2-1

The Role of the Circadian Transcription Factor D-site binding protein (DBP) in the Regulation of Energy Metabolism in Skeletal Muscle.

Jane Reznick, Elaine Preston, Donna Wilks, Susan Beale, Nigel Turner & **Gregory Cooney**.

Diabetes and Obesity Research Program, Garvan Institute of Medical Research, Darlinghurst, NSW 2010.

Disruption of the molecular circadian clock has recently been linked to metabolic disease. The D-site binding protein (DBP) is a clock 'output' transcription factor that displays a robust circadian rhythm. Factors such as DBP are believed to be responsible for regulating circadian expression of metabolic and other functional genes, but only a small number of DBP target genes in liver have been described so far and there has been no investigation into the role of DBP in the skeletal muscle. We investigated whether changes in DBP expression would have downstream effects on metabolic pathways in rat skeletal muscle by overexpressing DBP in the right tibialis cranialis (TC) muscle of rats through *in vivo* electroporation. The left leg was electroporated with an empty vector as a negative control. Tissues were collected 7 days post-electroporation at zt 0, 6, 12 and 18 (zt 0 and 12 being lights on and off respectively). qPCR was used to determine gene expression of several core clock genes and genes known to be expressed with a circadian rhythm that are functionally related to energy metabolism in muscle. Immunoblotting was also used to investigate changes in protein content.

Electroporation of the DBP construct achieved a constant expression of *dbp* across the entire 24 hour period at a level approximately 10-15 fold higher than the endogenous peak expression at zt 12 ($p<0.01$, $n=6$). The gene was also overexpressed at the protein level as determined by immunoblotting. We investigated core clock genes *per1* and *bmal1* expression in DBP overexpressing TC muscles at zt 0, 6, 12 and 18 by qPCR analysis. Overexpression of DBP did not have a significant effect on the amplitude or the temporal expression of *per1* or Bmal1 expression compared to muscle of the control leg. We also investigated key metabolic genes that are known to cycle in the skeletal muscle such as *pgc1alpha*, *pdk4* and *ucp3*. These transcripts cycled with a 2-4 fold difference between the peak and the trough of their expression. DBP overexpression in the right leg did not effect the expression of these metabolic genes.

In summary, constant overexpression of DBP across 24 hours did not affect the core molecular clock and had little effect on candidate functional genes *pgc1alpha*, *pdk4* and *ucp3*. This suggests that DBP alone is not critical in orchestrating the required temporal expression of cycling metabolic genes in muscle.

2-2

"That which doesn't kill you, does you good"- Role of Translational Control in Type-2 Diabetes.

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A universal response of eukaryotic cells to environmental stress is to phosphorylate the eukaryotic translational initiation factor, eIF2α and temporarily halt general protein translation. This allows cells to redirect available energy towards expressing stress proteins that aid the cells in resolving or coping with the stress. However, the prolonged inhibition of protein synthesis is incompatible with cell viability. Thus, most stresses induce the protein, GADD34, which assembles a cellular serine/threonine phosphatase that functions in a feedback loop to dephosphorylate eIF2α and reinitiate protein translation. As uncontrolled or excessive protein synthesis also overloads the protein folding machinery in the endoplasmic reticulum (ER), cells also do not tolerate the prolonged GADD34 expression, which has the potential to trigger cell death. Hence, cells possess multiple mechanisms for enhancing the degradation of the GADD34 protein, allowing full recovery of cells from stress. Mutations in components of this stress response pathway have implicated errors in protein processing in aberrant insulin synthesis and secretion and reduced viability of pancreatic b-cells. Thus, the focus of our current studies is on establishing the role of GADD34 in b-cells and its potential contribution to insulin resistance and the onset of type-2 diabetes.

Following on prior observations that GADD34 protein turnover was differentially modulated in different cell types, we have investigated the mechanisms that regulate cellular GADD34 protein levels. Our studies have identified a number of determinants, including N-terminal αNH2-ubiquitination that promotes GADD34 degradation by the 26S proteasome. We also identified a role for a membrane-binding domain in facilitating GADD34 protein turnover. More recent studies utilized mass spectrometry of GADD34 from stressed cells to identify multiple phosphorylated serines, threonines and tyrosines. Focusing on tyrosine phosphorylation, our studies suggested GADD34 phosphorylation by Src family tyrosine kinase(s) and substrate-trapping mutant tyrosine phosphatases identified a candidate tyrosine phosphatase. Mutations that impaired ubiquitination, membrane localization or tyrosine phosphorylation stabilized GADD34 and enhanced its ability to promote protein translation and exacerbate protein misfolding. Together, our data hint at a highly complex regulation of cellular GADD34 that in turn defines the ability of cells to synthesize proteins in the face of nutritional and oxidative stress. Based on these findings, current work using GADD34 knockout mice is aimed at establishing the role of GADD34 in insulin synthesis and b-cell survival under experimental conditions that trigger insulin resistance and diabetes.

2-3

Elucidating the biological role of the orphan nuclear receptor, RORalpha: regulation of adiposity and glucose tolerance.

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The Nuclear Hormone Receptor (NR) superfamily encode ligand dependent DNA binding proteins that translate nutritional, metabolic and patho-physiological signals into gene regulation. Orphan NRs belong to the family on the basis of homology; however, the agonists/hormones that regulate their action have not been identified. The significance of NRs in human health and wellbeing is underscored by the curative efficacy of pharmacopeia that target dysfunctional hormone signalling in the context of inflammation, dyslipidemia, insulin resitance/diabetes, endocrine diseases, reproductive disorders and cancer. NRs control lipid, glucose and energy homeostasis in a cell, tissue and organ specific manner, and aberrant NR signalling results in metabolic disease. Many of the 'orphans' are expressed in skeletal muscle (and other major mass metabolic tissues) with burdensome energy loads. Skeletal muscle accounts for ~35% of energy expenditure, and the majority of glucose and fat disposal. We have been particularly interested in understanding the role of the retinoic acid receptor-related orphan receptor (ROR) α1 in the regulation of adiposity, and glucose tolerance

We have previously demonstrated that a naturally occuring mutation in staggerer (sg/sg) mice, with decreased and dysfunctional RORα(1 and 4) expression in all tissues, leads to reduced adiposity. Moreover, we have shown these mice are resistant to diet induced obesity. Recently, we have over-expressed a truncated RORα1 lacking the ligand binding domain (that attenuated RORα1 signalling) in the skeletal muscle of transgenic mice (Tg-hRORαlΔDE). Illumina mediated expression profiling of sg/sg and Tg-hRORαlΔDE mice coupled to ingenuityfunction and pathway analysis indicated that RORα signalling was involved in: (i) Lipid and carbohydrate metabolism, cardiovascular and metabolic disease; (ii) NR signalling and, (iii) Akt-AMPK signaling. These significant changes in gene expression lead to aberrant Akt2AMPK signalling, and changes in blood glucose, glucose tolerance, insulin stimulated phosphorylation of Akt, and glucose uptake in the sg/sg and transgenic heterozygous TgRORα1ΔDE animals (S. Raichur et al. 2010, Nucl. Acids Res., in press). Furthermore, changes in AMPK activity lead to differential effects on lipogenesis, and fatty acid oxidation.

2-4

A Central Role of STAT3 in Homeostasis and Diabetes.

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Our laboratory is interested in the physiological roles STAT3 in maintaining body Homeostasis. We have created mice with a neural-specific disruption of STAT3 ($STAT3N^{-/-}$). Using these mice we have shown that neuronal STAT3 has an essential role in regulation of body homeostasis. We found that these $STAT3N^{-/-}$ mice had neonatal lethality but some survived mice were hyperphagic, obese, diabetic, and infertile through regulation of genes such as POMC (*PNAS 101. v 13, p4661–4666*). Recently, we have focused at discovery of possible epigenetic regulation mediated through STAT3 signaling pathway during development as well as in cytokine and stress responses. We found that a number of potential factors that participate in epigenetic programming, might be direct or indirect targets of STAT3 regulation. Detailed description of our findings will be presented.

2-5

The Transcriptional Coactivator Cited2 Regulates Hepatic Gluconeogenesis by Controlling PGC1-α Activity.

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Hepatic gluconeogenesis is critical in the adaptation to fasting conditions and contributes to fasting hyperglycemia under hepatic insulin resistance in diabetes. Many transcription factors and coregulators cooperatively induce and maintain the gluconeogenic program during fasting. It has been reported that the cAMP-responsive element-binding protein binding protein (CBP)/p300-binding transcriptional coactivator Cited2 binds hepatocyte nuclear factor 4α (HNF4α) and plays a crucial role during liver development. Despite the physiological importance of HNF4α and CBP in controlling gluconeogenesis, the mechanisms by which Cited2 regulates gluconeogenesis remain unknown.

We investigated the role of Cited2 in hepatic gluconeogenesis by using gain- and loss-of-function approaches *in vitro* and *in vivo*. In hepatocytes, the overexpression of Cited2 resulted in a 2-fold increase in cAMP-induced expression of gluconeogenic genes such as *G6Pase* and *Pepck*, leading to a 2-fold increase in glucose levels in the media. Cited2 deletion in hepatocytes attenuated cAMP-dependent induction of gluconeogenic genes. Hepatic expression of Cited2 *in vivo* increased gluconeogenic gene expression and blood glucose levels under both fasting and fed conditions. cDNA microarray analysis revealed that Cited2 overexpression *in vitro* enhanced cAMP-dependent induction of genes regulated by peroxisome proliferator–activated receptor γ coactivator-1α (PGC-1α such as *Ppara*, *Cpt1a*, *Cyp17a1*, as well as *G6Pase* and *Pepck*. Therefore, we next examined the effect of Cited2 on gluconeogenic gene expression induced by PGC-1α *in vitro*. Overexpression of Cited2 enhanced PGC-1α -induced gluconeogenic gene expression, but Cited2 deletion attenuated the expression. These data strongly suggest that Cited2 upregulates PGC-1α function and the molecular mechanism underlying the upregulation is currently being investigated.

2-6

Replication of Genome-Wide Association Signals of Type 2 Diabetes in Han Chinese in a Prospective Cohort.

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Background A recent genome-wide association study for type 2 diabetes in Han Chinese identified several novel genetic variants. We examined whether these variants predict progression to diabetes in a large prospective family-based Chinese cohort. We also investigated their associations with quantitative trait measures to explore the mechanism by which these variants affect glucose homeostasis.

Methods Eight single nucleotide polymorphisms (SNPs) near the *PTPRD*, *SRR, MAF/WWOX*, and *KCNQ1* genes were genotyped in 1,136 subjects of Chinese origin from the Stanford Asia-Pacific Program for Hypertension and Insulin Resistance (SAPPHIRe) study. Their effects on incidence of diabetes and quantitative measures of insulin secretion and sensitivity at baseline and over time were assessed.

Results Over an average follow-up period of 5.43 years, participants with the risk-conferring rs17584499 TT genotype in the *PTPRD* gene were more likely to progress from non-diabetes to diabetes than were non-carriers. The risk-conferring T allele was associated with a significantly greater increase in homeostasis model assessment of insulin resistance (HOMA-IR) over time. *PTPRD* gene expression in human adipose tissues was negatively associated with fasting insulin levels and HOMA-IR. At baseline, the risk-conferring rs2237892 C and rs2237897 C alleles in the *KCNQ1* gene were associated with lower homeostasis model assessment of β-cell (HOMA-β) and lower first-phase insulin response. The risk-conferring rs7192960 C allele near the *MAF/WWOX* gene was associated with lower HOMA-βand second-phase insulin response.

***Conclusio*n** *PTPRD* genetic variant seems to be associated with progression to diabetes in Chinese, probably through enhanced insulin resistance. Genetic variants near the *KCNQ1* and *MAF/WWOX* gene are associated with reduced insulin secretion.

2-7

Histone Modifications Determine Positive and Negative Hormonal Regulation of the TSHα Gene in the Pituitary.

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Currently little is known about the chromatin modifications that occur on the target gene promoters regulated by nuclear hormone receptors. Thyroid hormone (T_3) represses TSHα gene expression via thyroid hormone receptors (TRs) which belong to the nuclear hormone receptor superfamily, whereas thyrotropin releasing hormone (TRH) activates via the TRH receptor, a G protein-coupled receptor. We thus studied regulation of the TSHα gene by cAMP and T_3 using chromatin immunoprecipitation (ChIP) assays in stably-transfected rat pituitary GH3 cells containing the human TSHα promoter. Interestingly, cAMP selectively increased histone H4 acetylation whereas, as previously reported, T_3 induced histone H3 acetylation. In particular, cAMP increased H4K5 and H4K8 acetylation and decreased H4K20 trimethylation, modifications associated with transcriptional activation. T_3 increased H3K9 and H3K18 acetylation and H3K4 trimethylation; however, it also decreased H3K27 acetylation and increased H3K27 trimethylation which are associated with transcriptional repression. Of note, cAMP recruited pCREB, CBP/p300, and PCAF to the promoter whereas T_3 caused dissociation of NCoR/SMRT and HDAC3. Overexpression of a dominant negative mutant thyroid hormone receptor (TR) from a patient with the clinical syndrome of resistance to thyroid hormone (RTH) led to diminished T_3-dependent negative regulation and partially blocked histone H3 modifications of the TSHα promoter. Currently we are investigating the effects of 5-azacytidine and trichostatin A in rescuing the expression of endogenous TSHα and TSHα genes which are normally silenced in GH3 cells. In summary, our findings show that distinct non-overlapping histone modifications determine positive versus negative transcriptional regulation, and integrate opposing hormonal and intracellular signals, at the TSHα promoter. A mutant TR from a patient with RTH exerted dominant negative activity by blocking the histone modifications induced by T_3 on the TSHα promoter and likely contributes to the inappropriate TSH production observed in RTH.

3-1

Role of Stra13 in Insulin Regulation of Hepatic Lipid Metabolism.

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In the liver, insulin stimulates the synthesis of fatty acids thorough the up-regulation of the transcription factor SREBP1c. The mechanism how insulin regulates the expression of SREBP1c is still ambiguous, however. We have now found that hepatic expression of Stra13, a member of bHLH family transcription factors, was stimulated by insulin both *in vitro* and *in vivo*. In primary cultured hepatocytes, overexpression of Stra13 resulted in the up-regulation of SREBP1c, and the reduction of Stra13 with the use of short hairpin RNA (shRNA) inhibited insulin-induced expression of SREBP1c, indicating that Stra13 contributes to insulin-induced expression of SREBP1c. Stra13 was found to enhance and suppress the promoter activity of the SREBP1c gene by binding to an E-Box in the promoter region and to the intron1 of the SREBP1c gene, respectively. Insulin prevents the binding of Stra13 to the suppressor region and promotes it to the enhancer region. In obese KKAy mice, the hepatic abundance of Stra13 as well as of SREBP1c was greater than those of control mice. The reduction of Stra13 in the liver with the use of an adenovirus vector encoding shRNA of Stra13 decreased the expression of SREBP1c along with that of the downstream genes of SREBP1c including stearoyl-CoA desaturase1 or fatty acid synthase. The reduction of hepatic Stra13 in KKAy mice markedly ameliorated hypertriglyceridemia. These results suggest that Stra13 controls hepatic lipid metabolism thorough the regulation of SREBP1c and that the Stra13 pathway serves as a potential therapeutic target of dyslipidemia of obese individuals.

3-2

Molecular Characterization of Seipin and Its Mutants: Implications for Seipin in Triacylglycerol Synthesis.

Weihua Fei, Hui Li, Guanghou Shui, Chris Bielby, Ximing Du, Andrew J. Brown, Markus Wenk, Peng Li and **Honquan Yang**

The human lipodystrophy gene product Berardinelli-Seip Congenital Lipodystrophy 2 (BSCL2)/seipin has been implicated in adipocyte differentiation, lipid droplet (LD) formation and motor neuron development. However, the cellular function of seipin and its disease-causing mutants remains to be elucidated. Here, we characterize seipin and its mis-sense mutants: N88S/S90L (both linked to motoneuron disorders) and A212P (linked to lipodystrophy). Knockingdown seipin significantly increases the steady state level of triacylglycerol (TAG). In contrast, overexpression of seipin reduces oleate incorporation into TAG and the steady state level of TAG, causing impaired formation of lipid droplets. Expression of the A212P mutant, however, had little effect on LD biogenesis. Interestingly, expression of N88S or S90L causes the formation of many small lipid droplets, reminiscent of seipin deficient cells. This dominant-negative effect may be due to the N88S/S90L-induced formation of aggresomes where wild type seipin can be trapped. Importantly, co-expression of seipin and the N88S or S90L mutant can significantly reduce the formation of aggresomes. Lastly, we demonstrate that seipin can interact with itself and its mutant forms. Our results provide important insights into the biochemical characteristics of seipin and its mis-sense mutants, and suggest that seipin may function to inhibit lipogenesis.

3-3

Drosophila Model of Lipodystrophy.

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Disorders such as obesity and lipodystrophy cause ectopic lipid accumulation in non-adipose tissues, leading to lipotoxicity which has health-threatening consequences. The exact underlying mechanisms for ectopic lipid accumulation remain elusive. We will report the analysis of a *Drosophila* model of the most severe form of lipodystrophy, Berardinelli-Seip Congenital Lipodystrophy 2, which is caused by mutations in the gene *BSCL2/Seipin*. Our study indicates a tissue-autonomous mechanism of ectopic lipid accumulation in lipodystrophy.

3-4

Mechanism of Inflammation-Induced Insulin Resistance: Reliance of Toll-Like Receptor-4 (TLR4) Action on Ceramide Synthesis Reveals Roles for Saturated Fatty Acids.

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Obesity is associated with an enhanced inflammatory response that exacerbates insulin resistance and contributes to diabetes, atherosclerosis, and cardiovascular disease. One mechanism accounting for the increased inflammation is the amplification of innate immunity pathways (i.e. toll-like receptor-4 signaling, TLR4) by saturated fatty acids, an event which is essential for lipid-induced insulin resistance (Shi et al., Journal of Clinical Investigation, 116:3015). Using in vitro and in vivo systems to model lipid induction of TLR4-dependent inflammatory events in rodents, we determined that TLR4 activation alters the manner in which skeletal muscle and macrophages utilize fatty acids, diverting them away from oxidation and into sphingolipids (i.e. ceramides) that antagonize insulin action. This metabolic reprogramming, which can be attributed to an I?K?-dependent upregulation of genes driving ceramide biosynthesis, is dispensable for the induction of inflammatory cytokines, but is essential for the development of insulin resistance. These findings place sphingolipids such as ceramide at the nexus of signaling networks linking inflammatory agonists and ectopic lipids to the antagonism of insulin action.

3-5

Adipocyte Fatty Acid Binding Protein as a Link between Obesity and Cardiovascular Diseases.

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Obesity is associated with increased risk of metabolic and cardiovascular diseases. Adipose tissue secretes many adipokines that may play a role in mediating obesity-related disease. Adipocyte fatty acid binding protein (A-FABP) is an adipokine highly expressed in adipose tissue, but also expressed in macrophages, especially upon stimulation by oxidized low density lipoprotein and Toll-like receptor. In mouse models, genetic deletion of A-FABP results in partial protection from hyperglycaemia and dyslipidaemia associated with diet-induce obesity or genetic obesity (ob/ob). Furthermore, genetic deletion of A-FABP or the use of a selective A-FABP inhibitor in the atherosclerosis-prone apolipoprotein E-deficient (apoE-/-) mice result in significant reduction in atherosclerosis. In humans, genetic mutation of A-FABP at the promoter –T87C results in reduced risks of type 2 diabetes, hypertriglyceridaemia and cardiovascular disease. We have demonstrated that A-FABP circulates in high concentrations, and circulating levels correlate with adipose tissue expressions. We found that serum A-FABP correlated significantly with adverse cardiovascular risk factors, including obesity indices, fasting glucose, HOMA-IR, blood pressure, triglyceride and inversely with high-density lipoprotein. Serum A-FABP was predictive of the development of the metabolic syndrome over 5 years, and type 2 diabetes over 10 years. Genetic analysis revealed association of a promoter variant rs3834363 with the number of metabolic risk factors, as well as persistent metabolic syndrome over 12 years in Chinese. In view of the close relationship between dysglycaemia, dyslipidaemia, the metabolic syndrome and atherosclerotic disease, we have also explored the relationship of serum A-FABP with atherosclerosis and clinical cardiovascular diseases in human. We found independent associations of serum A-FABP with carotid intimal thickness and coronary artery calcium score, both being surrogate markers of atherosclerosis. Furthermore, in case-control studies, we observed significantly higher serum A-FABP levels in subjects with coronary artery disease and the effects were independent of conventional cardiovascular risk factors. We also observed significantly elevated serum A-FABP in subjects with ischaemic stroke, whether measured in acute stroke setting or in convalescent serum taken 6 months after stroke. Furthermore, serum A-FABP taken early in the course of the acute stroke was predictive of 3-month mortality, independent of age and clinical severity score. These studies suggest that A-FABP may have a pathogenic role in atherosclerotic disease in humans, as seen in rodents, and may be a key mediator of obesity-related cardiovascular diseases.

3-6

Chromatin Dynamics in Adipogenesis.

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Obesity and its related diseases such as diabetes increasingly are responsible for significant economic and social burdens in established and emerging countries. As such, understanding the molecular mechanism that controls adipose (fat) cell differentiation would greatly enhance our ability to solve these problems. Adipogenesis is a complex physiological process that requires concerted regulation of gene expression by various adipogenic factors. Among these regulators are many histone modifying enzymes and chromatin remodelers, suggesting that epigenetic mechanisms play essential roles in modulating adipogenesis. To better understand the epigenetic control of adipogenesis, we examined the dynamic changes of a number of key histone modifications during adipogenesis. Patterns of both active and repressive histone marks at several key adipogenic genes will be described and their correlation with gene transcription will also be discussed.

4-1

Identification of Specific Actin Filaments that Regulate Glucose Clearance and Insulin Secretion in the Mouse.

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Defects in two exocytic processes, glucose uptake and insulin secretion, is linked to the onset of Type 2 diabetes. In the former process, the glucose transporter, GLUT4, is trafficked in specialized vesicles to the surface membranes, while insulin secretion occurs through fusion of insulin-containing granules with the pancreatic β-cell surface. Cell culture experiments have shown that the actin cytoskeleton plays critical roles in both these processes. We have identified specific actin filaments in mouse tissue containing the cytoskeletal tropomyosin (Tm) isoform, Tm5NM1. To understand the function of these actin/Tm filaments *in vivo* we have created tissue-wide Tm5NM1 transgenic (Tg) mice. Whole body glucose and insulin tolerance tests and *in vivo* glucose uptake measurements in skeletal muscle and adipose tissue revealed that the Tg mice have increased glucose clearance in part due to increased insulin sensitivity. Gene expression analysis on adipose tissue from the Tg mice detected an increase in expression of genes involved in GLUT4 trafficking (Sec 8, Myo1c) and actin filament turnover (RhoGEFs, RhoGAPs and Rho GTPase binding proteins). This suggests that Tm5NM1 is affecting glucose uptake via altering the actin filament populations that regulate the GLUT4 trafficking pathway. Further experiments on pancreatic islets isolated from the Tm5NM1 Tg mice revealed a significant increase in basal insulin secretion. This provides the first *in vivo* evidence in mice of a role of actin in GLUT4 trafficking and insulin secretion.

4-2

Dynamic Analysis of Type 2 Diabetic Progression with Proteomic Approach.

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Type 2 Diabetes is a typical chronic disease that needs many years for progressing, and a typical systems-deficiency that involves metabolic dysfunctions of many different tissues of the body. Understanding the molecular properties of diabetic progression at systems-level is a big challenge in the systems-biology era. We reason that blood plasma proteome is an ideal “window” for observing global and dynamic progression of type 2 diabetes, since the circulation system is basic “highways” of the body for transporting all kinds of metabolic related molecules and, importantly, the plasma proteome is considered as the most comprehensive and largest version consisting of many different proteins that are secreted, or shed, or leaked from cells and tissues throughout the body. Here we present a dynamic analysis of diabetic rat- plasma proteome from pre-diabetic stage to diabetic stage by combining mass spectrometric and mathematical techniques. Based on computing analysis of 553 overlapped plasma proteins between diabetic rat, Goto-Kakizaki (GK) rat, and non-diabetic rat, Wistar rat, we first time demonstrate that GK rats preserved a larger entropic values than Wistar rats at systems-level throughout time. Furthermore, using our newly developed computing approach, we characterize 14 featured plasma proteins either significantly overrepresented or underrepresented all the time as well 112 dynamic plasma proteins significantly differentiated from time to time during the diabetic progression.

4-3

Oxidative Stress and β-Cell Death – Protective Role of The Selenoprotein SEPS-1.

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Selenoprotein S (SEPS1) is a novel glucose-regulated selenoprotein that has been proposed to have antioxidant properties. Indeed, an *in vitro* study has shown that SEPS1 can protect pancreatic β-cells from oxidative stress-induced death. To determine whether SEPS1 can protect against diabetes *in vivo* we generated a transgenic mouse that highly over-expresses SEPS1 in the β-cell. The transgenic mice developed mild hyperglycaemia (9.8±0.4 vs 14.6±0.9 mM, p<0.001). Glucose-mediated insulin secretion was significantly lower in transgenic compared with control mice (AUC: 13.0±1.8 vs 30.0±2.2 ng/mlx30 min, p<0.0005). Of significance the transgenic mice were protected against alloxan-induced diabetes (200 mg/kg ip). Following alloxan treatment plasma insulin levels (0.6±0.1 vs 0.02±0.01 ng/ml, p<0.05), pancreatic insulin content (0.028±0.004 vs 0.004±0.002 ng/□g prot. p<0.005), islet insulin staining (112±24 vs 48±10 a.u., p<0.05) and glucose-mediated insulin release (AUC: 11.0±1.8 vs 3.0±1.0 ng/mlx30 min, p<0.005) were significantly higher in transgenic compared with littermate control mice. Similarly, following streptozotocin treatment (210 mg/kg i.p.) transgenic mice had significantly lower plasma glucose (13.0±1.0 vs 38.8±3.1 mM, p<0.0005) and higher plasma insulin levels (0.7±0.2 vs 0.01±0.003 ng/ml, p<0.005) compared with control mice. In contrast, after high fat feeding (60%) transgenic mice were glucose intolerant (AUC: 3054±102 vs 1534±71 mMx120 min, p<0.005), displaying lower glucose-mediated insulin release (AUC: 135±14 vs 272±30 ng/mlx120 min, p<0.005) than control mice. The basal level of reactive oxygen species (CM-H_2DCFDA fluorescence) in isolated islets was lower in transgenic mice than control mice (26682±2285 vs 21254±1836 a.u., p<0.05), while the basal level of apoptosis was higher compared with control mice (0.09±0.01 vs 0.24±0.06 a.u., p<0.005).in transgenic islets under conditions. When treated with alloxanthe level of apoptosis significantly increased in control islets but not transgenic islets (0.23±0.041 vs 0.25±0.037 a.u.; p<0.005 neg untreated vs alloxan). Surprisingly, deletion of one copy of the Seps1 gene specifically in islet β-cells resulted in defective glucose-mediated insulin secretion (26.4±9.5 vs 55.3±6.1 ng/mlx30min, p<0.05). We therefore suggest that SEPS1 may have a specific antioxidant function in the β-cell and when up-regulated sufficiently could protect against oxidative stress-induced β-cell loss, but not against fat induced mechanisms of β-cell dysfunction.

4-4

Making a genetic diagnosis of mitochondrial diabetes – What's the point?

Rinki Murphy

Faculty of Medical and Health Sciences, The University of Auckland

Maternally inherited diabetes and deafness (MIDD) affects up to 1% of patients with diabetes but is often under-recognised by physicians. This case-based presentation illustrates why it is important to make an accurate genetic diagnosis, as there are implications for clinical investigation, diagnosis, management and genetic counselling. The patients described are real cases from the author's own practice and summarise the range of clinical phenotypes associated with MIDD and provide guidance on the optimal clinical management of these patients and their families. It is based on the review article Murphy et al. Diabet Med 2008; 25: 383-399.

4-5

Diabetes in China: the challenge now.

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China has made astonishing strides in economic development and emerged as a strong global partner during the past 30 years. While the rapid improvement of standard of living has also exposed the Chinese to new risks once thought to be the preserve of the west. The new estimates from a population-based national study conducted in 2008-2009 reported 92.4 million people with diabetes and 148.2 million people with pre-diabetes. China ahead of India now has become the country with the largest number of people with diabetes in the world (Fig. 1). When looking back the past 15 years, we can see a leap in the prevalence of diabetes in China, in which it increased markedly from 2.0% in 1995 to 5.5% in 2001 and to 9.7% in 2009. The rate of increase is much faster than the US, India, Japan and the UK.

Like many other developing counties, China has experienced dramatic socioeconomic and cultural transitions. The aging of the population, nutritional changes and increasingly sedentary lifestyles, with a consequent epidemic of obesity, have contributed to the national epidemic. We are caring about the huge population with diagnosed diabetes; we are also concerned about the number of people with undiagnosed diabetes.

As IDF pointed out, the prevalence of diabetes in China is a wake-up call for government and policy-makers to take action on diabetes. However, like many countries facing complex healthcare challenges, multiple institutional and attitudinal obstacles still exist to improving health care, and these barriers have created a substantial and growing gap between what we know and what we actually do. The top priority is to need to develop strategies assisting more primary health-care providers to recognize and treat diabetes; this is an important basis for national education of the disease prevention and treatment. Further, institutional collaborations between Chinese and international scientists are believed to be making an impact for promoting diabetes research and wider educational activities.

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4-6

Metabolic syndrome in Japan: Controversies about the Importance of Increased Waist Circumference.

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Obesity is defined as a condition of excessive body fat accumulation mainly in adipose tissue, which increases the risk of type 2 diabetes, hypertension, dyslipidemia and cardiovascular disease (CVD), and ultimately death. Body mass index (BMI) measures general obesity, but cannot take into account the distribution of body fat. In contrast, waist circumference is one of the indices of abdominal obesity. Considerable attention has been paid to the metabolic syndrome over the past two decades. At the same time, partially due to a lack of unified diagnostic criteria for the metabolic syndrome, heated arguments have arisen about the importance of increased waist circumference.

In Japan, under a national law, new health checkup and health care advice system with a particular focus on the metabolic syndrome has been introduced from April, 2008. Companies and local governments must measure waist circumference of Japanese people between the ages of 40 and 74 as part of their annual checkups. Therefore, the word “METABO” is an extremely popular term for metabolic syndrome in Japan.

Current widely used diagnostic criteria for the metabolic syndrome, including the Japanese original criteria published in 2005, adopt abdominal obesity as defined by waist circumference. The cut-off points, 85cm for men and 90cm for women at the level of the umbilicus decided by the Examination Committee of Criteria for the Metabolic Syndrome in Japan, were based on the original cross-sectional data from Japanese, and were the corresponding value for the visceral fat area (VFA) of 100cm^2 at the level of the umbilicus, rather than a specific value of BMI. The inverse relation between men and women in terms of the cut-off point for waist circumference has produced controversies.

Although waist circumference, relatively direct index of visceral obesity, is clearly associated with the accumulation of metabolic abnormalities, it is still unclear whether waist circumference itself can predict future CVD events more reliably than other obesity indices. At present, we are performing a nationwide survey to investigate the clinical validity of the Japanese criteria for the metabolic syndrome by integrating twelve cohort studies, through which some controversies are expected to be resolved.

4-7

The Novel Role of HERP in Insulin Secretion.

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The DBA/2 (D2) mouse is more susceptible to diabetes due to β-cell dysfunction than the C57BL/6J (B6J) strain. One distinctive feature of the D2 is that it hypersecretes insulin in response to glucose, both in vivo and in vitro and this seems to be independent of changes in insulin sensitivity. We have recently reported the insulin hypersecretion phenotype of the D2 is mediated by at least two genes, one of which is nicotinamide nucleotide transhydrogenase (*Nnt*), being 5-fold higher in the D2 when compared to B6J. To identify other gene(s) associated with insulin hypersecretion, the recombinant inbred BXD strain set was utilised, as their genome is derived from the B6J and D2. Our data show that 4 out of the 20 BXD lines tested hypersecreted insulin in response to glucose. A microarray analysis was then performed on the BXD low and high secretor strains to determine the differential gene expression profile. Five genes were differentially expressed in the high secretors and Real-Time PCR was performed to confirm the level of expression. These results showed that only 1 gene was consistently under-expressed in high secretor BXD strains and D2 islets. This gene is known as *Herpud1* or *Herp*. *Herp* encodes a 54 kDa protein called HERP which resides in the integral endoplasmic reticulum membrane. The physiological role of HERP in the pancreatic islet is currently unknown. To test the relationship between *Herp* and *Nnt*, *Herp* was either knocked down using siRNA or overexpressed in MIN6 cells. The down-regulation of *Herp* reduced *Nnt* expression while overexpression of *Herp* increased *Nnt* expression, indicating a role for *Herp* in the regulation of *Nnt*. Interestingly, when *Nnt* was overexpressed in MIN6 cells *Herp* expression was not affected. Furthermore, the down-regulation of *Herp* in D2 islets resulted in reduced glucose-mediated insulin secretion while it did not affect insulin release in B6J islets. Overexpression of *Herp* in B6N islets (a substrain of C57BL/6 with full-length *Nnt*) resulted in upregulation of *Nnt* and enhanced glucose-mediated insulin secretion but not in B6J islets. In conclusion, we have identified a second gene called *Herp* which controls insulin secretion by regulating Nnt. Whether *Herp* leads to β-cell dysfunction in response to a diabetes milieu remains to be determined.

5-1

Regulation of the ER stress sensor IRE1 in pancreatic α-cells.

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The unfolded protein response (UPR) plays a key role in cellular homeostasis through managing the protein folding capacity of the endoplasmic reticulum (ER) to cope with unfolded or misfolded proteins. Sensing increased protein folding demand in the ER lumen, inositol-requiring 1 (IRE1α), the ER transmembrane protein kinase/endonuclease, is activated through autophosphorylation to initiate a key signaling arm of the mammalian UPR pathways. To investigate the poorly understood mechanisms that regulate IRE1α signaling, we have found that in pancreatic β-cells and primary islets the scaffold protein RACK1 interacts with IRE1α in a glucose-stimulated or ER stress-responsive manner. Constitutively associated with protein phosphatase PP2A, RACK1 mediates the glucose-inducible assembly of a ternary IRE1α-RACK1-PP2A complex to inhibit glucose-stimulated IRE1α activation through dephosphorylation by PP2A, thereby attenuating IRE1α-dependent upregulation of insulin production. Conversely, ER stress-induced RACK1 dissociation from PP2A results in retained phosphorylation of RACK1-associated IRE1α molecules, suggesting RACK1's distinct action on IRE1α during ER stress. Furthermore, IRE1α is hyperactivated as a result of metabolic stress-associated reduction in RACK1 expression in the islets from obese *db/db* mice, and restored RACK1 overexpression leads to decreased IRE1α activation. Together, our findings demonstrate that RACK1 functions as a molecular switch to exert dynamic control of the IRE1α signaling platform. Chronic disruption of this RACK1-dependent feedback regulation of IRE1α may contribute to β-cell dysfunction in diabetes.

5-2

Inflammation and insulin resistance: cause or consequence?

GI Lancaster, CR Bruce, TL Allen, S Risis, C Yang, DC Henstridge, G Kowalski, HT Nicholls, and **MA Febbraio**

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The evidence linking inflammation and diabetes dates back more than a century with the finding that treatment with high doses sodium salicylate decreases glycosurea in patients presumed to have type 2 diabetes. Since this time, the link between obesity, chronic low grade inflammation and impaired insulin action has strengthened. There is little doubt that targeting inflammation is a sound therapeutic strategy since such interventions protects against insulin resistance and or type 2 diabetes in humans (1,2). However, whether inflammation is a primary cause of insulin resistance or an unwanted consequence that warrants treatment is uncertain. We have been investigating two models that indicate that inflammation is a consequence rather than a primary cause of dysregulated insulin action. The first is a genetically modified mouse model (dsRNA-dependent Protein Kinase (PKR) deficiency) that is protected from acute lipid induced inflammation. Paradoxically, this mouse has enhanced ectopic lipid expression in the liver and is markedly insulin resistant, primarily the result of a transcriptional program that increases both adipogenesis and the expression of important lipid transport and chaperone molecules key to the entry of lipid into cells. The second model is a careful high fat feeding time-course study in C57Bl6 mice, that indicates that glucose intolerance occurs extremely rapidly, due to increased ectopic lipid expression in the liver that preceedes any measures of inflammation. These data suggest that inflammation is secondary to ectopic lipid acumulation particular in the liver.

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5-3

Advanced Glycation End Products (AGEs) Increase Human Mesangial Foam Cell Formation by Increasing SCAP Golgi Modification

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Diabetic nephropathy caused by advanced glycation end products (AGEs) is associated with lipid accumulation in glomeruli. This study was designed to investigate whether N^{ε}-(carboxymethyl) lysine (CML) (one of AGEs family) increases lipid accumulation in human mesangial cells (HMCs) via LDL receptor pathway by increasing cholesterol sensor SREBP cleavage-activating protein (SCAP) transcription and its posttranslational modification in Golgi.

HMCs were treated by CML. Intracellular cholesterol content was assessed by Oil Red O staining and cholesterol enzymatic assay. Expression of mRNA and protein of molecules controlling cholesterol homeostasis in the treated cells was examined by real-time quantitative PCR and western blotting, respectively. Golgi enzymes activity was determined using enzyme-based method. SCAP translocation was detected by confocal microscopy.

CML increased cholesterol accumulation in HMCs. Exposure to CML led to abnormal translocation of SCAP from ER to Golgi even in the presence of a high concentration of LDL. The increased SCAP translocation carried more SREBP2 to the Golgi for cleavage and increased NH_2-terminal of SREBP2. This enhanced expression of LDL receptor and HMGCoA reductase which were blocked by inhibitors of Golgi mannosidase enzymes, suggesting that CML may affect SCAP glycolysations in Golgi. Furthermore, CML upregulated Golgi mannosidase activity, inhibiting SCAP degradation due to SCAP glycolysations.

Conclusions: AGEs (CML) increased lipid synthesis and uptake, thereby causing foam cell formation via increasing transcription and posttranslational modification of SCAP in HMCs. These data imply that Golgi enzymes inhibitors might have a potential renal protective role in prevention of mesangial foam cell formation.

5-4

LXR: Its Roles in Beta-cell Dysfunction

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Type 2 diabetes mellitus is characterized by hyperglycemia, insulin resistance, and defective insulin secretion. The accumulation of excess lipid in the pancreatic islets of obese subjects (with type 2 diabetes) has been implicated as being one of the main causes of β-cell dysregulation. However, it is still unclear how hyperlipidemia induces β-cell failure under hyperglycemic conditions of type 2 diabetes.

Liver X receptor (LXR), a sterol-activated nuclear hormone receptor, has been implicated in the cholesterol and fatty acid homeostasis via regulation of reverse cholesterol transport and *de novo* fatty acid synthesis. Further, LXR is also involved in immune responses including anti-inflammatory action and T cell proliferation. Although the functional roles of LXR in the liver, intestine, fat, and macrophage are well established, its role in pancreatic β-cells has not been clearly defined. In this presentation, I will present our recent data that chronic activation of LXR in β-cells would induce β-cell lipotoxicity, a key step in the development of type 2 diabetes mellitus. Moreover, I will discuss the posttranslational regulation of LXR upon its ligand.

5-5

Fibroblast Growth Factor 21 as a Novel Metabolic Hormone Integrating the Circadian Rhythm with Energy Homeostasis in Man.

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Fibroblast growth factor 21 (FGF21), produced predominantly from the liver, has been shown to have multiple beneficial effects on glucose and lipid metabolism in animal studies. First identified during a high throughput screening for agents capable of increasing glucose uptake in 3T3-L1 adipocytes, its systemic administration to rodents and nonhuman primates with obesity and diabetes resulted in weight loss, alleviation of insulin resistance and hepatosteatosis, as well as lowering of blood glucose and lipid levels. Transgenic overexpression of human or mouse FGF21 in mice also protected against metabolic disorders associated with genetic and dietary obesity. In diet-induced obese rats, FGF21 increased hepatic insulin sensitivity and energy expenditure through its actions on the central nerve system. As hepatic expression of FGF21 in mice is induced by fasting or by consumption of ketogenic diet through the activation of PPARα, FGF21 has been proposed as a key regulator in coordinating carbohydrate and fatty acid metabolism during the progression from fasting to starvation. However, due to the lack of a reliable commercial assay for FGF21, reported changes on its circulating levels in response to fasting and refeeding from animal and human studies have been scarce and inconsistent. We have recently developed highly sensitive chemiluminescence immunoassays (CLIA) for both mouse and human FGF21. We have demonstrated a paradoxical increase in serum FGF21 levels in obese humans which correlate with adiposity, fasting insulin and triglycerides, but negatively with HDL cholesterol levels. An independent association is found between serum FGF21 level and the metabolic syndrome, over and above the effect of individual components of the syndrome. Moreover, FGF21 expression in subcutaneous fat correlates well with its circulating levels in humans. In db/db obese mice, FGF21 mRNA expression is increased in the liver and adipose tissues, suggesting the presence of a compensatory increase or resistance to FGF21, in both obese humans and mice. More recently, we have shown that circulating FGF21 level in humans is tightly controlled by the circadian clock, rising from midnight to a peak level in the early morning, and then declining to its basal level early in the afternoon. The magnitude of the nocturnal rise in circulating FGF21 level is significantly blunted in obese subjects. The 24-hr oscillatory pattern of circulating FGF21 resembles those of free fatty acids (FFA) and cortisol, but is opposite to those of insulin and glucose. Our in vitro findings suggest that the diurnal rhythm of circulating FGF21 can be attributed, at least in part, to the changes in FFAs. These findings would support the role of FGF21 as an important metabolic regulator that integrates the circadian rhythm with energy homeostasis in humans. (This study was supported by the Hong Kong Research Grant Council (CRF HKU03/09C)

5-6

Inflammation induced by Diet-Disrupted Gut Microbiota and Development of the Metabolic Syndrome in Mouse Model.

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Both genetic variations and diet-disrupted gut microbiota can predispose animals to metabolic syndromes (MS). Our first work assessed the relative contributions of host genetics and diet in shaping the gut microbiota and modulating MS-relevant phenotypes in mice. Together with its wild-type counterpart, the *Apoa-*Ⅰ knockout mouse, which has impaired glucose tolerance (IGT) and increased body fat, was fed a high fat (HFD) or normal chow diet (NC) for 25 weeks. DNA fingerprinting and bar-coded pyrosequencing of 16S rRNA genes were used to profile gut microbiota structures and identify key population changes relevant to MS development by Partial Least Square Discriminate Analysis. Diet changes explained 57% of the total structural variation in gut microbiota, while genetic mutation accounted for no more than 12%. All three groups with IGT had significantly different gut microbiota relative to healthy wild-type/normal chow-fed animals. Sixty-five species-level phylotypes were identified as key members with differential responses to changes in diet, genotype, and MS phenotype. Most notably, gut barrier-protecting *Bifidobacterium* spp. were nearly absent in all animals on high fat diet, regardless of genotype. Sulphate -reducing, endotoxin-producing bacteria of the family, *Desulfovibrionaceae,* were enhanced in all animals with IGT, most significantly in the wild-type/high fat diet group, which had the highest calorie intake and the most serious MS phenotypes. Thus, diet plays a dominating role in shaping gut microbiota and changes of some key populations may transform gut microbiota of wild-type animals into a pathogen-like entity relevant to development of metabolic syndromes, despite a complete host genome. Then we investigated the relationship diet-disrupted gut microbiota and aging. Calorie restriction (CR) without malnutrition can expand healthy lifespan by attenuating chronic inflammation underlying aging and age-related diseases. Inflammation provoked by antigens from gut microbiota such as endotoxin may cause obesity and metabolic diseases. With a life-long study in mice, here we show that CR may elongate healthy longevity by significantly reducing antigenic burden of the host via a structurally more balanced gut microbiota. Mice of the same gender and genetic background were fed ad libitum on high-fat (HFD) or low fat diet (LFD), with 30% CR or voluntary exercise. Maximum lifespan of these animals showed a striking plasticity ranging from the shortest 118.8±1.5 weeks (HFD) to the longest 185.5±1.6 weeks (LFD,CR). LFD,CR had the lowest body fat content and highest insulin sensitivity. Life long trajectory analysis showed a significant impact of CR on gut microbiota structure. Partial Least Square regression between midlife gut microbiota and lifespan ($R=0.54$, $P<0.0001$) identified 53 phylotypes most related with healthy longevity. Most notably, LFD,CR had the highest gut barrier protecting bacteria such as Lactobacilli and Bifidobacteria but drastically decreased opportunistic pathogens such as Enterobactereacae, TM7, Streptococcaceae etc. Lipopolysaccharide-binding protein (LBP), a biomarker linking endotoxin load and inflammation, was the lowest in LFD,CR, and highest in HFD group. CR may prolong healthy lifespan by rendering gut microbiota to be much less inflammation-provoking. Minimizing lifelong antigenic burden from gut microbiota to the host may become a highly effective target for anti-aging nutritional interventions.

6-1

Investigating the Effect of Drugs Targeting the PI 3-kinase Pathway on Glucose Metabolism.

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The three isoforms of class-Ia PI-3-kinase (p110 α,β,δ) play a key role in mediating the actions of a wide range of growth factor receptors, including the insulin receptor. Therefore it is hardly surprising that hyperactivation of this pathway appears to play a critical role in many tumours. This has lead to a rush to develop novel inhibitors of PI 3-kinases (by our group and others) as a potential treatment for cancer. One of the most likely side effects of such drugs is predicted to be an effect on insulin signalling and thus on glucose metabolism so it is important to study these in detail. We have synthesized a range of drugs that are known to selectively inhibit class-IA PI 3-kinase and tested their effects on glucose metabolism in rats. As different drugs are metabolised at different rates in vivo, this first required a pharmacokinetic analysis of drug levels in the rats to identify appropriate doses to use. Using this information we have performed studies that show that the broad spectrum PI 3-kinase inhibitors ZSTK474, PI103 and BEZ235 all significantly impair glucose tolerance and insulin tolerance and result in increases in insulin levels while also stimulating hepatic glucose output. A similar pattern was seen with PIK75, a drug that is selective for the p110α isoform, but was not seen with TGX 221 or IC87114, isoform selective inhibitors of p110β and p110δ respectively. Together with data in adipocyte and muscle models this provides strong evidence that it is the p110α isoform of PI 3-kinase that plays a central role in regulating insulin action.

6-2

Visfatin/eNampt Controls Energy Balance through Central SIRT1-independent Mechanisms.

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Visfatin, a visceral adipose tissue-derived adipokine, is recently identified as an extracellular nicotinamide phosphoribosyltransferase (eNampt), a key enzyme of NAD biosynthesis. In the present study, we investigated a potential involvement of Visfatin/eNampt in central regulation of energy balance. Here we show that administration of Visfatin and Nampt product NMN caused anorexia and weight loss. In contrast, hypothalamic injection of Nampt inhibitor FK866 led to hyperphagia and antagonized the effects of exogenous Visfatin, suggesting an important role for hypothalamic Nampt activity in body weight metabolism. Consistently, Visfatin and NMN had inhibitory effects on the transcriptional activities of orexigenic neuropeptide Y (NPY) and *Agouti*-related protein (AGRP), which were opposed by FK866 treatment. Furthermore, the effects of Visfatin and NMN were independent of NAD-dependent deacetylase SIRT1. Our findings firstly demonstrate a novel anorexigenic and weight-reducing effects of Visfatin/eNampt, which are mediated through SIRT1-independent transcriptional regulation of NPY and AGRP.

6-3

The Adaptor Protein APPL2 Suppresses Insulin-evoked Inhibition of Hepatic Gluconeogenesis by Blocking the Membrane Targeting of APPL1 and Akt**.**

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The multi-domain adaptor protein APPL1 potentiates insulin-stimulated activation of the protein kinase Akt by promoting the plasma membrane translocation of Akt. However, little is known about the physiological functions of APPL2, a homolog of APPL1 that share the same domain organization. Here we show that APPL2 acts as a negative regulator of insulin-stimulated signaling pathway leading to the suppression of hepatic glucose production. Transgenic expression of APPL2 blocks insulin-evoked Akt activation and suppression of gluconeogenic genes, thereby inducing hyperglycemia and insulin resistance in mice. Under the basal condition, APPL2 and APPL1 are present predominantly as a heterodimer through the interaction between their NH2-terminal BAR domains. Insulin stimulation triggers the dissociation of the APPL1-APPL2 heterodimer and promotes the membrane translocation of both Akt and APPL1, but not APPL2, and this effect is blocked by overexpression of APPL2. X-ray crystallographic analysis of the BAR domain for these adaptor proteins shows a distinct 3-D structure between the APPL1 homodimer and APPL1-APPL2 heterodimer. The BAR motif of APPL1 displays a typical structural feature of N-Bar super-family – a well packed, crescent shaped dimer, which is in favor of membrane targeting. By contrast, the APPL1-APPL2 heterodimer forms a much flattened conformation, in which the diameter of curvature is significantly stretched compared to the APPL1 homodimer. These findings suggest that APPL2 suppresses insulin-evoked Akt activation through heterodimerization with APPL1 and blockage of Akt targeting to plasma membrane.

6-4

Preadipocyte factor-1 (Pref-1/Dlk1) Inducts Ductal Cell Transdifferentiation into β-cells through ERK-FOXO1 and Akt Pathways.

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Preadipocyte factor-1/Delta like 1 homologue (Pref-1/Dlk 1) is widely expressed in embryonic tissues, whereas in adult and postnatal, its expression is limited. Although it's involved in cell development, differentiation and regeneration in various tissues, the function of Pref-1 is not cleared yet in pancreas. In our previous study, we investigated Pref-1 expressed cells during pancreas development and regeneration. To extend our understanding for the role of Pref-1, we analyzed the mechanism of pancreatic cells after Pref-1 treatment. Purified soluble Pref-1 (Pref-1-mFc) treatment increased Akt, ERK1/2 and FOXO1 phosphorlylation. ERK1/2 and FOXO1 phosphorylation were blocked by PD98059, but Akt phosphorylation was not affected. Pref-1 induced FOXO1 phosphorylation was not regulated by phospho-Akt inhibition. The activation of Pref-1 increased PDX1 and insulin, whereas decreased FOXO1. Proteins from PANC1 cell lines were separated by 2-DE and identified by MALDI-TOF, some proteins were found that about protective effect against hypoxia-induced cell apoptosis and Rab GTPase-activating protein which might be related transport vesicle formation and AKT phosphorylation in Pref-1 overexpressed condition. Also, Pref-1 increased synaptophysin that was synaptic vesicle protein and neuro-endocrine cell marker. We conclude that Pref-1 expression might play an important role for the regeneration and differentiation of endocrine pancreas through phosphorylation of ERK1/2, FOXO1 and Akt signalings.

6-5

Dissecting the Final Stages of GLUT4 Vesicle Trafficking.

William. E. Hughes

Garvan Institute of Medical Research

The insulin-stimulated trafficking of the GLUT4 glucose transporter to the plasma membrane in muscle and fat tissue constitutes a central process in blood glucose homeostasis. The transport, tethering, docking and fusion of GLUT4 vesicles with the plasma membrane represent the most distal steps in this pathway and have been recently shown to be key targets of insulin action. However, it remains unclear how insulin influences these processes to promote the insertion of the glucose transporter into the plasma membrane.

We have been using high frequency total internal reflection fluorescence microscopy (TIRFM) to understand the distal processes controlling GLUT4 trafficking, particularly the transport, attachment and fusion processes. Using TIRFM in combination with probes that can distinguish between vesicle transport and fusion steps we found that defective actin remodelling is accompanied by normal insulin-regulated accumulation of GLUT4 vesicles close to the PM but the final exocytotic fusion step is impaired. These and our latest discoveries regarding the novel role for actin at the plasma membrane will be presented.

6-6

A Novel Regulatory Cascade for Controlling CRTC2 Activity.

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CREB regulated transcriptional coactivator 2 (CRTC2) is a fasting-induced transcriptional regulator for hepatic glucose production in vivo. The activity of CRTC2 is primarily regulated via hormone-dependent modification on its serine 171 residue, which determines the intracellular localization of this protein. AMPK family of kinases has been shown to be the major kinases to catalyze the phosphorylation event. However, the involvement of a potential protein phosphatase in this process has been elusive.

Here we show that mammalian orthologues for *Caenorhabditis elegans* SMK-1, SMEKs, function as regulatory subunits for protein phosphatase 4 (PP4) that enhances hepatic gluconeogenesis. We found that over-expression of SMEK promotes elevations in plasma glucose with increased hepatic gluconeogenesis, while knockdown of hepatic SMEK proteins improves hyperglycemia by promoting CRTC2 phosphorylation in mouse models of insulin resistance. Constitutive activation of hepatic CRTC2 due to the insulin resistance is linked with hyperglycemia during diabetic status, and modulation of SMEK activity could propose a novel mechanism for the maintenance of glucose homeostasis in this setting.

6-7

Biomarker discovery: from SNPs to CpGs.

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Over 28 million CpG dinucleotides are present in the human genome. Many of these CpGs are clustered in so-called CpG islands (CGIs) or nearby CpG island shores with close proximity to promoters. CpG methylation may have a direct impact on gene expression regulation. CpG methylation is both dynamic (regulated) and stable (heritable). Thus, CpG methylations may be superior markers for identifying genes involved in normal development and disease pathogenesis. However, the potential of using CpGs instead of SNPs for genome-wide association studies (GWAS) is largely a fantasy due to lack of cost-effective methods for analyzing the entire human methylome.

In this talk, I will highlight the current status in whole-genome methylation analysis. I will further discuss the practical considerations in using CpGs for GWAS. Examples will be given to show how CpG methylation biomarkers can be used in molecular diagnostics.