Symposium on Molecular and Physiological Aspects of Diabetes Mellitus

Karolinska Institutet, November 13, 2015
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
</table>
| 09.00 - 09.10 | WELCOME NOTE  
Juleen Zierath                                                                 |
| 09.10 - 09.20 | THE 4D TYPE 2 DIABETES PROJECT: STANDARDIZED CARE PROCESS, A PLATFORM FOR EXCELLENT CARE AND RESEARCH  
Eva Toft, Ersta Hospital |
| 09.20 - 09.55 | THE KIDNEY IN DIABETES  
Sally Marshall, Institute of Cellular Medicine, Newcastle University, UK |
| 09.55 - 10.30 | THE DIABETES EPIDEMIC: FACT AND FICTION  
Sarah Wild, Centre for Population Health Sciences, University of Edinburgh, UK |
| 10.30 - 11.00 | COFFEE BREAK |
| 11.00 - 11.35 | GENES AND METABOLISM: WHAT LESSONS HAVE WE LEARNED SO FAR?  
Inês Barroso, Wellcome Trust Sanger Institute, Hinxton, UK |
| 11.35 - 12.10 | INSULIN RESISTANCE IN CARDIOVASCULAR DISEASE DEVELOPMENT  
Markku Laakso, School of Medicine, University of Eastern Finland, Kuopio, Finland |
| 12.10 - 13.10 | LUNCH |
| 13.10 - 13.45 | METABOLIC DYSREGULATION AND INSULIN ACTION: HUMAN PHENOTYPIC CONSIDERATIONS  
William T. Cefalu, Pennington Biomedical Research Center, New Orleans, USA |
| 13.45 - 14.20 | OBESITY-RELATED INSULIN RESISTANCE: A SYSTEMS APPROACH  
Daniel P. Kelly, Sanford Burnham Prebys Medical Discovery Institute, Orlando, USA |
| 14.20 - 14.50 | COFFEE BREAK |
| 14.50 - 15.25 | TAKING CARE OF THE GUT MICROBIOTA TO CONTROL DIABETES AND RELATED METABOLIC DISEASES  
Nathalie Delzenne, Metabolism and Nutrition Research Group, Louvain Drug Research Institute, Catholic University of Louvain, Belgium |
| 15.25 - 16.00 | AGING OF THE AUTOPHAGIC SYSTEM: METABOLIC CONSEQUENCES  
Ana Maria Cuervo, Albert Einstein College of Medicine, New York, USA |
|            | CLOSING REMARKS |
THE KIDNEY IN DIABETES

SALLY MARSHALL
Institute of Cellular Medicine, Newcastle University, UK

Recently, the number of people with diabetes developing end-stage kidney disease (ESRD) has increased exponentially, so that diabetes is now the single commonest cause of ESRD requiring dialysis or transplantation worldwide. This increase is accounted for by individuals with type 2 diabetes with multiple co-morbidities. The incidence of ESRD in type 1 diabetes is stable or possibly declining.

Classical diabetic nephropathy is a clinical syndrome which develops over many years. The hallmark is gradually increasing proteinuria, initially a selective albuminuria but laterally unselected loss of proteins. Blood pressure rises in parallel with the albuminuria. Glomerular filtration rate (GFR) falls gradually, but is unlikely to be clearly abnormal (<60 ml/min/1.73m²) until unselected proteinuria is present. Cardiovascular risk also increases as albuminuria rises, and independently as GFR falls. This clinical phenotype is associated with specific renal histological changes in all layers of the glomerular filtration barrier. There is loss of the endothelial glycocalyx, glomerular basement membrane thickening, mesangial matrix expansion and podocyte changes and loss.

There is a genetic influence on the development of diabetic nephropathy and its associated cardiovascular disease. The disease is likely to be polygenic, perhaps with some genes influencing initiation and others progression of the kidney disease. There are also likely to be susceptibility and protective factors. Metabolic and haemodynamic factors drive the disease. Hyperglycaemia upregulates a number of vasoactive factors, growth factors, and signalling molecules. In the glomerulus there is local upregulation of angiotensin II, which reduces afferent and, to a lower degree, efferent arteriolar tone, thus altering glomerular capillary auto-regulation. The resulting increase in glomerular pressure and the consequent disproportionate transmission of the systemic pressure to the glomerular circulation leads to glomerular damage. These metabolic and haemodynamic factors work synergistically to drive glomerular damage, and the clinical and histological phenotype. Currently, blood glucose and blood pressure control are the most important factors in preventing and delaying progression of diabetic nephropathy. Use of inhibitors of the renin-angiotensin system, which reduce intraglomerular pressure, is particularly important. Many novel agents directed against metabolic and haemodynamic abnormalities are in development.

A small minority of people with type 1 diabetes and a substantial number of those with type 2 diabetes who develop ESRD do so without having significant albuminuria. Small biopsy studies have demonstrated that these individuals are much less likely to have classical histological features of diabetic nephropathy but instead show changes in keeping with hypertensive and vascular damage. Obesity and lipid damage may also contribute. This is likely to be a different disease to classical diabetic nephropathy. Thus, careful definition of the clinical phenotype, including quantification of albuminuria and stage of kidney disease, is essential for research studies.

THE DIABETES EPIDEMIC: FACT AND FICTION

SARAH WILD
Centre for Population Health Sciences, University of Edinburgh, UK

Whether or not there is an epidemic of diabetes remains controversial and depends on the definition of an epidemic. However, despite the fact that accurate estimates are not available for all countries, it is clear that the numbers of people in the world with diabetes and prevalence of diabetes is increasing and that changes over time differ between populations. The total number of people with diabetes is influenced by the number of people in the world and their demographic characteristics, the number of new cases and the survival of people with diabetes. Key demographic characteristics that influence risk of type 2 diabetes incidence include age and ethnicity with older age and non-European ancestry ethnicity being associated with higher risk. Male sex is generally associated with higher diabetes risk but may not be observed in countries in which obesity prevalence is markedly higher in women than men. The number of new cases is influenced by the distribution of risk factors, particularly obesity for type 2 diabetes, but also by diagnostic criteria and the extent of screening as type 2 diabetes may be asymptomatic and remain undiagnosed for many years. In developed countries survival of people with diabetes has increased in recent years due to a combination of factors including declining smoking prevalence and improved treatment of hypertension and dyslipidaemia. Few countries are able to provide accurate estimates of time trends in incidence of diabetes but the available data suggest that while overall incidence has not increased over time in recent years the United States (2008-2012), in Denmark (2004-2007), in Israel (2006-2012) or in Scotland (2004-2013) that there is evidence that trends in incidence vary by age, sex, ethnicity and socio-economic status in some countries and overall incidence continued to increase in Canada (1997-2003) and Italy (2001-2007). Increasing prevalence of diabetes and reductions in mortality of people with diabetes from cardiovascular disease result in increasing burden of other complications of diabetes on both individuals and health services. Development and implementation of effective approaches to primary and secondary prevention are required, with the former approach requiring major societal changes.
GENES AND METABOLISM: WHAT LESSONS HAVE WE LEARNED SO FAR?

INÉS BARROSO  
Wellcome Trust Sanger Institute, Hinxton, UK

In 2007, the genome-wide association study (GWAS) era began in earnest with the successful identification of the first loci influencing type 2 diabetes and obesity risk that were robustly replicated. Since then, hundreds of loci influencing type 2 diabetes and obesity risk, as well as relevant quantitative traits (e.g. BMI, glucose and insulin levels), have been identified. These successes have been empowered by the aggregation of data across multiple cohorts and datasets, through meta-analyses conducted within the context of large international consortia. In parallel, efforts focused on identification of mutations causative of rare extreme phenotypes have revealed additional genes that impact energy balance and glucose homeostasis. In my talk, I will summarise what we have learned during this journey: what we have found, what we have not found and what new biology we have learned. Is the glass half-empty, or half-full, and where do we go from here?

INSULIN RESISTANCE IN CARDIOVASCULAR DISEASE DEVELOPMENT

MARKKU LAAKSO  
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The prevalence of diabetes mellitus will likely increase globally from 371 million individuals in 2013 to 552 million individuals in 2030. This epidemic is mainly attributable to type 2 diabetes mellitus (T2DM), which represents about 90-95% of all cases. Cardiovascular disease is the leading cause of mortality among individuals with diabetes mellitus, and >50% of patients will die from a cardiovascular event—especially coronary artery disease, but also stroke and peripheral vascular disease. Classic risk factors such as elevated levels of LDL cholesterol and blood pressure, as well as smoking, are risk factors for adverse cardiovascular events in patients with type 1 diabetes mellitus (T1DM) and T2DM to a similar degree as they are in healthy individuals. Patients with T1DM develop insulin resistance in the months after diabetes mellitus diagnosis, and patients with T2DM typically develop insulin resistance before hyperglycaemia occurs. Many mechanisms related to insulin resistance are likely to initiate accelerated atherosclerosis, and hyperglycaemia additionally increases the risk of vascular complications. The effects of insulin resistance and hyperglycaemia on the risk of cardiovascular disease are largely tissue-specific and pathway-specific. Impaired endothelial function, low-grade inflammation, AGEs, thrombosis, fibrinolysis and modifications of lipoprotein particles increase the risk of cardiovascular events suggesting that both insulin resistance and hyperglycaemia substantially contribute to vascular complications in T1DM and T2DM. The mechanisms leading to vascular complications are, therefore, largely similar in both types of diabetes mellitus. Lifestyle changes, weight loss, a healthy diet and regular exercise—all known to reduce insulin resistance and improve glycaemic control—should be the basis of treatment of all individuals with diabetes mellitus to prevent cardiovascular complications.

METABOLIC DYSREGULATION AND INSULIN ACTION: HUMAN PHENOTYPIC CONSIDERATIONS

WILLIAM T. CEFALU  
Pennington Biomedical Research Center, New Orleans, USA

Many factors are postulated to modulate substrate metabolism and insulin action in humans. Specifically, many factors controlling metabolic flexibility (MF), the ability of the body to switch from fat to carbohydrate oxidation in response to feeding or with insulin administration, are being actively investigated. "Metabolic flexibility" (MF) is characterized by increased fat oxidation in skeletal muscle during fasting conditions and the ability to switch from fat to carbohydrate oxidation in response to a meal or insulin. However, an attenuation in metabolic inflexibility is observed in individuals with obesity and type 2 diabetes mellitus. The reduced MF observed in the individuals with type 2 diabetes mellitus has been mainly explained by the glucose disposal rate and it has been reported that the glucose disposal rate, baseline fasting RQ, and steady-state plasma free fatty acid concentrations were important contributing factors to MF. Obesity-related insulin resistance is also associated with accumulation of lipid intermediates in skeletal muscle and liver as the capacity of the adipose tissue to store lipids as triglycerides is exceeded. These "ectopic lipids" interfere with insulin signaling, either directly or indirectly via currently undefined mechanisms. Factors controlling metabolic flexibility on a whole body level will be reviewed in addition to the contributing role of lipid intermediates in the process.
OBESITY-RELATED INSULIN RESISTANCE: A SYSTEMS APPROACH

DANIEL P. KELLY
Center for Metabolic Origins of Disease, Sanford Burnham Prebys Medical Discovery Institute, Orlando, USA

Insulin resistance drives the pathogenesis of many end-organ complications of type 2 diabetes. We are interested in delineating the pathogenic mechanisms involved in the development of insulin resistance in heart and skeletal muscle relevant to cellular lipotoxicity. Significant evidence has established a correlation between the accumulation of myocyte neutral lipid, muscle insulin resistance, and cardiac dysfunction. However, the regulatory “crosstalk” between alterations in myocyte lipid homeostasis and insulin resistance is incompletely understood.

Previous studies have largely taken a biased (candidate) approach to this problem. We have embarked on an unbiased systems-based approach to address this question using chemical biology and genomic profiling strategies in order to identify mechanisms and new potential therapeutic targets. Cell-based small molecule high throughput screens were conducted in lipid-loaded myocytes. One of the molecular probes identified in this screen, SBI-477, inhibits triglyceride synthesis and activates mitochondrial fatty acid oxidation in primary human skeletal myotubes. In addition, SBI-477 enhances insulin-independent and -dependent glucose uptake by activating components of the insulin signaling pathway in the absence of insulin. Unbiased transcriptomic profiling and query of the Connectivity Map database revealed that SBI-477 inhibits expression of two negative regulators of insulin signaling, TXNIP and ARRDC4. The mechanism whereby TXNIP levels are downregulated involves inhibition of the action of the transcription factors MondoA and the related factor, ChREBP. MondoA loss-of-function recapitulates the effects of SBI-477 on myocyte insulin signaling, glucose uptake, and myocyte neutral lipid levels. These results provide proof-of-concept for a chemical biology screening approach to unveil mechanisms involved in obesity-related insulin resistance. The pathways identified downstream of SBI-477 represent candidate therapeutic targets aimed at muscle insulin resistance and diabetic cardiac dysfunction.

TAKING CARE OF THE GUT MICROBIOTA TO CONTROL DIABETES AND RELATED METABOLIC DISEASES

NATHALIE DELZENNE
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Each human intestine harbours hundreds of trillions of bacteria which constitute a complex and dynamic ecosystem referred to as the gut microbiota. An increasing number of data obtained during the last ten years have shown changes in gut bacterial composition or function in type 2 diabetes patients (called dysbiosis), but also in patients presenting metabolic disease associated with obesity (non alcoholic steato-hepatitis (NASH), or cardiovascular disease). Both alterations of the gut barrier function (implicating a drop in key bacteria, like Akkermansia muciniphila i.e.) and the increase in dietary lipid intake, allow the translocation of some components of bacteria – such as lipopolysaccharides (LPS) – which are promoting the metabolic alterations in the intestine, the liver, and the adipose tissues, contributing to insulin resistance and hyperglycemia, namely by modulating Myd88 activation. Novel approaches based on the modulation of the gut microbiota (e.g., probiotic, prebiotic and faecal transfer) could be of these new therapies to the management of type 2 diabetes. Among them, the administration of dietary fibers with prebiotic properties (fructans, glucans, resistant starch…) appear as particularly interesting, since it allows, through changes in the gut microbiota composition and activity, to improve key intestinal functions (mucus production, endocrine function - i.e. glucagon-like peptide 1 and 2 release- or immune function), and lessen systemic inflammation, adiposity, and hyperglycemia. Moreover, our recent data show that dietary fructans are able to counteract steatosis and NO-related vascular dysfunction in a mice model of cardiovascular disease (Apo E-/- mice depleted in n-3 polyunsaturated fatty acids), independent on any effect on inflammation and adiposity. Few intervention studies with prebiotic fibers have been reported in diabetic, NASH or obese patients, but the data published until now suggest that the biochemical mechanisms involved in the improvement of health shown in animal models is relevant in humans. Adequate nutrition for prevention and/or management of diabetes should take into account the interactions of nutrients with the gut microbiota in the next future.
AGING OF THE AUTOPHAGIC SYSTEM: METABOLIC CONSEQUENCES

ANA MARIA CUERVO
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Autophagy mediates the digestion of cytosolic material in lysosomes for quality control purposes and it becomes an alternative source of energy to assure cellular survival in the energetically-demanding conditions that associate to stress. We found that autophagy can actively contribute to the mobilization of intracellular lipid stores and that this becomes a defensive mechanism against nutritional stress. Growing evidence supports that functionality of the autophagy system is compromised with age and that intact autophagy is required to sustain longevity, in part through its contribution to the organism energetic balance.

In this talk, I will provide examples from ongoing studies on this dual relation between autophagy and glucose and lipid metabolism. We have focused on two mechanisms by which autophagy regulates cellular energy: through the mobilization/recycling of intracellular energy stores and through the selective degradation of key metabolic enzymes. We have found that the relevance of these two functions is different dependent on the tissue and organ, as well as on the time in which autophagy dysfunction is inflicted. We propose that age-dependent or diet-induced changes in autophagic activity could be behind some of the metabolic malfunction characteristic of aging. I will discuss some of our current efforts to chemically modulate autophagy activity to enhance the cellular response to metabolic stress.
POSTER ABSTRACTS

Paternal high-fat diet consumption reprograms the gametic epigenome and transgenerationally affects the metabolism of offspring

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Background and Aims: Genetic and non-genetic (environmental) factors are strongly implicated in obesity. Increasing evidence indicate that paternal obesity is associated with epigenetic inheritance of metabolic disorders in the next generations. However, a direct evidence of the molecular carriers remains elusive. We hypothesized that paternal high-fat diet (HFD) feeding transgenerationally alters the gametic epigenome, affecting the metabolism of the offspring. Materials and methods: F0 male rats fed either HFD or chow diet for 12 weeks were mated with chow-fed dams to generate F1 and F2 offspring. Motile spermatozoa were isolated from F0 and F1 breeders to determine DNA methylation and small non-coding RNA (snRNA) expression pattern by deep sequencing. Results: Newborn offspring of HFD-fed founders had reduced body weight and decreased beta-cell mass when compared to offspring from chow-fed breeders. F1 and F2 adult females from F0 founders fed a HFD were glucose intolerant and resistant to HFD-induced weight gain. No major phenotype was observed in male offspring, suggesting sex-specific responses. HFD-fed F0 and their F1 male offspring presented similar changes in the epigenome of spermatozoa, showing altered DNA methylation and snRNA expression profile when compared to respective controls. Altered expression of the miRNA let-7c in sperm of F0 and F1 breeders was also observed in the white adipose tissue of female offspring, leading to a transcriptomic shift in let-7c putative target genes. Conclusion: Our results indicate that chronic HFD consumption transgenerationally reprograms the gametic epigenome, which may be the molecular mechanism involved in the transmission of environmentally-induced metabolic dysfunction to the next generations.

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IGFBP-1 as a sensor for insulin sensitivity in liver and pancreatic β-cells

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Background and aims: Insulin-like growth factor protein-1 (IGFBP-1) is one of the six known binding proteins that modulate the bio distribution, bioactivity and bioavailability of the IGF-I and IGF-II proteins. However it is the only binding protein that is regulated by acute changes in nutrition and cellular stress. Insulin is the predominant inhibitor if IGFBP-1 and negatively regulates the expression and secretion of the IGFBP-1 protein under normal physiologic conditions. However, in conditions of stress and intensive exercise this correlation is lost possibly due to hepatic insulin resistance or due to factors that can directly stimulate IGFBP-1. High fat diet as a chronic metabolic stress induces characteristic derangement in cellular physiology and metabolism and leads to insulin resistance. However, the mechanism of short duration of high-fat diet induced insulin resistance and in particular hepatic insulin resistance is not clearly known. In the current study we looked at the early changes in the cellular physiology by subjecting young animals to western diet for a short period with a focus on the role of IGF system in combating the metabolic stress. Material and methods: We subjected the 5-week old male and female C57Bl/6CR mice to a 7-week diet intervention with a high-fat/high-sucrose diet (HF/HSD) and studied various parameters. We monitored the body weight during the whole time course of the diet study and after diet intervention we characterized the insulin and glucose homeostasis and determined IGFBP-1 levels in different metabolic conditions (starvation, sleep phase and wake phase). We also conducted intraperitoneal insulin tolerance tests and morphological studies in the liver. Results: After the diet intervention, HF/HSD-treated animals developed overweight, dyslipidemia, hyperglycemia, and presented significantly higher serum insulin and glucagon levels as compared to control diet fed mice. Insulin and glucose homeostasis impairments detected in HF/HSD mice were concomitant with a severe increase in the serum IGFBP-1 levels. Furthermore, animals
on HF/HSD experienced insulin intolerance in the intraperitoneal insulin tolerance test together with an evident deterioration on liver morphology. **Conclusion:** Altogether these results suggest that IGFBP-1 plays an essential role for liver and insulin sensitivity and that an intact cross talk between IGFBP-1 and insulin in liver and β-cells is required for the prevention of the early onset of metabolic diseases.

**An overactive Dll4-Notch1 loop impairs wound healing in diabetes**

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Diabetic foot ulcerations represent a major medical, social and economic problem. Therapeutic options are restricted due to a poor understanding of the pathogenic mechanisms. The Notch pathway plays a pivotal role in cell differentiation, proliferation and angiogenesis, processes that are profoundly disturbed in diabetic wounds. Notch signaling is activated upon interactions between membrane-bound Notch receptors (Notch 1-4) and their ligands (Jagged 1-2; Delta-like 1, 3, 4), resulting in cell/context-dependent outputs. Here, we report that Notch signaling is activated by hyperglycemia in diabetic skin and plays a specific pathogenic role. Local inhibition of Notch signaling in experimental wounds markedly improves healing exclusively in diabetic animals but not in non-diabetic animals. High glucose levels activate a positive Delta-like 4 (Dll4)-Notch1 feedback loop that contributes to impaired wound healing in diabetes. Using loss-of-function genetic approaches, we demonstrate that Notch1 inactivation is sufficient for inhibiting the repressive effects of the Dll4-Notch1 loop on wound healing in diabetes, thus making Notch1 signaling an attractive therapeutic target for the treatment of diabetic foot ulcerations.

**Intermittent fasting according to the 5:2 method is beneficial in overweight subjects with and without type 2 diabetes**

Neda Rajamand Ekberg, Björn Englund and Kerstin Brismar

**Background and aim:** Cardiovascular disease (CVD) is a major contributor to global morbidity and mortality. Preventable risk factors are the main cause of CVD, one of which is overweight. Weight reduction improves several CVD risk markers; however, adherence to weight reducing interventions is often poor. Alternatives to existing weight reducing interventions are therefore warranted. Intermittent fasting (IF) has demonstrated promising results during short term intervention, however, long term studies on IF is needed. The aim of this study was to assess potential effects of IF according to the 5:2 method on CVD risk markers in overweight subjects with (T2DM-group) and without (ND-group) type 2 diabetes. **Material and Methods:** Overweight (BMI≥25 kg/m2) subject with (n=12) and without (n=24) type 2 diabetes were recruited to the study. During a 6 month period subjects ate according to the 5:2 method. Outcome measurements of CVD risk markers included body weight reduction and CVD associated risk markers (anthropometrical measurements, blood pressure, insulin resistance, glucose intolerance and lipid profile). **Results:** Body weight decreased significantly (P<0.001) in both groups (5.3% in ND-group and 7.0% in T2DM-group) during the 6 months study period. CVD associated anthropometrical measurements improved in both groups. Systolic blood pressure, insulin resistance, glucose intolerance and lipid profile improved significantly only in the T2DM-group. **Conclusions:** IF according the 5:2 method is effective in order to reduce weight and improve CVD associated risk markers in overweight subjects with and without type 2 diabetes.
The role of FXYD1 protein in energy metabolism

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Background: The FXYD protein family consists of 7 members sharing a common FXYD motif. The FXYD1 protein, also known as phospholemman is a potent regulator of Na,K-ATPase (NKA) activity. Moreover, in rat adipocytes, prevention of FXYD1 phosphorylation blocks insulin-stimulated GLUT4 translocation (1). We have previously shown increased FXYD1 protein content precedes the onset of insulin resistance in rats fed a high fat diet, and exercise training attenuates this increase (2). In humans, exercise leads to increase in FXYD1 protein phosphorylation (3). Together, these data indicate that FXYD1 might be a novel regulator of glucose metabolism. Aim: To investigate the role FXYD1 in glucose and lipid metabolism. We hypothesize that FXYD1 may act as a specific scaffold molecule or a molecular brake for key enzymes and membrane transporters involved in energy metabolism. Methods: Glucose uptake, GLUT4 cell surface abundance and lipolysis were measured in differentiated 3T3-L1 adipocytes. Whole body FXYD1 knockout mice were generated by homologous recombination (4) and studied at 20-25 weeks of age. Mice underwent an intraperitoneal glucose tolerance test, indirect calorimetry, and hyperinsulinemic-euglycemic clamp for whole body glucose uptake. Isolated skeletal muscles were studied in vitro under basal, insulin-stimulated conditions and after contraction. Results: siRNA-mediated FXYD1 silencing increased glucose uptake and lipolysis in cultured 3T3-L1 adipocytes. Glucose tolerance was enhanced in FXYD1 KO mice compared to wild-type mice. Whole body glucose uptake was increased in skeletal muscle and brown adipose tissue. FXYD1 KO mice have reduced (50%) spontaneous locomotion compared to wild-type mice. Extensor digitorum longus (EDL) muscles from FXYD1 KO mice were fatigue prone, as demonstrated by a reduced ability to sustain work compared with wild-type mice. Furthermore, lactate production during muscle contraction was increased in isolated skeletal muscle from FXYD1 KO mice. Adrenergic beta 3 specific stimulation increased heat production and increased lipolytic activity in FXYD1 KO mice. Additionally, basal NKA activity was increased in intact EDL and soleus muscle from FXYD1-KO mice. Conclusion: FXYD1 ablation results in increased glucose uptake, decreased locomotion and increased lactate production, possibly by increasing anaerobic glycolysis. Additionally FXYD1 ablation leads to increased heat production during beta adrenergic stimulation, possibly due to increased cAMP signaling. In conclusion, we propose that FXYD1 plays a pivotal role in energy homeostasis by regulating both energy utilization and contractile properties of skeletal muscle.

References:

Modulation of IGFBP1 protein expression by Metformin

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Aim: The insulin like growth factor binding protein-1 (IGFBP-1) is one of the six identified binding proteins for Insulin-like growth factor-1 (IGF-1) and -II. IGFBP-1 complexes with IGF-1 and modulates IGF-1 functions by altering its bioavailability, distribution, and bioactivity thereby influencing glucose homeostasis. Low levels of circulating IGFBP-1 are associated with risk of type 2 diabetes (T2D) and fasting levels of IGFBP-1 predict insulin sensitivity. Lifestyle changes increases IGFBP-1 and decreases risk of T2D. Fasting enhances IGFBP-1 levels and there is a close correlation between glucoseogenesis and IGFBP-1 production in healthy subjects. The antidiabetic biguanide drug metformin acts by suppressing hepatic glucose production. Also metformin has been shown to decrease the risk of T2D. In this study we explored the in-vitro effect of metformin on IGFBP-1 expression in both normoglycemic and hyperglycemic conditions.

Material and methods: HepG2 cells were treated with different concentrations of metformin both in presence of high glucose (30 mM) and in normal glucose (5 mM). Treated cells were lysed for protein and RNA extraction. Western blot analysis was done to study the protein expression and quantitative PCR was performed to study gene expression.

Results: Metformin (1 mM and 2mM) treatment in unstarved cells for 24h significantly enhanced the expression of IGFBP-1 mRNA and protein in both normoglycemic and hyperglycemic conditions compared to the controls. At the same time the expression of gluconeogenic genes PEPCK and G-6-P were found to be decreased in both normoglycemic and hyperglycemic conditions compared to controls.

Conclusion: This is the first study to show that metformin modifies the expression of IGFBP-1 protein in hepatic cells. Since IGFBP-1 levels are low in prediabetic patients our results and others suggest that enhancing IGFBP-1 expression could be an additional factor in reducing the risk for diabetes.
The 4D Type 2 diabetes project: Standardized care process, a platform for excellent care and research

Eva Toft, Ersta Hospital

4D is a collaboration program between Karolinska Institutet and the Stockholm County Council (SLL) in order to create better conditions for health care and research. One of the four D (diagnoses) is type 2 diabetes. The diabetes project involves screening for prediabetes and diabetes in primary care in parallel with the development of a model for biobank-sampling with well-characterized patients from the screening-program. Periodic sampling of both the controls of persons with prediabetes and type 2 diabetes, respectively, is planned in order to find biomarkers of disease and complications. There are large variations in health outcomes for people with diabetes between the > 200 primary care units in SLL. Socioeconomic status among care recipients can explain the variation only to a small extent and factors attributable to the organization of care at different levels is recently found to be of major importance. In the project a standard process has been developed for how evidence-based care should be performed by a healthcare team in primary care, from diagnosis to follow-up including collaboration with other healthcare providers. A web-based improvement tool, DiaCert, for self-assessment and quality improvement work has been developed and tested. Support by a smartphone application, that can meet the needs of both patients and health care as well as facilitate the participation in research, is in preparation.

Gene regulation of insulin resistance in human white adipose tissue

*These first two authors contributed equally to this work
**These last two authors contributed equally to this work

Insulin resistance is associated with obesity, but up to one third of obese subjects display a metabolically healthy phenotype with insulin sensitivities comparable with non-obese individuals [1]. The mechanism by which insulin regulates the expression of genes is not completely understood. Recent studies carried out in two of the major insulin target tissues, skeletal muscle and adipose tissue, emphasize that the hormone insulin influences the expression of a large number of genes involved in specific signal pathways, which are altered in obesity. We investigated adipose gene regulation under fasting and hyperinsulinemic conditions in both, insulin resistant (IRO) and insulin sensitive (ISO) obese individuals.

Abdominal subcutaneous white adipose tissue (sWAT) was obtained before and after two-hour hyperinsulinemic euglycemic clamp in a well phenotyped cohort of 46 obese subjects subdivided into 20 ISO and 26 IRO. The sWAT RNA was analyzed using 5’ Cap Analysis Gene Expression (CAGE) expression profiling [2]. CAGE is sequencing the 5’end of the mRNA and allows the genome-wide identification of transcription starting site (TSS) and their corresponding promoter regions through which RNA transcription is regulated. Moreover, CAGE permits the identification and quantification of other regulatory elements such as gene enhancers [3].

In the entire cohort, hyperinsulinemic euglycemic clamp induced a pronounced alteration in gene expression. In particular, most of the genes were down regulated and associated with the insulin signaling pathway.

During fasting conditions, genes involved in insulin signaling, fatty acid biosynthesis and pyruvate metabolism were significantly different between ISO and IRO. However, this divergence was not maintained after the hyperinsulinemic clamp. The global gene expression pattern of ISO individuals became almost indistinguishable from IRO arguing against the concept of healthy obesity. Moreover, we also analyzed the changes in gene expression upon hyperinsulinemia in all obese subjects based on their M-values highlighting that subjects with high M-values displayed the largest insulin-induced changes in global transcription profiles. Lastly, in an ongoing analysis we preliminarily identified 47,378 enhancer candidates active in white adipose tissue.

Studies on Host-Pathogen Interactions with Relevance for Understanding the Etiopathogenesis of Type 1 Diabetes

Emma Svedin, Sebastian Kapell, Renata Utorova, Virginia Stone, Erna Domsgen & Malin Flodström Tullberg
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Type 1 diabetes (T1D) is rapidly increasing on a global level. At present, there are no preventative treatments for the disease. Limited understanding of the mechanisms behind disease development blocks progress in this research area and as such there is a critical need for new knowledge.

Recently, several research groups have analyzed the beta cell mass in islet remnants of T1D patients. Collectively, these studies have challenged the simplified view that beta cells are completely lost during development of T1D. Indeed, a large proportion of the patients still have insulin-positive beta cells. In addition, many of the patients with remaining beta cell mass had beta cells that stained positive with an antibody to an enterovirus protein. These observations are in line with epidemiological data and clinical findings suggesting that infections with common cold viruses (mainly those by enteroviruses, such as members of the Coxsackie B virus family) can trigger T1D.

Our research group has during the last 13 years focused much of our efforts on providing an increased understanding on how infections with enteroviruses may be involved in T1D. Here we present some of our ongoing studies in this research area. We expect that our studies will lead to increased understanding of T1D disease mechanisms. Such information may provide clues for the design of preventative or curative therapies (e.g. antivirals) that could be tested in a clinical setting.

Genomics, citromics, epigenomics and transcriptomics to address molecular details of type 2 diabetes

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Analysis of the genome, cistrome, epigenome and transcriptome provide opportunities to, in a non-hypothesis driven approach, provide molecular details of the development of type 2 diabetes in different tissues in samples from animal models and humans. Genomic analysis includes the association of genetic variation with disease. The cistrome refers to the global analysis of transcription factor binding to DNA in the context of chromatin and can be explored to assess the binding of transcription factors important for insulin signaling under various conditions. In epigenomics, the correlation and causative role of epigenetic modifications for type 2 diabetes can be investigated. In recent years significant attention has been given to the correlation and potential causal role of the epigenetic modification DNA-methylation in type 2 diabetes and how the epigenome can be modulated. Furthermore, correlations between epigenetic modifications and gene expression, the transcriptome, is an area of intense investigation.

BEA – the core facility for Bioinformatics and Expression Analysis is a national genomic service facility at the Karolinska Institute - offers access to an extensive repertoire of genomic technologies to support research projects. Specifically, BEA provides services and consultation for genomic analyses based on the Affymetrix, Agilent, Illumina platforms for microarray analysis, high throughput sequencing and qPCR. This includes a number of different types of gene expression and transcriptome analyses in standard and custom formats for different model organisms, epigenetic analysis including bisulfite DNA-methylation and miRNA analysis and genome wide SNP and copy number variation analysis. The services range from experimental planning to extensive bioinformatics support.

For further details about the core facility and examples of genomics, citromics, epigenomics and transcriptomics analysis to reveal molecular details of type 2 diabetes, please visit our poster at the Diabetes Symposium or go to http://www.bea.ki.se.
Regulation in brown adipose tissue thermogenesis through the receptor Alk7

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ALK7 is a receptor for activin B and other members of the TGFβ ligand superfamily, including nodal and GDF-3. ALK7 is mainly expressed in white and brown adipose tissue (WAT and BAT, respectively), pancreatic islets and selected brain regions. Interestingly, we have found that Alk7 is highly and selectively expressed in mature brown adipocytes. Brown fat dissipates energy as heat and has become a promising target for alleviating metabolic disease. Usage and storage of nutrients are indispensable for thermogenesis in brown adipose tissue (BAT).

Here, we describe and compare two transgenic mice models: ALK7 knock-out (ALK7^{-/-}) and ALK7 UCP1^{+} conditional knock-out (Alk7 fx/fx, UCP1 Cre^{+}). While the specific deletion of ALK7 in BAT decreases lipid accumulation and renders the tissue unable to fully increase UCP1 expression under thermogenic conditions (postnatal stage, HFD and cold), simultaneous deletion of ALK7 in BAT, white adipose tissue (WAT) and brain in Alk7 knock-out mice has a synergistic effect and produces a strong decrease in UCP1 expression on adult mice at room temperature (RT) that is not observed in conditional mutants. Moreover, we report that specific deletion of Alk7 in BAT produces severe hypothermia when nutrient availability is restricted through fasting.

According to our observations, we conclude that Alk7 induces fuel storage and energy dissipation in BAT, and may act as a sensor that allows the brown adipocyte to adapt metabolically depending on the external availability of nutrients.

Increased fat cell size – a major phenotype of subcutaneous white adipose tissue in non-obese Type 2 diabetes

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Aims/hypothesis: We aimed to elucidate the impact of fat cell size and inflammatory status of adipose tissue on the development of type 2 diabetes in non-obese individuals.

Methods: We characterized subcutaneous abdominal adipose tissue by examining stromal cell populations by 13-color flow cytometry, measuring expression of adipogenesis genes in the progenitor cell fraction and determining lipolysis and adipose secretion of inflammatory proteins in 14 non-obese type 2 diabetic men and 13 healthy controls matched for age, gender, body weight and total fat mass.

Results: Diabetic individuals had larger fat cells but neither stromal cell population frequencies nor adipose lipolysis or secretion of inflammatory proteins differed between the two groups. However, in the entire cohort fat cell size correlated positively with the ratio of M1/M2 macrophages, TNF-α secretion, lipolysis, and insulin resistance. Expression of adipogenesis and adipose morphology regulators (BMP4, CEBPa, PPARy and EBF1) correlated negatively with fat cell size.

Conclusions/interpretations: We show that a major phenotype of white adipose tissue in non-obese type 2 diabetes is adipocyte hypertrophy, which may be mediated by an impaired adipogenic capacity in progenitor cells. Consequently, this could have an impact on adipose tissue inflammation, release of fatty acids, ectopic fat deposition and insulin sensitivity.
In vivo imaging of pancreatic islets transplanted into the eye to study diabetes progression

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Background and aims: Accurate monitoring of both beta cell mass and function is essential in the diagnosis and staging of diabetes. However, due to their size and anatomical location within the pancreas, studying beta cells in vivo is challenging. It was hypothesized that longitudinal confocal imaging of pancreatic islets following transplantation into the anterior chamber of the eye (ACE) would be a suitable method to monitor diabetes progression in vivo.

Methods: We used a diphtheria toxin receptor mouse model (RIP-DTR) that allows for the selective destruction of beta cells to induce diabetes. Following diphtheria toxin (DT) administration, changes in islet mass and beta cell function were assessed over a period of four days in RIP-DTR mice carrying RIP-DTR islets engrafted into the ACE. Next, pancreatic beta cell mass was determined using optical projection tomography imaging of the whole mount insulin-stained and tissue-cleared pancreas.

Results: Following DT delivery, a gradual decrease in islet mass was observed. In the RIP-DTR pancreas, a similar degree of destruction was observed compared to what was found in islets engrafted into the eye. Hyperglycemia was confirmed around 72 hours after treatment. Interestingly, the mice showed impaired glucose handling before a significant loss of beta cell mass was observed. This indicates that in the RIP-DTR mouse loss of beta cell function occurs prior to loss of beta cell mass.

Conclusion: Transplantation of islets into the ACE allows for longitudinal monitoring of beta cell mass dynamics and can serve as a valuable tool to assess the progression of diabetes.

Genetic evidence for a prominent role of APOC3 in the regulation of plasma triglyceride levels

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Increased plasma triglyceride (TG) levels have been long recognized as an independent risk-factor for the development of cardiovascular disease (CVD). Increased plasma TG levels are also the main characteristic of diabetic dyslipidemia and constitute the proposed link between type 2 diabetes mellitus (T2D) and the increased cardiovascular risk of diabetic patients. Twin-studies demonstrated that >50% of the variation in plasma TG concentration is related to genetic heritability, but only a small fraction of the genetic heritability has thus far been identified. However, it was recently reported that the rs138326449 loss-of-function variant of apolipoprotein C3 (APOC3) is associated with markedly reduced plasma TG concentrations and reduced risk of CVD. In addition, it was found that APOC3 inhibitors reduce the plasma TG concentration in animal models and in humans, suggesting that APOC3 is potentially an important regulator of plasma TG levels. Here, we used biochemical and genetic methods to analyze in more detail the nature of the relationship between the plasma APOC3 and TG concentrations in large cohorts (n>8.000) of healthy, middle-aged subjects. The plasma APOC3 concentration was measured in all subjects using the Meso Scale Discovery method. More than 25-fold variation in plasma APOC3 concentration was observed in all cohorts. Significant, independent relationships were observed between the plasma APOC3 and TG levels in all cohorts, with Spearman correlation coefficients varying between 0.5-0.7. A twin-study demonstrated that 32% of the variation in plasma APOC3 concentration is related to genetic heritability. As expected, the low-frequency rs138326449 loss-of-function variant of APOC3 explained <0.5% of the variation in circulating APOC3 level in healthy subjects. Mendelian randomization methods were subsequently used to dissect the contribution of known genetic loci associated with the plasma TG concentration to the variation in circulating APOC3 levels. Genetic variants in the APOA5 (rs10466588), GCKR (rs1260326) and LPL (rs11991231) loci exhibited highly significant relationships with the plasma TG concentrations but showed only weak relationships with the plasma APOC3 concentration, indicating that variation in the plasma TG concentration is of minor importance for the regulation of circulating APOC3 levels. In contrast, the rs138326449 loss-of-function variant of APOC3 was associated with markedly reduced plasma TG levels. These observations suggest that factors governing the plasma APOC3 concentration play a prominent role in the regulation of the plasma TG concentration.
PGC-1α1 stabilizers as a novel class of therapeutic agents to treat metabolic and neuromuscular disease

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The peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) regulates genes involved in energy metabolism and is expressed in energy-demanding tissues like fat, muscle, liver and brain. Activation of PGC-1α has been suggested to have beneficial effects on whole-body metabolism by driving browning of adipose tissue and improving skeletal muscle fuel handling. PGC-1α1 activation can be achieved by several mechanisms; however, a limiting step for activation is the short half-life of the protein.

We have designed and validated a high-throughput screening platform to identify PGC-1α1 protein stabilizers from a small molecular compound library. This platform is based on a stable cell line developed to express an EGFP-PGC-1α1 fusion protein with the same short half-life as the endogenous protein. Validation of positive hits was performed using cultured brown adipocytes. First by western blot to confirm PGC-1α1 protein stabilization and then by quantitative real-time PCR to measure PGC-1α1 target gene activation. Promising compounds were further validated in mouse primary myotubes and mouse primary liver cells. A selection of compounds was also tested for their ability to induce mitochondrial respiration. Based on the activity in validation assays, the pharmacokinetic characteristics of the compounds and stability in vitro and in vivo we selected the most promising compounds to test for efficacy in mice. Candidates that stabilize PGC-1α1 protein in vivo will be tested in different disease models to evaluate their potential as therapeutic agents.
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