2012 APDO SYMPOSIUM

Asia-Pacific Diabetes and Obesity Study Group
Joint with Korean Endocrinology Society

Apr 20(FRI) ~ 21(SAT), 2012

Grand Hilton Seoul Hotel Convention Center
Seoul, Korea

Organizer:
Dr. Jae Bum Kim (Korea)

Regional Representatives:
Dr. David James (Australia)
Dr. Youfei Guan (China)
Dr. Karen Lam (Hong Kong)
Dr. Masato Kasuga (Japan)
Dr. Jae Bum Kim (Korea)
Dr. Peter Shepherd (New Zealand)
Dr. Weiping Han (Singapore)
Dr. Lee-Ming Chuang (Taiwan)

Supported by CRI MEST SNU KES

Center for Adipose Tissue Remodeling
Seoul National University
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2012 APDO SCHEDULE

■ Thursday, April 19, 2012 ■

18:00 – Welcome Dinner

■ Friday, April 20, 2012 ■

08:30 – 08:45 Opening Remark

08:45 – 09:30 Keynote Lecture1
Molecular Dysfunctions Linking Obesity to Type 2 Diabetes
Michael P. Czech, *USA*

09:30 – 10:30 ■ Session 1 **Chair : Karen Lam, *Hong Kong***

09:30-09:45 Real time imaging of exocytosis in intact islets of Langerhans
Peter Thorn, *Australia*

09:45-10:00 Suppression of islet fibrosis by GLP-1
Kun Ho Yoon, *Korea*

10:00-10:15 Regulation of Pancreatic Beta Cell Mass through ER stress
Tomokazu Matsuda, *Japan*

10:15-10:30 Crosstalk between cAMP-Epac signalling pathway and insulin granules in pancreatic β-cells
Xiaosong Ma, *China*

10:30 – 10:50 Coffee Break

10:50 – 12:20 ■ Session 2 **Chair : Wataru Ogawa, *Japan***

10:50-11:05 Islet hormone secretion regulation and diabetes
Weiping Han, *Singapore*

11:05-11:20 Role of PDKs in Metabolic Syndrome
In-Kyu Lee, *Korea*

11:20-11:35 FABP4 is an insulinotropic adipokine that facilitates compensatory hyperinsulinaemia during obesity
James Cantley, *Australia*

11:35-11:50 Liver X receptor beta(LXRβ) and water homeostasis regulation
Youfei Guan, *China*

11:50-12:05 Transcriptional control of hepatic gluconeogenesis
Seung Hoi Koo, *Korea*

12:05-12:20 FGF21 in the regulation of lipolysis
Karen Lam, *Hong Kong*

12:20 - 13:30 Lunch

13:30 – 14:45 ■ Session 3 **Chair : Youfei Guan, *China***

13:30-13:45 Targeting inflammation in cancer and metabolism
Vinay Tergaonkar, *Singapore*

13:45-14:00 A continued saga of Boc5, the first non-peptidic glucagon-like peptide-1 receptor agonist
Ming-Wei Wang, *China*

14:00-14:15 SNARE and Traffic : role of Vamp8/endobrevin in regulated exocytosis
Wanjin Hong, *Singapore*

14:15-14:30 How Do Antipsychotic Drugs Cause Diabetes?
Greg Smith, *New Zealand*

14:30-14:45 A novel actin filament class that regulates glucose uptake and GLUT4 trafficking
Peter Gunning*, Australia*

14:45 – 15:00 Coffee Break

15:00 – 16:15 ■ Session 4 **Chair : Peter Shepherd, *New Zealand***

15:00-15:15 CITED2: a new player in hormonal regulation of hepatic gluconeogenesis
Michihiro Matsumoto, *Japan*

15:15-15:30 Sphingolipid metabolism and ER stress
Tae Sik Park, *Korea*

15:30-15:45 Inositol Polyphosphate signaling in growth and metabolism
Seyun Kim, *Korea*

15:45-16:00 A Role of Hypothalamus in Amino Acid Sensing and Regulation of Metabolism
Feifan Guo, *China*

16:00-16:15 Orphan Nuclear Receptor ERRgamma and Liver Metabolism
Hueng Sik Choi, *Korea*

16:15 - 16:30 Coffee Break

16:30 – 18:10 ■ Session 5 **Chair : Greg Cooney, *Austrailia***

16:30-16:45 Class IIa histone deacetylases link oxidative stress with metabolic remodelling
Sean McGee, *Australia*

16:45-17:00 Roles of Novel Variants of PGC-1α in the Regulation of energy metabolism
Wataru Ogawa, *Japan*

17:00-17:15 Discovery of a novel glucose sensing pathway in β-cells in which glucose Upregulates β-catenin signalling via cAMP and Protein Kinase A
Peter Shepherd, *New Zealand*

17:15-17:30 Adipose-selective deletion of SirT1 causes peripheral insulin resistance by inducing adipose tissue inflammation in mice
Aimin XU, *Hong Kong*

17:30-17:45 Change of insulin secretion, gut hormone and incretin effect after gastro-intestinal metabolic surgery for the treatment of Diabetes
Wei-Jei Lee, *Taiwan*

17:45-18:10 Dissecting steps involved in GLUT4 trafficking
David James, *Australia*

18:10 – Welcome Reception

■ Saturday, April 21, 2012 ■

08:30 – 09:15 Keynote Lecture 2
Physiological role of autophagy and its regulation mechanism
Noboru Mizushima, *Japan*

09:15 – 10:30 ■ Session 6 **Chair : Min Seon Kim, *Korea***

09:15-09:30 Adipose tissue as therapeutic target in restoring endothelial function in diabetes
Yu HUANG, *Hong Kong*

09:30-09:45 Oxidative phosphorylation function in the control of adipose function
Minho Shong, *Korea*

09:45-10:00 The role of seipin in adipocyte differentiation and lipid droplet formation
Rob Yang, *Australia*

10:00-10:15 Oxidative stress in adipose tissues
Atsunori Fukuhara, *Japan*

10:15-10:30 Acetyl-L-Carnitine and Insulin Resistance
Moon Kyu Lee, *Korea*

10:30 – 10:45 Coffee Break

10:45 – 12:30 ■ Session 7 **Chair : Weiping Han, *Singapore***

10:45-11:00 Type 2 Diabetes: the Alzheimer's disease of the periphery?
Mark Febbraio, *Australia*

11:00-11:15 Mechanistic Study of Islet Injury Induced by Hyperuricemia
Dongming Su, *China*

11:15-11:30 PPARγ, phosphorylation and the anti-diabetic PPARγ ligands
Jang Hyun Choi, *Korea*

11:30-11:45 Impaired insulin signaling in the endothelial cells reduces insulin-induced glucose uptake by the skeletal muscle
Tetsuya Kubota, *Japan*

11:45-12:00 Hypothalamic Neuron Cilia and Energy Metabolism
Min Seon Kim, *Korea*

12:00-12:15 Mechanism of Inflammation-Induced Insulin Resistance : Reliance of Toll-Like Receptor-4(TLR4) Action on Ceramide Synthesis Reveals Roles for Saturated Fatty Acids
Scott A. Summers, *Singapore*

12:15-12:30 Fat Cell Size Determines Insulin Sensitivity and Inflammatory Responses
Jae Bum Kim, *Korea*

12:30 – 14:00 Lunch

Michael P. Czech, Ph.D.

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Molecular Dysfunctions Linking Obesity to Type 2 Diabetes

Insulin signaling to metabolic pathways is disrupted in obesity, contributing to glucose intolerance and the metabolic syndrome. Such dysfunctions in adipose tissues, particularly deficiencies in certain adipokines as well as in fatty acid esterification, lipogenesis and lipid storage promote systemic lipotoxicity and insulin resistance in liver and muscle. Using siRNA screens to search for genes that negatively impact insulin signaling in adipocytes, we identified the transcriptional co-repressor RIP140 (J Clin Invest, 2006), the lipid droplet protein FSP27/CIDEC (JBC, 2007, PNAS 2008) and the protein kinase Map4k4(PNAS 2006, Nature 2009) as novel suppressors of lipid metabolism. In adipocytes, we observed that Map4k4, independent of the JNK pathway, downregulates PPARg protein levels and the expression of SREBP1c and ChREBP, key transcription factors that control genes encoding lipogenic enzymes. Similarly, adipose specific Map4k4 deficiency in mice increases their capacity for triglyceride storage without causing insulin resistance on a high fat diet. Remarkably, Map4k4 is also required in at least two other adipose-resident cell types—macrpohages and endothelial cells--in their capacity to control adipocyte insulin signaling to lipogenesis. Macrophage expression and secretion of IL-1 and TNF-a, potent inhibitors of insulin action, are attenuated upon Map4k4 silencing. Endothelial cell activation and expression of surface adhesion proteins, required for macrophage infiltration into adipose tissue, are similarly compromised upon Map4k4 silencing in vitro and in endothelial cell-specific Map4k4 deficiency in vivo. Taken together, Map4k4 represents a novel signaling node in three adipose tissue cell types--adipocytes, macrophages and endothelial cells—that acts in each cell type to promote insulin resistance and dysfunctional adipocyte lipid storage.

Memo

Peter Thorn, Ph.D.

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Real time imaging of exocytosis in intact islets of Langerhans

At a cellular level the biochemical pathways that link glucose sensing in pancreatic β cells to the secretion of insulin are well understood (1). However, in the physiological context it is not clear how glucose controls/coordinates secretion of insulin from the many hundreds of β cells within the islets of Langerhans (2).

In principle all β cells could contribute to insulin secretion with glucose-dependence conferring enhanced secretion from each β cell. Alternatively, single cells of different glucose sensitivity could sequentially be recruited within the islet to generate increasing amounts of insulin secretion. Here we have set out to determine the basis of glucose sensing from β cells within intact isolated mouse pancreatic islets. To do this we have used the two-photon imaging of whole islets developed by Kasai (3).

We identified individual exocytic events within individual cells, induced by a range of different glucose concentrations. Of these factors we found the most important is the increased recruitment of β cells.

References

1.Henquin, J.C., et al. Hierarchy of the beta-cell signals controlling insulin secretion. European *Journal of Clinical Investigation* 33, 742-750 (2003).

2.Grodsky, G.M. Threshold distribution hypothesis for packet storage of insulin and its mathematical modeling. *Journal of Clinical Investigation* 51, 2047- (1972).

3.Takahashi, N., et al. Fusion pore dynamics and insulin granule exocytosis in the pancreatic islet. *Science* 297, 1349-1352 (2002).

Memo

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Suppression of islet fibrosis by GLP-1

Pancreatic islet fibrosis might be to lead the progressive beta-cell loss and dysfunction in type2 diabetes. Pancreatic stellate cells (PSCs) are known to be related to pancreatic fibrosis and inflammation, and are the result of extracellular matrix (ECM) protein synthesis. So far there was no report which demonstrates the effects of GLP-1 on pancreas stellate cell activation and proliferation. Recently we observed the activated PSCs expressed the GLP-1 receptor. Therefore, we investigated the effect of GLP-1 analogue on activation and proliferation of PSCs *in vivo* and *in vitro*.

GLP-1 receptor expression was strongly induced with high glucose stimulation in PSCs. The protein level of α-SMA was increase by high glucose however attenuated by Ex-4 treatment. After 9 days culture of PSCs in high glucose containing medium, the expressions of CTGF and type 1 collagen synthesis were significantly increased and also effectively attenuated by Ex-4 treatment. Islet fibrosis in OLETF rats was significantly attenuated by exendin 4 treatment than control group or glucose matched insulin treated group. The data suggesting the effects of exendin-4 on high glucose induced PSCs activation and the prevention of islet destruction by fibrosis in the animal model of type 2 diabetes mellitus.

Memo

Tomokazu Matsuda, M.D., Ph.D.

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Regulation of Pancreatic Beta Cell Mass through ER stress

Pancreatic β cell failure is thought to underlie the progression from glucose intolerance to overt diabetes, and endoplasmic reticulum (ER) stress is implicated in such β cell dysfunction. We (1) showed that the transcription factor CCAAT/enhancer-binding protein β (C/EBPβ) accumulates in the islets of diabetic animal models as a result of ER stress before the onset of hyperglycemia. The accumulation of C/EBPβ in pancreatic β cells reduced the abundance of the molecular chaperone GRP78 (BiP) as a result of suppression of the transactivation activity of ATF6α and thereby increased the vulnerability of these cells to excess ER stress. Our results thus indicate that the accumulation of C/EBPβ in pancreatic β cells contributes to β cell failure.

Next, we examined the effects of incretin on ER stress related β cell failure. Vildagliptin, a dipeptidyl peptidase-4 inhibitor, is representative of a new class of antidiabetic agents that act through increasing the expression of glucagon-like peptide-1. Diabetic pancreatic β cell-specific C/EBPβ transgenic mice exhibit decreased β cell mass associated with increased apoptosis, decreased proliferation, and aggravated ER stress. This agent also markedly increased β cell mass, improved aggravated ER stress, and restored attenuated insulin/insulin-like growth factor 1 signaling. A decrease in pdx1 expression was also observed in β cells isolated from our mouse model, but this was also restored by vildagliptin treatment. Vildagliptin elicits protective effects on pancreatic β cells and has potential for preventing the progression of type 2 diabetes.

1. Matsuda T, et al. Ablation of C/EBPβ alleviates ER stress and pancreatic β cell failure through the GRP78 chaperone in mice. J. Clin. Invest 120,115-126(2010).

Memo

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Crosstalk between cAMP-Epac signaling pathway and insulin granules in pancreatic β-cells

Incretins such as GLP-1 enhance glucose-stimulated insulin secretion by the generation of intracellular cAMP. This is accomplished by G-protein-coupled activation of adenylatecyclase. cAMP is involved in multiple signaling pathways in pancreatic β-cells, among which one is the activation of protein kinase A (PKA). Several activities in the β-cells are stimulated by activation of this pathway, including enhancement of electrical activity, increasing influx of Ca2+ through voltage-dependent Ca2+-channels and potentiation of exocytosis. Another pathway by which cAMP can increase insulin secretion is through exchange protein directly activated by cAMP (Epac2). This Epac2-mediated stimulation of insulin secretion involves enhancement of the exocytotic machinery. In this presentation, I will discuss the cellular mechanisms underlying Epac2-stimulated insulin secretion.

Memo

Weiping Han, Ph.D.

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Islet hormone secretion regulation and diabetes

Neurotransmitters, neuropeptides and hormones are released through regulated exocytosis of synaptic vesicles (SVs) and large dense core vesicles (LDCVs), a process that is controlled by calcium. Synaptotagmins are a family of type 1 membrane proteins that share a common domain structure. Most synaptotagmins are expressed in brain and endocrine cells, and some of these synaptotagmins bind to phospholipids and calcium at the levels that trigger regulated exocytosis of SVs and LDCVs. Here I will present our recent mouse genetic and physiological studies in identifying calcium-sensing proteins that are responsible for calcium-dependent hormone secretion, and how these studies may help in understanding the pathogenesis of diabetes.

Memo

In-Kyu Lee, M.D., Ph.D.

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Role of PDKs in Metabolic Syndrome

Regulation of the activity of the pyruvate dehydrogenase complex (PDC) is critical for disposal of excess glucose, fuel selection by tissues, and conservation of substrates for glucose synthesis. A dominant role for PDK4 in down regulation of PDC activity is suggested by the remarkable up regulation of PDK4 during fasting. This hypothesis is supported by reduced fasting levels of blood glucose levels and three carbon gluconeogenic substrates in PDK4 knockout ($PDK4^{-/-}$) mice. Relative to PDK4, less evidence has existed for an important role for PDK2 in glucose homeostasis. Although ubiquitously expressed, PDK2 is only modestly increased by fasting, and PDK2 deficiency has no effect upon fasting blood glucose levels in chow fed mice. However, in studies with mice fed a high fat diet for 16 weeks (12-13 mice per group), PDK2 deficiency reduced body weight (WT vs $PDK2^{-/-}$, 49.8±0.8 vs 42.8±1.2 g, Mean±SE, $P<0.001$), lowered fasting blood glucose levels (WT vs $PDK2^{-/-}$, 179.8±8.8 vs 152.7 mg/dL, $P<0.05$) improved glucose tolerance (area under curve (mg/dL x min), Wt vs $PDK2^{-/-}$, 31626±1897 vs 21123±1093, $P<0.001$), and reduced liver fat relative to wild type C57BL/6J mice. Sterol-regulatory-element-binding protein-1c (SREBP1c), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS) were significantly reduced in the liver of $PDK2^{-/-}$ mice compared with wild type mice. Interestingly, epididymal fat weight/body weight (%) increased in $PDK2^{-/-}$ mice compared to wild type mice (Wt vs $PDK2^{-/-}$, 3.93±0.39 vs 5.59±0.25, $P<0.01$). GLUT4 and hexokinase II were increased in adipose tissue of $PDK2^{-/-}$ mice, suggesting increased glucose metabolism in the epididymal fat of these mice. Furthermore, the inflammatory cytokine tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) were significantly decreased while adiponectin was increased in epididymal fat tissue of $PDK2^{-/-}$ mice, suggesting improved adipose tissue function. These results suggest an important role for PDK2 in regulating lipid metabolism in the liver and adipose tissue.

Memo

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FABP4 is an insulinotropic adipokine that facilitates compensatory hyperinsulinaemia during obesity.

Obesity rates are increasing at epidemic proportions, and this trend is accompanied by a cluster of diseases including type2 diabetes, cardiovascular disease and cancer. A common feature of obesity is hyperinsulinaemia, enabling glycaemic control to be maintained despite increasing adiposity and insulin resistance. A longstanding question is: what mechanisms coordinate the beta cell response to obesity and drive adaptive hyperinsulinaemia? We have recently identified the adipocyte secreted Fatty Acid Binding Protein 4 (FABP4) as having insulinotropic properties. We have found that recombinant or adipose secreted FABP4 potentiates insulin secretion, and insulin reciprocally regulates FABP4 secretion, forming the molecular basis of an endocrine loop coordinating the beta cell response to obesity. In humans, serum FABP4 levels correlate specifically with GSIS and adiposity, but not insulin sensitivity, supporting the role of our proposed endocrine loop in the physiological adaptation to obesity. In addition, we have recently identified 2 SNPs in the FABP4 gene that significantly associate with type2 diabetes in humans, suggesting that alterations in this adaptive endocrine loop may contribute to diabetes risk.

Memo

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Liver X receptor beta(LXRβ) and water homeostasis regulation

As members of the metabolic nuclear receptor family, liver X receptors (LXRs) are well-known for their essential roles in multiple metabolic processes. Increasing evidence suggests that LXRβ is critical in regulating immune function, nervous system development and osteogenesis. Recent studies have demonstrated that LXRβ was abundantly expressed in the kidney and was the predominant subtype of LXR receptors expressed in renal medulla, especially renal collecting ducts. LXRβ was also found to be highly expressed in the hypothalamus. The present study reveals a key role for LXRβ in the etiology of diabetes insipidus (DI). Given free access to water, LXRβ-/- but not LXRα-/- mice exhibit polyuria (abnormal daily excretion of highly diluted urine) and polydipsia (increased water intake), both features of DI. LXRβ-/- mice responded to 24-h dehydration with a decreased urine volume and increased urine osmolarity. In order to determine whether the DI was of central or nephrogenic origin, we examined the responsiveness of the kidney to arginine vasopressin (AVP). An intraperitoneal injection of AVP to LXRβ-/- mice revealed a partial kidney response, i.e., there was no effect on urine volume, but there was a partial return of the urine osmolality to normal. The persistent high urine volume after AVP was traced to a reduction in aquaporin-1 expression in the kidney of LXRβ-/- mice. Since LXRβ-/- mice do respond to AVP, the DI appeared to be due to a defect in central production of AVP. In the brain of wild-type mice, LXRβ was expressed in the nuclei of magnocellular neurons in the supraoptic and paraventricular nuclei of the hypothalamus. In LXRβ-/- mice there was a marked decrease in expression of AVP in the magnocellular neurons as well as in urine collected over a 24-h period. Moreover, the LXR agonist (GW3965) elicits an increase in urine osmolality suggesting that LXRβ receptor in wild-type mice. Taken together, the present study provides novel insights into a critical role of LXRβ in water homeostasis regulation by maintaining normal urine concentrating ability. LXRβ may therefore represent a potential target for treating certain diseases with urine concentrating defects.

Memo

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Sphingolipid metabolism and ER stress

In the setting of obesity and type 2 diabetes mellitus ectopic disposition of lipids may be a cause of heart failure. Clinical studies have clearly shown a correlation between accumulation of triglycerides and heart dysfunction. In this process, it is likely that there are also changes in the contents of sphingolipids. One specific sphingolipid, ceramide, may cause cardiac dysfunction and treatments that reduce ceramide in animals with lipid-induced heart diseases have proven to be beneficial. To determine whether the *de novo* synthetic pathway is important for heart ceramide levels and cardiac function, we created cardiomyocyte-specific deficiency of Sptlc2, a subunit of serine palmitoyltransferase. While we found reduced ceramide levels in the heart of heart-specific Sptlc2 deficient (hSptlc2 KO) mice, phospholipids and acyl CoAs contained increased saturated long chain fatty acids. Altered lipid composition leads to cardiac dysfunction via activation of ER stress in hSptlc2 KO mice. Many changes in the lipidome found in these mice further illustrate the complexity of heart lipid metabolism. These results and those obtained from other models suggest that a single lipid is unlikely to be changed in isolation and that lipotoxicity is likely to be caused by a number of processes.

Memo

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FGF21 in the regulation of lipolysis

Fibroblast growth factor 21 (FGF21), a hormone secreted predominantly from the liver, has been reported to play a regulatory role in glucose and lipid metabolism in animals. The adipocyte appears to be its major target of action where it modulates the expression of genes involved in lipolysis and induces insulin-dependent glucose uptake through the enhancement of GLUT1 expression. Our clinical studies suggest that it is also a metabolic hormone in humans with its circulating levels exhibiting a circadian rhythm. The nocturnal rise in serum FGF21 level, reaching a peak in the early morning, and its increase on fasting, are reminiscent of the changes in circulating growth hormone (GH), well known for its physiological role in the regulation of lipolysis. On the other hand, the 24 hours oscillation in serum FGF21 levels resembles that of circulating free fatty acids (FFA), and unsaturated fatty acids induce a time-dependent expression of FGF21 gene expression in human hepatocytes. These observations have prompted us to investigate for the physiological role of FGF21 in lipolysis and its interaction with GH in this regard. In our recent studies, we found that a single bolus injection of FGF21 could acutely reduce basal and isoproterenol-stimulated serum FFA and glycerol levels in both C57 and FGF21 knockout (KO) mice, with a greater suppression of lipolysis being seen in the KO mice which also exhibited higher basal serum FFA and glycerol levels. Data from explant studies in mouse and human white adipose tissue suggested that this physiological role of FGF21 in regulating basal and stimulated lipolysis is mediated through its Akt-dependent activation of phosphodiesterase 3B (PDE3B), leading to a reduction in cAMP levels and decreased phosphorylation of the hormone sensitive lipase (HSL) at ser660. The FGF21 KO mice also demonstrated a greater magnitude and duration of lipolysis in response to acute GH administration, suggesting that FGF21 also inhibits GH-induced lipolysis. Interestingly GH administration in vivo stimulated the hepatic FGF21 expression in C57 mice leading to elevated serum FGF21 levels, an effect which could be blocked by pretreatment with the lipolysis inhibitor niacin. Further in vitro studies showed that GH had no direct effect on FGF21 expression in the liver but stimulated the hepatic expression of FGF21 indirectly through FFA release from adipocytes and subsequent PPAR-αactivation in hepatocytes. The above studies, taken together, would support a physiological role of FGF21 in the regulation of lipolysis, in concert with other hormones such as GH.

Acknowledgement: These studies were supported by CRF 03/09, RGC, Hong Kong.

Memo

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Targeting inflammation in cancer and metabolism

Inflammation involving the innate and adaptive immune systems is a normal response to infection. However, it is now known that when allowed to continue unchecked, chronic inflammation is a key underlying cause for the development of autoimmune disorders, neurodegenerative diseases, metabolic syndromes such as diabetes and cancer. The annual cost and expenses associated with metabolic diseases and cancers runs in hundreds of billions dollars worldwide, and the market for diabetes drugs alone is estimated to be tens of billions of dollars. With the change of world economic order and rise of Asia this figure is only set to rise. What is more alarming is that obesity (caused by metabolic syndrome), itself is a huge risk factor for many ailments including cancer development. Males with BMI of 35-40 are 4 fold more prone to developing certain cancers, especially that of the liver. It is thus that tremendous effort and expanding interest from academic institutes and pharmaceutical/biotech companies is driving the development of a range of anti-inflammatory therapies. Although a variety of safe and effective anti-inflammatory agents have been developed and have been available in the market for decades, the development of more cost effective but potent drugs has been a pipe dream. The most important hurdle lies in developing agents that show minimal side effects (since blocking inflammation is also detrimental to normal physiology).

Our lab studies a transcription factor called NFkB which is a master regulator of inflammation. Indeed deregulated activity of NFkB precedes and is causally linked to chronic inflammation and the development of several human ailments including metabolic syndromes and cancers. However, given that NFkB signaling is also essential for many housekeeping cellular and developmental events in normal human beings, simply blocking NFkB to curb inflammation is not an option (several drugs designed to do this did not reach the clinic because of toxicity issues). Hence deciphering the regulation of NFkB signaling is crucial to understanding the mechanism and role of uncontrolled/unwanted NFkB activity seen in human ailments and in developing better and safer anti-inflammatory drugs. I will describe our current understanding of the pathway and our efforts to identify targets that will help develop drugs which will block NFkB/inflammation more selectively and not generically and hence may have less side effects.

Memo

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A continued saga of Boc5, the first non-peptidic glucagon-like peptide-1 receptor agonist

Glucagon-like peptide-1 (GLP-1)-based therapy presents a promising option for treating type 2 diabetes. However, there are several limitations relative to the peptidic GLP-1 mimetics currently on the market or under development. This concern has led to a continued interest in the search for non-peptidic agonists for GLP-1 receptor (GLP-1R). Here, we briefly review the discovery, characterization and current status of a novel class of cyclobutane-derivative-based non-peptidic agonists for GLP-1R, including Boc5 and its newly discovered analogue WB4-24. Although the oral bioavailability of such compounds still poses great challenges, the progress made so far encourages us to identify a truly ‘druggable’ small molecule agonist for GLP-1R.

Memo

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SNARE and Traffic: role of Vamp8/endobrevin in regulated exocytosis

Protein transport between different compartments of the secretory and endocytic pathways are mediated by vesicles/carriers as the shuttling intermediates. After budding, translocation and tethering, the vesicles will fuse with the target compartment, which is catalyzed by the SNARE family of proteins. We have been studying mammalian SNAREs and established that VAMP8/endobrevin is a major vesicle-associated SNARE (v-SNARE) important for regulated secretion of diverse physiological events such as exocrine secretion and vasopressin-stimualted surface mobilization of water channel (AQP2).

Memo

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How Do Antipsychotic Drugs Cause Diabetes ?

The second generation antipsychotic medicines such as clozapine and olanzapine are the most effective treatment for schizophrenia and as such they are widely used drugs. However, one of the side effects of the drugs is obesity and metabolic derangements characteristic of Type-2 diabetes. Therefore these drugs could be making a significant contribution to the increased incidence of Type-2 diabetes in recent times. It was long assumed that these metabolic defects were secondary to the obesity that developed but our work in rats found that these drugs acutely induce defects in glucose metabolism. However, these differ from those seen in type-2 diabetes in that they appear to be driven by hyposecretion of GLP-1 and hypersecretion of glucagon that causes a subsequent increase in hepatic glucose release and thus a flow on effect on insulin secretion. We are now in the process of replicating these studies in humans and will report the preliminary results of these studies at the APDO meeting.

Memo

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A novel actin filament class that regulates glucose uptake and GLUT4 trafficking.

A primary defect in Type 2 diabetes is alterations to glucose uptake in skeletal muscle and adipose tissue. Insulin-stimulated glucose uptake requires the trafficking of GLUT4-containing vesicles from intracellular stores to the cell surface. We have identified a novel population of actin filaments defined by the cytoskeletal tropomyosin (Tm) isoform Tm5NM1. Analysis of Tm5NM1 transgenic (Tg) mice suggests these filaments play a role in glucose uptake. In Tm5NM1 Tg mice, whole body glucose clearance and insulin-stimulated glucose uptake into white adipose tissue (WAT), skeletal muscle and heart was increased. This was specific to Tm5NM1, as glucose clearance was unaltered in mice expressing an alternative Tm, Tm3. Gene expression profiling (Illumina microarray), quantitative RT-PCR and Western blot analysis of WAT from the Tm5NM1 Tg mice detected an increase in genes involved in actin filament turnover and GLUT4 trafficking, including myosin motors (MyoIc, $P < 0.05$) and components of the exocyst complex (Sec8, $P < 0.005$). In keeping with Tm5NM1's reported role in stabilising actin filaments, there was a 30% increase ($P = 0.019$) in filamentous actin (detected by phalloidin staining) in Tg WAT. In 3T3L1 adipocytes, insulin-stimulation (30 min.) resulted in a shift in Tm5NM1 localisation to the plasma membrane, consistent with a role in GLUT4 trafficking. This effect was specific to Tm5NM1, as insulin had little impact on the localisation of other Tm isoforms. Finally, in 3T3-L1 adipocytes stably expressing Tm5NM1, there was an increase in insulin-stimulated GLUT4 movement to the plasma membrane compared to vector control cells. We propose that Tm5NM1 actin filaments promote recruitment of GLUT4 trafficking machinery that in turn results in enhanced insulin-stimulated GLUT4 translocation to the plasma membrane and glucose uptake.

Memo

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CITED2: a new player in hormonal regulation of hepatic gluconeogenesis

During fasting, induction of hepatic gluconeogenesis is important to ensure energy homeostasis in response to energy demand. Such induction is dysregulated in type 2 diabetes, resulting in the development of fasting hyperglycemia. Hormonal and nutrient regulation of metabolic adaptation during fasting is mediated predominantly by the transcriptional coactivator PGC-1α in concert with various other transcriptional regulators including the transcription factors FoxO1, HNF-4α, GR, and PPARα as well as the histone acetyltransferase CBP. Although CITED2 interacts with many of these molecules, the role of this protein in regulation of hepatic gluconeogenesis has been unknown. Here we show that CITED2 is required for the regulation of hepatic gluconeogenesis through PGC-1α. The abundance of CITED2 was increased in the liver of mice either by fasting or in association with type 2 diabetes, and it was increased in cultured hepatocytes by glucagon–cAMP–protein kinase A (PKA) signaling. CITED2 inhibited the acetylation of PGC-1α by blocking its interaction with the acetyltransferase GCN5. The consequent down-regulation of PGC-1α acetylation resulted in an increase in its transcriptional coactivation activity and increased expression of gluconeogenic genes. The interaction of CITED2 with GCN5 was disrupted by insulin in a manner dependent on phosphoinositide 3-kinase (PI3K)–Akt signaling. Our results thus reveal that CITED2 functions as a transducer of glucagon and insulin signaling in the regulation of PGC-1α activity associated with the transcriptional control of gluconeogenesis, and that this function is mediated through modulation of GCN5-dependent PGC-1α acetylation.

Memo

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Transcriptional control of hepatic gluconeogenesis

Postprandial insulin is critical in suppressing hepatic glucose production to maintain euglycemia. Insulin-dependent activation of Akt contributes this process in part via inhibiting FoxO1-dependent hepatic gluconeogenesis by direct phosphorylation and subsequent cytoplasmic exclusion. Previously, it was shown that protein arginine methyltransferase-1 (PRMT1) dependent modification of FoxO1 interferes with Akt-mediated inhibition in cultured cells or in the nematode, suggesting that this modification of FoxO1 might be critical in its transcriptional activity. In this presentation, we will discuss our recent data regarding the impact of arginine methylation of FoxO1 on hepatic glucose metabolism in vivo.

Memo

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Inositol polyphosphate signaling in growth and metabolism

Inositol is a naturally occurring glucose isomer and a key nutrient of the human diet. When levels of inositol are extremely low, disturbances such as diabetic changes, anxiety disorders, and hypercholesterolemia ensue. Inositol phosphates(IP) as signaling messengers mediate various physiological processes such as growth and apoptosis. IPMK(inositol polyphosphate multikinase) was first identified as Arg82, a transcriptional regulator in yeast. In mammals, IPMK generates inositol tetrakisphosphate(IP4) and IP5 and thus acts upstream of the IP6 kinase. IPMK is also a physiologically important PI3-kinase that forms PIP3 which activates Akt-dependent signaling pathways. IPMK, in a catalytically independent fashion, activates mammalian target of rapamycin(mTOR) in response to essential amino acids. In addition, IPMK regulates glucose signaling to AMP-activated kinase(AMPK) in a pathway whereby glucose activates phosphorylation of IPMK at tyrosine-174 enabling the enzyme to bind to AMPK and regulate its activation. These findings imply that IPMK participates in a network regulating growth factor– and nutrient-mediated signaling.

Memo

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A Role of Hypothalamus in Amino Acid Sensing and Regulation of Metabolism

We recently discovered that mice repress fatty acid and triglyceride synthesis in the liver and mobilize fat stores from abdominal adipose tissue maintained on a diet deficient for an essential amino acid leucine. While exploring molecular mechanisms underlying, we found that CNS leucine plays an important role in regulating fat loss under leucine deprivation. Furthermore, leucine deprivation-decreased fat mass is controlled by unregulated corticotrophin-releasing hormone expression in the hypothalamus and activated sympathetic nervous system activity. Our observations thus provide novel and important insights concerning CNS leucine as a signaling moleuclar in the regulation of energy homeostasis.

Memo

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Orphan Nuclear Receptor ERRgamma and Liver Metabolism

Glucose homeostasis is maintained by the balance between hepatic glucose production by the liver and glucose utilization by muscles and adipose tissue. Under fasting, gluconeogenesis is strongly stimulated by enhancing the transcription of gluconeogenic genes via the cAMP axis by glucagon but is inhibited by insulin under feeding. Dysregulation of glucose metabolism is associated with insulin resistance and diabetes. Estrogen receptor-related receptor gamma (ERRγ)□ is a member of the NR3B subfamily of the nuclear receptor superfamily. To date, the target genes and physiological functions of ERRγ in the liver remain unclear. Here, we show that the hepatic expression of ERRγ is induced under fasting in normal mice and in diabetic mice that exhibit elevated gluconeogenesis. Transient transfection assays reveal that the overexpression of ERRγ increases the transactivity of PEPCK promoter in HepG2 cell line. Electrophoretic mobility shift assays (EMSAs) and chromatin immunoprecipitation (ChIP) assays demonstrate that ERRγ binds directly to both ERRE1 and ERRE2 on PEPCK promoter. Adenoviral-mediated expression of ERRγ increases mRNA levels of key gluconeogenic genes, such as phosphoenolpyrubate carboxykinsae(PEPCK) and glucose-6-phosphatase(G6Pase) both *in vitro* and *in vivo*, and raises blood glucose levels. Conversely, knockdown of ERRγ with Ad-RNAi construct disrupts both PEPCK and G6Pase gene expression induced by Forskolin in rat primary hepatocyte. These results implicate ERRγ as a novel downstream mediator of cAMP axis and as a key regulator of hepatic gluconeogenesis under fasting state.

Memo

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Class IIa histone deacetylases link oxidative stress with metabolic remodelling

Altered metabolism in skeletal muscle is implicated in the pathogenesis of diseases such as obesity and type 2 diabetes. Impaired transcription of metabolic genes, oxidative stress and lipid accumulation have all been proposed as factors involved in altered muscle metabolism and the development of insulin resistance.

We have recently been investigating the role of the class IIa histone deacetylases (HDACs) in these responses. The class IIa HDACs are known to developmentally regulate muscle phenotype and we have found that their protein expression is increased in the oxidative muscle of diabetic mice. Insults that induce metabolic dysfunction in muscle, such as palmitate and TNFα, increase the class IIa HDACs and this appears to be oxidative stress dependent. Increasing class IIa HDAC expression in cells suppresses a large program of metabolic genes that results in mitochondrial dysfunction, impaired substrate oxidation, insulin resistance with respect to metabolic flexibility and accumulation of a variety of lipid species.

To investigate whether the class IIa HDACs might be a potential therapeutic target to reverse the metabolic phenotype of muscle in metabolic disease, we genetically inactivated the class IIa HDACs in cells. This increased oxidative metabolism and mitochondrial function and protected against the oxidative stress response induced by both palmitate and TNFα. We are currently designing novel compounds to selectively inhibit the class IIa HDACs as a new therapeutic strategy for metabolic diseases.

Together, these data suggest that oxidative stress-mediated increases in the class IIa HDACs leads to metabolic remodelling that could impact on insulin action and reveal the class IIa HDACs as potential therapeutic targets to combat metabolic disease.

Memo

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Role of novel variants of PGC-1α in the regulation of energy metabolism

Peroxisome proliferator–activated receptor γ coactivator–1α (PGC-1α) is a transcriptional coactivator that regulates various metabolic processes. We and others recently identified two alternative splicing variants of PGC-1α, transcription for which is initiated at an exon located ~14 kbp upstream of the previously identified exon 1 (*1*–*3*). While the abundance of canonical PGC-1α (here designated PGC-1αa) was greater than that of splicing variants (here designated PGC-1αb and PGC-1αc) in skeletal muscle under static conditions, the amounts of PGC-1αb and PGC-1αc were markedly increased in response to exercise. To characterize the function of these alternative variants, we generated mice that lack both PGC-1αb and PGC-1αc (PGC1αb/c-null mice). PGC1αb/c-null mice were found to manifest obesity and insulin resistance. Although the abundance of total PGC-1α in skeletal muscle under static conditions was unaltered, increases in the amount of total PGC-1α and in the expression of genes related to fatty acid metabolism in muscle, increase in energy expenditure as well as the decrease in fat mass induced by exercise were markedly attenuated in the mutant mice. These results suggest that the exercise-induced up-regulation of PGC-1α, for which the alternative variants are responsible, rather than the abundance of PGC-1α under static conditions, is important for the control of fat mass and insulin sensitivity.

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Memo

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Discovery of a novel glucose sensing pathway in β-cells in which glucose upregulates β-catenin signalling via cAMP and Protein Kinase A

β-cells have the ability to respond to changes in glucose levels and there is increasing evidence that the β-catenin is involved in regulating β-cell function. Therefore we looked to see if the β-catenin signalling pathway could be regulated by glucose in β-cells. We find that β-catenin signalling is regulated by changes in glucose levels in INS-1E β-cells as we see a glucose dependent increase in levels of β-catenin in the cytoplasm and the nucleus of these cells. The β-catenin target gene Cyclin D1 also increased and this rise was completely dependent on β-catenin. This was associated with increased phosphorylation of β-catenin□on Ser552, which is known to stabilise the molecule and increase its transcriptional activity. This was not due to AMP-kinase but forskolin and cell permeable cAMP analogues do stimulate this phosphorylation. Therefore we looked to see is cAMP and PKA might be involved in the glucose effect. We find glucose caused sustained increases in cAMP in the β-cells and inhibitors of adenylate cyclase and PKA signalling blocked the effects of glucose on β-catenin signalling. Finally, siRNA knockdown of PKA blocks the effects of glucose on β-catenin□ signalling. Together this indicates that INS-1E β-cells possess a pathway by which changes in glucose can regulate β-catenin and subsequently Cyclin-D1 using a pathway that requires the activation of PKA. We find knockdown of knockdown of β-catenin also regulates insulin secretion raising the possibility that this new pathway might also play a role in regulating whole body insulin responses to rises in glucose levels.

Memo

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Adipocyte-selective deletion of SirT1 causes peripheral insulin resistance by inducing adipose tissue inflammation in mice

SIRT1 is an NAD^+ dependent histone deacetylase that counteracts ageing-related metabolic deterioration through its pleiotropic actions on multiple targets. In adipocytes, SiT1 is implicated in regulating PPARγactivity and production of several adipokines. To explore the physiological role of SirT1 in adipocytes in regulating insulin sensitivity, we generated adipocyte-selective SirT1 knockout (AKO) mice by crossing $Sirt1^{flox/flox}$ mice with aP2-Cre mice. AKO mice and wild type (WT) controls had similar levels of food intake and body weight gains. However, adipocyte-specific deletion of SIRT1 resulted in a marked acceleration of both high fat diet- and ageing-induced glucose intolerance and systemic insulin resistance with obvious effects on adiposity. These changes were accompanied by impaired insulin-evoked Akt signaling and increased ectopic lipid accumulation in both skeletal muscle and liver. Adipose tissue in AKO mice exhibited significantly increased number of macrophage infiltration as well as enhanced conversion from a anti-inflammatory M2 to a pro-inflammatory M1 phenotype as compared to wild-type controls. In primary adipocytes, deletion of SirT1 led to increased expression of the pro-inflammatory chemokine MCP1, but reduced expression of adiponectin and the Th2 cytokine interleukin-4 (IL4). Furthermore, co-culture analysis demonstrated that the conditioned medium from AKO adipocytes caused a dramatic elevation of migration and M2 to M1 conversion of macrophages, and such changes were partially reversed by replenishment of IL4 or adiponectin, or by treatment with an antagonist of MCP-1. These findings demonstrate that adipocyte SirT1 is a key player of peripheral insulin sensitivity, by controlling the secretion of several chemokines and adipokines involved in macrophage infiltration and polarization. (*Acknowledgement: Hong Kong Collaborative Research Fund (HKU4/CRF/10R*)

Memo

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Change of insulin secretion, gut hormone and incretin effect after gastrointestinal metabolic surgery for the treatment of Diabetes

Owing to the pathogenic role of insulin resistance and beta-cell dysfunction in type 2 diabetes (T2DM), the clinical manifestation of this disease is extremely complex and is often associated with obesity. In recent years, gastrointestinal metabolic surgery, which was originally developed to treat morbid obesity, has been found to have therapeutic potential for diabetes. However, the underlying mechanisms of the therapeutic application of this technique are not fully known. Recent studies have found that laparoscopic sleeve gastrectomy or gastric bypass resulted in remission of poorly medically controlled non-severe obese T2DM up to 50-90% at 2 years after surgery. The effect is related to the decrease of insulin resistance, increase of early insulin response and total insulin secretion to glucose load. Both procedures can dramatically improve the incretin effect in T2DM patients after surgery. Although the incretin effect improved similarly in both groups, gastric bypass resulted in higher diabetes remission than sleeve gastrectomy. Gut hormone study has shown that both procedures had GLP-1 effect after surgery but sleeve gastrectomy had a better ghrelin reduction where gastric bypass had a better reduction in CCK and PP reducing effects. These studies have shown that duodenum exclusion do play a role in diabetes remission after gastrointestinal metabolic surgery.

Memo

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Dissecting steps involved in GLUT4 trafficking

Insulin regulation of GLUT4 trafficking plays a central role in peripheral actions of insulin. The stepwise molecular dissection of this process is essential in pinpointing key regulatory nodes that contribute either to insulin regulation or to insulin resistance. Here we present a novel ratiometric GLUT4 construct, rGLUTpHluor, that permits interrogation of this trafficking pathway at the level of individual cells and with subcellular resolution. We propose a workflow for streamlined analysis of GLUT4 trafficking; beginning with techniques that permit large-scale screens to identify compounds/proteins that influence GLUT4 trafficking, and progressing to methods to pin-point the step in GLUT4 trafficking affected by each manipulation. We provide novel data using TIRFM to show that insulin regulates the fusion of GLUT4 vesicles with the PM in a non-linear burst-like manner. Using our high throughput approach, we identified significant intercellular heterogeneity in insulin regulated GLUT4. Intriguingly, each cell has a unique highly reproducible response insulin stimulation.

Memo

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Physiological role of autophagy and its regulation mechanism

Macroautophagy (simply referred to as autophagy) is one of the major degradation pathways in the cell. In autophagy, intracellular components are sequestered by autophagosomes and then degraded upon fusion with lysosomes. Yeast genetic studies have identified more than 30 autophagy-related (*ATG*) genes. Many of these genes are conserved in higher eukaryotes, which allow us to perform genetic analysis of autophagy in mammals. Using autophagosome-indicator mice (GFP-LC3 mice) and various autophagy-deficient mouse models, we have shown that autophagy is important for maintenance of the amino acid pool during starvation and neonatal periods, preimplantation development as an amino acid supplying system, and intracellular protein quality control to prevent neurodegeneration. We also show using mice with systemic mosaic deletion of *Atg5*, in which only a small population of cells were autophagy-defective in every tissue, that autophagy is important to prevent spontaneous tumorigenesis in the liver. We are also analyzing other new mouse models, which suggest that autophagy has previously unappreciated roles in various developmental and physiological processes.

Autophagy is generally upregulated by nutrient starvation both in vitro and in vivo. Under nutrient rich conditions, the amino acid and insulin signaling pathways suppress autophagy through activation of mTORC1. mTORC1 directly associates with the ULK1 complex, which is the most upstream autophagy complex including ULK1, mAtg13, FIP200 and Atg101, and inactivates this complex. mTORC1 dissociates from the complex following starvation, leading to translocation of the ULK1 complex to the ER-associate autophagosome formation site, where it activates downstream Atg proteins to generate the autophagosome. The involvement of mTORC1 in nutrient-dependent autophagy regulation has also been observed in vivo. Furthermore, we recently found that autophagy is suppressed in denervated muscles through proteasome-dependent mTORC1 activation. These data suggest that mTORC1 is a major regulator of autophagy in vivo as well as in vitro.

Memo

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Adipose tissue as therapeutic target in restoring endothelial function in diabetes

Adipose tissue contributes to the regulation of vascular tone. Chronic inflammation of adipose tissue leads to vascular dysfunction, due to a diminished production of vasoprotective cytokines and increased release of inflammatory cytokines by adipocytes. However, the role, if any, of adipose tissue in vascular benefits of anti-diabetic drugs is largely unclear. PPARγ agonists improve insulin sensitivity indiabetes. In collaboration with the University of Hong Kong, our recent study demonstrates that adiponectin serves as a link in PPARγ- mediated amelioration of endothelial dysfunction in diabetes. Treatment with PPARγ agonist rosiglitazone restores endothelial function in *db/db* diabetic mice and diet-induced obese micebut not in adiponectin-deficient *db/db* mice. PPARγ activation elevates the release of adiponectin in fat explants and restores endothelium-dependent relaxations in diabetic and obese mouse aortas. This restoration is reversed by PPARγ antagonist, anti-adiponectin antibody or in *adiponectin*$^{-/-}$and not observed in arteries from *PPARγ*$^{+/-}$ mice. Subcutaneous fat transplantation demonstrates that the benefit of PPARγ activation in *db/db* mice is transferable. Inhibitors of AMPK, PKA, or adenylyl cyclase reverse the effect of adiponectin to restore endothelial function in diabetic mice. The vascular benefit of PPARγ activation is transferable by fat transplantation. To conclude, adipocyte-derived adiponectin is a prerequisite for PPARγ-mediated improvement of endothelial function in diabetes. (supported by Hong Kong CRF and GRF grants)

Memo

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Oxidative phosphorylation function in the control of adipose function

The OXPHOS system in mammalian mitochondria is composed of at least 89 polypeptides whose genes are located in either nuclear or mitochondrial DNA (mtDNA). The human mtDNA genome encodes only 13 proteins that form OXPHOS subunits, but they provide an essential framework for the assembly of OXPHOS complexes in the inner membrane of the mitochondria. Although substantial progress has been achieved in understanding the molecular mechanisms underlying the biogenesis of mtDNA encoded polypeptides, how these OXPHOS subunits are translated and integrated into the inner membrane remains to be determined. Here, we show that mitoribosome associated factors (MAFs) are required for the translation and subsequent insertion of OXPHOS polypeptides into the mitochondrial inner membrane. Loss of MAFs exhibit a complete loss of OXPHOS functions characterized by the disappearance of OXPHOS subunits and complexes. These changes are associated with marked defects in the translation of mitochondrial OXPHOS subunits. Adipose and endothelial-specific disruption of MAFs in mice resulted in striking cell and tissue specific degenerative phenotypes including endothelial dysfunction and systemic insulin resistance. These data indicate that MAFs plays a critical role in the formation of the mammalian mitochondrial OXPHOS system and may provide new opportunities for understanding human mitochondrial and metabolic diseases.

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Memo

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The role of seipin in adipocyte differentiation and lipid droplet formation

Seipin, the human Berardinelli-Seip congenital lipodystrophy 2 gene product, regulates adipocyte differentiation and lipid droplet (LD) formation. The molecular function of seipin, however, remains to be elucidated. We use yeast, fly, mammalian cells and mice as model systems to study the role of seipin and its orthologues in lipid metabolism. Our results suggest that seipin functions in the metabolism of phospholipids, and that seipin deficiency causes accumulation of lipid intermediates and alters the composition of membrane phospholipids. These changes result in tissue-specific abnormalities upon seipin dysfunction, such as defective adipocyte development and clustered LDs in fibroblasts.

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Memo

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Oxidative stress in adipose tissues

Obesity is considered a state of chronic inflammation of adipose tissue with increased production of pro-inflammatory cytokines. Recent reports have suggested that such chronic inflammation of adipose tissue is partly mediated by various cellular stresses including endoplasmic reticulum stress, hypoxia and oxidative stress. Oxidative stress plays an important role in obesity-related metabolic diseases. Glutathione peroxidase (GPx) is an antioxidant enzyme down-regulated in adipose tissue of obese mice. We measured cellular GPx activity, glutathione (GSH) contents, GSH/GSSG ratio, and mRNA expression of gamma-glutamylcysteine synthetase (gamma-GCS), a rate-limiting enzyme for de novo GSH synthesis, in adipose tissue of control and ob/ob mice, and in 3T3-L1 adipocytes treated with insulin, H2O2 or TNF-alpha. Furthermore, we investigated the effects of GPx inhibition with a specific GPx inhibitor or RNA interference against GPx, and reduced GSH on insulin signaling in 3T3-L1 adipocytes. Ob/ob mice showed not only a decrease in cellular activity of GPxs, but also an increase in gamma-GCS expression, resulting in increased GSH contents in adipose tissue. These alterations in glutathione metabolism were also observed during differentiation of 3T3-L1 cells and their exposure to insulin or H2O2. Inhibition of GPx activity, addition of GSH, and H2O2, resulted in impaired insulin signaling in 3T3-L1 adipocytes. These results suggest that decreased GPx activity and increased gamma-GCS expression lead to over-accumulation of GSH, which might be involved in the pathogenesis of insulin resistance in obesity.

Memo

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Acetyl-L-Carnitine and Insulin Resistance

Carnitine is studied extensively in part because of the important role it plays in fatty acid oxidation and energy production and because it is a well-tolerated and generally safe therapeutic agent. Mitochondrial dysfunction could lead to the state of insulin resistance, defects in insulin secretion and is associated with the development of diabetic complications. However, it is still unknown whether acetyl-L-carnitine (ALC) is beneficial in metabolic syndrome and if so, the mechanisms. The present study was designed to evaluate the effects of acetyl-L-carnitine on fatty acid oxidation, energy production and insulin resistance in C57BL/6J mice fed on a high-fat diet (HFD). The HFD feeding yielded overweight phenotype, glucose intolerance, hyperinsulinaemia, and hypertrophied islets and reduced liver peroxisome-proliferator activated receptor alpha (PPAR-alpha) expression concomitant with enhanced sterol-regulatory element binding protein-1 (SREBP-1) expression. Conversely, ALC treatment resulted in significant weight loss, a reversal of insulin resistance and islet hypertrophy, and alleviated hepatic steatosis. Both oxygen consumption and the expression of complexes in oxidative phosporylation were significantly increased after treatment with ALC for 8 weeks. The treated mice also showed higher expressions of the genes related to mitochondrial energy metabolism (PGC-1α) consistent with the increased mitochondrial biogenesis and energy expenditure in a dose-dependent manner. ALC treatment increased the phosphorylation of Akt at Ser^{473}, AMP-activated protein kinase (AMPK) at Thr^{172} and carnitine palmitoyltransferase (CPT)-1 and suppressed IRS-1 phosphorylation at Ser^{307}. These data suggest that ALC may play an important role in improvement of insulin resistance through the effects on mitochondria function, especially fatty acid oxidation and energy production.

Memo

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Type 2 Diabetes: the Alzheimer's disease of the periphery?

Alzheimer's disease (AD) and Type 2 diabetes (T2D) share many common features including common susceptibility genes, and increasing prevalence with age. Moreover, the risk of developing AD is doubled in patients with T2D. Interestingly, obesity is a common risk factor for the development of these two diseases and "lipotoxicity" is a hallmark of both AD and T2D, ultimately leading to protein aggregation. For the past decade, we (1,2) and others (3) have been studying the role of molecular chaperone proteins in the treatment of metabolic disease. Interestingly, molecular chaperones are also important in AD (4,5). Specifically, we have been studying the role of the inducible form of the 70kDa family of heat shock proteins, namely heat shock protein 72 (HSP72). We have identified an essential role of HSP72 in preventing obesity-induced insulin resistance, using both loss of function and gain of function genetic mouse models and, via the use of small molecule activators of HSP72 currently in human clinical trials for T2D. These data will be discussed and the clinical utility for molecules that activate molecular chaperone proteins in the treatment of obesity-related diseases will be evaluated.

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Memo

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Mechanistic Study of Islet Injury Induced by Hyperuricemia

Hyperuricemia is strongly associated with type2 diabetes. In our current study, uric acid stimulation in vitro resulted in a decrease in the insulin secretion and an increase in apoptosis of pancreatic beta cells. The injury of beta cell induced by uric acid is associated with increased production of nitric oxide and enhanced sumoylation of MafA.

Memo

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PPARγ, phosphorylation and the anti-diabetic PPARγ ligands

Obesity is a major risk factor of Metabolic Syndromes such as type 2 diabetes, dyslipidemia and cardiovascular disease. In addition, it is now clear that increasing rates of obesity are contributing to increases in the incidence and mortality of certain cancers. To date, the number of patients with diabetes is rapidly increasing and reaching to infectious disease level. The world health organization (WHO) announced that in 2030, 0.3 billion people will become patients with diabetes. Therefore, understanding the molecular pathways that link adipose tissue biology to this staggering array of pathologies is scientifically and clinically crucial. The nuclear receptor PPARγ is a master regulator of adipose cell differentiation and development. It is also the functioning receptor for the thiozolidinedione (TZD) class of anti-diabetic drugs such as rosiglitazone or pioglitazone. Here we showed that obesity induced in mice by high-fat feeding activates the protein kinase Cdk5, and this results in phosphorylation of PPARγ at Ser273 in adipose tissues. This modification of PPARγ does not alter its adipogenic capacity, but leads to dysregulation of a large number of genes whose expression is altered in obesity, including a reduction in the expression of the insulin-sensitizing adipokine, adiponectin. Unexpectedly, the phosphorylation of PPARγ□ by Cdk5 is blocked by anti-diabetic PPARγ ligands, such as rosiglitazone and MRL24. This inhibition works both *in vivo* and *in vitro*, and surprisingly, is completely independent of classical receptor transcriptional agonism. Similarly, inhibition of PPARγ□ phosphorylation in obese patients by rosiglitazone is very tightly associated with the anti-diabetic effects of this drug. More recently, we have developed novel synthetic compounds that have a unique mode of binding to PPARγ, completely lack classical transcriptional agonism and block the Cdk5-mediated phosphorylation in cultured adipocytes and in insulin-resistant mice. Moreover, one such compound, SR1664, has potent anti-diabetic activity while not causing the fluid retention and weight gain that are serious side effects of many of the PPARγ drugs. Unlike TZDs, SR1664 also does not interfere with bone formation in culture. These data illustrate that Cdk5-mediated phosphorylation of PPARγ is involved in the pathogenesis of insulin-resistance, and new classes of anti-diabetes drugs can be developed by specifically targeting the Cdk5-mediated phosphorylation of PPARγ.

Memo

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Impaired insulin signaling in the endothelial cells reduces insulin-induced glucose uptake by the skeletal muscle

In subjects with type 2 diabetes and obesity, insulin delivery and insulin-dependent glucose uptake by the skeletal muscle are known to be delayed and impaired (1-2). However, the mechanisms involved in such delay and impairment are not yet clearly understood. We demonstrate that impaired insulin signaling in the endothelial cells causes attenuation of the insulin-induced capillary recruitment and insulin delivery, which, in turn, reduces glucose uptake by the skeletal muscle. Moreover, improvement of insulin signaling in the endothelial cells completely reversed the reduction in the capillary recruitment and insulin delivery, and, as a result, significantly restored glucose uptake by the skeletal muscle in the high-fat diet-fed and endothelial-cell-specific insulin receptor substrate2-knockout mice (3). We conclude that impaired insulin signaling in the endothelial cells is a major mechanism of skeletal muscle insulin resistance in type 2 diabetes and obesity. Improving endothelial insulin signaling may serve as a novel therapeutic strategy for ameliorating skeletal muscle insulin resistance.

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3) Kubota, T., et al. Impaired insulin signaling in the endothelial cells reduces insulin-induced glucose uptake by the skeletal muscle. Cell Metab. 13: 294-307 (2011)

Memo

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Hypothalamic Neuron Cilia and Energy Metabolism

The primary, non-motile cilium is a specialized organelle found at the almost every eukaryotic cells. The cilium consists of 9+0 microtubule-based axoneme, an extension of mother centriole, in a plasma membrane sheath. Recently, the cilium has become the focus of intensive studies for its role in the transduction of extracellular signals and in a constellation of genetic disorders. Interestingly, human genetic ciliopathies (Bardet-Biedel Syndrome (BBS) and Alström syndrome, etc) commonly manifest obesity and diabetes mellitus. Similarly, mice deficient BBS protein-2,-4, and -6 display obese phenotype and leptin resistance. These mice develop central leptin resistance before the establishment of obesity, suggesting that BBS proteins are critical for central leptin signaling pathway. On the other hand, defective ciliogenesis in hypothalamic neurons causes hyperphagia and obesity, indicating that cilia of hypothalamic neurons play an important role in the maintenance of normal energy metabolism.

In my talk, I will present our recent data suggesting that leptin actively regulates cilia length of hypothalamic neurons, which may be an important determinant of leptin sensitivity in hypothalamic neurons.

Memo

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

Obesity is associated with an enhanced inflammatory response that exacerbates insulin resistance and contributes to diabetes, atherosclerosis, and cardiovascular disease. One mechanism accounting for increased inflammation is the activation 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. Using in vitro and in vivo systems to model lipid induction of TLR4-dependent inflammatory events in rodents, we determined that TLR4 is an upstream signaling component required for saturated fatty acid-induced ceramide biosynthesis. Moreover, we found that this increase in ceramide production, which is associated with an IkKb-dependent upregulation of genes driving ceramide biosynthesis, is dispensable for the induction of inflammatory cytokines, but is essential for TLR4-dependent 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.

Memo

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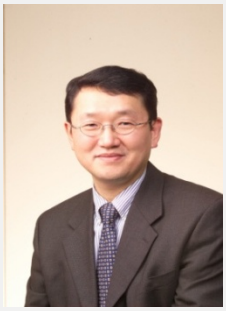

Fat Cell Size Determines Insulin Sensitivity and Inflammatory Responses

Adipose tissue (AT) plays an important role in control of whole body energy homeostasis by storing energy source in the form of triglycerides (TGs) and supplying energy to other tissues by releasing fatty acids (FAs). It also serves as an endocrine organ by producing a variety of hormones such as leptin, resistin, and adiponectin. The increase of AT is one of key features in obesity. In adults, increased AT is resulted from hypertrophic adipocytes. Recent data suggest that increased infiltration of immune cells into AT would induce insulin resistance and AT inflammation. However, the correlation between changes of adipocyte morphology and function in AT dysregulation has not been clearly established. In 3T3-L1 adipocytes, we have generated lipid-overloaded hypertrophic adipocytes with FFAs and investigated their characteristics. Compared to normal fat cells, lipid-overloaded hypertrophic adipocytes suppressed insulin dependent glucose uptake and insulin signaling, accompanied with increased expression of inflammatory genes. These observations suggest that FFAs-induced lipid overloaded adipocytes, which might mimic hypertrophic adipocytes in obese subjects, would be a good in vitro model system for studying adipocyte dysfunction.

Memo

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