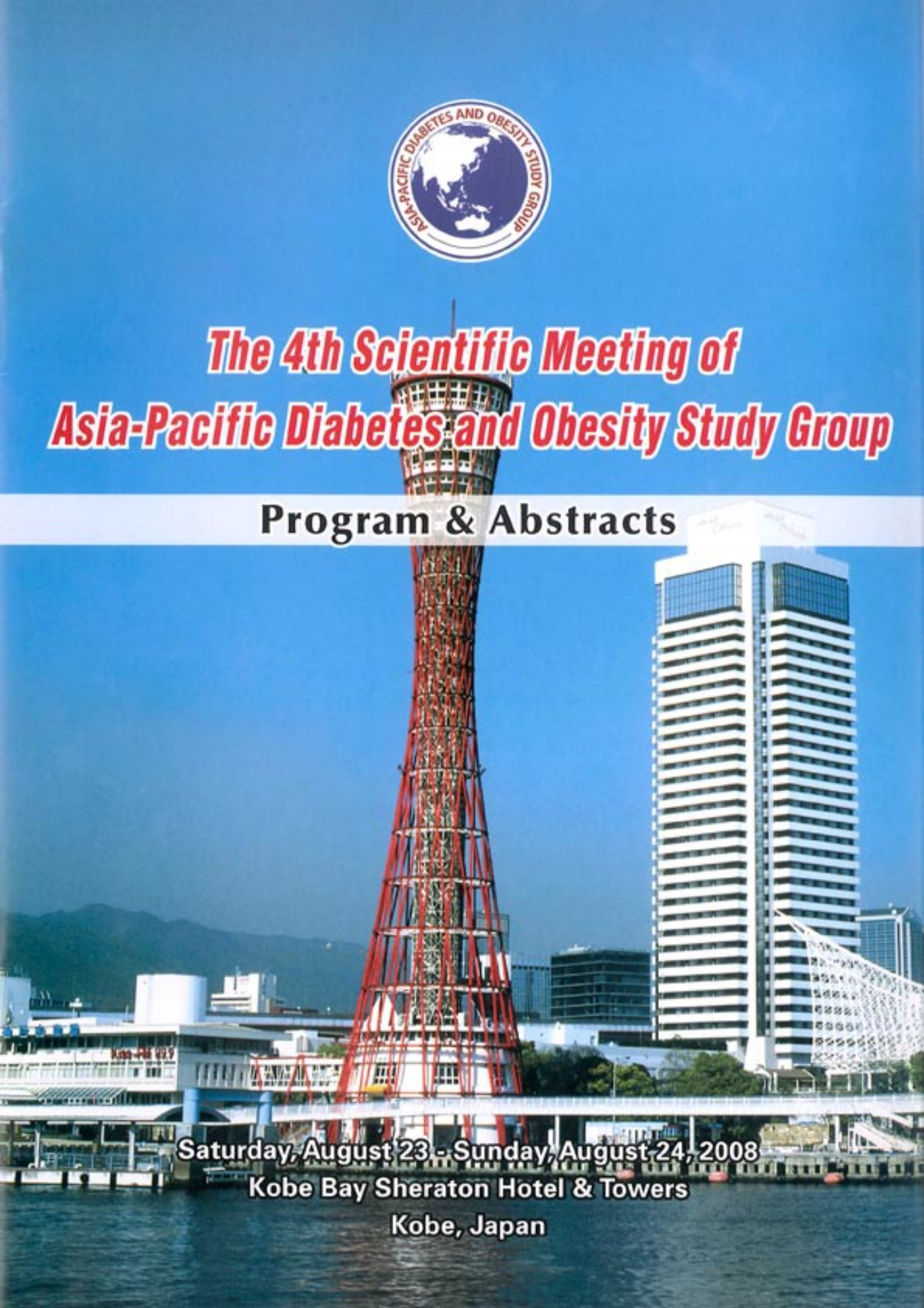


The 4th Scientific Meeting of Asia-Pacific Diabetes and Obesity Study Group

Program & Abstracts

Saturday, August 23 - Sunday, August 24, 2008

Kobe Bay Sheraton Hotel & Towers

Kobe, Japan

The 4th Scientific Meeting of Asia-Pacific Diabetes and Obesity Study Group

Program & Abstracts

Welcome Massage

It is a great pleasure for us to extend a warm welcome to all participants of the 4th Scientific Meeting of the Asia-Pacific Diabetes and Obesity Study Group. The purpose of this scientific meeting is twofold: firstly to bring together basic scientists, clinicians and educators interested in diabetes to report on the most recent advances in their areas of research and clinical work, and secondly to promote active participation and more collaboration amongst all those actively working in the field of diabetes throughout the Asian-Pacific region.

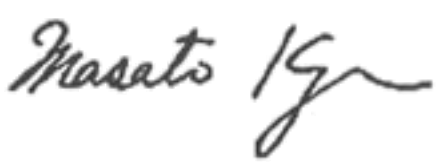
The Organizing Committee is confident that the format, content and exceptionally high level of presentations planned for this 4th meeting will continue the high standards that have been the hallmark of the three previous very successful meetings. The impressive background of scientific, clinical, and educational reports presented in the earlier programs has resulted in the APDO Scientific Meeting being increasingly recognized as an important and not-to-be-missed educational and social event. We feel sure that this will ultimately result in helping us achieve our goal of promoting diabetes awareness and optimal clinical management throughout the Asian region. With this in mind we have developed a program that continues to shed light on the most important issues confronting all working in the field of diabetes. While it is unfortunate that the incidence of diabetes continues to grow, we are fortunate in having a committed group of doctors, researchers and diabetes workers who recognize this problem and have devoted their time and abilities to halting this spread.

Highlighting the increasing depth of this meeting is the fact we have presentations from experts representing all corners of the Asia-Pacific region. The program begins with the APDO Study Group lecture, which will be presented by Kazuki Yasuda, Japan, reporting important genetic research into diabetes susceptibility. Toward the end of the meeting we are fortunate to hear from Mark Febbraio, Australia, who will present the APDO Special Lecture on hierarchical regulation of inflammation induced insulin resistance. Between these sessions, participants will be able to choose from a wide array of interesting oral and poster presentations covering a diverse and informative range of topics.

As part of the experience of attending this meeting, we pride ourselves on developing a relaxed and informal atmosphere, in which all participants feel comfortable to mix and talk about all issues relevant to diabetes. Please take this opportunity to discuss findings with presenters, as it is through ongoing dialogue that further developments as well as national and international relationships are built. Our goal is that by the end of the meeting all participants will have added to their diabetes knowledge and that they will then return to hospitals and research facilities throughout the Asian-Pacific region with added enthusiasm and skills to be used to fight this deadly disease. Please help us achieve this goal.

David E James, BSc, PhD

Professor and Director
Diabetes & Obesity Research Program
Garvan Institute of Medical Research
Sydney, Australia



Masato Kasuga, MD, PhD

Director-General
Research Institute
National Medical Center of Japan
Tokyo, Japan

GENERAL INFORMATION

● DATES

Saturday, August 23- Sunday, August 24, 2008

● VENUE

Kobe Bay Sheraton Hotel & Towers
2-13 Koyocho-naka, Higashinada-ku, Kobe 658-0032 Japan
Tel: +81-(0)78- 857-7000

● 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 23 and dismantle it after the reception.
2. Remaining posters will be taken away by the secretariat.

PROGRAM

August 23

◆PROGRAM◆

Saturday, August 23

13:00-13:10 ■ Opening Remarks

David James, *Australia*

13:10-14:00 ■ Session 1: APDO Study Group Lecture

- chair: **David James**, *Australia*

The hunt for type 2 diabetes susceptibility genes in Japanese
Kazuki Yasuda, *Japan*

14:00-15:15 ■ Session 2: Oral presentation

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

- co-chairs: **Haruhiko Osawa**, *Japan*
  Gregory Cooney, *Australia*

[Oral 2-1] Common Variation in the *FTO* Gene Confers Risk of Obesity and Modulates Body Mass Index in the Chinese Population
Yi-Cheng Chang, *Taiwan*

[Oral 2-2] Common variants in *GCKR* and *GCK* genes were associated with type 2 diabetes, obesity and their related traits in a Chinese Han population
Xu Lin, *China*

[Oral 2-3] Structural Mechanisms underlying the Inhibitory Effects of Angiopoietin-like Protein 4 on Lipoprotein Lipase Activities
Yu Wang, *Hong Kong*

[Oral 2-4] Deletion of AMPK β1 enhances hepatic insulin sensitivity and prevents high-fat diet induced obesity
Gregory R Steinberg, *Australia*

[Oral 2-5] Heme Oxygenase-1 overexpression prevents endoplasmic reticulum stress by high glucose in INS-1 cells
Kyu Chang Won, *Korea*

15:15-15:30 Coffee Break

15:30-17:00 ■ Session 3: Oral presentation

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

- co-chairs: **Kazuyuki Tobe**, *Japan*
  Yasuhiko Minokoshi, *Japan*

[Oral 3-1] The Nutritional Geometry of Obesity: the protein leverage hypothesis
Stephen J. Simpson, *Australia*

[Oral 3-2] Indoline derivatives as weight loss agents
Kathy Mountjoy, *New Zealand*

[Oral 3-3] Novel Anorexigenic Effect of Clusterin (Apolipoprotein J)
Min-Seon Kim, *Korea*

[Oral 3-4] Daytime feeding reduces energy expenditure and alters glycogen metabolism in liver and muscle of rats
Gregory J Cooney, *Australia*

[Oral 3-5] Natural AMPK Regulator Berberine Improves Energy Homeostasis
Jae Bum Kim, *Korea*

[Oral 3-6] Molecular mechanisms mediating calcium control of hormone secretion
Weiping Han, *Singapore*

17:00-17:15 Coffee Break

17:15-18:45 ■ Session 4: Oral presentation

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

• co-chairs: **Iichiro Shimomura,** *Japan*
Wataru Ogawa, *Japan*

[Oral 4-1] Endothelial cell dysfunction in diabetes
Yu Huang, *Hong Kong*

[Oral 4-2] Increased activity and expression of NADPH oxidase 4 stimulated by glucose induces oxidative stress in vascular endothelial cells
Jian Li, *China*

[Oral 4-3] Vascular lipotoxicity as a mechanism of endothelial dysfunction in the metabolic syndrome
Michio Shimabukuro, *Japan*

[Oral 4-4] The cellular function of seipin, a protein associated with congenital generalized lipodystrophy
Hongyuan Yang, *Australia*

[Oral 4-5] The orphan nuclear hormone receptor, RORalpha, regulates adiposity and insulin signaling
George E.O. Muscat, *Australia*

[Oral 4-6] A novel protein phosphatase CIPP contributes to suppression of AMPK activity in the arcuate hypothalamus in response to leptin by inhibiting CaMKKβ activity
Yasuhiko Minokoshi, *Japan*

18:45-19:15 ■ Session 5: Poster Session & Refreshments

[Poster 1] Ginsenoside Re improves insulin resistance through inhibition of JNK and NF-κB

Xiao-Ying Li, *China*

[Poster 2] Klotho is a target gene of PPAR-γ

Nanping Wang, *China*

[Poster 3] GLUT12 - linking nutrient overloard, insulin resistance and type 2 diabetes?

Suzanne Rogers, *Australia*

[Poster 4] FSP27 contributes to efficient energy storage in white adipocytes by promoting unilocular lipid droplet formation

Yoshikazu Tamori, *Japan*

[Poster 5] Essential Role of Translational Control in β Cell Survival under Stress Conditions

Hisamitsu Ishihara, *Japan*

[Poster 6] The role of Preadipocyte factor-1 (Pref-1/Dlk1) expressing pancreatic cells; as a pancreatic protodifferentiated cells

Marie Rhee, *Korea*

19:15- ■ Welcome Reception

PROGRAM

— August 24 —

◆PROGRAM◆

Sunday, August 24

8:00-8:20 ■ **Takeda Morning Session**

8:20-9:50 ■ **Session 6: Oral presentation**

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

- co-chairs: Yoshihiro Ogawa, *Japan*
  Jae Bum Kim, *Korea*

[Oral 6-1] Regulation of insulin sensitivity by adipose tissue macrophage
Isao Usui, *Japan*

[Oral 6-2] Regulation of obesity and insulin sensitivity by Cide proteins
Peng Li, *China*

[Oral 6-3] Lipin1, a novel mediator for TORC2 induced hepatic insulin resistance
Seung-Hoi Koo, *Korea*

[Oral 6-4] Central Role of the Transcription Factor FoxO1 in Hepatic Glucose Metabolism
Michihiro Matsumoto, *Japan*

[Oral 6-5] FoxO1 plays important roles in pancreatic cell differentiation and cell type specification
Tadahiro Kitamura, *Japan*

[Oral 6-6] Dynamic functional relay between insulin receptor substrate-1 and -2 in hepatic insulin signaling during fasting and feeding
Naoto Kubota, *Japan*

9:50-10:50 ■ **Session 7: Invited Lecture**

- chiar: **Masato Kasuga,** *Japan*

Glut2-dependent glucose sensors and the control of energy homeostasis
Bernard Thorens, *Switzerland*

10:50-11:05 Coffee Break

11:05-12:20 ■ Session 8: Oral presentation

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

- co-chairs: **Susumu Seino,** *Japan*
  Karen SL Lam, *Hong Kong*

[Oral 8-1] Intracelluar mechanism of cytoprotective effect of Rosiglitazone in Cuclosporine A-induced pancreatic beta-cell dysfunction
Ji-Won Kim, *Korea*

[Oral 8-2] Roles of AMP-activated protein kinase and oxidative stress in hypoxic injury to pancreatic β cell
Ki-Ho Song, *Korea*

[Oral 8-3] Mechanistic links between lipid metabolism, protein trafficking, endoplasmic reticulum stress and apoptosis in pancreatic beta cell failure
Trevor Biden, *Australia*

[Oral 8-4] Differential exocytic roles between Rab27a effectors expressed in pancreatic beta cells
Tetsuro Izumi, *Japan*

[Oral 8-5] New insights into the formation and function of caveolae
Robert G. Parton, *Australia*

12:20-13:20 ■ Lunch

13:20:14:10 ■ Session 9: APDO Special Lecture

- chair: **Takashi Kadowaki,** *Japan*

Identification of a novel hierarchical regulator of inflammation induced insulin resistance
Mark Febbraio, *Australia*

14:10-15:40 ■ Session 10: Oral presentation

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

- co-chairs: **Youfei Guan,** *China*
  Peter Shepherd, *New Zealand*

[Oral 10-1] Identification of two natural compounds from traditional Chinese medicine that increase adiponectin production by adipocytes and alleviate diabetes in mice

Karen SL Lam, *Hong Kong*

[Oral 10-2] Direct evidence that Class-IA PI 3-kinase activity may not be required for Insulin stimulated glucose uptake in Muscle: potential role for PIKfyve

Claire Chaussade, *New Zealand*

[Oral 10-3] The Role of Phospholipids in Controlling the Exocytosis of Insulin and GLUT4

William E. Hughes, *Australia*

[Oral 10-4] Targeted suppression of hepatic lipogenic enzyme ATP-citrate lyase protects against fatty liver and ameliorates hyperglycemia

Yong Liu, *China*

[Oral 10-5] Development of therapeutic strategies to reduce 11βHSD1 / steroid induced effects on obesity and glucose metabolism

Jon Whitehead, *Australia*

[Oral 10-6] Role of hepatic and muscular IL-6/ Stat3 signaling in regulation of glucose metabolism

Hiroshi Inoue, *Japan*

15:40 ■ Closing Remarks

Masato Kasuga, *Japan*

ABSTRACTS

Bernard Thorens, Ph.D.

Department of Physiology and Center for Integrative Genomics
University of Lausanne

● EDUCATION AND TRAINING

1974	Baccalaureate in Sciences College of Geneva, Switzerland
1979	Master in Biochemistry University of Geneva, Switzerland
1984	Ph.D. in Sciences University of Geneva, Switzerland

● POSITION AND EMPLOYMENTS

1985-1986	Maitre-assistant, Department of Pathology, Faculty of Medicine, University of Geneva
1986-1991	Postdoctoral fellow, Dr. H.F. Lodish's laboratory, Whitehead Institute for Biomedical Research, Cambridge, USA
1991-1998	START fellow, Assistant Professor, Institute of Pharmacology and Toxicology, University of Lausanne, Switzerland
1998-2002	Associate Professor, Institute of Pharmacology and Toxicology, University of Lausanne, Switzerland
2002-present	Professor of Physiology, Department of Physiology and Center for Integrative Genomics, University of Lausanne, Switzerland

● HONORS

1988	Marios Balodimos Award, Massachusetts Affiliates/ American Diabetes Association
1995	Friedrich Miescher Award of the Swiss Biochemical Society
1996	Apollinaire Bouchardat Award. Journées de Diabétologie de l'Hôtel-Dieu, Paris
1996	Prix Jaubert. Awarded by the Faculty of Sciences, University of Geneva
1999	Sarda Farriol Award, Sarda Farriol Foundation, Barcelona, Spain
2002	Cloëtta Award, Foundation Pr. Dr. Max Cloetta, Switzerland

● OTHER EXPERIENCE AND PROFESSIONAL MEMBERSHIPS

1996-2002	Medical Science Review Committee, Juvenile Diabetes Research Foundation International
2000-2004	Member of the Editorial Board, The Journal of Biological Chemistry
1998-2000	Member of the Editorial Board, American Journal of Physiology
2005-	Editorial Board Member, Endocrinology
2005	Programme National de Recherche sur de diabète, France, President of the Scientific Advisory Board
2005-2006	Member of Review Committee, Agence Nationale de la Recherche (Physiology), France

Glut2-dependent glucose sensors and the control of energy homeostasis

Bernard Thorens
University of Lausanne, Switzerland

The balance between food intake and energy expenditure determines the body weight. The equilibrium between these two physiological mechanisms is controlled, in large part, by the hypothalamus. This structure integrates information on food absorption and stored energy levels. This information is delivered by hormones or nutrients acting directly on sensitive hypothalamic neurons, or through activation of afferent neuronal pathways. Over the recent years, we have investigated the role that glucose plays as a signal to regulate feeding and energy homeostasis. We have tested the hypothesis that central glucose sensors share similarities with pancreatic beta cells, in particular that glucose uptake and metabolism is required for their function. In the beta-cells, glucose uptake is controlled by the glucose transporter Glut2 and inactivation of its gene leads to suppressed glucose-stimulated insulin secretion. Using mice with global inactivation of the Glut2 gene, but which express a transgenic glucose transporter in their beta-cells to normalize insulin secretion, we evaluated whether Glut2-dependent central glucose sensors were involved in the control of feeding and energy expenditure. We showed that absence of Glut2 expression led to abnormal feeding behavior, reduced energy expenditure, cold intolerance and increased susceptibility to fast-induced torpor. The defect in thermoregulation can be linked to a reduction in the stimulation of brown adipose tissue by the sympathetic nervous system secondary to a reduction in leptin sensitivity of the hypothalamic NPY and POMC neurons. Identification of Glut2-expressing cells in the brain indicated that arcuate nucleus neurons did not express Glut2, but that arcuate nucleus neurons were connected to nerve terminals from glucose sensitive and Glut2-expressing neurons. Our data indicate that glucose controls the leptin sensitivity of NPY and POMC neurons in an indirect manner, through Glut2-expressing neurons located at other sites. They define a neuronal circuit involved in the control by glucose of energy homeostasis.

The hunt for type 2 diabetes susceptibility genes in Japanese

Kazuki Yasuda

Department of Metabolic Disorder,
Research Institute, International Medical Center of Japan

Type 2 diabetes mellitus is one of the most common diseases in the world, and is caused by both genetic and environmental factors. Some genetic factors causing monogenic from of diabetes have been identified including MODY genes, while most of the susceptibility genes for more common, polygenic type of diabetes have been only poorly described. Hundreds of candidate genes have been investigated. Recent progress in human genomic sciences and technologies has made genome-wide association studies (GWAS) possible This approach is highly comprehensive and hypothesis-free method, and has proved to be amazingly successful in a lot of diseases including type 2 diabetes. We started in 2002 a GWAS for type 2 diabetes in Japanese using 100K SNPs from Japanese SNP database, as part of the national project called "The Millennium Genome Project". After multi-stage analysis, we obtained a very promising novel gene with odds ratio (OR) of around 1.4, comparable to that of TCF7L2. Replication analyses using Japanese and other East Asian panels reproduced the association, and this gene seemed a trans-ethnic susceptibility gene for type 2 diabetes. I will introduce our results in this project, compare with other GWASs of type 2 diabetes in Caucasians which have been most successful, and would like to emphasize the need for GWAS in different populations.

Identification of a novel hierarchical regulator of inflammation induced insulin resistance

Febbraio MA., Lancaster GI, Sadler A, Nicholls HT, Skiba B, Williams BRG

Baker IDI Heart and Diabetes Institute, Melbourne, Australia

Evidence is emerging linking insulin resistance to inflammation. It is now known that inflammation results in the secretion of inflammatory cytokines such as TNF-α from adipocytes and/or macrophages which result in signalling through TNF receptors to activate serine threonine kinases such as JNK and IKK-β in metabolic tissues. In addition, obesity can lead to increased deposition of lipid species such as DAG and ceramide which can also activate JNK and IKK-β in liver and/or skeletal muscle, leading to insulin resistance. The importance of both JNK and IKK-β in insulin resistance is highlighted by the observation that genetic disruption of these pathways in mice confers protection against obesity-induced insulin resistance. While IKK, JNK and TNF-α, have been established as important 'end-point' mediators of obesity-induced insulin resistance, the initial trigger that activates these inflammatory molecules is unclear. While ROS (Houstis et al., Nature 440, 944-948, 2006), endoplasmic reticulum (ER) stress (Ozcan et al., Science 306, 457-4612004), and toll-like receptor activation (Shi et al., J Clin Invest 116, 3015-3025, 2006) are key proximal signals central to obesity-induced insulin resistance, how these cellular events are initiated and how they promote cellular inflammation is not known. We have recently identified a novel regulator of inflammation that is sensitive to ROS, ceramide and TNF-α and which is both necessary and sufficient to activate both JNK and IKK-β to promote insulin resistance. This novel regulator will be discussed in this presentation.

2-1

Common Variation in the *FTO* Gene Confers Risk of Obesity and Modulates Body Mass Index in the Chinese Population

Yi-Cheng Chang[1], Pi-Hua Liu[2], Wei-Jei Lee[3], Tien-Jun Chang[4], Yi-Der Jiang[4], Hung-Yuan Li[4], Shan-Shan Kuo[4], Kuang-Chin Lee[4], Lee-Ming Chuang [4,5] *

*Corresponding author

[1] Department of Internal Medicine, National Taiwan University Hospital Yunlin Branch, Yunlin, Taiwan;
[2] Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan;
[3] Department of Surgery, Ming-Sheng General Hospital, Taoyuan, Taiwan
[4] Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan;
[5] Graduate Institute of Clinical Medicine, National Taiwan University Medical College, Taipei, Taiwan;

Objective: Genetic variants in the *FTO* gene have been associated with obesity and type 2 diabetes in European populations. We aimed to test the role of FTO genetic variants in obesity and type 2 diabetes in the Chinese population.

Methods: We genotyped 19 common single nucleotide polymorphisms (SNPs) spanning from the 3' end of the neighboring *RPGRIP1L* gene to the 5' flanking region of the *FTO* gene. We analyzed their associations with obesity (638 cases and 1,610 controls), type 2 diabetes (759 cases and 784 controls), and obesity-related quantitative metabolic traits (including fasting glucose, insulin, cholesterol, triglycerides, uric acid, C-reactive protein and blood pressure) in non-diabetic subjects.

Results: Among the 19 SNPs, the rs9939609 A allele was strongly associated with obesity (p= 7.0×10^{-4}) and body mass index (BMI) (p= 0.0024) in the Chinese population. The odds ratio for obesity was 2.60 (95% CI: 1.24-5.46; p=0.011) for the AA genotype and 1.32 (95% CI: 1.05-1.66; p= 0.018) for the AT genotype as compared to the TT genotype. Each additional copy of the rs9936609 A allele was associated with a BMI increase of ~ 0.37 kg/m^2. The rs9939609 A allele was substantially less common in the Chinese population than in the European population (12.6% *vs.* 45%), corresponding to a lower population-attributable risk of 8.7 %. We did not find significant associations between the 19 SNPs and type 2 diabetes in the Chinese population. We also failed to identify their associations with the obesity-related metabolic traits.

Conclusions: Genetic variation in the *FTO* gene is strongly associated with obesity and BMI in the Chinese population. The risk variant is less common in the Chinese population but its effect size on BMI is comparable to that in the European population.

2-2

Common variants in *GCKR* and *GCK* genes were associated with type 2 diabetes, obesity and their related traits in a Chinese Han population

Xu Lin[1], Qibin Qi[1], Huaixing Li[1], Ying Wu[1], Ruth J.F. Loos[2], Frank B. Hu[3], Liang Sun[1], Ling Lu[1], Xingwang Ye[1], Lihua Chen[1]

[1] Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Graduate School of the Chinese Academy of Sciences, Shanghai 200031, China.
[2] MRC Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK.
[3] Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts

OBJECTIVE

The *GCKR* rs780094 and *GCK* rs1799884 (-30G>A) polymorphisms have been reported to be associated with type 2 diabetes in populations of European origin. We aimed to replicate these observations in a population-based sample of Chinese Hans.

RESEARCH DESIGN AND METHODS

The *GCKR* rs780094 and *GCK* rs1799884 variants were genotyped and tested for associations with type 2 diabetes, obesity and related traits in 3210 unrelated Chinese Hans, comprising 424 individuals with type 2 diabetes, 878 with impaired fasting glucose and 1908 with normal fasting glucose.

RESULTS

Consistent with previous observations in Europeans, the A-allele of the *GCKR* rs780094 variant was associated with reduced risk of type 2 diabetes (OR 0.85[0.73-1.00], $P = 0.05$), and with reduced risk of combined IFG/type 2 diabetes (OR 0.86[0.77-0.96], $P = 0.0056$). The *GCKR* rs780094 A-allele also showed a trend toward reduced risk of obesity (BMI $\geq$ 28 kg.m^{-2}) (OR 0.88[0.75-1.02], $P = 0.09$), and a lower risk of overweight (BMI $\geq$ 24 kg.m^{-2}) (OR 0.87[0.78-0.96], $P = 0.0061$). Consistently, the protective A-allele of *GCKR* rs780094 was significantly associated with decreased fasting glucose ($P = 0.017$), increased HOMA-B values ($P = 0.017$), as well as with lower BMI ($P = 0.020$) and waist circumference ($P = 0.013$). The *GCK* rs1799884 A-allele exhibited significant association only with decreased HOMA-B value ($P = 0.0005$), but not with type 2 diabetes or any other related traits. We found no synergistic interaction between *GCKR* rs780094 and *GCK* rs1799884 on type 2 diabetes and related traits, but individuals with increasing numbers of risk alleles from both *GCKR* rs780094 and *GCK* rs1799884 tended to have significantly lower HOMA-B value ($P = 5.8 \times 10^{-5}$) in the combined analysis. Importantly, the associations with type 2 diabetes, IFG, HOMA-B and fasting glucose remained significant after adjustment for BMI, but the associations with type 2 diabetes and IFG disappeared after adjusting for HOMA-B.

CONCLUSIONS

Our results suggest that *GCKR* rs780094 alone or in combination with *GCK* rs1799884 contribute the risk of type 2 diabetes and obesity in the Chinese Han population. The effect on type 2 diabetes of these SNPS is likely mediated through impaired beta-cell function, but not through BMI.

2-3

Structural Mechanisms underlying the Inhibitory Effects of Angiopoietin-like Protein 4 on Lipoprotein Lipase Activities

Yu Wang, Ming-hon Yau, Karen S.L. Lam, Herbert J.L. Zhang, Donghai Wu and Aimin Xu

Department of Pharmacology, The University of Hong Kong, Hong Kong, China

Lipoprotein lipase (LPL) is a key enzyme involved in the clearance of chylomicrons and very low density lipoproteins (VLDL) from plasma and thus plays a pivotal role in the regulation of lipid metabolism. Recent studies had shown that two members of the angiopoietin-like protein (Angptl) family, Angptl3 and Angptl4, are capable of inhibiting LPL by directly binding to the enzyme via their N-terminal coiled-coil domains (CCDs). In this study, we showed that recombinant CCDs of human Angptl3 and 4 differentially inhibited the LPL activities and that disulfide bonds-mediated oligomerization determined the maximum inhibitory effect of Angptl4. Sequence alignment revealed a consensus 12-amino acid motif located within an α-helix close to the N-termini of Angptl3 and Angptl4, but not presented in other Angptl family members. Secondary structure prediction indicated that on the same face of the α-helix there existed three polar amino acids, namely His^{46}, Gln^{50} and Gln^{53} in human Angptl4. Substitution of all three residues simultaneously with alanines completely abolished the LPL inhibitory effect of Angptl4-CCD. Injection of C57BL/6J mice with the wild-type Angpl4-CCD, but not the mutated protein, resulted in an elevation of the plasma triglyceride levels. Protein chips-based studies demonstrated that mutations at these positions block the interactions between Angptl4-CCD and LPL. Besides, a synthetic peptide (15 amino acids) consisting of the consensus sequence motif inhibited LPL *in vitro* but the peptide with alanine substations at the three polar residues did not have this effect. In summary, these results demonstrated that the 12-amino acid consensus motif, especially the three polar residues, of Angptl4-CCD might play pivotal roles in mediating its negative regulatory effects on LPL activities through its direct interaction with LPL.

2-4

Deletion of AMPK β1 enhances hepatic insulin sensitivity and prevents high-fat diet induced obesity

Gregory R Steinberg, Nicolas Dzamko, Bryce JW van Denderen, Andrea L Hevener, Jane Honeyman, Sandra Galic, Duncan J Campbell and Bruce E Kemp

St. Vincent's Institute of Medical Research, 41 Victoria Parade, Fitzroy, Victoria 3065, Australia

The AMP-activated protein kinase (AMPK) is an evolutionarily conserved serine/threonine protein kinase that functions as a metabolic regulatory enzyme. AMPK functions as an αβγ heterotrimer of which seven different isoforms exist (α1, α2, β1, β2, γ1, γ2 and γ3), generating a family of AMPK heterotrimers that mediate unique tissue specific functions. Despite an absolute requirement for the AMPK β subunits in heterotrimer formation the physiological role of the AMPK β subunit isoforms is unknown; therefore, we generated AMPK β1 null mice and examined their metabolic characteristics. Genetic disruption of the AMPK β1 subunit resulted in tissue-specific defects (liver>hypothalamus>adipose>heart> skeletal muscle) in phosphorylation of AMPK on Thr172, and AMPK activity with a 90% reduction in liver as a consequence of a dramatic loss in AMPK α1 and AMPK α2 protein. Due to the lack of AMPK in the liver we hypothesized that AMPK β1 null mice would have impaired liver fatty acid metabolism and hyperglycaemia. On a control high-carbohydrate diet food intake, body mass, serum parameters and hepatic lipids and gluconeogenic gene expression were normal. However, surprisingly AMPK β1 null mice had enhanced insulin sensitivity during an insulin tolerance test an effect which was attributed to increased hepatic insulin sensitivity as assessed during a hyperinsulinemic-euyglycaemic clamp or in isolated hepatocytes. We then placed mice on a high fat diet to see if the increased hepatic insulin sensitivity would persist. Within as little as 3 weeks on a high fat diet AMPKβ1 null mice had reduced body mass compared to wildtype littermates an effect which could be attributed to reduced food intake. Consistent with reductions in body mass AMPK β1 null mice had enhanced glucose and insulin tolerance, reduced adipose tissue mass and liver triglyceride and increased hepatic insulin sensitivity. In conclusion these studies have demonstrated the surprising finding that the deletion of the AMPK β1 isoform increases hepatic insulin sensitivity and protects mice from diet induced obesity.

2-5

Heme Oxygenase-1 overexpression prevents endoplasmic reticulum stress by high glucose in INS-1 cells

Kyu Chang Won, Jun Sung Moon, Ji Eun Lee, Ji Sung Yoon, Yong Woon Kim, Hyoung Woo Lee

Department of Internal Medicine, College of Medicine, Yeungnam University, Daegu, Korea

Heme oxygenase-1 (HO-1) is a cytoprotective protein that catalyzes the degradation of heme to biliverdin, iron, and carbon monoxide (CO). And, endoplasmic reticulum (ER) stress is induced by adverse metabolic conditions in pancreatic b cells. We wanted to evaluate a protective role for HO-1 in pancreatic islets against ER stress by high glucose conditions. In the present study, we found ER stress induced by oxidative stress via high glucose conditions decreased by overexpression of HO-1 in INS-1 cells. The intracellular peroxide levels of INS-1 cells measured by flow cytometry were increased in the high glucose (30 mM) media compared to normal glucose (5.6 mM) media ($p<0.05$). The insulin mRNA levels, MafA, PDX-1 and glucose stimulated insulin secretion (GSIS) were decreased in high glucose media compared to 5.6 mM glucose media ($p<0.05$). ER stress was induced by high glucose condition via oxidative stress in INS-1 cells. We used HO-1 adenovirus for overexpression. HO-1 overexpression decreased ROS, increased insulin mRNA, PDX-1, MafA and preserved the GSIS in the islets at a high glucose condition ($p<0.05$). These results suggest that HO-1 seems to mediate the protective response of pancreatic islets against the ER stress induced by high glucose condition via oxidative stress.

3-1

The Nutritional Geometry of Obesity: the protein leverage hypothesis

Stephen J. Simpson, David Raubenheimer

The University of Sydney (Simpson)
Massey University (Raubenheimer)

Background: The role of protein in the obesity crisis has, until recently, been largely ignored. This is for two reasons. First, protein provides the minor part of the human energy budget. Second, protein intake has remained far more constant over time and across populations than either fat or carbohydrate, both as a percentage of energy in the diet and in terms of absolute amounts eaten. Hence, while the obesity epidemic has spread, protein intake has remained relatively unchanged – giving the impression that protein cannot be responsible.

Method: We use state-space models (the Geometric Framework) developed from extensive animal studies to postulate a key role for protein appetite in the obesity epidemic, and provide supporting evidence from experimental, nutritional survey, and animal studies.

Results: Protein is the most satiating macronutrient group for humans and is the most tightly regulated post-absorptively. Results from comparative studies of other vertebrates, human experiments, and population-level data strongly suggest that the response of humans when faced with imbalanced diets is to prioritise protein intake. Hence, when the percent protein in the diet is low, non-protein energy is overeaten, whereas when dietary % protein is high, energy intake is limited: in both cases, absolute intake of protein is maintained near constant.

Conclusion: We show how, paradoxically, it may be because protein comprises a relatively small component of the human diet and is tightly regulated that it has sufficient leverage over human ingestive behavior to explain obesity. Focusing on this leverage over intake clarifies the role of dietary protein in the development of obesity, provides a possible means of ameliorating the problem, and explains the effectiveness of high-protein diets as weight loss regimes.

3-2

Indoline derivatives as weight loss agents

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Despite the high prevalence of obesity and the many advances in our understanding of how it develops, current therapies have persistently failed to achieve long-term success. We found sustained and significant weight loss in mice following a single intraperitoneal injection at ~ 50 days of age of novel indoline derivatives and the weight loss was not accompanied by any apparent toxicity. The initial serendipitous lead (a prodrug, SN 28127, designed to be selectively activated by reduction in tumour tissue to release a cytotoxic alkylating agent) caused 15 -25% weight loss in four different strains of mice (C3HeN, A^{vy}/a, a/a and NZG). Food and water intake were transiently increased in the drug treated mice but were then indistinguishable from the intakes for DMSO treated control mice, despite the drug treated mice weighing significantly less than control mice 160 days post drug injection. The weight loss was accompanied by significant (~ 75%) fat mass loss and reduced plasma leptin and insulin levels. Analogues of SN 28127 were designed to find out which aspects of the molecule are required for the weight loss effect. Our most potent analogue, SN 29220, induced significant body weight reduction (>20% compared to DMSO treated control mice) for at least two months following a single treatment at a well-tolerated dose (<10% MTD). SN 29220 reversed high fat diet induced obesity and diabetes in C57BL/6J mice. Bio-distribution of radiolabelled SN 29220 showed that it does not cross the blood brain barrier and ~5% of the injected dose remained in mouse tissues 40 days post injection, preferentially accumulating in pancreas, spleen, liver and adipose tissues. Its mechanism of action is currently unknown.

3-3

Novel Anorexigenic Effect of Clusterin (Apolipoprotein J)

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The hypothalamus plays a key role in regulation of body weight and energy metabolism. One of the critical factors influencing these processes is the anorexigenic hormone leptin. Clusterin (apolipoprotein J), identified as a plasma leptin-binding protein, is expressed in the hypothalamus. Here we report that central administration of clusterin significantly reduces food intake and body weight, whereas suppression of hypothalamic clusterin expression increases both. Leptin enhances hypothalamic clusterin expression. Moreover, inhibition of clusterin expression blocks leptin-induced early anorexia, suggesting that clusterin is an important downstream mediator of leptin in the hypothalamus. Like leptin, clusterin activates signal-transduction-activated-transcript-3 (Stat3) in the hypothalamic arcuate nucleus and boosts the effect of leptin on Stat3. Importantly, the abilities of leptin and clusterin to decrease food intake and to activate Stat3 are significantly impaired when binding of clusterin to endocytic clusterin receptors is inhibited. These findings indicate that clusterin is a novel anorexigenic molecule that constitutes leptin's signaling pathway in the hypothalamus.

3-4

Daytime feeding reduces energy expenditure and alters glycogen metabolism in liver and muscle of rats

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Glucose and fatty acid metabolism in animals and humans follows a diurnal rhythm linked to the day/night cycle and the availability of food. These rhythms are co-ordinated by the suprachiasmatic nucleus (SCN) of the hypothalamus. The molecular basis of this circadian clock is an autoregulatory transcriptional/translational feedback loop which can be modulated in the SCN by direct input of the light/dark cycle via retinal afferents. Although metabolic rhythms in peripheral tissues (liver, adipose and muscle) are regulated by hormonal and neural outputs from the SCN, these tissues also possess the core molecular clock mechanisms suggesting that local regulation of circadian rhythms is possible.
To determine the role of feeding pattern in controlling diurnal energy metabolism in liver and muscle, groups of chow and fat-fed rats were only allowed access to food during the 12 hours of the light or dark period. Changes in diurnal energy expenditure, circulating hormones, glycogen content, lipid metabolites and gene expression were measured in liver and muscle at 3 hour intervals across the 24 hour cycle. Allowing rats to feed only during the daylight reduced total energy expenditure and reversed the diurnal variations in glycogen content and circadian gene expression in liver of chow-fed rats. In fat-fed rats changes in glycogen content were reversed but gene expression changes were not. In muscle daytime feeding blunted circadian gene expression without altering the phase of expression and increased glycogen content in the muscle. These results suggest that a loss of synchronization in tissue circadian gene expression as a result of changes in time of feeding and type of diet could have significant consequences for energy metabolism and the co-ordination of metabolic pathways in different organs.

3-5

Natural AMPK Regulator Berberine Improves Energy Homeostasis

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AMP-activated protein kinase (AMPK) plays critical roles in regulating whole body energy homeostasis. Recently, we and others reported that berberine (BBR) exerts anti-obesity and anti-diabetic effects in obese and diabetic rodent models through the activation of AMPK. Here, we show that BBR alleviates fatty liver and improves plasma and hepatic lipid profiles of obese mice through by activating AMPK in liver and skeletal muscle. In obese *db/db* mice BBR reduced liver weight and hepatic triglyceride and cholesterol. Inhibition of AMPK activity in the liver and skeletal muscle abolished the beneficial effects of BBR on lipid metabolism. BBR-induced activation of AMPK in hepatocytes in culture provoked ACC phosphorylation/inactivation, lowered cellular triglyceride and cholesterol levels and increased fatty acid oxidation. In addition to its direct activating effect on AMPK activity in the liver and skeletal muscle of obese mice, BBR acts centrally via indirect neural signaling from the central nervous system to skeletal muscle, thereby decreasing malonyl-CoA and increasing fatty acid oxidation gene expression.

3-6

Molecular mechanisms mediating calcium control of hormone secretion

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Synaptotagmins, a family of 15 type 1 membrane proteins, share a common domain structure. Each protein is composed of a short N-terminal sequence, followed by a transmembrane sequence, a linker of various length and two calcium-binding C_2-domains. Since its initial characterization and molecular cloning, the synaptotagmin family has been a subject of intense investigations for the past 15 years. Experimental data from biochemical, genetic and electrophysiological studies have established that synaptotagmin-1, -2 and -9 function as calcium sensors during the fast phase of neurotransmitter release. Recently, we demonstrated that synaptotagmin-7 serves as a high-affinity calcium regulator controlling insulin secretion. In the present talk, I will discuss the potential role of synaptotagmin-7 in regulating neuroendocrine functions.

4-1

Endothelial cell dysfunction in diabetes

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Endothelial dysfunction is prevalent in diabetes mellitus, being closely associated with the development of cardiovascular diseases. The pathophysiology of endothelial dysfunction in diabetes mellitus is complex. It has become apparent that excessive production of reactive oxygen species (ROS) contributes to cardiovascular complications of diabetes mellitus. Increasing evidence suggests that antioxidant therapy could delay the onset of diabetes-associated complications. However, clinical trials of antioxidant therapy in diabetic patients produce mixed results. It is therefore important to identify the sources of superoxide anions ($O_2^{\cdot-}$) in diabetes mellitus. Our present study shows a pathogenic role of angiotensin II type 1 (AT_1) receptor in the development of oxidative stress-related endothelial dysfunction in $lepr^{-/-}$ db/db mouse aorta, which is related to the activation of NAD(P)H oxidases and generation of $O_2^{\cdot-}$. The increases in the angiotensin II level in diabetic arteries were indicated indirectly by a significantly enhanced expression of angiotensin converting enzyme, while the reduced endothelial nitric oxide (NO) bioavailability was reflected by a decreased phosphorylation of eNOS at ser-1177. Low NO bioavailability may contribute to an increased production of ROS. This study has provided useful insights into new therapeutic strategies against the development of endothelial dysfunction in diabetes.

4-2

Increased activity and expression of NADPH oxidase 4 stimulated by glucose induces oxidative stress in vascular endothelial cells

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Hyperglycemia is one of major characteristics of diabetic patients. Long-term diabetic patients are often accompanied by multiple complications. Oxidative stress was known to be involved in the development of diabetic complications such as atherosclerosis. *In vitro* studies have revealed that high levels of glucose could lead to excessive production of reactive oxygen species (ROS), resulting in oxidative stress in vascular endothelial cells. Furthermore, in vivo studies suggested that the production of ROS, such as superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), was increased during the pathogenesis and progression of atherosclerosis.

ROS are second messengers in regulating vascular function and cell growth. The potential sources of ROS in endothelial cells include NADPH oxidase (NOX), cytochrome p-450, xanthine oxidase, uncoupled NO synthase and lipoxygenase. However, ROS in vascular endothelial cells are mostly derived from NOX. gp91phox, the complex enzyme composed of five subunits including cytosolic components (p47phox, P67phox and Rac-1) and cytomembrane components (p22phox and NOX2), was firstly found in phagocyte cells. In recent years, the novel gp91phox homologues of NOX families such as NOX1, NOX3, NOX4 and NOX5 have been identified in different organs and vascular cells. Latest studies indicated that NOX4 is the most important enzyme in the ROS generation in vascular endothelial cells. Our investigations showed that high concentration of glucose could upregulate the expression of NOX4 and stimulate the production of ROS in HUVECs. Preincubation with DPI, the specific inhibitor of NOX could reverse those effects. Furthermore, the regulation of NOX4 in those processes was investigated. The results showde that p47phox was translocated to nuclear membrane from cytoplasm to mediate the activation of NOX4 and the generation of ROS in glucose-treated HUVECs. Moreover, PKC could activate p47phox translocation. To assess the involvement of NOX4 in oxidative damage in HUVECs, we repressed the level of NOX4 mRNA by transiently transfecting NOX4-siRNA into HUVECs. Analysis by real-time PCR revealed that the amount of NOX4 mRNA was down-regulated by 50% in NOX4-siRNA-transfected HUVECs. Moreover, down-regulation of NOX4 significantly reduced the level of ROS and prevented HUVECs from apoptosis. Finally, our results indicated that mechanisms of ROS-dependent signaling inclued the activation of p38MAPK and the nuclear translocation of NF-κB.

In conclusion, our results provide the molecular evidence that NOX4-derived ROS could play an important role in oxidative stress of vascular endothelial cells induced by high concentration of glucose.

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4-3

Vascular lipotoxicity as a mechanism of endothelial dysfunction in the metabolic syndrome

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Vascular endothelial dysfunction has been demonstrated in obesity,but the molecular basis for this link has not been clarified. We examined the role of free fatty acids(FFA)on vascular reactivity in the obese fa/fa Zucker diabetic fatty(ZDF)rat. Vasodilator response to acetylcholine was decreased in aortic rings of ZDF than lean +/+ rats. A 2-wk treatment with a reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor, apocynin, improved the response in ZDF. Vascular reactive oxygen species (ROS) levels and NADPH oxidase activity in aorta were increased in ZDF rats but were decreased.
In in vitro cell culture, intracellular ROS signal and NADPH oxidase subunit mRNA were increased by palmitate, but NADPH oxidase inhibitor or pitavastatin inhibited it. In conclusion, FFA-induced NADPH oxidase subunit overexpuression and ROS production could be involved in the endothelial dysfunction seen in obese ZDF rats.

4-4

The cellular function of seipin, a protein associated with congenital generalized lipodystrophy

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Lipid droplets (LDs) are emerging cellular organelles that are of crucial importance in cell biology and human diseases. We present here our screen of ~4700 *Saccharomyces cerevisiae* mutants for abnormalities in the number and morphology of LDs; we identify 17 *fld* (for few lipid droplets) and 116 *mld* (for many lipid droplets) mutants. One of the *fld* mutants (*fld1*) is due to deletion of *YLR404W*, a previously uncharacterized open reading frame. Cells lacking *FLD1* contain strikingly enlarged ("supersized") LDs, and LDs from *fld1*Δ cells demonstrate significantly enhanced fusion activities both *in vivo* and *in vitro*. Interestingly, expression of human seipin, mutations in which are associated with Berardinelli-Seip Congenital Lipodystrophy and motoneuron disorders, rescues LD-associated defects in *fld1*Δ cells. Lipid profiling reveals alterations in acyl chain compositions of major phospholipids in *fld1*Δ cells. These results suggest that an evolutionarily conserved function of seipin in phospholipid metabolism and LD formation may be functionally important in human adipogenesis. New data on the role of mammalian seipin will also be presented.
Ref: Fei et al., J Cell Biol. 2008 Feb 11; 180: 473-82. Epub 2008 Feb 4.

4-5

The orphan nuclear hormone receptor, RORalpha, regulates adiposity and insulin signaling

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Homozygous *staggerer* mice (*sg/sg*) in a C57Bl/6J background, are characterized by dyslipidemia, dysfunctional and decreased expression of the orphan nuclear hormone receptor, RORalpha. We very recently [Lau P et al (2008) J Biol Chem. 2008 Apr 25, in press, Epub ahead of print] demonstrated that dyslipidemia in *sg/sg* mice is associated with decreased hepatic expression of SREBP-1c (and downstream lipogenic target genes) and the reverse cholesterol transporters, ABCA1 and ABCG1. In addition, we showed the *sg/sg* mice were characterised by reduced adiposity (associated with decreased fat pad mass and adipocyte size). Furthermore, the lean phenotype in *sg/sg* mice is characterized by significantly increased expression of PGC-1α, PGC1β and lipin1 mRNA in liver, white and brown adipose. Moreover, a significant 4-fold increase in β2-adrenergic receptor mRNA in brown adipose is observed. Finally, dysfunctional RORα expression protects against diet-induced obesity. Following a 10-week high fat diet, wildtype but not *sg/sg* mice exhibited ~20% weight gain, increased hepatic triglycerides, notable white and brown adipose accumulation. In summary, these changes in gene expression (that modulate lipid homeostasis) in metabolic tissues are involved in decreased adiposity, and resistance to diet-induced obesity in the *sg/sg* mice. Ongoing studies have undertaken food intake, fecal output, serum leptin, insulin, cytokine expression, glucose and insulin tolerance tests coupled to expression profiling studies to further probe the function of the orphan nuclear receptor. Our current data indicates that RORalpha is involved in the regulation of the insulin signaling pathway. In conclusion, we demonstrate this orphan nuclear receptor is a key modulator of adiposity, insulin signaling, and an important regulator of the pathways that are operate in the context of diet induced metabolic disorders. We suggest that selective ROR modulators may have utility in the treatment of obesity, and glucose intolerance.

4-6

A novel protein phosphatase CIPP contributes to suppression of AMPK activity in the arcuate hypothalamus in response to leptin by inhibiting CaMKKβ activity

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Leptin and adiponectin are adipokines that regulates body-energy metabolism. We have previously shown that leptin suppresses food intake by inhibiting α2AMPK activity in the arcuate hypothalamus (ARH), while it stimulates fatty acid oxidation in skeletal muscle by activating muscle AMPK. In contrast, adiponectin increases food intake by activating α2AMPK activity in the ARH. Here, we report that a novel protein serine/threonine phosphatase CIPP (CaMKK/AMPK cascade-inhibitory protein phosphatase) suppresses α2AMPK activity in the ARH in response to leptin by dephosphorylating Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ) and inhibiting its activity. CIPP expresses in mouse ARH, human brain and human neuroblastoma cell line, SH-SY5Y cells, but not in skeletal muscle. In mouse ARH and SH-SY5Y cells, leptin activates Akt and thereby phosphorylates CIPP, then recruiting to CaMKKβ. In contrast, adiponectin activates AMPK in the ARH and SH-SY5Y cells via independent mechanism of CaMKK and CIPP. These results provide a putative mechanism by which leptin and adiponectin reciprocally regulate AMPK activity in the ARH and food intake, whereas both adipokines activates AMPK in skeletal muscle.

6-1

Regulation of insulin sensitivity by adipose tissue macrophage

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Diet-induced obesity causes a phenotypic switch in adipose tissue macrophages from an anti-inflammatory M2 state to a pro-inflammatory M1 state. PPARγ signaling in monocytes was recently reported to play an important role in alternative activation of monocyte to M2 macrophage. In this study, we firstly examined the effects of PPARγ agonists on the phenotype of macrophage in epididymal fat of high fat-fed mice. Pioglitazone and telmisartan, PPARγ agonists, decreased the number of M1 macrophage and selectively increased some markers of M2 macrophage, including interleukin-10 (IL-10). To examine the involvement of the increased expression of IL-10 in the improved insulin resistance by PPARγ agonists, we directly overexpressed IL-10 using adenovirus vector (Ad-hIL-10) in high fat-fed mice. Insulin sensitivity assessed by glucose- or insulin-tolerance test was improved by Ad-hIL-10 infection without any effect on the amount of food intake and body weight. Markers of M2 macrophage in epididymal fat tissue were significantly higher in IL-10 overexpressing mice, while M1 macrophage markers were not significantly altered. The expressions of G6Pase and PEPCK in liver were down-regulated, and the expressions of genes involved in OXPHOS and fatty acid metabolism in skeletal muscle were up-regulated in Ad-hIL-10-infected mice. Taken together, M2 macrophages in adipose tissue may contribute, at least in part, to the improvement of insulin sensitivity by PPARγ agonists, for which the expression of IL-10 play an important role.

6-2

Regulation of obesity and insulin sensitivity by Cide proteins

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Adipose tissues can be divided into brown adipose tissue (BAT) and white adipose tissue (WAT). Both BAT and WAT contain abundant amount of lipid and can serve as energy storage organ. BAT plays a unique role in energy expenditure by uncoupling oxidative phosphorylation and dissipating energy as heat to maintain core body temperature in animals when exposed to cold. The primary role of WAT is to store energy in the form of triglycerides (TAG) in lipid droplets and immobilize the energy in time of needs such as starvation. Adipose tissue can also serve as an endocrine organ to secret crucial hormones such as Leptin and Adiponectin for the control of whole-body energy homeostasis. Cide proteins, including Cidea and Fsp27 (Cidec in human), were originally identified by their sequence homology to the N-terminal region of DNA fragmentation factor DFF40/45. While Cidea is expressed at higher levels in BAT, Fsp27 mRNAs and proteins were detected in WAT and BAT. To understand the physiological role of Cide proteins, we generated *Cidea* and *Fsp27*-null mice by homologues recombination. Interestingly, we observed that mice deficient in *Cidea* or *Fsp27* exhibited higher energy expenditure and were resistant to high-fat-diet induced obesity and diabetes. Here, we will compare the detail phenotype of *Cidea* and Fsp27 null mice and analyze the underlying mechanism of Cide proteins in the regulation of the development obesity and diabetes.

6-3

Lipin1, a novel mediator for TORC2 induced hepatic insulin resistance

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TORC2 plays a major role in the regulation of glucose production from liver. Insulin inhibits hepatic gluconeogenesis by blocking nuclear entry of TORC2 in SIK2-dependent manner. Dysregulation of this process during insulin resistance greatly perturb hepatic glucose metabolism, thus promoting hyperglycemia in rodents. Here we show a novel mechanism for TORC2-mediated hepatic insulin resistance. Hyperactivation of TORC2 enhances expression of Lipin1, an enzyme for diacylglycerol (DAG) synthesis in hepatocytes. TORC2 is shown to be directly involved in the transcriptional activation of lipin1 gene by transient transfection assay and chromatin immunoprecipitation analysis. While increased expression of wild type would enhance DAG production and promote insulin resistance in hepatocytes, shRNA-mediated knockdown of hepatic Lipin1 ameliorate hyperglycemia and insulin resistance by reducing PKCε activation in *db/db* diabetic mice. Finally, we were able to observe that S171A TORC2-mediated insulin resistance is blunted by concomitant knockdown of hepatic Lipin1, implicating the importance of this enzyme in perturbation of insulin signaling in liver. These data suggest that dysregulation of TORC2 activity would further exacerbate insulin resistance and may promote type II diabetes via induction of Lipin1 pathway.

6-4

Central Role of the Transcription Factor FoxO1 in Hepatic Glucose Metabolism

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We have previously reported that FoxO1 promotes hepatic glucose production via increased expression of PEPCK and G6Pase in vitro and in vivo by gain-of function approaches. However, the effects of FoxO1 ablation on glucose metabolism have not been explored. In this study, we examined the effects of loss of FoxO1 in hepatic glucose metabolism using liver-specific FoxO1 knockout mice (L-Foxo1KO) and FoxO1 siRNA adenovirus in mouse hepatocytes. In L-Foxo1KO mice, hepatic expression of G6Pase, PEPCK and PGC1α decreased by nearly 60%, resulting in a 40% reduction of blood glucose levels (107.5 ± 5.5 vs 68.2 ± 10.1) and hepatic glycogen content than control mice. Pyruvate-induced rise of plasma glucose was impaired in L-Foxo1KO mice. This was associated with a substantial increase of glycogen content after fasting and increased glucose tolerance during GTT. To examine whether Foxo1 is in the insulin signaling pathway, we introduced the conditional Foxo1 mutations in the background of Insulin receptor knockout mice. L-Foxo1 KO mice lacking insulin receptors (IRKO) showed increased survival, decreased blood glucose levels and hepatic TG content, and increased hepatic glycogen compared to IRKO mice. In FoxO1 knockdown hepatocytes, both basal and cAMP-induced glucose production were reduced concomitant with the decreased expression of G6Pase, PEPCK and PGC1α genes. These data indicate that 1) Foxo1 is the critical insulin-responsive transcription factor regulating glucose production via PGC1α and gluconeogenic genes. 2) Foxo1 lies downstream of the insulin receptor pathway.

6-5

FoxO1 plays important roles in pancreatic cell differentiation and cell type specification

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Insulin-producing β cells are central to the pathogenesis of diabetes. Understanding the mechanisms governing their ontogeny may offer strategies for their somatic replacement. We show that FoxO1 displays a unique distribution pattern during pancreatogenesis, with broad expression at E14.5, restriction to endocrine progenitors at E17.5, and limitation to β cells post-natally. This finding prompted us to explore FoxO1's role in the developing pancreas. Here we show that transgenic mice expressing constitutively active FoxO1 under the Pdx1 promoter display severe hypoplasia of pancreatic acinar cells, marked increase in duct-like structure and abnormal islet architecture. These findings resemble the phenotype of Notch1 transgenic mice, and are consistent with our demonstration that FoxO1 cooperates with Notch to regulate differentiation (*Kitamura T et al., JCI 2007*). In addition to mature β cells, FoxO1 is expressed in a rare population of pancreatic duct-associated cells. We hypothesized that these cells represent adult pancreatic endocrine cell progenitors. Accordingly, conditional FoxO1 knockout in pancreatic progenitors (using Pdx1-cre mice), but not in endocrine progenitors (using Neurogenin3-cre) or fully differentiated β cells (using insulin-cre), resulted in a selective increase of juxta-ductal β cells. We conclude that FoxO1 plays important roles in pancreatic cell differentiation and cell type specification. FoxO1-positive cells may represent a source of adult pancreatic cells with endocrine-like differentiation potential.

6-6

Dynamic functional relay between insulin receptor substrate-1 and -2 in hepatic insulin signaling during fasting and feeding

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Insulin receptor substrate (Irs)1 and Irs2 exhibit high structural homology, both are abundantly expressed in the liver, and these proteins are thought to be responsible for transmitting insulin signaling from the insulin receptor to the intracellular effectors in the regulation of glucose and lipid homeostasis. In this study, we generated liver-specific Irs1-knockout mice as well as liver-specific Irs2-knockout mice, to investigate the physiological roles of hepatic Irs1 and Irs2 in the regulation of glucose metabolism. Moreover, we also generated liver-specific Irs1/Irs2 double-knockout mice in an attempt to elucidate whether hepatic insulin signaling is exclusively mediated by Irs1 and Irs2. We found that Irs1 and Irs2 function in a distinct and reciprocal manner in the regulation of glucose homeostasis during fasting and after refeeding. The PI3K activity associated with Irs2 began to increase during fasting and reached its peak immediately after refeeding, before decreasing rapidly thereafter; on the other hand, the PI3K activity associated with Irs1 began to increase a few hours after refeeding and reached its peak thereafter, suggesting that Irs2 mainly functions during fasting and immediately after refeeding, and Irs1 functions primarily after refeeding. In fact, liver-specific Irs1-knockout mice failed to exhibit insulin resistance during fasting, but showed insulin resistance after refeeding; conversely, liver-specific Irs2-knockout mice displayed insulin resistance during fasting, but not after refeeding. Moreover, liver-specific Irs1/Irs2 double-knockout mice developed diabetes and exhibited insulin resistance under both during fasting and after refeeding. Taken together, we propose the concept of the existence of a dynamic relay between Irs1 and Irs2 in hepatic insulin signaling during fasting and feeding, and shed light on the ramifications of the insulin signaling mechanism in metabolic regulation.

8-1

Intracelluar mechanism of cytoprotective effect of Rosiglitazone in Cuclosporine A-induced pancreatic beta-cell dysfunction

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We performed this study to clarify the effects of rosiglitazone (RGTZ) on cyclosporine A (CsA) induced beta-cell dysfunction and death. Male Sprague Dawley rats were allocated into four groups (each group of n=10 and treated by four weeks): (a) Control group (vehicle only) (b) RGTZ (3mg/kg) group (c) CsA (15mg/kg) group. (c) CsA + RGTZ group. Glucose tolerance test, Insulin tolerance test, total graft mass, beta cell mass and insulin gene expression in isolated islets were determined. For in vitro study, the expressions of insulin mRNA and ER stress marker after treatment with CsA and/or RGTZ were analyzed by RT-PCR and MTT assay in MIN6 cell line. The deterioration of glucose tolerance by CsA was significantly protected and the fasting insulin levels were also increased by combine treatment of RGTZ. However insulin sensitivity determined by insulin tolerance test was not different between 4 groups. At 4 weeks after treatment, the relative volume, absolute mass of beta-cells in pancreas, and total pancreas weight were significantly decreased by CsA treatment while combine treatment of RGTZ preserved beta-cell mass. There was no difference of beta-cell mass between control and RGTZ group. CsA induced cell death and reduced expression of insulin gene. However, these results, beta-cell dysfunction by CsA, were rescued by RGTZ treatment. Moreover induction of the expression level of CHOP and sXBP-1 by CsA treatment were reduced by RGTZ treatment. These findings demonstrate that RGTZ signaling directly modulates the ER stress response leading to promotion of beta-cell adaptation and survival. We propose that RGTZ might function as a key molecule, which protect the glucolipotoxicity-induced β-cell dysfunction by CsA in vivo and in vitro.

8-2

Roles of AMP-activated protein kinase and oxidative stress in hypoxic injury to pancreatic β cell

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In islet transplantation, a substantial part of the graft becomes nonfunctional due to several factors including hypoxia. Recently, we found that nitric oxide synthase-nitric oxide is activated in pancreatic β cells under hypoxic state (Transplantation 85:323, 2008). Hypoxia is also known to increase AMP-activated protein kinase (AMPK) activity in many types of cells. However, the role of AMPK is not clear in β cells.

INS-1 rat-derived insulin-secreting cells were incubated in an anaerobic chamber. The viability of INS-1 cell was markedly decreased within 6 hr, accompanied by increase in activated caspase-3 expression and TUNEL-positive cells. Phosphorylated-AMPK, and phosphorylated acetyl-CoA carboxylase were upregulated with time. Production of reactive oxygen species (ROS) was also increased. Pretreatment with an antioxidant (N-acetylcystein) or an AMPK inhibitor (compound C) attenuated ROS production and apoptosis during hypoxia. Apoptotic cells in the graft, 24 hr after transplanting mouse islets under the kidney capsule, were markedly decreased by pretreatment of the islets with N-acetylcystein or compound C.

In conclusion, AMPK activation and oxidative stress might be associated with β cell apoptosis under hypoxia.

8-3

Mechanistic links between lipid metabolism, protein trafficking, endoplasmic reticulum stress and apoptosis in pancreatic beta cell failure

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There is growing evidence that fatty acids (FAs) promote endoplasmic reticulum (ER) stress, and that this contributes to the loss of pancreatic beta cell mass seen in humans and in animal models of Type 2 diabetes (Laybutt et al, Diabetologia 2007). This process is highly selective for saturated FAs, but the precise identity of the lipid species that induces ER stress, and the cellular mechanisms whereby this occurs, are poorly understood.
Here we demonstrate using clonal MIN6 beta cells overexpressing a GFP-tagged and temperature sensitive mutant of the VSVG virus protein, that the saturated FA palmitate slows ER to golgi protein trafficking. This is not observed with the unsaturated FA, oleate. Importantly, a strong chemical inducer of ER stress, thapsigargin, was also a relatively poor inhibitor of protein trafficking, suggesting that the effects of palmitate are cause and not a consequence of ER stress. In separate studies we employ tracer labeling and mass spectroscopy to demonstrate that palmitate-induced ER stress and apoptosis are associated with enhanced flux through the sphingolipid metabolite ceramide.
Overexpression studies to reduce cellular ceramide content also protected MIN6 cells against both apoptosis and ER stress.
These studies suggest that lipo-apoptosis in pancreatic beta cells is associated with generation of specific sphingolipid metabolites that cause a defect in ER to golgi trafficking. The resulting protein overload in the ER triggers apoptosis secondary to ER stress. This mechanism is potentially relevant to the failure of pancreatic that occurs in Type 2 diabetes during periods of increased secretory demand.

8-4

Differential exocytic roles between Rab27a effectors expressed in pancreatic beta cells

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The small GTPase Rab27a and its effector granuphilin is involved in the docking of insulin granules to the plasma membrane. Although the two proteins form a endogenous complex in pancreatic beta bells and seem to play coordinated roles in insulin exocytosis, beta cells of ashen mice that lack functional Rab27a exhibit markedly different phenotypes from those of granuphilin-null beta cells. Although granuphilin specifically acts on the granule underneath the plasma membrane, Rab27a mainly acts on those in a more distal area. There findings suggest that Rab27a plays another role through its effectors other than granuphilin. In fact, we found that a couple of Rab27 effectors called exophilins are expressed in beta cells. In particular, exophilin7 was expressed to a significant level and formed an endogenous complex with Rab27a in pancreatic beta cells. We will present distinct characteristics between exophilin7 and granuphilin and discuss the property necessary for their granule-docking ability.

8-5

New insights into the formation and function of caveolae

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Caveolae are flask-shaped pits of the cell surface, an abundant morphological feature of many mammalian cells. Caveolins, the major proteins of caveolae, play a crucial role in the formation of caveolae. Mutations in caveolins are associated with breast cancer and with a number of muscle diseases, including limb girdle muscular dystrophy. We have studied how caveolin-lipid interactions generate the unique architecture of the caveolar domain by studying caveolae formation in caveolin-null fibroblasts by light and electron microscopy upon expression of mammalian caveolins (caveolin-1 and caveolin-3), specific mutants of these proteins, or non-mammalian caveolins (from *C elegans* and the honey bee, *Apis mellifera*). We are also analysing the function of caveolins in mammalian and non-mammalian (zebrafish, *Danio rerio*) cells. We have shown an unexpected link between caveolae and lipid droplets, the major storage depot for lipids in mammalian cells. Caveolin can move between the cell surface and lipid droplets in response to high fatty acid levels. In addition, caveolin mutants, which show constitutive association with lipid droplets, disrupt cholesterol regulation. The importance of caveolin in lipid regulation *in vivo* is shown by our studies of liver regeneration. We have shown transient reversible association of caveolins with lipid droplets during the regeneration process. In the absence of caveolin, lipid droplet formation is inhibited and liver regeneration is perturbed due to a specific block in the cell cycle[1]. Our recent studies have identified a new family of coat proteins which regulate caveolae formation. PTRF-cavin family members regulate association of caveolin with caveolae and identify a cellular mechanism to regulate caveolar and non-caveolar functions of caveolins[2]. These studies have implications for understanding the distinct cell-type specific roles of caveolin in disease conditions.

References; 1) Fernandez et al, 2006. Science, 313:1628-32. 2) Hill et al, 2008. Cell 132:113-124.

10-1

Identification of two natural compounds from traditional Chinese medicine that increase adiponectin production by adipocytes and alleviate diabetes in mice

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Adiponectin is an adipocyte-derived hormone with insulin-sensitizing, anti-inflammatory and anti-atherosclerotic properties, demonstrated in animal studies. In humans, hypoadiponectinemia is found in obesity and has been identified as an independent risk factor for diabetes and its cardiovascular complications. It is therefore envisaged that pharmacological interventions targeting at the enhancement of adiponectin production might hold promise for the treatment and/or prevention of these diseases. We have recently identified two natural compounds that possess such an activity, from the medicinal herb Radix Astragali. These two natural compounds, structurally belonging to triterpene saponins, increased adiponectin production primarily by enhancing the secretion of this hormone in adipocytes. They had no obvious effects on adipogenesis or the production of a panel of other major adipokines. Furthermore, an additive effect on inducing adiponectin production was observed between the two compounds and rosiglitazone, a thiazolidinedione insulin-sensitizer. Chronic administration of these two natural compounds in mice with diet-induced or genetic obesity significantly increased the circulating levels of total adiponectin, with a selective effect on high molecular weight adiponectin. These changes were accompanied by the amelioration of hyperglycemia, glucose intolerance and insulin resistance. In summary, our results suggest that the anti-diabetic property of Radix Astragali described in traditional Chinese medicine might be mediated, at least in part, by the triterpene saponin-type molecules, through the induction of adiponectin.

10-2

Direct evidence that Class-IA PI 3-kinase activity may not be required for Insulin stimulated glucose uptake in Muscle: potential role for PIKfyve

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For the last 15 years it has been assumed that insulin stimulation of PI 3-kinase is necessary for the effects of insulin on GLUT-4 translocation and on glucose transport. These conclusions have been largely based on the use of two chemical inhibitors, LY294002 and wortmannin, which acutely block insulin stimulation of glucose transport. As insulin activates the class-IA PI 3-kinases (p85/p110 it has been widely assumed that this class of PI3-kinase is mediating the effects on glucose transport. Currently genetic evidence for this is lacking, as the results from the p110 knockout mouse models are equivocal. Further, wortmannin and LY294002 are broad-spectrum inhibitors of PI3Ks and do target several other lipid kinases. To address this question in more detail we have synthesized a range of chemical inhibitors that more selectively target different isoforms of PI 3-kinase. We previously characterized those inhibitors and used them to dissect the role of each isoform in insulin-induced phosphorylation of PKB in a wide range of cell lines (Chaussade *et al., Biochemical J.*, 2007). Here we will report the effects of these on insulin stimulated glucose transport in 3T3-L1 adipocytes and in freshly isolated soleus muscle from rats. The results we obtained provide the first strong pharmacological evidence that insulin stimulated class-IA PI 3-kinase activity may not be necessary for insulin mediated increases in glucose uptake in muscle and that PIKfyve activity is required for this process in both adipocytes and skeletal muscle.

10-3

The Role of Phospholipids in Controlling the Exocytosis of Insulin and GLUT4

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Regulated exocytosis consists of a variety of budding, translocation, coating/uncoating, targetting, docking and fusion processes. Whilst the roles of proteins in these events are becoming clearer the contribution of membrane phospholipids is less well established. We have used a combination of biochemical and cell biological approaches to study the roles of phospholipids in the later steps of exocytosis of insulin from pancreatic beta cells and of GLUT4 in muscle cells.

Phospholipase D derived phosphatidic acid is required for glucose stimulated insulin release and appears to participate in the mechanism of vesicle-plasma membrane fusion. We propose that PLD and phosphatidic acid regulate fusion by affecting membrane curvature.

PI3kinase derived phosphatidylinositol 3-phosphate is required for efficient insulin stimulated GLUT4 exocytotic translocation to the plasma membrane. We propose that phosphatidylinositol 3-phosphate contributes to vesicular trafficking by affecting trafficking/movement of GLUT4 storage vesicles in close proximity to the plasma membrane.

10-4

Targeted suppression of hepatic lipogenic enzyme ATP-citrate lyase protects against fatty liver and ameliorates hyperglycemia

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Lipogenic dysregulation underlies the pathogenic development of obesity, hyperlipidemia and insulin resistance. ATP-citrate lyase (ACL) is a key lipogenic enzyme that catalyzes the critical reaction linking cellular glucose catabolism and lipid synthesis, converting cytosolic citrate to acetyl-CoA. Acetyl-CoA is further converted to malonyl-CoA, the essential precursor for fatty acid biosynthesis. However, the metabolic connections of ACL to the derangements of lipid and glucose metabolism remain largely unclear. Here we show that ACL, as a peripheral effector target of leptin, was markedly suppressed with accompanying onset of hyperleptinemia in response to high-fat diet challenge in wild type, but not in leptin receptor-deficient *db/db* mice. Moreover, ACL expression was selectively deregulated in the liver of *db/db* mice, paralleled by abnormally elevated expression of hepatic PPARγ. Importantly, liver-specific ACL knockdown in *db/db* mice via adenovirus-mediated RNAi prominently reduced the hepatic contents of both acetyl-CoA and malonyl-CoA, and dramatically repressed the hepatic expression of PPARγ. The metabolic alterations emanated from ACL deficiency resulted in substantial suppression of the entire lipogenic program and consequently, protection from hepatic steatosis. This, in turn, led to marked improvement in systemic glucose metabolism, largely attributable to down-regulated gluconeogenic program in the liver and alleviated insulin resistance in the muscle. These results thus demonstrate that hepatic ACL mediates crucial physiological actions by leptin in the control of both lipid and glucose homeostasis, and that targeting hepatic ACL is a promising therapeutic strategy in the treatment of fatty liver and hyperglycemia associated with impaired leptin signaling.

10-5

Development of therapeutic strategies to reduce 11βHSD1 / steroid induced effects on obesity and glucose metabolism

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Glucocorticoids (steroids) have a significant effect to decrease cellular glucose uptake, thus promoting hyperglycaemia. This is observed clinically as a side-effect of steroid therapy; in the rare disease state of Cushing's syndrome; and in obesity where increased 11β hydroxysteroid dehydrogenase type 1 (11βHSD1) activity produces high cortisol levels in key tissues including adipose, muscle and liver.

We hypothesise that strategies to reduce steroid-induced hyperglycaemia will have clinical utility in settings both of steroid use (for conditions including asthma, arthritis and transplant) and of metabolic dysfunction associated with obesity. Such strategies include inhibition of 11βHSD1 activity and reversal of steroid induced cellular events that lead to impairment of cellular glucose uptake (and hence hyperglycaemia). We have taken two complementary approaches to test this hypothesis.

We have shown that 11βHSD1 activity in human adipose tissue is significantly increased proportional to BMI ($R^2 = 0.29$, $n = 18$, $p = 0.03$) and waist circumference ($R^2 = 0.39$, $n=18$, $p = 0.01$). Further, activity is significantly greater in omental than subcutaneous adipose tissue (0.37 +/- 0.03 vs 0.19 +/- 0.02 nmol cortisol/mg adipose/hr, $n=18$, $p = 0.001$). Moreover, pharmacological inhibition of 11βHSD1 activity significantly reduces differentiation of primary human preadipocytes ($p < 0.05$). This data highlights the candidate status of 11βHSD1 as a therapeutic target.

In parallel, we have investigated steroid (Dex/cortisol) effects on glucose uptake in human adipose and other relevant cell types. We have demonstrated a significant 50% reduction of both basal and insulin-stimulated glucose uptake ($p < 0.01$). This effect is mediated, at least in part, by the glucocorticoid receptor. Steroids induce a significant 40-50% reduction in plasma membrane (PM) expression of GLUT1 and GLUT4 ($p < 0.05$). Further work identified two candidate molecules/abnormalities that may be involved in the reduced glucose uptake. Firstly, steroid induces a significant 40-50% reduction in basal and insulin-stimulated phosphorylation of AS160 ($p < 0.05$), an AKT substrate that has a key role in GLUT trafficking to the PM. Second, steroid induces a 2-4 fold increase in FOXO1 protein - a metabolic transcriptional regulator that has a role in reducing insulin sensitivity. Current work seeks to investigate the efficacy of strategies to reverse the abnormalities in AS160 and FOXO1 by steroid, with respect to glucose uptake.

10-6

Role of hepatic and muscular IL-6/ Stat3 signaling in regulation of glucose metabolism

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Objective- We have previously demonstrated that the IL-6/STAT3 pathway plays a physiological role in the suppression of hepatic glucose production as an effector of the brain insulin action. However, evidence suggests that proinflammatory cytokines including IL-6 contributes to the development of insulin resistance in obese individuals. We thus evaluated the role of IL-6 and STAT3 in the regulation of glucose metabolism both in lean and obese animals.

Results- In db/db mice, circulating levels of IL-6 was robustly increased in response to feeding or glucose administration, which was associated with the phosphorylation of STAT3 both in the liver and skeletal muscle. Such increase in the levels of IL-6 was not observed in, and the phosphorylation of STAT3 was only detected in the liver of lean control mice. Administration of neutralizing antibodies to lean mice impaired insulin-induced suppression of hepatic glucose production, indicating that IL-6 is required for this action of insulin. In contrast, the antibodies to IL-6 increased whole body glucose utilization rate, and ameliorated hyperglycemia of db/db animals. Moreover, liver-specific disruption of STAT3 deteriorated glucose tolerance whereas skeletal muscle-specific disruption of STAT3 ameliorated glucose intolerance of db/db mice.

Conclusion- These results suggest that, whereas IL-6 is important for insulin-induced suppression of hepatic glucose production, the increase in the cytokine in obese animals contributes to the development of insulin resistance in skeletal muscle, and that such bilateral functions, a normal physiological function in the liver and a pathological function in skeletal muscle, are mediated by STAT3 in the respective organs.

5-1

Ginsenoside Re improves insulin resistance through inhibition of JNK and NF-κB

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Ginsenoside Re(Re), a compound derived from Panax ginseng, shows an antidiabetic effect. However the molecular basis of its action remains unknown. We investigate insulin signaling, and anti-inflammation effect by Re in 3T3-L1 adipocytes and in high fat diet rats to dissect its anti-hyperglycemic mechanism. Glucose uptake is measured in 3T3-L1 cells and glucose infusion rate (GIR) determined by clamp in high fat diet (HFD) rats. The insulin signaling cascade, including IRβ, IRS-1, PI3K, Akt and AS160, and Glut4 translocation are examined. Furthermore, JNK MAPK and NF-κB signaling cascade are also assessed. The results show Re increases glucose uptake in 3T3-L1 cells and GIR in HFD rats. The activation of insulin signaling by Re is initiated at IRS-1 and further passes on throguh PI-3K and downstream signaling cascade. Moreover, Re demonstrates an impressive suppression of JNK and NF-κB activation and IκBα degradation. In conclusion, Re reduces insulin resistance in 3T3-L1 adipocytes and HFD rats through inhibition of JNK and NF-κB activation.

5-2

Klotho is a target gene of PPAR-γ

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Klotho is an anti-aging gene whose expression is regulated by many stimuli. Here we examined the transcriptional regulation of the klotho gene by peroxisome proliferator activated receptor-γ (PPAR-γ). The PPAR-γ agonists thiazolidinediones increased both klotho mRNA and protein expression in HEK293 cells and several renal epithelial cell lines. The induction was blocked by PPAR-γ antagonists or small-interfering RNA-mediated gene silencing of PPAR-γ, suggesting a PPAR-γ-dependent mechanism. Chromatin immunoprecipitation and gel shift assays found a noncanonical PPAR-responsive element within the 50-flanking region of the human klotho gene with promoter-reporter assays further confirming transcriptional functionality. Moreover, thiazolidinediones or adenovirus-mediated overexpression of PPAR-γ increased klotho expression in mouse kidneys while renal klotho expression was attenuated in mice treated with PPAR-γ antagonists. These results demonstrate that klotho is a target gene of PPAR-γ.

5-3

GLUT12 - linking nutrient overload, insulin resistance and type 2 diabetes?

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The glucose transporter protein GLUT12 may perform a specialised physiological role that is activated by hyperglycaemia. GLUT12 is expressed in skeletal muscle, heart and fat and undergoes regulated translocation from a basal, intracellular location to the cell surface. The key stimulus for GLUT12 translocation is glucose. We developed an *in vitro* model to correlate GLUT12 targeting to the cell surface with functional glucose transport. Using exofacially-tagged cDNA and neutralising antibodies, we demonstrated that GLUT12 translocates to the cell surface where it mediates glucose-responsive glucose transport. Significantly, this response was acutely inhibited by rapamycin, the specific inhibitor of mTOR (Endocrinology, 2008: 149:917-924).

The mTOR pathway is a central regulator of cell metabolism and growth, responding to nutrient and energy levels. We predicted that if GLUT12 were regulated by glucose in heart, fat and skeletal muscle – this GLUT would play a role in glucose homeostasis. We prepared differentiated, primary human myocyte cultures. In 5 mM glucose, confocal immunofluorescence localised GLUT12 to the cytoplasm. When exposed to high (25 mM) glucose, a distinct redistribution to the cell surface was observed. Correspondingly, glucose uptake increased following growth in 25 mM glucose. Glucose-regulated targeting and transport was inhibited by rapamycin.

The GLUT12 promoter exhibits a carbohydrate response element (CRE) consensus sequence, thus we considered whether GLUT12 was also transcriptionally activated by elevated glucose. Levels of GLUT12 mRNA in differentiated primary human myocytes were increased six-fold following four hours exposure to high glucose. To further these findings, we investigated the expression of GLUT12 in the New Zealand Obese (NZO) mouse model. NZO mice exhibit polygenetic inheritance of obesity, insulin resistance and hyperglycaemia. Real-time PCR analyses revealed significant (up to seven fold) increases in GLUT12 mRNA in the heart and skeletal muscle of NZO animals, in both fed and fasted states, when compared to controls.

We have made the novel discovery that glucose stimulation and rapamycin sensitive mTOR signalling control GLUT12 targeting and glucose transport. Normally, mTOR is activated by amino acids and glucose and downstream action mediates cell growth. In the presence of nutrient overload, such as in the obese state, the mTOR pathway is overactive and is implicated in insulin resistance. The mechanisms that link these processes are not well understood but are thought to include inhibition of insulin receptor signalling. We hypothesise that with balanced nutrient intake, GLUT12 mediated glucose transport contributes to metabolic homeostasis. In nutrient overload, GLUT12 would drive deregulation of the mTOR axis.

5-4

FSP27 contributes to efficient energy storage in white adipocytes by promoting unilocular lipid droplet formation

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White adipocytes contain large unilocular lipid droplets that occupy most of the cytoplasm. Here we show that fat-specific protein of 27 kDa (FSP27) is required for unilocular lipid droplet formation in white adipose tissue (WAT). FSP27 localizes to lipid droplets in white adipocytes, and ablation of FSP27 in mice resulted in the formation of multilocular lipid droplets in these cells. FSP27-deficient mice were also protected from diet-induced obesity and insulin resistance and manifested an increased metabolic rate as a result of increased mitochondrial biogenesis in WAT. Depletion of FSP27 in cultured white adipocytes resulted in the formation of numerous small lipid droplets, increased lipolysis, and decreased triacylglycerol storage. In contrast, forced expression of FSP27 in COS cells promoted the formation of large lipid droplets. Our results suggest that FSP27 contributes to efficient energy storage in WAT by promoting the formation of unilocular lipid droplets, thereby restricting lipolysis and subsequent oxidation of free fatty acids. In addition, the nature of lipid accumulation in WAT appears to be associated with obesity and whole-body insulin sensitivity.

5-5

Essential Role of Translational Control in β Cell Survival under Stress Conditions

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[Background] Apoptotic cell death is triggered when internal and external insults overwhelm cellular capacity to counteract such stress stimuli. Pancreatic β cells are especially prone to endoplasmic reticulum stress and oxidative stress, since their continuous and abundant insulin production generates an ER burden and their active mitochondrial metabolism can result in high production of reactive oxygen species. Translational control is an important strategy by which cells cope with stress conditions. There are two means by which cells regulate the rate of translation: inhibition of eukaryotic initiation factor 2 (eIF2) through phosphorylation of the α-subunit of eIF2 (eIF2α) by eIF2α kinases and inhibition of eIF4E by eIf4E-binding proteins (4E-BPs). An important role of eIF2α kinases has been revealed by the phenotypes of mice with eIF2α kinases' mutations. We studied the roles of 4E-BP1, an extensively studied isoform of 4E-BPs, in β cells under stress conditions.

[Results] 4E-BP1 expression was found to be increased under ER stress through ATF4 in MIN6 cells and mouse islets. Arsenite-induced oxidative stress increased 4E-BP1 expression and decreased 4E-BP1 phosphorylation, resulting in activation of 4E-BP1, in MIN6 cells. 4E-BP1-deficient MIN6 cells and islets were vulnerable to both ER and oxidative stress. Furthermore, *Eif4ebp1*-deletion worsened glucose homeostasis in high-fat diet-fed mice and genetic mouse models of diabetes.

[Conclusions] These data indicate that 4E-BP1-mediated translational control contributes to β cell survival during both ER and oxidative stress. We propose that dual control of mRNA translation is essential for maintenance of β cell mass in diabetes.

5-6

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

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Preadipocyte factor-1/Delta like 1 homologue (Pref-1/Dlk 1) is a type I membrane protein that has six epidermal growth factor (EGF)-like repeats in its extracellular domain and a short cytoplasmic domain. It is widely expressed in embryonic tissues, whereas in adult and postnatal, its expression is limited. Although Pref-1 is involved in both differentiation and growth of β-cells, the mechanism and function are not cleared yet. To extend our understanding for the role of Pref-1 during pancreas development and regeneration, we observed and analyzed Pref-1 expression in embryonic and adult partial pancreatectomized (Px) rat pancreases, neonatal pig pancreas and monolayer cultured porcine neonatal pancreatic cell clusters (NPCCs) over time. 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 Px, 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 NPCCs, Pref-1 expression was peaked at day 3 to 4, then gradually disappeared until day 7 as Px rat pancreas. Most of Pref-1(+) cells were co-stained with pancytokeratin. Purified soluble Pref-1 (Pref-1-mFc) treatment increased ERK1/2 phosphorylation whereas decreased FOXO1 phosphorlylation in a time-dependent manner in PANC-1 cells. We also observed that the activation of Pref-1 increased PDX1 and decreased FOXO1 expression. We conclude that Pref-1 expression was regained in adult pancreatic cells during proliferation and regeneration and regulates phosphorylation of ERK1/2, FOXO1 and expression of PDX-1. Pref-1 might be a useful marker for the pancreatic protodifferentiated cells.