animal experiments. In literature review we found that most of published studies on fetal pancreas transplantation were performed in 1980s and 1990s. In recent years research on human fetal stem cells have been revived and some clinical trials have been started in different conditions such as ALS, diabetes, spinal cord injury and cirrhosis. The aim of this presentation is to describe the potential applications of fetal stem cells in islet regeneration. We also briefly explain about our experience of clinical grade fetal pancreatic stem cell manufacturing in our GMP facility.

IT - Establishing a cGMP-compliant Facility for Stem Cell and Pancreatic Islet Manufacturing: Our Experience in Iran

Babak Arjmand*1,2, Hamid Reza Aghayan1,2, Bagher Larijani1, Parisa Goodarzi3
1- Endocrinology and Metabolism Research Institute, Chronic Diseases Research Center, Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences, Tehran, 14114, Iran
2- Chronic Diseases Research Center, Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences, Tehran, 14114, Iran
3- Brain and Spinal Cord Research Center, Tehran University of Medical Sciences, Tehran Iran

It has been predicted that one of the greatest increase in prevalence of diabetes will happen in the Middle East bear in the next decades. The aim of standard therapeutic strategies for diabetes is better control of complications. In contrast, some new strategies like cell and gene therapy have aimed to cure the disease. In recent years, significant progress has occurred in Stem cell and beta-cell replacement therapies with a progressive improvement of short-term and long term outcomes. In year 2005, considering the impact of the disease in Iran and the promising results of the Edmonton protocol, the funding for establishing a current Good Manufacturing Practice (cGMP) stem cell and islet processing facility by Endocrinology and Metabolism Research Institute was approved by Tehran University of Medical Sciences. Several stem cell and islet manufacturing processes were performed following establishment of cGMP facility and recruitment of all required equipments for process validation and experimental purpose. Finally, the first successful clinical islet isolation and transplantation was performed in September 2010. In spite of a high cost
of the procedure it is considered beneficial and may prevent long term complications and the costs associated with secondary cares. Furthermore, we provide human stem cells from different sources for various clinical applications. Now, our purpose is to describe our experience in setting up a cGMP stem cell and islet manufacturing facility for clinical transplantation.

**Session 5: New Treatments of Type 1 and 2 Diabetes**

**16:30-18:45**

**IT - Restoration of Pancreatic Beta Cell Mass and Function by GLP-1 Gene Delivery for Diabetes**

*Salih Sanlioglu*

*Gene and Cell Therapy Center of Akdeniz University Hospitals and Clinics, Antalya, Turkiye 07058*

Glucagon-like peptide-1 (GLP-1) is an incretin hormone generated through post-translational processing of proglucagon fragment. It is an insulin secretagogue released from intestinal L-cells in response to nutrient digestion. Antidiabetic functions of GLP-1 include but not limited to enhancement of beta cell function, stimulation of beta cell proliferation and differentiation as well as induction of satiety by delaying gastric emptying ultimately leading to weight loss. Since patients with Type 2 Diabetes (T2DM) manifested impaired GLP-1 secretion, GLP-1 peptide has been regarded as a novel therapeutic agent for diabetic patients. Unfortunately, its short half-life due to quick degradation by dipeptidyl-peptidase 4 (DPP-4) renders it unfit for therapeutic use as a subcutaneously administered drug. Therefore, DPP-4 resistant forms of these drugs; the synthetic GLP-1-receptor agonists exenatide, and liraglutide were developed with half-lives of 2 and 12 h, respectively (1). However, these antidiabetic agents still require daily injection to demonstrate full efficacy. Because patient compliance is an important component of diabetes management, once or twice daily injection may represent a significant hurdle for adoption of any therapeutic agent. Thus, to avoid frequent injections or larger quantities needed for the compensation of the short biologic activity of GLP-1, viral or non-viral vector gene delivery methods were developed to supply a constant bioactive GLP-1 production and secretion in vivo (2).

Initial plasmid-based gene delivery techniques only mediated transient effects on insulin secretion and blood glucose levels (3). This was mainly attributed to the inherent nature of plasmid-based gene delivery method providing short-term gene expression along with an absence of a secretory signal within GLP-1 encoding sequence. Among the viral vectors tested, adenoviral vectors were very efficient in transducing a wide range of tissues with an ability to infect both dividing and non-dividing cells, to produce high titer yield and accommodate large transgenes (4). However, adenovirus-transduced cells were quickly cleared by the immune system due to antigenicity to adenovirus encoded viral peptides severely limiting the longevity of transgene expression. Not to mention, repeated administration of the vector was not feasible due to the presence of neutralizing antibodies. On the contrary, Adeno Associated Virus (AAV) has not been associated with any disease or pathology in humans despite its limited packaging capacity. Unlike adenovirus, lack of an immune response against the AAV vector and its ability to form double-stranded extra chromosomal (episomal) genomes allowed long term vector persistence in vivo. Thus, AAV vectors were able to supply long-term transgene expression in pancreatic beta cells (5). The use of a cell type-specific promoter (e.g., insulin promoter) was instrumental in restricting transgene expression in target tissues. By this token, intra-islet production of GLP-1 generated a localized environment capable of significantly improving islet function and survival even in the absence of high levels of circulating GLP-1. Meal regulated GLP-1 secretion from islets augmented glucose stimulated insulin secretion and contributed to the maintenance of islet health prior to degradation with DPP-4. Consequently, beta cell function and mass were successfully restored in monogenic and polygenic animal models of
diabetes using GLP-1 gene delivery. Tissue specificity was also achieved using epitope targeting by way of pseudo-typing or use of alternative serotypes of AAV vectors (AAV8).

Since gene delivery approaches involving GLP-1 were very effective in both pre-diabetic and fully diabetic animals, this approach might be a good alternative to constant infusions or daily injections of GLP-1 peptide. Although GLP-1 gene delivery approaches using double stranded AAV (dsAAV) vectors have yielded some successful results, lentivirus vectors targeting pancreas with glucoregulatory function might be a better option to deploy against diabetes considering the long-term beneficial neuroprotective and/or cardioprotective effects of GLP-1. Despite the fact that GLP-1 gene therapy approaches were mostly conducted in small rodent models of T2DM, designing of future clinical trials requires the testing of antidiabetic potential of GLP-1 gene delivery in larger animal models (such as cats, dogs, pigs and even primates).

Financial support: This work is supported by grants from Akdeniz University Scientific Research Administration Division and the Scientific and Technological Research Council of Turkey (TUBITAK-112S114).

References:

IT - Immunotherapy of Type 1 Diabetes

Anne Cooke

Department of Pathology, University of Cambridge, Tennis Court Rd, Cambridge CB2 1QP

Type 1 diabetes is an autoimmune disease where the insulin producing pancreatic β cells are destroyed by the immune system. Individuals with Type 1 diabetes require exogenous insulin administration to maintain glucose homeostasis. However, this is not a cure for Type 1 diabetes and there is great interest in devising a cure for this disease which like Type 2 diabetes is also increasing in incidence in many countries.

A cure for this autoimmune disease would require that the immune system should be tolerised to islet antigens so that T cells would no longer mediate β cell destruction. If the individual has established disease then it may also require some therapeutic approach to facilitate recovery of the destroyed β cell mass. Immunotherapeutic approaches using defined islet antigens have not proved successful in reversing ongoing autoimmunity. Monoclonal antibody therapy to target T cells has shown some improvement in β cell function in a subgroup of newly diagnosed Type 1 diabetic patients. Improved biomarker identification to enable the clinician to identify “at risk” groups may lead to improved outcomes of therapeutic intervention. Additionally combining T cell targeted approaches with ther agents to scavenge pro-inflammatory cytokines may provide better outcomes. The pros and cons of different approaches will be discussed in this lecture.
Oral Presentations

(Abstracts are listed by presentation order)
**OP-01  WNT4 REGULATES CANONICAL WNT SIGNALLING BY BLOCKING THE INCREASE OF ACTIVE BETA CATENIN INTO THE NUCLEUS**

Bowen A, Whatmore J, Kos K and Welters HJ
Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK

Introduction and Objective: The canonical Wnt signalling pathway plays an important role in beta cell growth and function; however the role of the non-canonical pathway in beta cell biology is less well understood. Wnt4, a non-canonical Wnt ligand is abundantly expressed in mouse and human pancreatic islets. The objective of this study is to investigate the function of Wnt4 in beta cells.

Methods: The rat beta cell line INS-1 was used to investigate the effects of Wnt4 and Wnt3a. Cell growth was assessed by counting viable cell numbers. Western Blotting was used to measure active and total beta-catenin protein levels in nuclear and cytoplasmic cell fractions.

Results: Wnt3a increased both nuclear and cytoplasmic protein levels of active beta-catenin by 2 fold indicative of canonical Wnt activation (p<0.05). Wnt4 had no significant effect on active beta-catenin levels alone but blocked the Wnt3a mediated increase in both the cytoplasm (results not shown) and nucleus (Fig 1). Interestingly both Wnt3a and Wnt4 increased total beta-catenin levels in the nucleus. As expected Wnt3a treatment of INS-1 cells increased cell growth. These effects were not observed by treatment with Wnt4 alone, however when co-treated with Wnt3a, Wnt4 blocked Wnt3a mediated growth (Fig 2).

Conclusion: This data suggests Wnt4 may play a role in regulating canonical Wnt signalling in beta cells by preventing the build-up and movement of cytoplasmic active beta-catenin into the nucleus. Active beta-catenin rather than phosphorylated beta-catenin can then act as a transcriptional activator in the nucleus resulting in increased cell growth.

**OP-02  BETA CELLS AND THE BENEFICIAL EFFECT OF GLUTEN-FREE DIET IN ANIMAL MODELS OF TYPE 1 DIABETES**

Karsten Buschard
Bartholin Institute, Rigshospitalet, Copenhagen, Denmark

Studies have documented that type 1 diabetes (T1D) is a diet-influenced disease. The Bartholin Institute was first to show that gluten-free diet markedly lowers diabetes incidence in non-obese diabetic (NOD) mice (from 64% to 15%). Many others have confirmed these findings subsequently, and also in BB rats, gluten-free diet protects against diabetes. Eight percent of T1D patients also develop celiac disease, and they far most commonly acquire T1D before diagnosis of celiac disorder, possibly due to the termination of gluten exposure following this disease. Recently, we published a case report of a newly diagnosed T1D patient that was prescribed a gluten-free diet shortly after diagnosis and who has now been without need of insulin for 30 months. The mechanisms seem to involve the beta cells in several ways. 1. A 33-mer-gliadin fragment, which is found in blood after oral intake, has direct effects on the beta cells by closing potassium channels and thereby enhancing the insulin-production. 2. In isolated islets, a gluten-free diet induced a higher expression of specific NKG2D ligands. 3. Stimulation of C57/BL6 islets with gliadin, significantly increased secretion of CCL2. 4. Gliadin changes the response to cytokine challenge in INS-1E cells towards more necrosis. Besides the direct beta-cell effects, gliadin changes the activity of NK cells and the cytokine profile of involved T cells. The results call for a trial of gluten-free diet in pre-T1D persons.
OP-03  SOLUBLE TRAIL (TNF-RELATED APOPTOSIS-INDUCING LIGAND) TREATMENT INDUCES PROLIFERATION IN BOTH PRIMARY RAT PANCREATIC BETA CELLS AND MOUSE PANCREATIC BETA CELL LINE

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Therapeutic approaches to increase functional beta cell mass in diabetic patients are of great interest since loss of pancreatic beta cell mass and function contribute to development of both type-1 and type-2 diabetes. TRAIL (TNF-Related Apoptosis-Inducing Ligand), which is an apoptosis inducer in a wide variety of tumor cells, has also proven to promote survival and proliferation in various cell types, such as vascular smooth muscle cells. Furthermore, TRAIL is claimed to protect pancreatic beta cells against cytokine-related harm. To investigate whether TRAIL could induce proliferation of beta cells, rat pancreatic islets were isolated and dispersed into single cells, then treated with recombinant sTRAIL (0, 1, 10ng/ml) for 48 hours. Cultures of dispersed rat islets contained ~73% beta cells (insulin+), ~16% alpha cells (glucagon+), ~5% fibroblast cells (vimentin+), and ~5% other cells (only DAPI+). sTRAIL treatment did not induce apoptosis in dispersed rat islet cell cultures as examined by AnnexinV/PI staining, but tended to increase viability compared to the untreated group, revealed by WST-1 assay. Insulin-Ki67 double stainings revealed that sTRAIL treatment increased proliferation of beta cells ~1.5 fold compared to the untreated group (0.41% vs 0.28%, P<0.05). Increased proliferation was also observed in Min6 beta cells 48 hours after sTRAIL treatment. In conclusion, TRAIL does not induce apoptosis in primary rat beta cells and mouse beta cell line, while increasing viability and proliferation. TRAIL might be a candidate molecule to prevent beta cell loss and/or replenish beta cell mass.

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OP-04  PREMATURE WEANING EXACERBATE DIABETES RESULTING FROM A LATER INJURY TO PANCREATIC BETA CELLS

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It is established that events during fetal development may lead to metabolic disease during adult life (the Barker hypothesis). However it is less clear how environmental factors during early postnatal development affect adult metabolism. Among such factors, weaning stands out as an abrupt dietary change from high fat milk to high carbohydrate food. Here we explore the impact of weaning on the severity of diabetes triggered by beta cell deficiency. We have previously shown that acute expression of a diphtheria toxin transgene in beta cells of adult mice results in diabetes, followed by spontaneous recovery due to beta cell regeneration. Strikingly, premature weaning of mice to normal rodent chow attenuates recovery of glucose homeostasis upon future beta cell ablation, by prevention of beta cell regeneration. Similarly, premature weaning of Akita mice, expressing a folding-defective mutation of the insulin gene, accelerates the development of diabetes. Premature weaning to high fat diet, mimicking the composition of maternal milk, partially rescues this phenotype suggesting a key role of food composition in the weaning process. We propose that full duration of suckling is important for beta cell maturation. The molecular mechanisms by which premature weaning affect glucose homeostasis and beta cell biology are under investigation.
Introduction: Type 1 diabetes results from auto-immune mediated destruction of insulin-secreting beta cells in the islets of Langerhans of the pancreas. A potential source of cells for the treatment of diabetes is embryonic stem cells (ESCs), due to their self-renewal capacity and pluripotency, have become a potential source of transplantable β-cells for the treatment of diabetes. Different strategies have been reported so far for derivation of insulin-positive cells from ESCs. Providing similar microenvironmental conditions as in vivo, functional differentiation of stem cells into desired cell types could be obtained in vitro. In this report we present an in-directed differentiation protocol in which mouse pancreatic islets induced differentiation of mouse ESCs to insulin producing cells (IPCs).

Materials and Methods: Novel in-directed co-culture differentiation protocol in which mouse pancreatic islets (mPIs) induced differentiation of mouse ESCs to IPCs was used. Beside this co-culture technique, chemical differentiation protocol that involved supplementing differentiation media with specific growth factors and/or inducers used as a positive control. The methods were compared by immune staining, gene expression and protein secretion analyses.

Results: IPCs were obtained within 30 days following in-direct co-culture. Differentiated mESCs were found to be positive for IPC specific markers. The results of immunocytochemical and gene expression analysis showed higher differentiation efficiency in co-culture group than chemical differentiation group. These results were confirmed by the functionality assay with ELISA.

Discussion and Conclusions: The interaction between ESCs and islets in co-culture induced differentiation by soluble paracrine factors. Co-culture allowed maximum crosstalk between the two cell types. These evidences indicated that PIs could be regarded as critical components of the ESCs niche. This approach would circumvent the need for pancreatic islet-stem cell co-culture and could potentially facilitate the production of functional IPCs for future in the treatment of diabetes.
Poster Presentations
**Aim:** Several studies indicate that bacterial dysbiosis leads to increase in gut-derived endotoxin which ultimately increase pancreatic endotoxin and exposure to islets possible contribute to the development of Type 1 diabetes. To study if Lipopolysaccharide (LPS) dominating bacterial dysbiosis can activate the bone marrow cells (BMCs) in islets and if acute phase protein; alpha-1-antitrypsin (AAT) plays a role in the down regulation, we aimed to identify cytokine gene expression in islets and cytokine release upon exposure to LPS and AAT.

**Methods:** Using NMRI mice, we isolated islets and BMCs. They were individually cultured and co-cultured and exposed to LPS and AAT. The alteration in gene expression in the islets was studied using quantitative PCR and cytokines in culture media were measured using Bioplex assay.

**Results:** The mRNA level of cytokines (IL-1ß, CXCL1, CXCL2, TNF-1ß, TNF-1, IL-6), insulin and glucagon measured in culture media were increased in islets exposed to LPS and in combination with AAT is synergistic in reversal of mentioned cytokines in islets in-vitro. When islets were co-cultured with BMCs, a further increase in IL-6, insulin and glucagon was observed at the protein level.

**Conclusion:** This study shows that the endotoxin, LPS, induces a direct inflammatory response in pancreatic islets and that BMCs modulate the inflammation. AAT can prevent some of the adverse effects of LPS.

**PP-02**  
A HOLISTIC APPROACH TO ELUCIDATE THE BIOLOGICAL MECHANISM OF TYPE 2 DIABETES  
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Type 2 diabetes, which is a metabolic disorder that is characterized by hyperglycemia (high blood sugar) in the context of insulin resistance and relative lack of insulin, is caused by a combination of lifestyle and genetic factors. Understanding the mechanisms of diseases and identification of specific biomarkers are grand challenges in regenerative and preventive medicine. Since solutions to these challenges require integration of data from different levels of biological organization, system biology perspective is needed. In the present study, transcriptome data from different tissues including beta cells and pancreatic islets of 96 patients were integrated with genomic and proteomic (protein-protein interaction) networks to understand the mechanism of type 2 diabetes. Identification of differential expressed genes, reconstruction and topological analysis of active protein-protein interaction sub-networks, and functional enrichment analyses indicated that (i) the genomic reprogramming depends on the type of tissue (i.e., the transcriptional response of beta cells was not identical to that of pancreatic islets), (ii) expression of 2-33% of the genome (17% in average) were affected at the transcriptome level, (iii) 160 reporter genes, pointing out the genetic mechanism underlying the disease, were identified, (iv) 17 proteins were determined as candidate biomarkers for type 2 diabetes, and (v) there exists a statistically significant association of type 2 diabetes with Alzheimer and Parkinson diseases. This study represents important clues on the biological mechanism of type 2 diabetes, and also provides several hypotheses for further experimental works.
PP-03  DOES NICOTINAMIDE AFFECT BETA-CELL REGENERATION OR APOPTOSIS IN NEONATAL DIABETIC RATS?
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Our aim was to observe the effect of Nicotinamide (PARP inhibitor) on beta-cell apoptosis and regeneration in newborn diabetic rats. Three groups were performed; the control group (1), STZ diabetic (100 mg/kg i.p on the second day after birth; n2-STZ) (2), 500mg/kg/day NA for 5 days(n2-STZ+NA) by starting from third day (3). The pancreatic tissue sections were immunostained with insulin, glucagon, somatostatin, pdx-1, ngn3, notch1, jagged1, active caspase-3 and PARP antibodies, double immunostained with insulin and PCNA antibodies. In situ hybridization was performed for insulin. TUNEL assay was used for apoptotic cells. Blood glucose levels were measured. The increase in blood glucose levels in n2-STZ+NA group was significantly decreased by NA treatment (p<0.01). The number of insulin/PCNA double-positive cells significantly increased in the n2-STZ+NA group compared with the other groups (p<0.001). n2-STZ group had lower number of insulin and pdx-1 positive cells compared to NA treated diabetic group in islets. The immunopositive insulin, pdx1 and ngn3 cells were located in small cell clusters or scattered in exocrine tissue and close to ducts in n2-STZ+NA. The ngn3 expression was not in the islets. There was significant difference between the numbers of notch1 and jagged1 immunopositive cells when the n2-STZ+NA group was compared with the other groups. PARP1, active caspase-3 (p<0.001) and TUNEL (p<0.001) positive cells increased in n2-STZ group compared to the other groups. In conclusion, we showed that NA treatment inhibits apoptosis via PARP1 inhibition, and stimulates duct epithelium or acinar cell differentiation into the beta cells via up regulation of ngn3 and pdx1, and down regulation of notch1.

PP-04  THE EFFECTS OF VILDAGLIPTIN ON BETA CELL APOPTOSIS AND NEOGENESIS IN STZ-DIABETIC NEWBORN RATS
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Vildagliptin (VG), an inhibitor of DPP-4, regulates plasma glucose levels and insulin secretion via GLP1. We aimed to observe the effects of short and long term VG treatment on beta cell regeneration, apoptosis, and islet morphology in newborn STZ-diabetic rats. Three groups were performed; (1) control, (2) diabetic (n2STZ) (STZ;100mg/kg,ip injected second day after birth), (3) treated (n2STZ+VG) (VG;60mg/kg/day,oral during 8/28 days), all groups were collected under short-and long-term groups. The tissue sections were immunostained using insulin, glucagon, somatostatin and PCNA antibodies. TUNEL method was used for apoptosis. Blood glucose (BG) levels were measured. BG levels significantly increased in n2STZ groups compared to the other groups. Insulin(+) cells were scattered in exocrine tissues and duct epithelia in the treated groups. Glucagon and somatostatin positive cells were increased within the islets in n2STZ groups compared to the other groups in both terms. The sizes of islets containing insulin (+) cells and their numbers were increased in n2STZ+VG groups compared to n2STZ groups in both terms. PCNA(+) cells in the islets of n2STZ+VG groups were significantly higher than the other groups in both terms. Apoptotic cell numbers in islets were lower in n2STZ+VG than n2STZ group in 10 days-old rats, apoptosis was observed within exocrine tissue and duct-epithelium in 30 days-old rats. The results show that Vildagliptin induces beta cell neogenesis from acinar cells or duct-epithelium by stimulating potential endocrine progenitor cells, reduces apoptosis in the islets, induces proliferation of islet cells by increasing the PCNA expression, and regulates morphological reorganization of the islets in the STZ-diabetic newborn rats. This study was supported by The Scientific Research Projects Coordination Unit of Istanbul University, the project no: BAP-28090.
PP-05  SEQUENCING ANALYSIS OF THE MODY GENES IN KOCAELI REGION

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Background: Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes mellitus that is caused by mutation in a single gene. Each different mutated gene causes a slightly different type of diabetes. The most common forms are HNF1A-MODY3, HNF4A-MODY1, HNF1B-MODY5 and GCK-MODY2, due to mutations in the HNF1A, HNF4A, HNF1B and GCK genes, respectively. Our aim was to screen MODY mutations in Turkish population who had DM.

Patients and Methods: 16 cases were diagnosed and analysed in Kocaeli University. We used sequencing to analyse whole exons of HNF1A, HNF4A, HNF1B, PDX1, INS and GCK genes.

Results: All patients carried HNF1A genetic changes. L17L, I27L, L459L, S487N, T515T, G288G, A98V and Pro291fsX316 were detected in HNF1A forms. -108/3G--4G polymorphism of PDX1 (IPF1) were detected in six patients, -108/4G-->4G polymorphism of PDX1 (IPF1) were detected in seven patients, -108/3G-->3G polymorphism of PDX1 (IPF1) were detected in three patients. One likely pathogenic substitution (c.872dupC, Pro291fsx316) in HNF1A was detected. We identified an alteration (R303L) in GCK in only one patient. There were no genetic changes detected in HNF4A, HNF1B, and INS genes.

Conclusions: Preliminary findings show that sequencing of the most common MODY genes is effectively applicable for diagnosis. Diagnostic power is thought to be increased with the scanning of MODY genes and with detection of rare mutations and polymorphisms.

PP-06  ENHANCED INS-1E CELL INSULIN GENE EXPRESSION ON A CHITOSAN-BASED POLYMERIC SURFACE

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Introduction: Pancreatic islet transplantation is a viable cell-based treatment alternative for patients with type 1 diabetes, especially for those who experience hypoglycemic episodes. While the procedure holds significant promise, the limited durability of graft function is still an important challenge that must be overcome. From the tissue engineering point of view, there is an ongoing search to identify platforms that will facilitate and enhance islet survival and function.

Objective: In the current study, we aimed to establish a biocompatible and biodegradable polymeric surface, which could later serve as a three-dimensional vehicle (scaffold) for islet cell transplantation.

Methods: A chitosan derivative was obtained by introducing functional groups to the structure and was combined with raw chitosan to coat cell culture plates. After being evaluated for cytocompatibility (i.e. direct contact, extraction-testing), INS-1E rat insulinoma cells were seeded on these treated surfaces with appropriate controls. The cells were analyzed for morphology, viability and insulin-1 and -2 gene expression.

Results and Conclusion: Cytotoxicity tests revealed that cell adhesion and growth on the chitosan-based surfaces were comparable to the conventional tissue culture polystyrene. INS-1E cells seeded on these treated surfaces preserved their morphology and growth characteristics. Preliminary results from quantitative PCR analysis indicate a 1.8-fold increase in expression of the gene for insulin 1 and a 4-fold increase for insulin-2. Taken together, our results suggest that this novel chitosan-based material is a promising candidate for use in transplantations and for establishing in vitro islet models.
Non-Obese Diabetic (NOD) mice are frequently preferred in Type 1 Diabetes (T1D) research. Non-Obese Diabetes-Resistant (NOR) mice are described as suitable control strains for NOD mice for research related to non-MHC genes. We showed previously that diabetic agent Streptozotocin (STZ) caused severe diabetic phenotype and poor survival in NOR mice, at 150 mg/kg dose while NOD mice had high survival rates*. Variations in GLUT2 expression is known to influence STZ susceptibility. Thus we compared GLUT2 expression levels in pancreas, liver and kidney tissues of NOD and NOR mice.

Insulin-glucagon stainings revealed that NOR mice had nearly half the beta cell content compared to NODs at day 2 of STZ application (NOR 66%; NOD 35%; n=3, p<0.05). Double immunofluorescence stainings for GLUT2 and insulin resulted in increased mean fluorescence intensities (MFI) shortly after STZ injection in NOR islets. MFIs were also 2-fold higher in NORs compared to NODs at day 4 (NOR 162.5±31.9; NOD 81.1±9.2 MFI). Intensity scoring revealed weak GLUT2 staining in NOD and NOR islets at day 0, but strong staining only in NOR islets at day 4 (NOR 3.0±0.0; NOD 0.75±0.25; n=3, p<0.05). Western Blotting in liver and kidney tissues of the same animals revealed significantly higher GLUT2 expression in livers of NOR mice compared to NODs.

These results suggest that NOR control strain is highly sensitive to STZ toxicity due to high GLUT2 expressions in pancreatic beta cells and liver, thus are not suitable for STZ-mediated applications.

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EFFECTS OF CD4+ AND CD8+ T CELLS DERIVED FROM TYPE 1 DIABETIC PATIENTS AND HEALTHY INDIVIDUALS ON HUMAN Β CELLS

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Introduction and Objectives: Worldwide, millions of people live with diabetes, and this number is increasing daily. Type 1 Diabetes, also called as "Juvenile Diabetes Mellitus or IDDM (Insulin Dependent Diabetes Mellitus)", is based on the insulin hormone deficiency, and mostly diagnosed in children and teenagers. Until today, many researches have been done on the mechanism of disease progress and many efforts have been spent to understand the cause-effect of diabetes. In the pathogenesis of type 1 diabetes, not only β cells, but the antigen presenting cells (APCs) and the immune system cells (CD4+ and CD8+ T cells, B cells and macrophages) in the same microenvironment are also playing a very important role. During the onset of diabetes type 1, it is well known that stimulated CD8+ T cells are responsible for β-cells' selective destruction. However, publications in recent years reported that the T cells obtained from healthy individuals contribute to β cell proliferation. Therefore, the effect of the CD4+ and CD8+ T cells from healthy individuals and type 1 diabetic patients on the proliferation of β cells and the alterations in the gene expression levels were aimed to investigate in vitro in this study. At the same time, we focused to investigate T cell proliferation and inflammation associated with levels in gene expression changes.
**Methods:** The peripheral blood samples from healthy volunteers (n=5) and type 1 diabetes patients (n=5) were collected, and CD4+ and CD8+ T cells were isolated by FACSaria Cell Sorter (BD Sciences). The T cells were co-cultured with human β cells line (1.1 B4) in ratio 1:1 for 24 h allowing cell-to-cell contact. After the co-culture, the cell viability and proliferation were analyzed by WST-1, and the gene expression levels of VCAM, HGF1, TGFB1, TNF, ICAM, IL1b and IP10 were estimated by RT-Real Time-PCR.

**Results:** Following the direct co-culture with healthy T cells the β cells proliferation was observed to increase, while the β cells proliferation was suppressed in the co-culture with the T cells from type 1 diabetic patients. Healthy and diabetic T cells significantly suppressed HGF-1 and VCAM-1 expressions in the β cells. On the contrary, the TNF-α and IL-1b expression levels in β cells increased.

**Conclusion:** The results of this study demonstrate the role of T cells in type 1 diabetes. Overall, the β cells proliferation was suppressed by T cells, while proinflammatory cytokine expressions were increased. Furthermore, the gene expressions focused in this study were increased, but HGF-1 expression was decreased, which might have important role in the proliferation of the β cells and the cell adhesion.

**PP-09 EFFECT OF MATERNAL HIGH FAT DIET ON OFFSPRING PANCREATIC ISLET**

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**Introduction:** Like other eating disorders the etiology of obesity and how obesogenic/metabolic traits are transmitted to the next generation remain to be unknown. Recent studies have shown that maternal obesity and/or maternal obesogenic diet consumption during pregnancy can affect pancreatic development causing later development of chronic degenerative diseases. For our experiment, we focused on offspring pancreatic islets functionality at 20 days of age.

**Materials and Methods:** Experiment was initiated with 5 weeks old total of 20 female Wistar rats. Female Wistar rats were fed with standard chow (SC) or high fat (HF) diet for 8 months prior to mating and during the gestational and lactational periods. 20 day old pancreatic islets were isolated by pancreatic duct injection of collagenase type V solution. Viability of islets was analyzed with propidium iodide (PI) and fluorocein diasetate (FDA). Islets were incubated in 3.3 mmol/l glucose for low glucose concentration. For high glucose; the islets were incubated in 16.7 mmol/l. The solutions (low glucose and high glucose) were collected for insulin ELISA assay.

**Results:** From 20 day old HF diet and control group offspring islets were isolated and glucose-induced insulin secretions were compared. Trend in insulin response between HF diet and SC diet groups were significantly different. HFD group offspring islets highly responded to low and high glucose with respect to control group offspring islets. Also stimulation indexes were compared and found to be significantly different at 24 and 72 hours.

**Conclusion:** Our primary results have shown that maternal diet can affect offspring pancreatic islet response to glucose and because of increased insulin level developmental insulin signaling can initiate metabolic deregulations.
Figure 1: Primary islet cell culture isolated from 20 day old high fat diet (HFD) and control group offspring. Isolated primary islet cell response to low glucose (3.3mmol/l) measured with Insulin ELISA assay after 0, 24, 48, and 72 hour. Each assay repeated 3 times.

Figure 2: Primary islet cell culture isolated from 20 day old high fat diet (HFD) and control group offspring. Isolated islet cells response to high glucose (16.7mmol/l) measured with Insulin ELISA assay after 0, 24, 48, and 72 hour. Each assay was repeated 3 times.
High & Low Glucose Total Stimulation Comparison

Figure 3: Primary islet cell culture isolated from 20 day old high fat diet and control group offspring. Isolated islet cells response to high glucose (16.7mmol/l) and low glucose (3.3mmol/l) measured with Insulin ELISA assay after 0, 24, 48, and 72 hour. Each assay repeated 3 times.

Islet Stimulation Index

Figure 4: Stimulation index (high glucose insulin response/low glucose insulin response) calculated for 20 day old high fat diet (hfd) and control group offspring after 0, 24, 48 and 72 hour.
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