The 5th Scientific Meeting of the

Asia-Pacific Diabetes and Obesity Study Group

Program & Abstracts

29 - 30 August 2009

Hotel GRAND PACIFIC LE DAIBA

TOKYO, JAPAN

The 5th Scientific Meeting of the

Asia-Pacific Diabetes and Obesity Study Group

Program & Abstracts

General Information

● Dates

Saturday, August 29- Sunday, August 30, 2009

● Venue

HOTEL GRAND PACIFIC LE DAIBA
2-6-1 Daiba, Minato-ku Tokyo 135-8701 Japan
Tel: +81-(0)3-5500-6711

● Organized by

Organizing Committee of Asia-Pacific Diabetes and Obesity Study Group

● Sponsored by

Takeda Pharmaceutical Company Limited

● Language

English is the working language of the meeting.

● Attire

Business casual attire is appropriate for all functions.

● Name Badge

You are requested to wear a name badge at all functions.

● Scientific Sessions

● Oral presentation

1. A 10- minute presentation is allotted to each speaker followed by 5-minute Q & A session.
2. Speakers are requested to come to the Slide Reception at latest 30 minutes prior to your session.

● Poster presentation

1. Your are requested to mantle a poster between 12:00-15:00 on August 29 and dismantle it after the reception.
2. Remaining posters will be taken away by the secretariat.

schedule-at-a-glance

Day 1 : Saturday, August 29

	B1F Palais Royal C	B1F Palais Royal D
12:00	Registration Starts	
13:00	Opening Remarks by David James	
13:10	13:10-13:45 **Session 1: APDO Study Group Lecture** Chair: David James / Speaker: Barry Halliwell	
13:45	13:45-14:45 **Session 2: Oral Presentation** Co-chairs: Tetsuro Izumi & Kun-Ho Yoon	
14:45	Coffee Break	
15:00	15:00-16:00 **Session 3: Oral Presentation** Co-chairs: Shun Ishibashi & Hongyuan Yang	
16:00	16:00-17:15 **Session 4: Oral Presentation** Co-chairs: Yoshihiro Ogawa & Aimin Xu	
17:15	Coffee Break	
17:30	17:30-18:30 **Session 5: Oral Presentation** Co-chairs: Ichiro Shimomura & Youfei Guan	
18:30		Photo Session
18:45		18:45-19:15 **Session 6: Poster Presentation**
19:15		19:15- Welcome Reception

schedule-at-a-glance

Day 2 : Sunday, August 30

	B1F Palais Royal C	B1F Chatelet
7:40		
	7:40-8:00 Takeda Morning Session	
8:00		
	8:00-9:15 **Session 7: Oral Presentation** Co-chairs: Takashi Kadowaki & Weiping Han	
9:15		
	9:15-10:15 **Session 8: Invited Lecture** Chair: Masato Kasuga / Speaker: Dr. Daniel Zeve	
10:15		
	Coffee Break	
10:30		
	10:30-11:45 **Session 9: Oral Presentation** Co-chairs: Wataru Ogawa & Heung-Sik Choi	
11:45		
	Closing Remarks by Masato Kasuga	
11:55		
12:00		12:00- Lunch

PROGRAM

— August 29-30 —

PROGRAM

◆ Saturday, August 29 ◆

13:00-13:10 ■ **Opening Remarks**

David James, *Australia*

13:10-13:45 ■ **Session 1: APDO Study Group Lecture**

Chair: David James, *Australia*

Antioxidants in health and disease — **Barry Halliwell**, *Singapore*

13:45-14:45 ■ **Session 2: Oral Presentation**

<10 minutes presentation +5 minutes Q&A session each>

Co-chairs: **Tetsuro Izumi**, *Japan*
Kun-Ho Yoon, *Korea*

Oral 2-1 Direct visualisation of peptide complement in cultured beta and alpha cells — **Christina Buchanan**, *New Zealand*

Oral 2-2 The role for Preadipocyte factor-1 (Pref-1/Dlk1) in pancreatic cells; as a pancreatic protodifferentiated cells — **Kun-Ho Yoon**, *Korea*

Oral 2-3 Differentiation of Enteroendocrine K-cells to Insulin-expressing Cells — **Ki-Ho Song**, *Korea*

Oral 2-4 Menin,Wnt/ β-catenin and pancreatic β cell proliferation — **Xiaoying Li**, *China*

14:45-15:00 **Coffee Break**

15:00-16:00 ■ **Session 3: Oral Presentation**

<10 minutes presentation +5 minutes Q&A session each>

Co-chairs: **Shun Ishibashi**, *Japan*
Hongyuan Yang, *Australia*

Oral 3-1 ADAMTS-7 Mediates Vascular Smooth Muscle Cell Migration and Neointima Formation in Balloon-injured Rat Arteries — **Wei Kong**, *China*

Oral 3-2 Change in P-glycoprotein and caveolin protein expression in capillaries of the brain striatum in New Zealand Obese mice with type 2 diabetes — **Huei-Ju Pan**, *Taiwan*

Oral 3-3 Relationship between Salt-Sensitivity and Thiazolidinedione-Induced Edema — **Akinobu Nakamura**, *Japan*

Oral 3-4 Rosiglitazone Attenuates Angiotensin II-induced Versican Expression in Rat Arteries and Smooth Muscle Cells — **Nanping Wang**, *China*

16:00-17:15 ■ **Session 4: Oral Presentation**

<10 minutes presentation +5 minutes Q&A session each>

Co-chairs: **Yoshihiro Ogawa**, *Japan*
Aimin Xu, *Hong Kong*

Oral 4-1 Molecular regulation of POMC promoter activity
Weiping Han, *Singapore*

Oral 4-2 Attenuation of diet-induced obesity, insulin resistance, and fatty liver in mice lacking the neuropeptide, α-calcitonin gene-related peptide: implications for the neuromuscular junction and sensory nerve axes
Kerry Loomes, *New Zealand*

Oral 4-3 Orphan nuclear receptor SHP and steatohepatitis **Hueng-Sik Choi**, *Korea*

Oral 4-4 Orphan Nuclear Receptor Small Heterodimer Partner Attenuates Renal Fibrosis in Obstructive Nephropathy **In Kyu Lee**, *Korea*

Oral 4-5 RabGAPs - a major node of signal regulation in vesicular transport
Jacqueline Stöckli, *Australia*

17:15-17:30 **Coffee Break**

17:30-18:30 ■ **Session 5: Oral Presentation**

<10 minutes presentation +5 minutes Q&A session each>

Co-chairs: **Iichiro Shimomura**, *Japan*
Youfei Guran, *China*

Oral 5-1 Characterisation of human acetyl CoA carboxylase 2 -a target for obesity therapeutics **Stuart Lance Macaulay**, *Australia*

Oral 5-2 Cholesterol redistribution under inflammatory stress associated with type 2 diabetes **Xiong Zhong Ruan**, *China*

Oral 5-3 Increased muscle mass by knocking out Myostatin protects against high-fat diet induced insulin resistance **Cheol Soo Choi**, *Korea*

Oral 5-4 Interleukin-6 protects against diet-induced insulin resistance independent of obesity **Mark Febbraio**, *Australia*

18:30-18:45 **Photo Session**

18:45-19:15 ■ **Session 6: Poster Presentation**

<Free Discussion>

Poster 1 The metabolic effects of antipsychotic medication in the rat
Greg Smith, *New Zealand*

Poster 2 Perilipin overexpression decreases adipocyte size and protects against diet-induced obesity and glucose intolerance **Hideaki Miyoshi**, *Japan*

Poster 3	Caveolin, caveolae, cholesterol and signal transduction	**Michelle Hill**, *Australia*
Poster 4	Ghrelin Upregulates UCP-2 and Inhibits Insulin Secretion	**Masahiro Nishi**, *Japan*
Poster 5	Suppression of PGC-1α (PPAR-gamma-coactivator-1α) normalizes the glucolipotoxicity induced decreased BETA2 gene transcription and improved glucose tolerance in diabetic rats	**Ji-Won Kim**, *Korea*
Poster 6	In vitro Transdifferentiation of the Human Hepatocytes into Insulin-Producing Cells using Adenoviral transduction	**Dong-Sik Ham**, *Korea*
Poster 7	Genetically Engineered K-cells Express Glucose-dependent Insulin Secretion in Diabetic Mice	**Sung-Dae Moon**, *Korea*
Poster 8	Dysregulated glutathione metabolism links to impaired insulin action in adipocytes	**Atsunori Fukuhara**, *Japan*
Poster 9	Genetic variants associated with obesity and obesity-related diabetes mellitus in Southern Chinese	**Annette WK Tso**, *Hong Kong*
Poster 10	Murine gamma herpes virus 68 infection promotes fatty liver and hepatic insulin resistance in C57BL/6J mice	**Xiong Zhong Ruan**, *China*
Poster 11	Beneficial effects of acute Inhibition of hepatic S6K1 signaling in obese animals	**Shuying Li**, *China*
Poster 12	Oxidative Stress Induced by Inhibition of Fatty Acid Synthesis in Liver Promotes Insulin Resistance through Phosphorylation of IRS1 by Erk1/2	**Toru Uchida**, *Japan*
Poster 13	PDK1-FoxO1 in AGRP neurons regulates energy homeostasis by modulating food intake and energy expenditure	**Jun Nakae**, *Japan*
Poster 14	Quantitative proteomic research and integrative analysis of endogenous S-nitrosated proteins in diabetic mouse liver	**Chang Chen**, *China*
Poster 15	Common PCSK1 Haplotypes are Associated with Obesity and Modulate Body Mass Index in the Chinese Population	**Yi-Cheng Chang**, *Taiwan*
Poster 16	Identification of specific actin filaments that regulate glucose clearance, insulin sensitivity and insulin secretion	**Peter Gunning**, *Australia*

19:15- Welcome Reception

◆ Sunday, August 30 ◆

7:40-8:00 ■ **Takeda Morning Session**

8:00-9:15 ■ **Session 7: Oral Presentation**

<10 minutes presentation +5 minutes Q&A session each>

Co-chairs: **Takashi Kadowaki**, *Japan*
Weiping Han, *Singapore*

Oral 7-1	Increasing fat oxidation in rats via acute activation of AMPK does not alter energy expenditure	**Gregory J. Cooney**, *Australia*
Oral 7-2	Selective inactivation of c-Jun NH2 terminal kinase (JNK) in adipose tissue is sufficient to alleviate metabolic disorders associated with dietary obesity in mice	**Karen Lam**, *Hong Kong*
Oral 7-3	The size of lipid droplets	**Hongyuan Robert Yang**, *Australia*
Oral 7-4	IRE-1 and HSP-4 play key roles in energy homeostasis via novel fasting-induced lipases in C. elegans	**Jae Bum Kim**, *Korea*
Oral 7-5	Adipogenesis licensing and execution are disparately linked to cell proliferation	**Jia-Rui Wu**, *China*

9:15-10:15 ■ **Session 8: Invited Lecture**

Chair: **Masato Kasuga**, Japan

Harnessing the power of stem cells to cure obesity and diabetes
Daniel Zeve, *USA*

10:15-10:30 **Coffee Break**

10:30-11:45 ■ **Session 9: Oral Presentation**

<10 minutes presentation +5 minutes Q&A session each>

Co-chairs: **Wataru Ogawa**, *Japan*
Hueng-Sik Choi, *Korea*

Oral 9-1	APPL1 enhances Insulin Sensitivity by counteracting the effect of the endogenous Akt inhibitor Tribble-3	**Aimin Xu**, *Hong Kong*
Oral 9-2	Role of CREBH in the regulation of hepatic gluconeogenesis	**Seung-Hoi Koo**, *Korea*
Oral 9-3	Regulation of hepatic glycolysis by FoxO1 via ChREBP O-glycosylation	**Tadahiro Kitamura**, *Japan*
Oral 9-4	Hepatic fructose-1,6-bisphosphatase: a novel nutrient sensor	**Sof Andrikopoulos**, *Australia*

Oral 9-5	Development of a Systemic and Targeted Screening Process for the Discovery of New Antidiabetic Molecules Derived from Traditional Chinese Medicines	**Jiming Ye**, *Australia*

11:45-11:55 **Closing Remarks**

Masato Kasuga, *Japan*

12:00 **Lunch**

ABSTRACTS

1

Antioxidants in health and disease

Barry Halliwell

Tan Chin Tuan Centennial Professor, Deputy President (Research and Technology), Professor of Biochemistry
National University of Singapore

Oxidative stress is widely thought to contribute to the pathology of human diseases, especially diabetes and the metabolic syndrome. However, "antioxidants" such as vitamin E have evidenced little clinical benefit in these and other conditions. My talk will explore the reasons for this paradox in the content of the generation and roles of reactive oxygen species *in vivo*, and the true biological effect of antioxidants.

Reference:

Halliwell B and Gutteridge JMC (2007) *Free Radicals in Biology and Medicine*. Clarendon Press, Oxford (fourth edition), UK.

2-1

Direct visualisation of peptide complement in cultured beta and alpha cells

Christina Buchanan, Arpita Malik, Garth Cooper

Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, New Zealand

The application of intact-cell mass spectrometry (ICM) by MALDI-TOF mass spectrometry to achieve direct protein-profiling of bacterial species is now well established. However this methodology has not to our knowledge been applied to the analysis of mammalian cells in routine culture. Here, we describe a novel application of ICM by which we have identified proteins in intact cells from two lines representative of pancreatic islet α and β cells. Adherent αtc1 clone 9 and βTC6 F7 cells were harvested into PBS using enzyme-free dissociation buffer before 1 μL of cell suspension was spotted onto MALDI plates. Cells were overlaid with sinapinic acid then washed with pure water before application of a final coat of sinapinic acid. Data in the 2,000-20,000 m/z range were acquired in linear mode on a Voyager DE-Pro mass spectrometer [1]. The entire process from cell harvest to peptide identification takes approx. 10 minutes, therefore the ease of use coupled with the rapid and direct nature of this analytical system, indicate its potential in a number of possible cell biology applications.

The proteins that ionised were composed in large part of peptide hormones known to be packaged into the secretory granules of the β and α cells respectively (See Table). Furthermore, this method identified the presence of oxyntomodulin in the α-cell line, a finding that has not been reported previously.

β cell hormones	*Expected m/z*	*Measured m/z*	*%Error*
Proinsulin II	9491.8	9494.5	0.03
Insulin II	5797.7	5797.8	0.00
Insulin I	5804.7	5803.2	-0.03
C-peptide II	3122.4	3122.3	0.00
C-peptide I	3134.4	3134.2	-0.01
Amylin	3922.4	3921.8	-0.02
Preptin	3949.4	3949.8	0.01
α cell hormones	**Expected *m/z***	**Measured *m/z***	**%Error**
Proglucagon	18626	Not detected	NA
Glucagon	3483.2	3483.2	0.00
GLP-1	4169.9	4170.0	0.00
Oxyntomodulin	4450.5	4450.5	0.00
GRPP	3441.5	3441.2	-0.01
MPF	9946.9	9948.7	0.02

Abbreviations: GLP, glucagon-like peptide; GRPP, glicentin-related polypeptide; ICM, intact-cell mass spectrometrry; MALDI, matrix-assisted laser-desorption/ionisation; MPF, major proglucagon fragment; MS, mass spectrometry; PBS, phosphate-buffered saline.

1 Buchanan, C. M., Malik, A. S. and Cooper, G. J. (2007) Direct visualisation of peptide hormones in cultured pancreatic islet alpha- and beta-cells by intact-cell mass spectrometry. Rapid Commun Mass Spectrom **21**, 3452-3458

2-2

The role for Preadipocyte factor-1 (Pref-1/Dlk1) in pancreatic cells; as a pancreatic protodifferentiated cells

Kun-Ho Yoon, Marie Rhee, Dong-Sik Ham, Ji-Won Kim

Diabetes Research Laboratory, Division of Endocrinology and Metabolism, Catholic Research Institutes of Medical Science, Catholic University of Korea, Seoul, Korea

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 Pref-1 has reported that is involved in both differentiation and growth of β-cells, the mechanism and function are not cleared yet. Herein we further investigated Pref-1 expression pattern and intracellular signaling mechanism during pancreas development and regeneration. In the rat embryonic pancreas at E20, Pref-1 expression was restricted only in the small ductules whereas was not observed at all in adult pancreas. After partial pancreatectomy (Px) of adult rat pancreases, Pref-1 was strongly regained in the small regenerative duct cells located in foci of regeneration while not expressed in common and main pancreatic duct, and then completely disappeared at 7 days. In monolayer cultured porcine neonatal pancreatic cell clusters (NPCCs), Pref-1 expression was regained in small duct cells and peaked at day 3 to day 4, then gradually disappeared until day 7 as Px rat pancreas. Most of Pref-1(+) cells were co-stained with pancytokeratin and PDX1. To extend our understanding for the role of Pref-1 in pancreas duct cells, we investigated the intracellular signaling mechanism of Pref-1 in pancreatic cells after Pref-1 treatment. Purified soluble Pref-1 (Pref-1-mFc) treatment increased ERK1/2 and FOXO1 phosphorlylation, and their phosphorylation were blocked by PD98059 in PANC-1 cells. We also observed that the activation of Pref-1 increased PDX1 and insulin gene expressions, whereas it decreased FOXO1 expression. However, we couldn't find any effect by Pref-1 treatment in INS1 cells. We conclude that Pref-1 expression was regained in adult pancreatic duct cells during proliferation and might play an important role for the regeneration and differentiation of endocrine pancreas through phosphorylation of ERK1/2 while Pref-1 haven't any effects on beta-cells.

2-3

Differentiation of Enteroendocrine K-cells to Insulin-expressing Cells

Ki-Ho Song, Esder Lee, Gyeong Ryul Ryu, Seung-Hyun Ko, Yu-Bae Ahn, Sung-Dae Moon

Department of Internal Medicine, College of Medicine, The Catholic University of Korea

Despite a recent breakthrough in human islet transplantation for treating type 1 diabetes, the limited availability of donor pancreas is still a major obstacle.
Endocrine cells within the gut epithelium (enteroendocrine cells) and pancreatic β cells share similar pathways of differentiation during embryonic development. Especially, K-cells that secrete glucose dependent insulinotropic peptide (GIP) after a meal, have been shown to express many of the key proteins found in β cells. Therefore, we hypothesize that K-cells can be differentiated to β cells because both cells have remarkable similarities in their embryonic development and cell phenotypes.
We obtained K-cell clones from heterogeneous STC-1 cells originating from an endocrine tumor of the mouse intestine. K-cells were found to express glucokinase, as reported previously, and GIP mRNA. Interestingly, K-cells expressed Pdx-1, NeuroD1, and MafA which are crucial transcription factors for development and function of β cells. And insulin mRNA and protein were detected after exendin-4 treatment and serum deprivation in K-cells. We are currently doing *in vitro* experiments to optimize a strategy of converting K-cells to β cells.
In conclusion, K-cells might be an attractive potential source of insulin-producing cells for treatment of type 1 diabetes.

2-4

Menin,Wnt/β-catenin and pancreatic β cell proliferation

Xiaoying Li, Yanan Cao, Ruixin Liu, Xiuli Jiang, Guang Ning

Shanghai Institute of Endocrinology and Metabolism, Rui-Jin Hospital, Shanghai Jiao Tong University School of Medicine

The multiple endocrine neoplasia type 1 (MEN1) gene encodes a tumor suppressor protein, denoted as menin. Mutations in MEN1 gene always lead to loss of function of menin, which is responsible for MEN1 including insulinoma. Recently, a number of studies have focused on menin for its role in pancreatic β cell proliferation and diabetes pathogenesis.
The canonical Wnt/beta-catenin signaling pathway has a critical role in cell proliferation, development and tumorigenesis. The inappropriate degradation or transportation of beta-catenin leads to activation of the Wnt pathway, which could be regulated by some tumor suppressors, eg, APC and WTX. Recent studies have indicated that Wnt pathway regulates pancreatic β cell proliferation and cell development. Recent investigations have demonstrated that changes in Men1 expression significantly affected pancreatic β cell growth. We found that beta-catenin was accumulated in the nuclei in the pancreatic beta cells in Men1 knockout mice and pancreatic tumors in MEN1 patients, which further activated Wnt pathway. Our study documented that menin could interact with beta-catenin, exported beta-catenin out of the nuclei via its two nuclear export signals (NES) domains and promoted beta-catenin degradation. We speculated that menin negatively regulated Wnt/beta-catenin signaling and the defects of menin lead to Wnt/beta-catenin signaling activation and pancreatic β cell proliferation.

3-1

ADAMTS-7 Mediates Vascular Smooth Muscle Cell Migration and Neointima Formation in Balloon-injured Rat Arteries

Wei Kong, Jingang Zheng, Xue Bai, Bo Liu, Chuan-ju Liu, Qingbo Xu, Yi Zhu, Nanping Wang, Xian Wang

Peking University Health Science Center, Department of Physiology and Pathophysiology

The migration of vascular smooth muscle cells (VSMCs) plays an essential role during the development of atherosclerosis and restenosis. Extensive studies have implicated the importance of extracellular matrix (ECM)-degrading proteinases in VSMC migration. A recently described family of proteinases, a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTs), is capable of degrading vascular ECM proteins. Here, we sought to determine whether ADAMTS-7 is involved in VSMC migration and neointima formation in response to vascular injury. ADAMTS-7 protein accumulated preferentially in neointima of the carotid artery wall after balloon injury. In primary VSMCs, ADAMTS-7 level was enhanced by the pro-inflammatory cytokine tumor necrosis factor α (TNF-α) and growth factor platelet-derived growth factor (PDGF)-BB. ADAMTS-7 overexpression greatly accelerated and small interfering RNA (siRNA) knockdown markedly retarded VSMC migration/invasion *in vitro*. In addition, luminal delivery of ADAMTS-7 adenovirus to carotid arteries exacerbated intimal thickening nearly sixfold 7 days after injury. Conversely, perivascular administration of ADAMTS-7 siRNA but not scramble siRNA to injured arteries attenuated intimal thickening by 50% at 14 days after injury. Furthermore, ADAMTS-7 mediated degradation of the vascular ECM cartilage oligomeric matrix protein (COMP) in injured vessels. Replenishing COMP circumvented the pro-migratory effect of ADAMTS-7 on VSMCs. Enforced expression of COMP significantly suppressed VSMC migration and neointima formation post-injury, which indicates that ADAMTS-7 facilitated intimal hyperplasia through degradation of inhibitory matrix protein COMP. ADAMTS-7 may therefore serve as a novel therapeutic target for atherosclerosis and postangioplasty restenosis.

3-2

Change in P-glycoprotein and caveolin protein expression in capillaries of the brain striatum in New Zealand Obese mice with type 2 diabetes

Huei-Ju Pan, Kuo-Chen Wu, Hsiang-Shu Yin, Mei-Ru Chen, Shao-Chun Lu, Chun-Jung Lin

Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan
School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan

Aims: To investigate the expression of P-gp and caveolins in capillaries of brain striatum in inbred mice with type 2 diabetes.

Main methods: Inbred mice with type 2 diabetes (male New Zealand obese; NZO) were compared with related mice without diabetes (female NZO and New Zealand White). Protein expression of P-gp and caveolins in capillaries of the brain striatum was examined by immunohistochemical analysis. P-gp efflux pump activity in the blood-brain barrier was measured by in vivo brain microdialysis. Regulation of P-gp and caveolins expression was examined in cultured adult rat brain endothelial cells (ARBEC).

Key findings: In capillaries of the brain striatum, expression of P-gp was higher but caveolins was lower, respectively, in mice with type 2 diabetes compared with non-diabetic mice. Brain extracellular concentrations of intravenously injected rhodamine 123 were more than 50-60% lower in type 2 diabetic mice. Insulin and PMA treatments significantly increased P-gp expression, whereas the same treatments decreased caveolin expression in ARBEC.

Significance: Protein expression of P-gp and caveolin can be regulated in animals with type 2 diabetes. These changes may be important in modulating P-gp activity in the BBB in type 2 diabetes.

3-3

Relationship between Salt-Sensitivity and Thiazolidinedione-Induced Edema

Akinobu Nakamura, Yasuo Terauchi
Department of Endocrinology and Metabolism, Yokohama City University, Japan

Although there is growing recognition that fluid retention and edema are common adverse effects of thiazolidinediones (TZDs), the underlying mechanisms remain unclear. To explain the relationship between salt-sensitivity and TZD-induced edema, we analyzed sodium excretion before and after administration of TZDs to 26 female subjects with type 2 diabetes. When a significant correlation was found between salt excretion and blood pressure, the patient was classified as salt-sensitive. Six patients were classified into a salt-sensitive group and 20 patients into a non-salt-sensitive group. After 8 weeks of pioglitazone administration, 5 patients had developed edema, and, surprisingly, all 5 subjects were salt-insensitive. Salt excretion after administration of pioglitazone was significantly lower in both edema and salt-sensitive groups, and the hematocrit was significantly lower after administration in the salt-sensitive group, but not in the edema group. In the subjects who developed edema, administration of TZDs caused fluid retention, and increased fluid in the intravascular space would be mobilized to extravascular space. On the other hand, administration of TZDs caused fluid retention in salt-sensitive subjects, however, increased fluid would be retained in the intravascular space. Consequently, they did not develop edema. In conclusion, TZD-induced edema would be caused not only by fluid retention but also by vascular hyperpermeability.

3-4

Rosiglitazone Attenuates Angiotensin II-induced Versican Expression in Rat Arteries and Smooth Muscle Cells

Nanping Wang, Yuanyuan Li, Hong Zhang, Guanghua Hao, Yeguang Chen

Institute of Cardiovascular Science and Diabetes Center, Peking University Health Science Center, Beijing 100191, China

Objective-The proteoglycan versican is one of several extracellular matrix (ECM) molecules that accumulate in lesions of atherosclerosis and restenosis. Versican is among the genes upregulated after vascular injury. Expression of versican is highly regulated by growth factors and cytokines. This study was to examine the effect of rosiglitazone on the expression of versican in vascular arterials and smooth muscle cells (SMCs) in response to angiotensin II (Ang II).

Methods and Results-Ang II induced expression of versican in rat aortic SMCs in a time- and dose-dependent manner. Rosiglitazone attenuated the Ang II-induced expression of versican. This inhibitory effect was abrogated by the pretreatment with PPARγ antagonist GW9662. Moreover, adenovirus-mediated overexpression of a constitutively active PPARγ inhibited Ang II-induced expression of versican mRNA level, indicating a PPARγ-specific effect. Sprague-Dawley rats were administrated with Ang II (300 $ng \cdot kg^{-1} \cdot min^{-1}$) via osmotic pumps with or without rosiglitazone (4 $mg \cdot kg^{-1} \cdot d^{-1}$) treatment for 7 days. Using real-time RT-PCR, western blotting and immunohistochemistry, we demonstrated that rosiglitazone significantly diminished Ang II-induced expression of versican and other extracellular matrix proteins in rat aorta and mesentric arteries. Overexpression of ALK5, the TGF-beta type I receptor kinase, increased the expression of versican in SMCs whereas overexpression of Smad2 abolished the suppressive effect of rosiglitazone on the Ang II induced versican expression, which indicated that rosiglitazone exerted the effect via inhibition of the ALK5/Smad2 pathway.

Conclusions-Rosiglitazone suppressed Ang II-induced expression of versican via inhibiting Smad2. Our results provided novel insight into the underlying mechanisms for a role of PPARγ in preventing Ang II-induced vascular remodeling.

4-1

Molecular regulation of POMC promoter activity

Weiping Han, Guoqing Yang, Chun-Yan Lim, Chao Li, Wei Ma, Xiuhui Lou

Laboratory of Metabolic Medicine, Singapore Bioimaging Consortium, Agency for Science, Technology and Research (A*STAR)

Leptin controls food intake and energy expenditure by regulating hypothalamic neuron activities. Leptin exerts its actions through complex signaling pathways including STAT3 phosphorylation, nuclear translocation, and binding to target gene promoter/cofactor complexes. Deficient or defective leptin signaling leads to obesity, which may be caused by insufficient leptin levels and/or resistance to leptin signaling. To understand the molecular mechanisms of leptin resistance, we established a cell-based system that allows investigation of POMC gene regulation by leptin. We show that phospho-STAT3 activates POMC promoter in response to leptin signaling through a mechanism that requires an SP1 binding site in the POMC promoter. Furthermore, FoxO1 binds to STAT3 and prevents STAT3 from interacting with the SP1/POMC promoter complex, and consequently, inhibits STAT3-mediated leptin action. Our study suggests that leptin action could be inhibited at a step downstream of STAT3 phosphorylation and nuclear translocation, and provides a potential mechanism of leptin resistance in which an increased FoxO1 antagonizes STAT3-mediated leptin signaling.

4-2

Attenuation of diet-induced obesity, insulin resistance, and fatty liver in mice lacking the neuropeptide, α-calcitonin gene-related peptide: implications for the neuromuscular junction and sensory nerve axes

Kerry Loomes, Christopher Walker, Xiaoling Li, Lynda Whiting, Shaoping Zhang, Rachel Danaher, Sarah Glyn-Jones, Anthony Hickey, Katya Ruggierio, Debbie Hay, Edward Kraegen, Anthony Phillips, Garth Cooper

School of Biological Sciences and Maurice Wilkin's Centre for Molecular Biodicovery, University of Auckland, Auckland, New Zealand; Diabetes and Obesity Research Program, Garvan Institute of Medical Research, Darlinghurst, New South Wales 2010, Australia

Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide that is expressed primarily in motor and sensory neurons in both central and peripheral nervous systems. CGRP belongs to the calcitonin family of peptides and exists as two αCGRP and βCGRP isoforms with overlapping tissue distribution. Of these, α-CGRP is co-produced in motoneurons together with the classical neurotransmitter, acetylcholine, and also in sensory nerves where its release is stimulated by capsaicin. Due to its well documented vasodilatory activities, α-CGRP has been previously ascribed roles in cardiovascular conditioning, neuromuscular junction development, and regulation of sympathetic outflow. However, its relevance in these processes is unclear in light of findings from three independently generated knockout mice lines that lack specific expression of α-CGRP. Here, we report on a study where α-CGRP-specific knockout mice (α-CGRP$^{-/-}$) were challenged chronically with two concurrent high fat diet regimens in order to explore whether CGRP might play a role in lipid homeostasis. Our findings showed that compared with control mice, α-CGRP$^{-/-}$ mice displayed a reduction in body weight, improved glucose handling and insulin sensitivity, and attenuated symptoms of fatty liver. We also found evidence for altered AMPK and ACC signaling and a potentiated mitochondrial adaptive capacity in liver and skeletal muscle in response to high fat feeding in α-CGRP$^{-/-}$ mice. We found no evidence for alterations in circulating inflammatory markers or thyroid function. These findings implicate a role for CGRP in the development of obesity and diabetes, possibly through site-of-action effects exerted through the neuromuscular junction and/or sensory nerves.

4-3

Orphan nuclear receptor SHP and steatohepatitis

Hueng-Sik Choi

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Plasminogen activator inhibitor type I (PAI-1) is a marker of the fibrinolytic system, and serves as a possible predictor for hepatic metabolic syndromes. Fenofibrate, a peroxisome proliferator-activated receptor α (PPARα) agonist, is a drug used for treatments of hyperlipidemia. Orphan nuclear receptor small heterodimer partner (SHP) plays a key role in transcriptional repression of crucial genes involved in various metabolic pathways. In this study, we show that fenofibrate increased SHP gene expression in cultured liver cells and in the normal and diabetic mouse liver by activating the AMP-activated protein kinase (AMPK) signaling pathway in a peroxisome proliferator-activated receptor α (PPARα)-independent manner. Administration of transforming growth factor β (TGFβ) or a methionine and choline deficient (MCD) diet to induce the progressive fibrosing steatohepatitis model in C57BL/6 mice was significantly reversed by fenofibrate via AMPK-mediated induction of SHP gene expression with a dramatic decrease in PAI-1 mRNA and protein expression along with other fibrotic marker genes. No reversal was observed in SHP null mice treated with fenofibrate. Fenofibrate exhibited a differential inhibition pattern on PAI-1 gene expression depending on the transcription factors inhibited by SHP.
Conclusion: By demonstrating that a PPARα-independent fenofibrate/AMPK/SHP regulatory cascade can play a key role in PAI-1 gene down regulation and reversal of fibrosis, our study suggests that various AMPK activators regulating SHP might provide with a novel pharmacologic option in treating hepatic metabolic syndromes.

4-4

Orphan Nuclear Receptor Small Heterodimer Partner Attenuates Renal Fibrosis in Obstructive Nephropathy

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The accumulation of extracellular matrix proteins is the key feature of chronic fibrotic kidney disease including diabetic nephropathy. Accumulating evidences suggest that plasminogen activator inhibitor type 1 (PAI-1) plays a key role in the development of TGF-beta-induced renal fibrosis. Previously, we demonstrated that small heterodimer partner (SHP) represses PAI-1 expression in liver by inhibition of TGF-β signaling pathway. Here, we examined whether SHP prevents renal fibrosis in unilateral ureteral obstruction (UUO)-induced renal fibrosis models and elucidate its mechanism in cultured renal cells. UUO markedly increased the expression levels of PAI-1, type 1 collagen and fibronectin. Moreover, in kidney of SHP-/- mice, the expressions of PAI-1, type 1 collagen and fibronectin were increased compared with those in kidney of wild type mice. Collectively, these data suggested that decreased expression of SHP plays a role in the development of renal fibrosis. Adenovirus-mediated overexpression of SHP in cultured rat mesangial cells (RMCs) and renal tubular epithelial, NRK-52E cells inhibited TGF-beta-stimulated PAI-1, collagen 1 type and fibronectin expressions. SHP inhibited TGF-beta and SMAD3-stimulated PAI-1 promoter activities and TGF-beta-stimulated Smad3 binding to its response consensus element on PAI-1 promoter. Moreover, upregulation of SHP level in kidney by adenovirus expressing SHP inhibited UUO-induced PAI-1, type 1 collagen and fibronectin expressions. This study shows that SHP prevents renal fibrosis *in vitro* and *in vivo*. The present study raises the possibility that SHP can be a target for the prevention of renal fibrosis.

4-5

RabGAPs - a major node of signal regulation in vesicular transport

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Rab GTPases play an essential role in regulating vesicle transport in eucaryotic cells. GTP loading of these proteins, catalysed by GEFs, enhances their ability to regulate vesicle fusion while GTP hydrolysis, catalysed by GAPs, inhibits this process. Strikingly GAPs appear to be central nodes of regulation since they undergo phosphorylation dependent regulation in response to multiple upstream protein kinases. Moreover, there are >50 distinct RabGAPs expressed in mammals. This raises the question does each RabGAP display distinct Rab binding specificity or are different RabGAPs capable of regulating many transport steps but in a manner that is regulated via discrete upstream regulation? Of particular interest to us is the Rab GAP TBC1D4 which is phosphorylated by the Ser/Thr kinase Akt on 4 sites in response to insulin and this regulation plays an essential role in the regulated movement of the glucose transporter GLUT4 to the cell surface in muscle and fat cells. To distinguish between the above possibilities we fused GAP domains of different RabGAPs to GLUT4 and tested their ability to inhibit insulin action in adipocytes. Fusion of the TBC1D4 GAP domain completely inhibited insulin action whereas the equivalent domain of an unrelated Rab GAP TBC1D16 had only a partial effect. These data are consistent with a model in which different GAPs display Rab specificity. To further test this model we performed a yeast two hybrid screen using a library of all mammalian Rab proteins and found that full length TBC1D4 interacted with two endosomal Rabs whereas TBC1D16 interacted with a Rab that controls ER/Golgi transport. Intriguingly TBC1D4 has been suggested to be the GAP for Rab10 however we found no interaction with Rab10 using the yeast two hybrid screen. Rather another RabGAP, TBC1D13 was found to interact with Rab10 and overexpression of TBC1D13 in adipocytes blocked insulin-stimulated GLUT4 translocation. These studies suggest that different RabGAPs control different vesicle transport steps and that these molecules may act in a cooperative manner to control membrane protein trafficking. In the case of GLUT4 we propose that both TBC1D4 and TBC1D13 regulate GLUT4 exocytosis in a sequential manner. These studies again were consistent with the model in that we observed a high degree of Rab binding specificity to different GAPs. For example, TBC1D16 bound only to Rab2, TBC1D13 to Rab 10 while TBC1D4 bound only to two Rabs.

5-1

Characterisation of human acetyl CoA carboxylase 2 -a target for obesity therapeutics

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Acetyl CoA carboxylase (ACC) 1 and 2 are required for fatty acid synthesis and regulation of fatty acid oxidation respectively. Both are targets of AMPK and are potential therapeutic targets for the treatment of obesity and type 2 diabetes. Difficulties in expression of full length proteins have hampered investigation of the enzymes as targets. Two recent reports described the expression of ACC1 and truncated ACC2 (Kim et al 2007 (1), Cheng et al 2007(2)). Here we describe the expression of full length human ACC2 and ACC1 with a C-terminal FLAG tag in insect cells and inhibitors of enzyme activity. Both proteins were active and had a Km of 12-15 μM for acetyl CoA. They were purified on soft-link avidin (Promega) or on FLAG MAb affinity resin. The yield of protein was modest, 0.2mg/L. The proteins were inhibited by low molecular weight compounds related to the cyclohexanedione family previously studied as potential herbicides, with the best of the inhibitors having IC_{50} of 200nM. To further investigate inhibition by related compounds, FLAG tagged carboxyltransferase (CT) domain was expressed and purified from insect cells. This protein expressed at much higher levels than the full length enzyme (25mg/L). A colorimetric assay measuring enzyme activity in the reverse direction was developed. Activity of this domain was inhibited by the same compounds that inhibited the full length enzyme. Crystallisation trials of this domain are in progress together with medicinal chemistry around the inhibitors. These compounds stimulated fatty acid oxidation in L6 muscle cells up to 2.5 fold confirming them as potential leads for therapeutic development.

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(2) Cheng D, Chu CH, Chen L, Feder JN, Mintier GA, Wu Y, Cook JW, Harpel MR, Locke GA, An Y, Tamura JK. Protein Expr Purif. 2007 Jan;51(1):11-21.

5-2

Cholesterol redistribution under inflammatory stress associated with type 2 diabetes

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Type 2 diabetes /obesity are increasingly accepted as a long-term, low-grade, inflammatory state (LLI) characterised by increased serum CRP and TNF levels. High risk of death from cardiovascular disease is also accompanied with type 2 diabetes while serum cholesterol levels are not high. This study aimed to examine whether activation of the inflammatory stress directly causes paradoxical association between serum cholesterol and cardiovascular events by redistributing cholesterol from the circulation to the tissues.

Homozygous 8-week old ApoE KO mice and ApoE/Scavenger receptor typeA/CD36 triple KO mice were fed a high fat atherogenic diet and were randomly assigned to receive either daily subcutaneous injections of 0.5 ml 10% casein (to induce LLI) or vehicle (controls). Animals were sacrificed after 8 weeks and terminal blood samples were taken for lipids, serum amyloid A (SAA), cytokines, glucose and insulin assays. The lipid accumulation in aorta, liver and kidney were determined by Oil Red O (ORO) staining and quantitated by computer-assisted image analysis. The gene and protein expression of molecules involved in cholesterol trafficking were examined by real-time PCR and Western Blot.

Blood levels of SAA (532±29.4 *vs* 258.4±30.5 ng/ml, $p<0.001$) and TNFα (37.4±5.1 *vs* 2.11 ±0.32 μg/ml, $p<0.001$) were higher, while total cholesterol (20.2±3.5 *vs* 40.7±9.9mmol/l, $p<0.05$), LDL-cholesterol (18.8±3.7 *vs* 34.82±7.5 mmol/l, $p<0.05$) and HDL cholesterol (7.3 ±0.9 *vs* 15.7±2.5 mmol/l, $p<0.001$) were lower in the casein-treated ApoE KO mice (n=7) compared to controls (n=7). Casein injection increased LDL receptor gene and protein expression in the liver and aorta, probably by disrupting the regulatory function of SREBP cleavage activating protein (SCAP), an intracellular cholesterol sensor. Lipid accumulation in the liver and aorta was more extensive in casein-injected animals (4.28±0.23 fold *vs* controls) despite lower blood lipid levels and caused liver insulin resistance. Increased lipid accumulation in the kidney was also observed by Oil Red O staining in casein-injected animals in a similar experiment. Interestingly, knockout of Scavenger receptor type A and CD36 in the ApoE KO mice did not reduce lipid accumulation in tissues, suggesting that SCAP/SREBP/LDL receptor pathway plays an important role in the inflammation-induced cholesterol redistribution.

In conclusion, inflammation both exacerbates liver steatosis/plaque formation and lowers serum cholesterol levels. Normal serum cholesterol does not necessarily exclude the importance of cholesterol in the progression of type 2 diabetes because inflammatory stress causes cholesterol redistribution. This suggests that there is no safe serum cholesterol level in the presence of positive inflammatory markers.

5-3

Increased muscle mass by knocking out Myostatin protects against high-fat diet induced insulin resistance

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Myostatin is a negative regulator of skeletal muscle mass and myostatin null (KO) mice have been shown to have a twofold increase in muscle mass. Skeletal muscle is a major organ responsible for glucose and lipid disposal, but the effect of increased muscle mass per se on fat oxidation and fat induced insulin resistance is not clear. The myostatin KO mice were heavier than wt mice and the increase in body weight is mainly attributed to a 2 fold increase in muscle mass because they have less fat, compared to Wt. Myostatin KO mice were resistant to high-fat diet induced obesity and hyperlipidemia. In addition, insulin sensitivity was dramatically increased in myostatin KO mice. Most notably, preventive effect on fat-induced obesity and insulin resistance by deficiency of myostatin was sustained with increasing age which was mostly attributable to doubled muscle mass. These data demonstrate that myostatin KO mice are insulin sensitive and protected against diet induced obesity and insulin resistance. These data suggest that myostatin deficiency (increased muscle mass) is an excellent therapeutic target for treatment of obesity and insulin resistance.

5-4

Interleukin-6 protects against diet-induced insulin resistance independent of obesity

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The role of interleukin (IL)-6 in the aetiology of insulin resistance is controversial. While IL-6 knockout mice (IL-6-/-) develop mature-onset obesity (1), whether these mice develop insulin resistance is not known. Accordingly, IL-6-/- and littermate control (CON) mice were fed a standard chow or high fat diet (HFD) for 12 wk. Consistent with the previous report (1), IL-6-/- mice fed a chow diet developed mature-onset obesity, which was not associated with hyperphagia. In addition, IL-6-/- mice fed this diet displayed insulin resistance as indicated by increased ($P<0.05$) fasting insulin, and impaired glucose (AUC IP GTT= 1355 ± 51 vs 1601 ± 64 mmol/L for CON and IL-6-/- respectively; $P<0.05$) and insulin (AUC IP ITT= 981 ± 51 vs 1205 ± 57 mmol/L for CON and IL-6-/- respectively; $P<0.05$) tolerance. Obesity-induced insulin resistance in IL-6-/- mice was associated with inflammation, since phosphorylation of JNK and IKK were elevated ($P<0.05$) in both liver and skeletal muscle relative to CON. The HFD markedly increased body weight and fat mass in both IL-6-/- and CON, but no differences were observed when comparing strains. Despite equivalent body mass, IL-6-/- mice were insulin resistance relative to CON, indicated by fasting hyperinsulinemia and impaired insulin tolerance (AUC IP ITT= 1261 ± 62 vs 1441 ± 68 mmol/L for CON and IL-6-/- respectively; $P<0.05$). These data demonstrate that an absence on IL-6 in mice leads to obesity, inflammation and insulin resistance. Moreover, when metabolically challenged with a high fat diet, IL-6-/- mice display exacerbated insulin resistance relative to littermate control mice in the absence of any differences in body mass. Our data demonstrate, for the first time, that endogenous IL-6 protects against the development of insulin resistance *in vivo*.

1. Wallenius V, et al. Interleukin-6-deficient mice develop mature-onset obesity. Nat Med 8:75-79, 2002.

6-1

The metabolic effects of antipsychotic medication in the rat

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Growing lists of human epidemiological studies have reported substantial increases of type-2 diabetes in individuals with schizophrenia. However, it remains unclear if the increased risk of diabetes is of etiological significance to schizophrenia or an off-target effect of antipsychotic medication. Second generation or atypical (newer) antipsychotic medication can offer important health benefits to people with Schizophrenia. Although a large amount of research has been undertaken to identify the mode of drug action in the central nervous system, off target effects on peripheral organs (i.e. the liver, muscle, and adipose tissue) have not been fully characterised. We have studied the metabolic effects of a range of antipsychotic medication in the rat and have started to systematically characterise alterations in major signalling molecules that may underlie the phenotype we have observed.

6-2

Perilipin overexpression decreases adipocyte size and protects against diet-induced obesity and glucose intolerance

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Perilipin A (PeriA) is the most abundant phosphoprotein on adipocyte lipid droplets (LDs) and has a crucial role in lipid storage and lipolysis. Perilipin null mice exhibit diminished adipose tissue, elevated basal lipolysis, reduced catecholamine- stimulated lipolysis, and increased insulin resistance. To understand the physiological roles of perilipin *in vivo*, we generated transgenic mice that overexpressed either human or mouse perilipin using the adipocyte-specific aP2 promoter/enhancer.
Phenotypes of female transgenic and wild-type mice were characterized both on chow and high fat diets. Variables measured included: body weight, adipose depot size, adipocyte size, lipolysis and measures of glucose/insulin homeostasis.
When challenged with a high fat diet, body weight, fat mass, and adipocyte size were significantly reduced in both transgenic lines. In addition, expression of oxidative genes was increased and lipogenic genes decreased in the adipose tissue of transgenic mice. Furthermore, basal and catecholamine- stimulated lipolysis was decreased, and glucose tolerance was significantly improved in transgenic mice fed a high fat diet.
Perilipin overexpression in adipose tissue protects against high fat diet induced adipocyte hypertrophy and increased fat mass resulting in improved glucose tolerance. Our findings provide important new insights in the role of perilipin in regulating adipose tissue and systemic metabolism.

6-3

Caveolin, caveolae, cholesterol and signal transduction

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Caveolin-1 is a membrane protein essential for the formation of caveolae, cell surface pits abundant in fibroblasts, endothelial cells and adipocytes. Caveolin-1 expression is up-regulated during adipocyte differentiation implicating an adipocyte-specific function. A key role for caveolin-1 in obesity and diabetes is demonstrated in caveolin-1 deficient mice, which show resistance to diet-induced obesity and insulin-resistance. Caveolin-1 and caveolae are proposed to modulate signal transduction from the plasma membrane, however, the exact mechanism is not clear and seemingly contradictory functions have been reported. Since caveolin-1 binds cholesterol and caveolae are a type of cholesterol-rich lipid rafts, we hypothesized that signalling through caveolin-1/caveolae is dependent on cholesterol. Cholesterol depletion by methyl-β-cyclodextrin causes release of the caveolar coat protein cavin-1/PTRF, and flattening of the caveolae structure. However, cholesterol-depletion affects cholesterol-enriched lipid rafts that also mediate signal transduction. To distinguish between caveolin-1, caveolae and lipid raft- mediated signal transduction, we used two different model systems with methyl-β-cyclodextrin treatment. Caveolin-1 null mouse embryonic fibroblasts lack caveolin-1 and caveolae, while prostate cancer PC3 cells express abundant caveolin-1 but lack caveolae due to lack of cavin-1/PTRF. The effect of cholesterol depletion on signal transduction was compared to wild type mouse embryonic fibroblasts, or PC3 cells that express cavin-1/PTRF to reconstitute caveolae. Our data show that cholesterol depletion differentially affects signaling depending on caveolin-1/caveolae status of the cell.

6-4

Ghrelin Upregulates UCP-2 and Inhibits Insulin Secretion

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Background and Aim: Ghrelin, the only peripheral orexigenic hormone, has been reported to be related with obesity and diabetes. It was found in our laboratory that Ghrelin could inhibit glucose-stimulated insulin secretion (GSIS) in mouse insulinoma cell line MIN6 via induction of IA-2β (Doi A. et al. PNAS 103:885,2006.). Recent studies revealed that ghrelin could activate AMPK and UCP2 in some tissues, and that ghrelin's orexigenic action on hypothalamic neuron was mediated by UCP2. UCP2 is expressed in various tissues including pancreatic β cells. Higher expression of UCP2 will lead to inhibition of GSIS via decreased ATP production. In this study, we have investigated the mechanism of ghrelin's inhibitory effects on GSIS, especially paying attention to the involvement of AMPK or UCP2.
Materials and Methods: MIN6 cells were stimulated by ghrelin under different conditions and GSIS was measured. The phosphorylation of AMPK was analysed by Western blot, and the expression of UCP2, IA-2 or IA-2β was analysed by quantitative RT-PCR.
Results: Ghrelin increased AMPK phosphorylation and UCP2 expression under both low and high glucose condition. Ghrelin's inhibitory effects on GSIS were blocked by siRNA for UCP2, while overexpression of UCP2 inhibits basal insulin secretion and GSIS both with and without ghrelin. The under- or overexpression of UCP2 has no impact on IA-2β expression, nor does IA-2β on UCP2.
Conclusion: Ghrelin's inhibitory effects on GSIS were mediated by UCP2 at least partially, and this pathway might be independent to IA-2β pathway.

6-5

Suppression of PGC-1α (PPAR-gamma-coactivator-1α) normalizes the glucolipotoxicity induced decreased BETA2 gene transcription and improved glucose tolerance in diabetic rats

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PPAR-gamma-coactivator-1α (PGC-1α) is significantly elevated in the islets of animal models of diabetes. However, the molecular mechanism has not been clarified. We investigated whether the suppression of PGC-1α expression protects against β-cell dysfunction *in vivo* and determined the mechanism of action of PGC-1α in β-cells. The studies were performed in glucolipotixicity-induced primary rat islets and INS-1 cells. *In vitro* and *in vivo* approaches using adenoviruses were used to evaluate the role of PGC-1α in glucolipotoxicity-associated β-cell dysfunction. The expression of PGC-1α in cultured β-cells increased gradually with glucolipotoxicity. The overexpression of PGC-1α also suppressed the expression of the insulin and β-cell E-box transcription factor (BETA2/NeuroD) genes, which was reversed by PGC-1α siRNA. BETA2/NeuroD, p300-enhanced BETA2/NeuroD, and insulin transcriptional activities were significantly suppressed by Ad–PGC-1α but were rescued by Ad–siPGC-1α. PGC-1α binding at the glucocorticoid receptor (GR) site on the BETA2/NeuroD promoter increased in the presence of PGC-1α. Ad–siPGC-1α injection through the celiac arteries of 90% pancreatectomized diabetic rats improved their glucose tolerance and maintained their fasting insulin levels. The suppression of PGC-1α expression protects the glucolipotoxicity-induced β-cell dysfunction *in vivo* and *in vitro*. A better understanding of the functions of molecules such as PGC-1α, which play key roles in intracellular fuel regulation, could herald a new era of the treatment of patients with type2 diabetes mellitus by providing protection from glucolipotoxicity, which is an important cause of the development and progression of the disease.

6-6

In vitro Transdifferentiation of the Human Hepatocytes into Insulin-Producing Cells using Adenoviral transduction

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Hepatocytes are excellent candidates for generating beta-cell surrogates for autologous transplantation, because liver and pancreas share a common bipotential precursor cell within the embryonic endoderm so that transdifferentiation of hepatocytes to beta cells is easier than other germ-line cells. In this study, we try to transdifferentiate human hepatocytes cell line (HepG2) to insulin producing cells on the basis of our rodent data.

In our previous study, we induce transdifferentiation of mouse primary hepatocytes into insulin producing cells by adenovirally transduction of Ad-PDX-1/VP-16, BETA2 and MafA. HepG2 cells were cultured in low serum and high glucose containing cytokines such as HGF, EGF after adenoviral transduction and formed pseudo-islet clusters by suspension cultures. Gene expression was determined cells were stained for insulin antibody. Insulin contents were measured by radioactive-immunoassay kit.

Real time qPCR analysis data shows that albumin expression was significantly decreased in transduced HepG2 cells while insulin expression was strongly observed. Other pancreatic gene also induced in transduced HepG2 cells but not in control HepG2. Consistent with PCR data, insulin was found in adenovirus-transduced group in immunohistochemical staining, but not in control group. Also, glucose stimulated insulin secretion was significantly increased at the low level in the transduced cells.

In conclusion, we shows that transdifferentiation of human hepatocytes was induced by adenoviral transduction, which could provide basis of searching for transplantable beta cell source for the treatment of diabetes mellitus.

6-7

Genetically Engineered K-cells Express Glucose-dependent Insulin Secretion in Diabetic Mice

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Background: Type 1 diabetes mellitus is caused by destruction of the pancreatic beta cells. Expression of insulin in non-β-cells to surrogate β-cells has been studied for the treatment of type 1 diabetes. The strategy for achieving glucose-dependent insulin secretion in non-β-cells relies on glucose-responsive transcription of insulin mRNA and constitutive secretory pathway. K-cells have been introduced as an ideal target for insulin gene therapy. In this study, the murine enteroendocrine cell line (STC-1) was genetically modified and observed glucose-regulated insulin production in diabetic mice.

Methods: Rat-preproinsulin (PPI) expression cassette transcriptionally controlled by the promoter of glucose dependent insulinotropic peptide (GIPP) is fused to pCEP4 vector containing the origin of replication (oriP) and Epstein-Barr virus nuclear antigen 1 (EBNA-1). pGIPP/PPI/CEP4 was constructed and transfected into STC-1. Pure K-cell was isolated from the clonal expansion by hygromycin treated selection of STC-1. K-cells was concurrently transplanted under the kidney capsule of streptozotocin-induced diabetic mice and observed blood glucose levels. In addition, we did immunohistochemistry of graft-bearing kidney and pancreas.

Result: Blood glucose levels of K-cell-transplanted diabetic mice decreased gradually to a normal range by 2 weeks after transplantation. Body weights of K-cell-transplanted diabetic mice increased after transplantation, whereas those of untreated control diabetic mice continued to decline. When glucose was injected intraperitoneally into K-cell-transplanted diabetic mice, blood glucose levels was increased at 30 minutes, and was restored to the normal range between 60 and 90 minutes after injection, although untreated control diabetic mice consistently showed hyperglycemia. To examine whether the transplanted K-cells produce insulin, kidney capsules containing the transplanted cells were removed, and sections were stained with anti-insulin antibody. We found insulin-positive cells in the kidney capsule from K-cell-transplanted diabetic mice.

Conclusion: We identified genetically engineered K-cells expressing glucose-dependent insulin secretion in diabetic mice. These results suggest that genetically modified insulin producing K-cells may act as surrogate β-cells to treat type 1 diabetes.

6-8

Dysregulated glutathione metabolism links to impaired insulin action in adipocytes

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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. However, the role of GPx in adipocytes is not elucidated. The objective of this study was to clarify the pathophysiological changes in GPx activity and glutathione metabolism, and their roles in the pathogenesis of insulin resistance in adipocytes. 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 (GPx1, 4 and 7), 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.

6-9

Genetic variants associated with obesity and obesity-related diabetes mellitus in Southern Chinese

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Introduction: The prevalence of obesity is growing in epidemic proportion, and is becoming a global health burden due to associated co-morbidities such as type 2 diabetes (DM) and cardiovascular diseases. Recent genome-wide association studies have identified genetic variants close to the fat mass- and obesity-associated gene *(FTO), NEGR1, SEC16B-RASAL2, TMEM18, SFRS10-ETV5-DGKG, GNPDA2, NCR3-AIF1-BAT2, LGR4-LIN7C-BDNF, MTCH2, BCDIN3D-FAIM2, SH2B1-ATP2A1, KCTD15,* and *MC4R* to be associated with obesity. However, these genome-wide association studies, as well as most replication studies involved subjects of Caucasian descent, and the implications of these variants with obesity in Chinese are not known. We have therefore examined the associations of the above genetic variants with obesity in a pilot case-control study and subsequently in a Southern Chinese population-based cohort under prospective follow-up. Furthermore, we also examined the associations of the *FTO* SNPs with DM in a subgroup of the population-based cohort.

Subjects and Methods: The pilot case-control study consisted of 150 obese subjects (BMI>30kg/m2) and 400 lean subjects (BMI<23kg/m2). The population-based cohort examined for association with body mass index (BMI) involved subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS). The subgroup examined for association of DM consisted of subjects with persistent normal glucose tolerance over a 6.4-year follow-up and subjects with type 2 diabetes from CRISPS, as cases and controls, respectively.

Results: In the pilot study, variants rs7647305, rs10938397, rs16952522, rs8050136, rs17782313, rs29941 were significantly associated with obesity. Furthermore, rs10938397, rs8050136 and rs17782313 were also significantly associated with BMI in the CRISPS cohort at both baseline and 6.4-year follow-up. The *FTO* SNPs were further examined for association with DM. Both rs16952522 and rs8050136 were significantly associated with DM, but the effects were ameliorated after adjustment for BMI. However, in an obese subcohort, the associations with DM were more prominent and remained significant after adjustment for age, sex and BMI.

Conclusions: Genetic variants, rs10938397, rs8050136 and rs17782313, of the *GNPDA2, FTO* and *MC4R* genes respectively, appeared to influence body weight in our population and may play a role in the predisposition to obesity in Southern Chinese. As the risk for DM is high in obese subjects, the role of *FTO* variants in the predisposition to obesity-related DM deserves closer attention.

6-10

Murine gamma herpes virus 68 infection promotes fatty liver and hepatic insulin resistance in C57BL/6J mice

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Murine gamma herpes virus 68 (MHV68) is a naturally occurring mouse pathogen that is homologous to both human herpes virus 8 and Epstein-Barr virus. This study was designed to determine the correlation between MHV68 infection and fatty liver/hepatic insulin resistance in C57BL/6J mice and to explore the underlying mechanisms.

Female C57BL/6J mice fed a high fat diet were randomly assigned to receive either MHV68 or PBS treatment. Glucose tolerance tests were performed to test insulin sensitivity of the animals. Serum was analyzed for lipids and cytokines. Liver was taken for histology and lipid analysis. Quantitative reverse transcription polymerase chain reaction and western blotting were used to measure the mRNA and protein levels of hepatic mammalian target of rapamycin (mTOR), ribosomal S6 kinase 1 (S6K1), insulin receptor substrate-1 (IRS-1), sterol regulatory element binding protein-1 (SREBP1), fatty acid synthase (FAS), acetyl CoA carboxylase (ACC).

Our data showed MHV68 infection promoted fatty liver, hypertriglyceridemia, insulin resistance, and hyperinsulinemia in association with elevated inflammatory cytokines. In the liver of MHV68 infected C57BL/6J mice, SREBP1, FAS, ACC expression was increased. MHV68 infection also inhibited total IRS-1 expression and increased serine phosphorylated IRS-1, which is correlated to the over activation of mTOR signaling pathway. Sirolimus, a specific inhibitor of mTOR, inhibited the MHV68-induced hepatic expression of the serine phosphorylated IRS-1 and increased the levels of total IRS-1, improved the MHV68-induced glucose intolerance.

Conclusion: In C57BL/6J mice, MHV68 infection promotes fatty liver and hepatic insulin resistance in which the mTOR pathway is involved.

6-11

Beneficial effects of acute Inhibition of hepatic S6K1 signaling in obese animals

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Although the mechanism by which obesity triggers insulin resistance is not fully understood, evidence suggests that S6 Kinase 1 (S6K1) contributes to the pathogenesis of this process. To evaluate potential therapeutic utility of the inhibition of S6K1 signaling, we attempted to acutely reduce the expression S6K1 in the liver of obese insulin resistance animals. Intravenous injection of an adenovirus vector encoding short haipin (sh) RNA of S6K1 (shS6K1) in db/db mice resulted in a decrease in the hepatic abundance of S6K1 mRNA by ~70%. This treatment increased the expression IRS1 and IRS2 proteins in the liver, in consistent with a notion that S6K1 contribute to the development of insulin resistance through affecting the abundance of IRS proteins. Hepatic expression of mRNA for gluconeogenic genes including G6Pase and PEPCK was suppressed by shS6K1, probably reflecting the amelioration of insulin resistance. Interestingly, hepatic lipid content in db/db mice injected with shS6K1 was less than that in control animals. The inhibition of S6K1 signaling in the liver is thus of potential therapeutic utility for obesity-related health disorders.

6-12

Oxidative Stress Induced by Inhibition of Fatty Acid Synthesis in Liver Promotes Insulin Resistance through Phosphorylation of IRS1 by Erk1/2

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Persistent hepatosteatosis due to high-calorie diets and/or hyperphagia are often associated insulin resistance and subsequent type 2 diabetes. The development of hepatosteatosis is induced by increased activity of several lipogenic factors including fatty acid synthase (FAS). Thus, the inhibitors of FAS are expected as therapeutic tools for obesity-related diseases. In order to analyze specific roles of FAS in developing hepatosteatosis and insulin resistance, we generated liver-specific FAS knockout ($L\_FAS^{-/-}$) mice. Although this knockout reduced triglyceride accumulation in liver of mice on a high-sucrose, high-fat (HSHF) diet or in leptin-deficient *ob/ob* background, it enhanced hepatic insulin resistance with decreased expression of insulin receptor substrate (IRS)-1 and IRS-2. In the liver of these mice, markers of oxidative stress such as protein carbonylation, expression of p53 and its regulated genes, and stress-related extracellular signal-regulated kinase (Erk) 1/2 activity were increased. The study using rat hepatoma FAO cells revealed treatment by FAS inhibitor C75 induced insulin resistance due to, at least in a part, oxidative stress-induced activation of Erk 1/2 and subsequent phosphorylation on Ser612 of IRS-1. Thus, oxidative stress induced by depletion of FAS products in liver enhanced insulin resistance despite amelioration of hepatosteatosis in mice. Our data raises a possibility that usage FAS inhibitors as therapeutic tools for obesity-related diseases induce oxidative stress and subsequent insulin resistance in liver during abundant carbohydrate consumption.

6-13

PDK1-FoxO1 in AGRP neurons regulates energy homeostasis by modulating food intake and energy expenditure

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The hypothalamic insulin receptor substrate-phosphatidylinositol-3-OH kinase pathway is one of several points of convergence and synergy in intracellular signaling pathways used by both insulin and leptin. Although 3-phosphoinositide-dependent protein kinase 1 (PDK1) in POMC neurons is known to regulate Pomc expression, little is known about the role of PDK1 in AgRP neurons for energy homeostasis. To address this issue, we generated AgRP neuron-specific PDK1 knockout (*AgRPPdk1*$^{-/-}$) mice. *AgRPPdk1*$^{-/-}$ mice showed decreased food intake and body weight with increased leptin sensitivity and an attenuated response to ghrelin. *AgRPPdk1*$^{-/-}$ mice were protected against high-fat diet-induced obesity due to decreased food intake, increased energy expenditure and increased locomotive activity. Selective expression of transactivation-defective FoxO1 (•256FoxO1) reversed the hypothalamic phenotype of *AgRPPdk1*$^{-/-}$ mice due to increased food intake and decreased locomotive activity. These data suggest that the signaling pathways through PDK1 and FoxO1 in AgRP neurons play important roles in energy homeostasis.

6-14

Quantitative proteomic research and integrative analysis of endogenous S-nitrosated proteins in diabetic mouse liver

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A body of evidence shows that S-nitrosylation plays important roles in cell signaling and disease. However, present methods do not provide an adequate quantitative integrative description of endogenous multiply S-nitrosylated proteins and their relationship with disease. In this study we developed a quantitative proteomic method named ICAT-switch by introducing isotope-coded affinity tag (ICAT) reagents into a biotin-switch, and used it to study S-nitrosylation in the liver of normal control C57/BL6 mice and type 2 diabetic KK-Ay mice. Sixty-eight proteins were identified as S-nitrosylation targets showing quantitative variation, some of which, including insulin-like growth factor binding protein 6, Arg/Abl-binding protein, hepatocyte pterin 4 alpha carbinolamine dehydratase/dimerization cofactor, pantophysin, aminolevulinate delta dehydratase, and aldo-keto reductase family 1 member C13 were shown to be S-nitrosylated for the first time and are known to be related with diabetes. Gene ontology enrichment results suggested that S-nitrosylated proteins are more abundant in amino acid metabolic processes. The network constructed for S-nitrosylated proteins by text-mining technology provided clues about the relationship between S-nitrosylation and diabetes. Our work provides a new approach for quantifying endogenous multiply S-nitrosylated proteins and investigating the dynamics of S-nitrosylation, and suggests that it is important to elucidate the integrative functions of S-nitrosylation in pathophysiological processes.

6-15

Common PCSK1 Haplotypes are Associated with Obesity and Modulate Body Mass Index in the Chinese Population

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Objective: *PCSK1* genetic polymorphisms have recently been associated with obesity in European populations. This study aimed to examine whether common *PCSK1* genetic variation is associated with obesity and related metabolic phenotypes in the Chinese population.

Methods: We genotypes 9 common tag single nucleotide polymorphisms (tagSNP) of the *PCSK1* gene in 1,094 subjects of Chinese origin from the Stanford Asia-Pacific Program for Hypertension and Insulin Resistance (SAPPHIRe) family study.

Results: Two SNPs in the *PCSK1* gene were nominally associated with risk of obesity in the SAPPHIRe cohort (P=0.01 and 0.04). A common protective haplotype in the *PCSK1* gene was strongly associated with reduced risk of obesity (22.99 % vs. 35.04 %, P=0.006), lower body mass index (BMI) (24.44±3.52 kg/m^2 *vs.* 25.61±3.65 kg/m^2, P=0.01), and smaller waist circumference (91.93±10.58 cm vs. 85.64±10.56 cm, P=0.006). Another common risk haplotype was associated with higher risk of obesity (38.75 % *vs.* 24.19 %, P=0.002). The global P-value for haplotype association with obesity was 2 x10^{-4}. We also identified a suggestive association of another *PCSK1* haplotype with fasting plasma glucose, insulin, triglycerides, high-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, and homeostasis model assessment of insulin resistance (HOMA-IR) (P=0.04, 0.03, 0.02, 0.04 and 0.02, respectively).

Conclusions: These data indicate common *PCSK1* genetic variants are associated with obesity and BMI in the Chinese population.

6-16

Identification of specific actin filaments that regulate glucose clearance, insulin sensitivity and insulin secretion

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The actin cytoskeleton is known to play critical roles in both the trafficking of GLUT4 to the plasma membrane and the release of insulin. We have identified novel actin cytoskeletons defined by the cytoskeletal tropomyosin (Tm) isoform, Tm5NM1. Immunoprecipitation and immunolocalisation studies indicate that Tm5NM1 interacts with syntaxin-4, a protein necessary for GLUT4 insertion into the plasma membrane. To understand the function of these actin/Tm filaments we have created tissue-wide Tm5NM1 transgenic (Tg) and knock-out (KO) mice. Glucose and insulin tolerance tests revealed that the Tg mice have increased glucose clearance in part due to increased insulin sensitivity. However, insulin caused a similar increase in Akt phosphorylation (Ser 473), the major upstream regulator of GLUT4 translocation, in skeletal muscle and adipose tissue from both Tg and KO mice. This suggests that Tm5NM1 is acting downstream of insulin signalling. Gene expression analysis on adipose tissue from the Tg mice detected an increase in expression of genes involved in GLUT4 trafficking and actin filament turnover. This suggests that Tm5NM1 is impacting on the GLUT4 trafficking pathway to increase glucose clearance in these mice. Further experiments on pancreatic islets isolated from the Tm5NM1 Tg mice revealed that in addition to enhanced insulin sensitivity these animals also displayed an increase in basal insulin secretion. In conclusion, we have identified novel actin/Tm filaments that may regulate glucose uptake and insulin secretion. Thus, the cytoskeletal Tms represent novel therapeutic targets for conditions of altered glucose uptake and insulin secretion, such as Type 2 diabetes and obesity.

7-1

Increasing fat oxidation in rats via acute activation of AMPK does not alter energy expenditure

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To investigate the relationship between fat oxidation and energy expenditure we examined whole-body energy metabolism in rats treated with compounds that stimulate fat oxidation via activation of the AMP-activated protein kinase pathway. Whole body energy expenditure (VO_2) and substrate oxidation (respiratory exchange ratio [RER]) were measured using an Oxymax indirect calorimetry system. Rats were dosed at 10am with vehicle (saline, n=9), metformin (500mg/kg, n=8), AICAR (250mg/kg, n=9) or the mitochondrial uncoupler, dinitrophenol (DNP; 30mg/kg, n=5). Other cohorts of similarly treated animals were sacrificed at 30, 60 and 90 minutes after drug administration to measure tissue metabolites and protein phosphorylation. All drugs increased the phosphorylation of AMPK and ACC and reduced the amount of malonyl CoA in muscle. In the 8-hours after dosing, rats treated with metformin, AICAR and DNP all displayed increased fat oxidation (RER 0.87-0.88, $P<0.001$) compared with vehicle-treated animals (RER 0.92). DNP was the only intervention that increased VO_2 (2058±28 ml/kg/hr, $P<0.001$) compared with vehicle-treated animals (1654±21 ml/kg/hr), with no difference observed for metformin or AICAR (1619±23 and 1681±20 ml/kg/hr respectively). To test if increased fat oxidation was associated with a decrease in oxidation of other substrates, palmitate and glucose oxidation was examined in isolated EDL muscle treated *ex vivo* with AICAR (2mM) or DNP (0.5mM). AICAR increased palmitate oxidation (+45%, $P<0.001$), but decreased glucose oxidation by 28% ($P<0.01$). In contrast DNP treatment caused a significant increase in the oxidation of both palmitate (+41%, $P<0.01$) and glucose (+77%, $P<0.01$). These results suggest that in the absence of any change in energy demand, acute increases in fat oxidation caused by activation of AMPK are accompanied by a concomitant decrease in the oxidation of other substrates, and not a change in energy expenditure.

7-2

Selective inactivation of c-Jun NH2 terminal kinase (JNK) in adipose tissue is sufficient to alleviate metabolic disorders associated with dietary obesity in mice

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Inflammation in adipose tissue has recently been proposed as a key mediator that links obesity and its related metabolic complications. The activity of c-Jun NH2 terminal kinase (JNK), an important player in inflammation, is markedly elevated in adipose tissue of obese rodents. However, the role of JNK activation in the initiation and/or perpetuation of adipose tissue inflammation and insulin resistance remains to be defined. To address this question, we have generated a transgenic mouse model with adipose tissue-specific expression of dominant negative (DN) JNK, and compared the phenotypic changes between the transgenic mice and their wild type littermates. Under the standard chow, there is no obvious difference in body weight gain, insulin sensitivity, and glucose and lipid profiles between the two groups of mice. By contrast, the DN JNK transgenic mice are partially protected from developing high fat diet-induced obesity, as evidenced by the decreased size of adipocytes in each anatomical location of fat tissues. More remarkably, the transgenic mice display an almost complete resistance to high fat diet-induced glucose intolerance, hyperglycemia, hyperinsulinemia as well as insulin resistance, and these metabolic changes are accompanied by a markedly decreased macrophage infiltration in adipose tissues, reduced production of pro-inflammatory adipokines and increased expression of adiponectin. In the liver tissue, high fat diet induces severe steatosis and increases the expression of gluconeogenic genes in wild type mice, whereas these pathological changes are almost undetectable in the DN JNK transgenic mice. These results suggest that selective inactivation of JNK alone is sufficient to counteract high fat diet-induced obesity and protect against its associated metabolic dysregulation in mice. The detailed mechanism whereby JNK inactivation decreases high fat diet-induced obesity is currently under active investigation in our laboratory.

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7-3

The size of lipid droplets

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Obesity is characterized by accumulation of adipocytes loaded with lipid droplets (LDs). By reverse genetic screening in yeast, we have previously identified a large number of gene products that regulate the size and number of cellular lipid droplets. In particular, we demonstrate that deletion of a previously uncharacterized gene, FLD1, results in the formation of "super-sized" lipid droplets (>50 times the volume of normal ones). We also provide evidence that Fld1p plays a role in regulating the metabolism of fatty acids and phospholipids, and that deletion of FLD1 leads to enhanced fusion of LDs. Interestingly, the mammalian homolog of Fld1p is seipin, whose malfunction causes the most severe form of human lipodystrophy.

As the size of LDs appears to be an important factor in determining the rate and location of lipolysis and the oxidation of fatty acids, we have recently identified additional yeast mutants that give rise to "super-sized" lipid droplets and characterized the role of these mutants in lipid metabolism. We have also extended our study to mammalian cells and examined the role of seipin and the newly discovered genes in the fusion of LDs. Our results suggest an important role for phospholipids in determining the size of cellular LDs.

7-4

IRE-1 and HSP-4 play key roles in energy homeostasis via novel fasting-induced lipases in *C. elegans*

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The endoplasmic reticulum (ER) is an organelle associated with lipid metabolism. However, the involvement of the ER in nutritional status-dependent energy homeostasis is largely unknown. Here we show that IRE-1, an ER protein involved in the unfolded protein response, and HSP-4, an ER chaperone, regulate the expression of novel adipose triglyceride lipase (ATGL)-related fasting-induced lipases, FIL-1 and FIL-2, for fat granule hydrolysis upon fasting in *C. elegans*. Interestingly, failure of *ire-1* and *hsp-4* mutant animals to hydrolyze intestinal fat granules during starvation impaired their motility, whereas glucose supplementation rescued the motility defect, implicating the importance of *ire-1/hsp-4*-dependent lipid homeostasis for energy supply from stored fat droplets during fasting. Taken together, our data suggest that the ER resident proteins IRE-1 and HSP-4 are key nutritional sensors that modulate expression of inducible lipases to maintain energy homeostasis.

7-5

Adipogenesis licensing and execution are disparately linked to cell proliferation

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The coordination of cell differentiation and proliferation is a key issue in the development process of multi-cell organisms and stem cells. Here we have provided evidence that the establishment of adipocyte differentiation of 3T3-L1 cells requires two processes: the licensing an adipogenesis gene-expression program within the contact-inhibition stage, and then the execution of this program in a cell-cycle-independent manner, by which the licensed progenitors are differentiated into adipocytes in the presence of inducing factors. The results showed that the differentiation licensing of 3T3-L1 cells during the contact-inhibition stage was mainly involved in epigenetic modifications such as DNA methylation or histone modifications, while the disturbance of these epigenetic modifications by DNA methylation inhibitors or RNAi transfection during the contact-inhibition stage significantly reduced adipogenesis efficiency. More importantly, if these licensed 3T3-L1 cells were re-cultured under undifferential conditions or treated only with insulin, this adipogenesis commitment could be maintained from one cell generation to the next, whereas the licensed program could be activated in a cell-cycle-independent manner once these cells were subjected to adipogenesis-inducing conditions. This result suggests that differentiation licensing and differentiation execution can be uncoupled and disparately linked to cell proliferation. Our findings deliver a new concept that the cell-fate decision can be subdivided into at least two stages, licensing and execution, which might have different regulatory relationships to cell proliferation. In addition, this new concept may provide a clue for developing new strategies against obesity.

8

Harnessing the power of stem cells to cure obesity and diabetes

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White adipose tissues (WAT) protect against traumatic and thermal insults and regulate metabolism and lifespan. Mammalian adipocytes develop, in coordination with the vasculature, in a burst during the post-natal period and throughout life. However, little is understood about the identity and anatomical location of adipocyte progenitors *in vivo*. Studying genetically marked mice, we identified and isolated proliferating adipogenic progenitors located in the adipose stromal-vascular (SV) compartment. The majority of adipocytes descend from a pool of these proliferating precursors already committed early in postnatal life. These progenitors reside in the mural cell compartment of blood vessels that supply adipose depots but not in vessels of other tissues. Thus, the adipose vasculature appears to function as a progenitor niche and may provide signals for adipocyte development.

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9-1

APPL1 enhances Insulin Sensitivity by counteracting the effect of the endogenous Akt inhibitor Tribble-3

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APPL1, an endosomal adaptor protein containing an NH2-terminal Bin/Amphiphiphysin/Rvs (BAR) domain, a central pleckstrin homology (PH) domain and a COOH-terminal phosphotyrosine binding (PTB) domain, was originally identified as an interacting partner of Akt in a yeast two-hybrid assay using Akt2 as a bait. However, the precise role of APPL1 in modulating Akt activity remains unclear. Here, we provide both ex vivo and in vivo evidence demonstrating that APPL1 is an important regulator of insulin-evoked Akt signaling cascade and glucose metabolism. In primary rat hepatocytes, insulin-stimulated activation of Akt and suppression of glucose production are enhanced by APPL1 overexpression, but are attenuated by APPL1 knockdown. In mice, down-regulation of APPL1 impairs insulin sensitivity and glucose tolerance, whereas adenovirus-mediated overexpression of APPL1 markedly attenuates diabetic phenotypes associated with both dietary and genetic obesity. In addition, transgenic mice with overexpression of APPL1 display markedly elevated Akt activation and glucose uptake in skeletal muscle upon insulin stimulation. APPL1 interacts with Akt and blocks the association of Akt with its endogenous inhibitor tribble 3 (TRB3) through direct competition. Overexpressing of TRB3 arrests Akt within the cytoplasm and prevents insulin-induced membrane translocation of Akt. By contrast, overexpression of APPL1 releases cytosolic Akt trapped by TRB3 and promotes Akt translocation to the plasma membrane and the endosomes for further activation. Taken in conjunction, these data suggest that insulin-mediated Akt activation is finely tuned by APPL1 and TRB3 through spatial regulation.
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9-2

Role of CREBH in the regulation of hepatic gluconeogenesis

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Endoplasmic reticulum-resident transcription factor families are involved in the control of various energy homeostasis. In this study, we report a novel role of ER-bound factor, CREBH, in the control of hepatic gluconeogenesis. CREBH is transcriptionally induced under fasting or by insulin resistance in a dexamethasone/PGC-1•-dependent manner. Hepatic expression of active CREBH induces transcription of PEPCK or G6Pase gene by binding to its enhancer site that is distinct from cAMP response element or NR binding sequences *in vivo*. Furthermore, depletion of hepatic CREBH reduces blood glucose levels significantly without altering expression of genes involved in the ER stress signaling cascades both in wild type and *db/db* diabetic mice. These data suggest a novel role for CREBH as a regulator for hepatic glucose metabolism in mammals.

9-3

Regulation of hepatic glycolysis by FoxO1 via ChREBP O-glycosylation

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Type 2 diabetes is characterized by insulin resistance and reduced glucose effectiveness. It's unclear whether the two mechanisms share a common pathogenesis. Hyperglycemia, acting through the hexosamine pathway, promotes O-glycosyltransferase (OGT)-dependent protein O-glycosylation. We show that carbohydrate response element binding protein (ChREBP), a glucose-regulated transcription factor, is O-glycosylated by OGT, leading to increased ChREBP levels, owing to decreased ubiquitination. Conversely, expression of O-GlcNAcase (GCA) in hepatocytes decreases ChREBP O-glycosylation, thus reducing its protein levels. Moreover, ChREBP O-glycosylation is regulated by fasting (low) and re-feeding (high) in mouse liver. The nutritional regulation of ChREBP O-glycosylation is impaired in livers of high fat-fed and db/db mice. We have also previously reported that FoxO1 controls hepatic glucose metabolism via the regulation of glycolytic (L-PK) and lipogenic (ACC) genes, two critical ChREBP target genes. We show here that FoxO1 overexpression inhibits ChREBP O-glycosylation in hepatocytes, thus reducing ChREBP levels. Conversely, conditional FoxO1 knockout in liver results in increased levels of O-glycosylated ChREBP, even in the fasted state. Chromatin immunoprecipitation reveal that recruitment of ChREBP to the L-PK promoter is suppressed by FoxO1, and increased in liver-specific FoxO1 knockout mice in both fasted and fed conditions. Taken together, our results suggest that O-glycosylation is an important mechanism to regulate ChREBP function, and that FoxO1's effects on glucose metabolism is mediated by ChREBP O-GlcNAc modifications. We propose that FoxO1 is the shared signaling element linking glucose- and insulin-activated pathways to regulate hepatic glucose metabolism. FoxO1 inhibition may increase glucose effectiveness.

9-4

Hepatic fructose-1, 6-bisphosphatase: a novel nutrient sensor

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The biochemical mechanisms involved in nutrient sensing and body weight regulation are not completely understood. Fructose-1,6-bisphosphatase (FBPase) is a regulatory enzyme in gluconeogenesis that has been shown to be up-regulated in the liver by obesity and fat. We have previously generated liver-specific FBPase transgenic mice with a 3-fold over-expression in the liver. These mice are 10% leaner than the negative littermates. We therefore proposed that hepatic FBPase may have an important role in body weight regulation. To investigate this further energy balance parameters including food intake, voluntary physical activity, resting energy expenditure (REE) and fat-pad masses were measured in transgenic and negative littermates. Circulating gut hormones, neuropeptide levels and fatty acid oxidation (FAO) parameters were also measured. Hepatic branch vagotomies and specific FBPase inhibitor intervention studies were also performed. The results showed that transgenic mice ate significantly less by 15% and had smaller fat-pad masses compared with negative littermates ($p<0.005$) with no differences in energy expenditure levels (activity or REE). This was associated with elevated FAO, increased levels of carnitine palmitoyltransferase-1a mRNA and increased concentrations of circulating cholecystokinin (CCK), gastrin and leptin. Hypothalamic AgRP and NPY mRNA were reduced in the transgenics ($p<0.05$). Both hepatic branch vagotomy and direct inhibition of FBPase in the transgenics normalised food intake, body weight and hypothalamic AgRP and NPY expression to those of the wild-type. Therefore, this data demonstrates that hepatic FBPase may be a novel nutrient sensor in regulating food intake as over-expression increases FAO and plasma CCK, gastrin and leptin and suppresses hypothalamic orexigenic neuropeptide levels.

9-5

Development of a Systemic and Targeted Screening Process for the Discovery of New Antidiabetic Molecules Derived from Traditional Chinese Medicines

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Natural products are a rich source for the discovery and development of new drugs. We have developed a highly targeted screening process to discover new antidiabetic agents derived from traditional Chinese medicine (TCM) natural products. Our studies focus mainly on the treatment of insulin resistance, a major metabolic defect of type 2 diabetes (T2D).

Our process started with evaluation of the TCM literature for implicated benefits for T2D and extensive search for reported hints linked to metabolic syndrome. Selected TCM ingredients were first tested in mice, where applicable, for their metabolic effects (eg GTT, VO_2 and RER) and the data were also used to guide the extraction of active compounds. Isolated compounds were then screened for their activity to stimulate GLUT4 translocation and glucose uptake in L6 myotubes or 3T3-L1 adipocytes. If necessary, derivatives were made from active compounds to improve the efficacy or drug-like properties. Potential leads were selected and measured their effects on PI3K/Akt and AMPK in those cells to determine the signaling pathway mediating the above effects. Compounds of interests were also used to identify biological targets. Finally, leads were selected and investigated for their efficacy in insulin resistant animal models and detailed mechanisms involved.

Using this systemic and targeted process we have identified two promising classes of compounds, berberines and cucurbitane triterpenoids. Both possess potent antidiabetic properties and activate AMPK but by different mechanisms. Berberine and its derivative dihydroberberine activate AMPK by inhibiting mitochondrial Complex 1 in a manner similar to metformin and rosiglitazone. Despite their potent efficacy on AMPK and GLUT4 translocation, however, the newly identified triterpenoids extracted from bitter melon have no effects on mitochondrial respiration, suggesting them as a new class of AMPK activators. Currently, studies are underway to identify the primary targets for these antidiabetic compounds and evaluate their roles in the original TCM for the treatment of T2D. Our results demonstrate that the targeted screening process we have developed is an effective approach to explore TCM for the discovery and development of new antidiabetic compounds.