Arranged by the Strategic Research Programme in Diabetes

Symposium on Molecular and Physiological Aspects of Diabetes Mellitus

Friday, November 9, 2012 at Nobel Forum, Wallenbergssalen
Karolinska Institutet, Nobels väg 1, Solna
Symposium on Molecular and Physiological Aspects of Diabetes Mellitus

9:00–9:10  WELCOME NOTE  
Juleen R. Zierath  
Chair: Anna Krook

9:10–9:45  MICRO RNA: PAST, PRESENT AND FUTURE  
Victor Ambros, Program in Molecular Medicine, UMass Medical School, USA

9:45–10:20  THE ROLE OF miRNAs IN METABOLISM  
Markus Stoffel, Institute for Systems Biology, ETH Zurich, Switzerland

10:20–10:50  COFFEE BREAK  
Chair: Ingo Leibiger

10:50–11:25  THE HUMAN PANCREATIC BETA-CELL: NORMAL PHYSIOLOGY AND IMPAIRMENT IN TYPE-2 DIABETES  
Patrik Rorsman, Oxford Centre for Diabetes, Endocrinology & Metabolism, University of Oxford, UK

11:25–12:00  THE GENETICS OF TYPE 2 DIABETES: WHAT HAVE WE LEARNED FROM GWAS?  
Jose Florez, Center for Human Genetic Research, Mass Gen Hospital/Harvard, USA

12:00–13:00  LUNCH  
Chair: Mikael Rydén

13:10–13:45  PATERNALLY INDUCED TRANSGENERATIONAL ENVIRONMENTAL REPROGRAMMING OF METABOLIC GENE EXPRESSION IN MAMMALS  
Oliver Rando, Department of Biochemistry & Molecular Pharmacology, UMass Medical School, USA

13:45–14:20  INACTIVITY-RELATED METABOLIC DISEASES: AN ENVIRONMENTAL AND GENETIC TUG-OF-WAR  
John Hawley, School of Medical Sciences, RMIT University, Melbourne, Australia

14:20–14:50  COFFEE BREAK  
Chair: Helena Edlund

14:50–15:25  ENDOPLASMIC RETICULUM STRESS, METAFLAMMATION AND INSULIN RESISTANCE  
Gökhan Hotamisligil, Department of Genetics and Complex Diseases, Harvard, USA

15:25–16:00  NOVEL LINKS BETWEEN AUTOPHAGY AND GLUCOSE METABOLISM  
Beth Levine, Howard Hughes Medical Institute and Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

16:00–16:10  CLOSING REMARKS  
Per-Olof Berggren

NOVEMBER 2012, AT NOBEL FORUM, WALLENBERGSALLEN  
KAROLINSKA INSTITUTET, NOBELS VÄG 1, SOLNA | 9:00–16:00 |

HOSTS  
Professor Juleen R. Zierath  
Department of Molecular Medicine and Surgery  
Department of Physiology and Pharmacology

Professor Per-Olof Berggren  
Department of Molecular Medicine and Surgery  
Department of Medicine

Professor Peter Arner

HOSTS  
Professor Juleen R. Zierath  
Department of Molecular Medicine and Surgery  
Department of Physiology and Pharmacology

Professor Per-Olof Berggren  
Department of Molecular Medicine and Surgery  
Department of Medicine

Professor Peter Arner
MICRO RNA: PAST, PRESENT AND FUTURE

Victor Ambros, RNA Therapeutics Institute, Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605

MicroRNAs are endogenous regulators of gene expression that act post-transcriptionally to regulate mRNA activity through antisense base pairing. Genes that produce microRNAs have been identified in animals, plants, and fungi. It appears that microRNAs have evolved independently in each of these clades, as components of the ubiquitous RNA interference (RNAi) gene silencing machinery. Many of the animal microRNA genes are evolutionarily ancient; the nucleic acid sequences of these microRNAs are completely conserved across essentially all animal phyla. Interestingly, even deeply conserved microRNAs display an amazing evolutionary fluidity in the repertoire of target genes that they regulate. Apparently, the roles of microRNAs in genetic regulatory networks can easily evolve, suggesting a significant role for microRNAs in evolutionary adaptation.

Our current understanding of the roles of microRNAs in animals is derived from the characterization of phenotypes associated with microRNA gene mutations, using a variety of experimental systems, including worms, flies, fish, mice, and human cells. Many research groups have uncovered roles for microRNAs in diverse processes including developmental cell fate determination, wound healing and regeneration, growth control, metabolic regulation, stress responses, immunity, and other host-pathogen interactions. A fascinating recent realization from these studies is that the functions of microRNAs in animal systems can appear to be subtle based on superficial inspection – yet after detailed study understood to be profound. Indeed, a common theme has emerged wherein microRNA mutant phenotypes can be modified by physiological conditions or stresses, indicating roles for microRNAs in buffering or modulating connections amongst broad gene regulatory networks.

Challenges for the future include obtaining a deeper understanding of microRNA function in complex animals, including humans. These advances will require integrated studies involving model organisms such as flies, worms and fish, together with mouse models, human cells, and whole genome sequence analysis of human pathologies. We need to understand much better how microRNAs are themselves regulated by modulatory cofactors and upstream signaling pathways, and how they are integrated into different genetic regulatory pathways in different contexts. Advancing our fundamental understanding of the mechanisms and regulation of microRNA biogenesis, stability, and activity should reveal novel perspectives on normal and disease biology, and help realize the promise of microRNA-based therapeutics, examples of which are being developed by academic and industry laboratories.
THE ROLE OF miRNAs IN METABOLISM
Markus Stoffel, Institute for Systems Biology, ETH Zurich, Switzerland

Defects in insulin signaling are among the most common and earliest events predisposing to the development of type 2 diabetes. MicroRNAs (miRNAs) are a new class of regulatory molecules influencing many biological functions including metabolism. In this talk I will discuss miR-103/107 and miR133 in insulin signaling and energy homeostasis.

In the first example data will be discussed showing that miR-103/107 expression is upregulated in obesity. Silencing of miR-103/107 leads to improved glucose homeostasis and insulin sensitivity. In contrast, gain of miR-103/107 function in either liver or fat is sufficient to induce impaired glucose homeostasis. Caveolin-1, a critical regulator of the insulin receptor is a direct target gene of miR-103/107. Cav-1 is upregulated upon miR-103/107 inactivation in adipocytes, concomitant with stabilization of the insulin receptor, enhanced insulin signaling, decreased adipocyte size and enhanced insulin-stimulated glucose uptake.

In the second example I will discuss the role of miR-133 in brown adipose tissue and in the control of energy homeostasis. I will present recent studies demonstrating that cold exposure and adrenergic stimulation inhibits expression of miR133a/b in a MEF2c-dependent manner to abrogate posttranscriptional silencing of Prdm16 and thereby enhance brown adipocyte differentiation and function. These findings demonstrate the central importance of two classes of miRNAs in insulin sensitivity and energy homeostasis and identify new targets for the treatment of type 2 diabetes and obesity.

THE HUMAN PANCREATIC BETA-CELL: NORMAL PHYSIOLOGY AND IMPAIRMENT IN TYPE-2 DIABETES
Patrik Rorsman, Matthias Braun, Stephan Collins, David Do and Resshma Ramracheya. The Radcliffe Department of Medicine, Oxford Centre for Diabetes, Endocrinology & Metabolism, University of Oxford, UK

The insulin-secreting beta-cell of the pancreatic islets plays a central role in the regulation of systemic glucose homeostasis. During the last 25 years there has been dramatic progress in our understanding of the glucose sensing of the beta-cell and how changes in plasma glucose are linked to stimulation or inhibition of insulin secretion (1). Like neurones, the beta-cell is electrically excitable and when exposed to insulin-releasing glucose concentrations, it depolarises and starts generating action potentials. Action potential firing is associated with stimulation of calcium entry and the resultant elevation of the cytoplasmic calcium concentration triggers fusion of the insulin-containing secretory granules with the plasma membrane culminating in the ‘delivery’ of insulin into the islet interstitium/capillaries. A key player in beta-cell electrical activity is the ATP-regulated potassium channel. This channel closes in response to a glucose-induced elevation of the intracellular ATP/ADP-ratio. It also represents the molecular target of the hypoglycaemic sulphonylureas.

However, the ‘consensus model’ of glucose-induced insulin secretion is based almost entirely on experimental data obtained in rodent beta-cells. With improved access to human pancreatic islets (as a by-product of clinical islet transplantation programmes), it is now possible to test the validity of the ‘consensus model’ in human beta-cells.

In my presentation I will present a revised model for glucose-induced insulin secretion in human beta-cells (2). With this background, I will proceed to consider the biphasic nature of glucose-induced insulin secretion and why it becomes impaired in type-2 diabetes and what we have learnt from in vitro studies of human islets from non-diabetic and type-2 diabetic organ donors (3). I will conclude by presenting novel data implicating defective expansion of the fusion pore (the connection between the granule lumen and the extracellular space) as a cause of type-2 diabetes.

References
THE GENETICS OF TYPE 2 DIABETES: WHAT HAVE WE LEARNED FROM GWAS?
Jose C. Florez, Center for Human Genetic Research, Mass Gen Hospital/Harvard, USA

Over the past five years there has been an explosion of genome-wide association studies (GWAS) for phenotypes related to type 2 diabetes and metabolism. These GWAS have occurred on the background of genotyping arrays populated by common single nucleotide polymorphisms (SNPs), deployed in various cohorts that have coalesced to form large international consortia. As a result, we have begun to accumulate lists of genetic loci that influence type 2 diabetes, related quantitative glycemic traits, adiposity, serum lipid levels and blood pressure. Genome-wide association findings have typically illustrated novel pathways, pointed toward fundamental biology, confirmed prior epidemiological observations, drawn attention to the role of β-cell dysfunction in type 2 diabetes, explained a fraction of disease heritability, tempered our expectations with regard to their use in clinical prediction, and provided possible targets for pharmacotherapy and pharmacogenetic clinical trials.

On the other hand, the causal variants have only been identified for a handful of these loci, and a substantial proportion of the heritability of these phenotypes remains unexplained. The latter is likely due to insufficient sample sizes to detect small effects, a nearly exclusive focus on populations of European descent, an imperfect capture of uncommon genetic variants, an incomplete ascertainment of alternate (non-SNP) forms of genetic variation, and the lack of exploration of additional genetic models. As the community embraces complementary approaches that include systematic fine-mapping, custom-made replication, denser genotyping arrays, platforms that focus on functional variation, next-generation sequencing techniques, and expansion to non-European populations, the coming years will continue to witness exponential growth in our understanding of the genetic architecture of metabolic phenotypes. Whether these findings will prove useful in disease prediction or therapeutic decision-making must be tested in rigorously designed clinical trials.

PATERNALLY INDUCED TRANSGENERATIONAL ENVIRONMENTAL REPROGRAMMING OF METABOLIC GENE EXPRESSION IN MAMMALS
Oliver Rando, Department of Biochemistry & Molecular Pharmacology, UMass Medical School, USA

Epigenetic information can be inherited through the mammalian germline and represents a plausible transgerational carrier of environmental information. To test whether transgenerational inheritance of environmental information occurs in mammals, we carried out an expression profiling screen for genes in mice that responded to paternal diet. Offspring of males fed a low-protein diet exhibited elevated hepatic expression of many genes involved in lipid and cholesterol biosynthesis and decreased levels of cholesterol esters, relative to the offspring of males fed a control diet.

These results, in conjunction with recent human epidemiological data, indicate that parental diet can affect cholesterol and lipid metabolism in offspring and define a model system to study environmental reprogramming of the heritable epigenome. I will also discuss ongoing efforts to understand the mechanistic basis for this phenomenon, using in vitro fertilization and epigenomic mapping in sperm.

INACTIVITY-RELATED METABOLIC DISEASES: AN ENVIRONMENTAL AND GENETIC TUG-OF-WAR
John A. Hawley, Exercise & Nutrition Research Group, School of Medical Sciences, RMIT University, Melbourne, Australia.

During the past 50 years, the prevalence of a cluster of interrelated chronic metabolic disease states including coronary heart disease (CHD), insulin resistance, type 2 diabetes mellitus (T2DM) and obesity has reached epidemic proportions (Shaw et al. 2010). The aetiological basis of this quartet of disorders is polygenic and highly dependent on the environment (i.e. existing genes interact with environmental factors to result in phenotypic expression of these diseases). However, since no new major human gene mutations have occurred in the latter half of the 20th century to cause the greater frequency of chronic metabolic diseases, then the increased incidence must principally be due to alterations in environmental conditions. One environmental factor to have changed dramatically in this time and strongly associated with a plethora of chronic metabolic disorders is the decline in daily physical activity.

Indeed, the increased prevalence of CHD, insulin resistance, T2DM and obesity and their strong association with inactivity has produced an “exercise deficient phenotype” in which individuals with a particular combination of disease-susceptible genes (i.e. risk factors) interact with undefined environmental conditions (e.g., level of physical activity) and cross a threshold of biological significance that results in overt clinical conditions. Evidence in support of this premise comes from studies in which multiple genes involved in aerobic metabolism are down-regulated in several metabolic states and may be linked to the pathogenesis of these disorders.
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(Mootha et al. 2003). While there is irrefutable proof that exercise training is an effective therapeutic intervention to increase insulin action in skeletal muscle from obese and insulin-resistant individuals (Hawley and Lessard 2008), recent evidence demonstrates that there is a large range within the population of observed improvements in response to exercise training: no matter which training parameter is studied, “nonresponders” are typically observed (Timmons 2011). Such variability in training-induced physiological adaptation provides a unique opportunity to examine the relationship between molecular responses to exercise and the magnitude of physiological change in humans. However, development of personalized exercise medicine applications will be difficult or even impossible without a proper understanding of gene-exercise interactions

References

ENDOPLASMIC RETICULUM STRESS, METAFLAMMATION AND INSULIN RESISTANCE

Gökhan S. Hotamisligil, MD, PhD. Harvard University, Boston, MA 02115, USA

Metabolism and immune response are closely integrated and proper coordination is essential for health and survival. Immune response is altered in the face of both malnutrition and overnutrition, the latter best exemplified in obesity. In the course of obesity, a state of nutrient and energy surplus, key metabolic sites such as adipose tissue, liver, pancreas and parts of the central nervous system exhibit chronic, low-grade, and persistent inflammation or metaflammation, which disrupt the proper metabolic responses and contribute to chronic metabolic pathologies such as insulin resistance and type 2 diabetes.

Obesity is also a state of ER stress and dysfunction in both experimental models and humans. Compromising ER function predisposes to metabolic disease and conversely, alleviation of ER stress by genetic or chemical means improve metabolic homeostasis both in humans and in experimental models. Interestingly, ER stress and the related responses are also closely linked to several inflammatory signaling networks, such as the kinases JNK, IKK, and more recently identified PKR. However, the triggering mechanisms for metabolic inflammation or the mechanisms giving rise to ER stress and dysfunction are not clearly understood and whether ER stress and inflammatory responses are causally linked to each other in disrupting metabolism remains unknown. In search for these mechanisms, we have taken a systematic approach to study how the different components of ER, such as lipids, proteins and calcium, act together to accommodate physiological dynamics and nutritional fluctuations in vivo and applied comparative polysome profiling in organelle fractions to systematically examine the translational status of every transcript.

These studies led to identification of lipid metabolism and related reprogramming of calcium homeostasis as critical drivers of ER dysfunction. We also determined that inflammatory environment impairs adaptive responses of ER and contributes to a malfunctioning unfolded protein response, particularly through the IRE1-XBP-1 branch. In this talk, I will present emerging data on these new developments as well as protein kinase R, a signaling molecule that reside at the intersection of nutrient and pathogen sensing and a vital component of inflammasome activation and metabolic regulation. Recently, we identified mechanisms leading to PKR activation in obesity and identified putative signals that link metabolic input to inflammatory and stress signaling. Potential intersection points between nutrients, ER and mitochondrial dysfunction, and metabolic control and novel translational and therapeutic opportunities emerging from these platforms that are applicable to human metabolic diseases will be discussed.
NOVEL LINKS BETWEEN AUTOPHAGY AND GLUCOSE METABOLISM
Beth Levine, M.D. Howard Hughes Institute and UT Southwestern Medical Center, Dallas, TX USA

The lysosomal degradation pathway, autophagy, exerts diverse functions in cellular, tissue, and organismal homeostasis. In this talk, two separate novel links between autophagy and glucose metabolism will be discussed, including (1) a role for autophagy in mediating the beneficial effects of exercise on glucose metabolism; and (2) a newly described autophagy gene that also functions in the endolysosomal degradation of the cannabinoid 1 receptor and in the control of glucose metabolism.

With respect to the first link, we found that exercise is a potent inducer of autophagy in multiple different mouse tissues, including skeletal muscle, cardiac muscle, liver, pancreas, adipose tissue, and brain. To investigate the role of exercise-mediated autophagy in vivo, we generated mutant mice that show normal levels of basal autophagy but are deficient in stimulus (exercise- or starvation)-induced autophagy. The mice (termed BCL2 AAA mice) contain knock-in mutations in BCL2 phosphorylation sites that prevent stimulus-induced disruption of the BCL2-Beclin 1 complex and autophagy activation. BCL AAA mice show decreased endurance and altered glucose metabolism during acute exercise, as well as impaired chronic exercise-mediated protection against high-fat-diet-induced glucose intolerance. Thus, exercise induces autophagy, BCL2 is a crucial regulator of autophagy in vivo, and autophagy induction may contribute to the beneficial metabolic effects of exercise.

With respect to the second link, we will describe in vitro studies demonstrating that a newly characterized autophagy gene also plays a genetically distinct function in the endolysosomal degradation of certain G protein-coupled receptors, including the delta opoid receptor and the cannabinoid 1 receptor. Mice lacking this autophagy gene have increased levels of brain cannabinoid 1 receptor, elevated food intake, and obesity and glucose intolerance. These studies highlight the functional and mechanistic diversity of autophagy proteins in different membrane trafficking pathways and the control of glucose metabolism.