2nd Scientific Meeting of the Asia-Pacific Diabetes and Obesity Study Group

Program & Abstracts

Saturday, August 26 – Sunday, August 27, 2006
Hotel Granvia Kyoto
Kyoto, Japan

2nd Scientific Meeting of the Asia-Pacific Diabetes and Obesity Study Group

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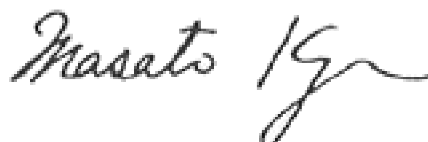
Welcome Message

Welcome to the 2nd Scientific Meeting of the Asia-Pacific Diabetes and Obesity Study Group. Following on the success of the first meeting, the Organizers have been able to prepare a program that encompasses the most important and thought-provoking areas of research occurring in this fast moving area. We extend a warm welcome to all participants, both new and returning, and feel sure you will not be disappointed with the range of topical presentations arranged for you. Diabetes continues to be a disease of increasing concern, growing in incidence and devastating effects throughout the world, affecting both developed and developing countries alike. This symposium is able to build upon the firm foundations laid down by last year's inaugural meeting, to further enhance knowledge and understanding of this disease. Faculty from throughout the Asian-Pacific region have been bought together to provide an international and regional perspective in order to help prepare therapeutic and preventative strategies aimed at reducing this disease.

The symposium begins with the first APDO Study Group lecture focusing upon cell biology of insulin stimulated glucose transport. This is an area at the forefront of research and one that we know will be of great interest to all participants. We are also fortunate to have a special Invited Lecture given by Dr. Vamsi Mootha, who will report on recent efforts using data from the human genome project to link mitochondrial dysfunction to diabetes, with the ultimate aim being the identification and development of new therapeutic strategies. In addition there is a selection of stimulating oral and poster presentations covering a diverse and informative range of topics. Participants also have the opportunity to discuss findings with presenters in a relaxed setting, and we urge you all to take advantage of this to raise any issues or queries you may have. It is through the interactive exchange of views that knowledge is disseminated, an integral part of ongoing developments in the field of diabetes research. Therefore please take the time to meet new and old friends while listening to the most up-to-date diabetes trends from specialists. Finally, we hope that you leave the symposium with greater understanding regarding the complexity of the problem, energized and enthusiastic to continue the fight against this disease.

David E James, BSc, PhD

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



Masato Kasuga, MD, PhD

Professor and Chairman
Dept. Of Diabetes, Digestive& Kidney Diseases
Kobe University Medical School
Kobe, Japan

FACULTY MEMBERS

● ORGANIZING COMMITTEE

Co-chairs

David E James, BSc, PhD
Professor and Director
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Sydney, Australia

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The University of Tokyo
Tokyo, Japan

Iichiro Shimomura, MD, PhD
Professor
Dept. of Metabolic Medicine
Graduate School of Medicine
Osaka University
Osaka, Japan

● INVITED SPEAKER

Vamsi K. Mootha, MD
Department of Systems Biology and Medicine
Harvard Medical School
Massachusetts General Hospital

GENERAL INFORMATION

● DATES

Saturday, August 26- Sunday, August 27, 2006

● VENUE

Hotel Granvia Kyoto

901 Higashi-Shiokoji-cho, Shiokoji Sagaru Karasuma-Dori, Shimogyo-ku, Kyoto 600-8216
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http://www.granvia-kyoto.co.jp

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

● Poster Presentation

1. You are requested to mantle a poster between 13:00-15:00 on August 26 and dismantle it after the reception.
2. Remaining posters will be taken away by the secretariat.

◆PROGRAM◆

Saturday, August 26

15:00 ■ Opening Remarks

Masato Kasuga, *Japan*

15:15-16:00 ■ Session 1 : APDO Study Group Lecture 1

• chair: **Masato Kasuga**, *Japan*

Cell biology of insulin stimulated glucose transport
David James, *Australia*

16:00-17:00 ■ Session 2 : Oral Presentation

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

• co-chairs: **Kerry Loomes**, *New Zealand*
Davis Ng, *Singapore*

[Oral 2-1] Regulation of hepatic glucose metabolism
Seung-Hoi Koo, *Korea*

[Oral 2-2] Brain insulin action regulates hepatic glucose production via STAT3 signaling in the liver
Wataru Ogawa, *Japan*

[Oral 2-3] The structure and function of the AMPK
Bruce Kemp, *Australia*

[Oral 2-4] Melanocortin peptides signal to regulate appetite, body weight and metabolism
Kathleen Mountjoy, *New Zealand*

17:00-17:15 Break

17:15-18:30 ■ Session 3 : Oral Presentation

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

• co-chairs: **Youfei Guan**, *China*
Karen SL Lam, *Hong Kong*

[Oral 3-1] Forkhead transcription factor FoxO1 in brown adipose tissue regulates energy expenditure through regulation of Pgc-1α and 4E-BP1 expression
Jun Nakae, *Japan*

[Oral 3-2] Involvement of apolipoprotein E in excess fat accumulation and insulin resistance
Yasushi Ishigaki, *Japan*

[Oral 3-3] Skp2 plays an essential role in adipocyte proliferation during the development of obesity
Hiroshi Sakaue, *Japan*

[Oral 3-4] Down-regulation of HDACs stimulates adipocyte differentiation
Jae Bum Kim, *Korea*

[Oral 3-5] Role of IRS-1 serine phosphorylation in the regulation of insulin signaling and glucose transport
Young-Bum Kim, *Korea*

18:30-19:15 ■ Session 4 : Poster Presentations & Refreshments

[P-1] Glucose and fat metabolism in adipose tissue of acetyl-CoA carboxylase 2 knockout mice
Won Keun Oh, *Korea*

[P-2] The role of adipose glycerol channel; aquaporin adipose/7
Norikazu Maeda, *Japan*

[P-3] Pioglitazone improves insulin sensitivity by increasing adiponectine secretion from fat tissue via reduced expression of SOCS3
Isao Usui, *Japan*

[P-4] Uncoupling protein 2 promoter polymorphism -866G/A affects peripheral nerve dysfunction in Japanese type 2 diabetic patients
Hiroshi Yamasaki, *Japan*

[P-5] Obesity and late-onset diabetes in the mice lacking *Sqstm1*/p62 associate with mitochondrial dysfunction
Jaekyoon Shin, *Korea*

[P-6] Expression of glutathione peroxidae 3 gene is regulated by peroxisome proliferator-activated receptor-γ
Kyong Soo Park, *Korea*

[P-7] Overall and early stage hypertrophy of β-cells in humans with type 2 diabetes
Jae-Hyoung Cho, *Korea*

[P-8] AMPK action; cell cycle progression and cell death in glucolipotoxicity
Ji-Won Kim, *Korea*

[P-9] The participation of PKCζ in insulin-medicated glucose transport in rat skeletal muscle L6 cells
Peter Tong, *Hong Kong*

19:15- ■ Welcome Reception

Saturday, August 27

07:30-08:00 ■ Morning Session

sponsored by Takeda Pharmaceutical Company Limited

08:00-08:45 ■ Session 5 : APDO Study Group Lecture 2

• chair: **David James**, *Australia*

Novel insulin and adiponectin actions on blood vessel and brain
Takashi Kadowaki, *Japan*

08:45-10:15 ■ Session 6 : Oral Presentation

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

• co-chairs: **Tao Xu**, *China*
Greg Cooney, *Australia*

[Oral 6-1] From QTL to candidate gene; a story told by a diabetes mouse model
Huei-Ju Pan, *Taiwan*

[Oral 6-2] Diabetes protein signature in human serum revealed by label-free and pathway-associated differential proteomics
Jia-Rui Wu, *China*

[Oral 6-3] Berberine activates AMPK and has beneficial metabolic actions in diabetic and insulin resistant states
Edward W Kraegen, *Australia*

[Oral 6-4] Dissecting the role of individual isoforms of PI 3-kinase in insulin signalling
Peter R Shepherd, *New Zealand*

[Oral 6-5] Physiological function of the unfolded protein response using single target gene analysis
Davis Ng, *Singapore*

[Oral 6-6] Inhibition of PKC epsilon as a novel strategy for treatment of type 2 diabetes, by reconstitution of glucose-induced insulin secretion and diminution of hepatic insulin clearance
Trevor J Biden, *Australia*

10:15-10:25 Break

10:25-11:40 ■ Session 7 : Oral Presentation

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

• co-chairs: **Kan Liao**, *China*
Tetsuro Izumi, *Japan*

[Oral 7-1] Role of cyclophilin B in ER stress and insulin processing
Sung Soo Kim, *Korea*

[Oral 7-2] Upregulation of islet beta-cell fructose 1,6 bisphosphatase results in insulin secretory dysfunction
Joseph Proietto, *Australia*

[Oral 7-3] RNA editing by ADAR2 is metabolically regulated in pancreatic islets and β-Cells
Yong Liu, *China*

[Oral 7-4] Crucial role for an evolutionarily conserved *cis*-regulatory region of *pdx1* in pancreas organogenesis and islet function
Yoshio Fujitani, *Japan*

[Oral 7-5] Inducible nitric oxide synthase (iNOS) plays an important role in hypoxic injury to pancreatic beta cells
Kun-Ho Yoon, *Korea*

11:40-12:40 Lunch (photo session)

12:40-13:30 ■ Session 8 : Invited Lecture

• chair: **Masato Kasuga**, *Japan*

Systems analysis of mitochondrial dysfunction in human diabetes
Vamsi K. Mootha, *USA*

13:30-14:15 ■ Session 9 : Oral Presentation

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

• co-chairs: **Kyung Soo Park**, *Korea*
Peter Tong, *Hong Kong*

[Oral 9-1] Adiponectin inversely correlates with triglyceride and high-sensitivity C-reactive protein but not with insulin or insulin resistance index in Japanese men
Mitsuhisa Komatsu, *Japan*

[Oral 9-2] The role of the macrophage in obesity induced insulin resistance
Mark Febbraio, *Australia*

[Oral 9-3] Overexpression of Monocyte Chemoattractant Protein 1 (MCP-1) in adipose tissues causes systemic insulin resistance
Nozomu Kamei, *Japan*

14:15-14:20 Break

14:20-15:20 ■ Session 10 : Oral Presentation

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

co-chairs: **Jon Whitehead**, *Australia*
Yoshihiro Ogawa, *Japan*

[Oral 10-1] Adipocyte-FABP as a novel player in the metabolic syndrome: clinical and functional studies
Karen SL Lam, *Hong Kong*

[Oral 10-2] Elevated circulating concentrations of lipocalin 2 may contribute to obesity-associated ectopic lipid accumulation and insulin resistance in mice
Aimin Xu, *Hong Kong*

[Oral 10-3] CXS741, a novel secreted protein involved in the pathogenesis of obesity and type 2 diabetes
Ken Walder, *Australia*

[Oral 10-4] Pathophysiological roles of adipokine network in metabolic syndrome
Toshimasa Yamauchi, *Japan*

15:20 ■ Closing Remarks

Takashi Kadowaki, *Japan*

15:25 ■ adjourned

ABSTRACTS

Session 8 : Invited Lecture p10

Session 2 : Oral Presentation p12

Session 3 : Oral Presentation p15

Session 6 : Oral Presentation p18

Session 7 : Oral Presentation p22

Session 9 : Oral Presentation p25

Session 10 : Oral Presentation p28

Session 4 : Poster Presentation p31

VAMSI K. MOOTHA, M.D.

Center for Human Genetic Research
Massachusetts General Hospital

● CURRENT POSITIONS

Assistant Professor, Department of Medicine, Massachusetts General Hospital
Assistant Professor, Department of Systems Biology, Harvard Medical School
Associate Member, Broad Institute of MIT and Harvard
Diplomate, American Board of Internal Medicine

● CLINICAL TRAINING

1998-2001	Brigham and Women's Hospital, Boston, MA Internship and Residency, Internal Medicine

● EDUCATION

1993-1998	Harvard Medical School, Boston, MA M.D. (*cum laude*) in the Harvard-MIT Division of Health Sciences and Technology
1989-1993	Stanford University, Stanford, CA B.S. (*with honors, with distinction*) in Mathematical and Computational Science
1985-1989	Kelly High School, Beaumont, TX Diploma (valedictorian)

● RESEARCH EXPERIENCE

2001-2004	HHMI Physician Postdoctoral Fellow, Whitehead Institute, Cambridge, MA
2001-2001	Visiting Scientist, MDS Proteomics, Odense, Denmark
1999-2001	Resident Research Fellow, Dana Farber Cancer Institute, Boston, MA
1995-1997	HHMI Research Scholar, Laboratory of Cardiac Energetics, NIH, Bethesda, MD

● SELECTED AWARDS AND FELLOWSHIPS

2004	John D. and Catherine T. MacArthur Foundation Fellowship
2004	Burroughs Wellcome Career Award in the Biomedical Sciences
1993	Phi Beta Kappa
1989	Westinghouse Science Talent Search Competition Semifinalist

● SELECTED ORAL PRESENTATIONS

2005	Nestle Lecture in Metabolism, Lausanne, Switzerland
2006	Massachusetts General Hospital, Department of Medicine Grand Rounds, Boston, MA
2006	NIH Wednesday Afternoon Lecture Series, Bethesda, MD

● JOURNAL REVIEWS

Science, Nature, Nature Genetics, Cell, New England Journal of Medicine

Systems analysis of mitochondrial dysfunction in human diabetes

Vamsi K. Mootha, M.D.
Department of Systems Biology and Medicine, Harvard Medical School, Massachusetts General Hospital

Mitochondria are spectacular organelles, serving as the center-stage for energy homeostasis, apoptosis, and dozens of biosynthetic pathways. Moreover, human mitochondria contain their own genome (mtDNA), representing a vestige of their bacterial ancestry. During the past 20 years it's become increasingly evident that this organelle plays a key role in the pathogenesis of numerous rare and common human diseases that span virtually all organ systems. Much of this progress has been possible thanks to advances in genome sequencing. In a landmark paper in 1981, Siv Andersson and colleagues at the Sanger Center reported the complete sequence of human mtDNA. Armed with this information, clinicians and geneticists quickly identified mutations in this tiny genome that give rise to devastating, maternally inherited metabolic diseases. Since then over 50 mutations in this tiny genome have been linked to human diseases. It's important to remember, however, that mtDNA encodes only 13 of the estimated 1500 proteins that are resident in human mitochondria. The remaining proteins are encoded by the nuclear genome, translated in the cytosol, and then imported into this organelle. Fortunately, in 2001 the International Human Genome Sequencing Consortium reported the draft sequence of the entire human genome.

In this talk I present some of our recent efforts to capitalize on the availability of the human genome sequence to systematically link mitochondria to human diabetes. First, we are using experimental and computational genomics to construct a protein *map* for this organelle. This protein parts list is permitting us to chart metabolic pathways resident within this organelle and is enabling us to quickly identify nuclear genes responsible for the maintenance of mitochondria. Second, we are using microarrays to *measure* the abundance of mitochondrial transcripts during normal developmental processes as well as in the common form of type 2 diabetes. We have developed computational strategies for monitoring concordant changes in the expression of mitochondrial genes to spotlight common human diseases that are characterized by dysfunction in this organelle. Third, using new computational techniques, we are developing *models* of mitochondrial biogenesis in healthy and in disease states. These models provide insights into mitochondrial remodeling and reveal targets for manipulating mitochondrial function.

Together the genomic approaches are enabling a systems-level approach to organelle pathogenesis and promise to identify novel therapeutic strategies for diabetes.

This work was funded by grants from the Howard Hughes Medical Institute, United Mitochondrial Disease Foundation, Burroughs Wellcome Fund, American Diabetes Association, the Smith Family Foundation, and the MacArthur Foundation.

2-1

Regulation of hepatic glucose metabolism

Seung-Hoi Koo, Ph.D.
Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine

Glucose levels are tightly controlled in mammals in part by regulated hepatic glucose production. During fasting conditions, glucagon promotes cAMP signaling in liver to activate gluconeogenesis, and a novel cAMP responsive CREB coactivator TORC2 is the primary factor for the regulation of this process. cAMP-induced dephosphorylation and nuclear localization of TORC2 prompts hepatic gluconeogenic gene expression by co-activating CREB on relevant promoters. Paradoxically, CREB/TORC2 signaling is also found to be critical to enhance hepatic insulin signaling by activating transcription of the key component of insulin signaling, insulin receptor substrate 2 (IRS2) gene. Knockdown of IRS2 expression in liver by shRNA leads to glucose intolerance as well as mild fasting hyperglycemia, suggesting the induction of IRS2 by CREB/TORC2 during fasting might be indeed crucial for glucose homeostasis. Furthermore, adenovirus-mediated hepatic IRS2 overexpression reduced fasting glucose levels.presumably due to the enhanced insulin signaling during fasting. Taken together, CREB/TORC2 signaling appears to be critical for maintaining fasting glucose homeostasis in liver by modulating genes in two opposite pathways.

2-2

Brain insulin action regulates hepatic glucose production via STAT3 signaling in the liver

Wataru Ogawa, Hiroshi Inoue, Yasuo Okamoto, Masato Kasuga
Kobe University Graduate School of Medicine

STAT3 regulates glucose homeostasis by suppressing the expression of gluconeogenic genes in the liver. The mechanism by which hepatic STAT3 is regulated by nutritional or hormonal status has remained unknown, however. Here we show that an increase in the plasma insulin concentration, achieved either by glucose administration or by intravenous insulin infusion, stimulates tyrosine phosphorylation of STAT3 in the liver. This effect of insulin was mediated by the hormone's effects in the brain, and the increase in hepatic IL-6 induced by the brain insulin action is essential for the activation of STAT3. The inhibition of hepatic glucose production and of expression of gluconeogenic genes induced by intra-cerebral ventriclar insulin infusion was impaired in mice with liver-specific STAT3 deficiency or in mice with IL-6 deficiency. These results thus indicate that insulin action in the brain suppresses hepatic glucose production by activating IL-6–STAT3 signalling in the liver.

2-3

The structure and function of the AMPK

BE Kemp[1,3], NL Dzamko[1], BJW van Denderen[1], GR Steinberg[1], MJ Watt[1], SL Macaulay[3], DJ Campbell[1], GS Lynch[2]

[1] St Vincent's Institute, Department of Medicine and [2] Department of Physiology, University of Melbourne
[3] CSIRO, Molecular & Health Technologies, Victoria, Australia

AMPK is a major metabolic regulatory enzyme and a therapeutic target for the treatment of obesity and type-2 diabetes through its ability to stimulate fat oxidation and glucose uptake into skeletal muscle as well as suppress glucose output from liver. AMPK is a αβγ heterotrimer with seven genes encoding the AMPK subunits (α1, α2, β1, β2, γ1, γ2, γ3). Skeletal muscle AMPK is activated in response to metabolic stress during exercise as well as by a number of hormones, including, adiponectin, leptin and CNTF. Recent characterization of AMPK α1, α2 and γ3 knockout mice has elucidated individual roles for the different AMPK subunits. Since the β subunit controls the assembly of the heterotrimer, as well as targeting to membranes and glycogen, it was of interest to generate AMPK β1 and β2 null mice to examine the individual roles of the AMPK β subunits. In skeletal muscle the AMPK β2 subunit is highly expressed and AMPK β2-containing heterotrimers account for essentially all AMPK activity. In AMPK β2 null mice there was marked suppression of AMPK α1 and α2 protein levels and ACC phosphorylation in skeletal muscle while AMPK β1 null mice had normal levels of AMPK α1 and α2. Consistent with the expression of AMPK α1 and α2, glucose uptake in isolated extensor digitorum longus (EDL) muscle in response to AICAR was markedly suppressed in AMPK β2 but not AMPK β1 null mice. These results support the concept that the AMPK β2 subunit is required for full AMPK activity in skeletal muscle and that AMPK β2 is essential for AMPK-stimulated glucose uptake, while demonstrating that AMPK β1 is not obligatory. In contrast, in liver, the AMPK β1 isoform predominates and AMPK β1-containing heterotrimers account for essentially all of the AMPK activity in this tissue. AMPK β1 null mice had a marked suppression of AMPK α1 and α2 in liver, an effect not observed in AMPK β2 null mice. Lastly, in cardiac ventricle from AMPK β2 null mice, AMPK α2 protein was decreased but AMPK α1 protein was normal. These results demonstrate that the AMPK β subunits are under separate genetic control and one is unable to compensate for the loss of the other.

2-4

Melanocortin peptides signal to regulate appetite, body weight and metabolism

Kathy Mountjoy

Department of Physiology, University of Auckland, Auckland, New Zealand

The melanocortin peptides derived from pro-opiomelanocortin (POMC) and their receptors play pivotal roles maintaining energy homeostasis through the regulation of appetite and metabolism. This is clearly demonstrated by human melanocortin-4 receptor (MC4R) and POMC gene variants, two knockout mouse models (MC3R and MC4R knockout mice) and the spontaneously occurring dominant agouti mutant mouse. POMC-derived peptides are downstream effectors of the leptin signal generated in peripheral fat reserves and acting in the hypothalamus to maintain energy homeostasis. The melanocortin peptides include ACTH, α-MSH, desacetyl-α-MSH, and β-MSH. Signals acting through neural MC3R and MC4R are critical for regulating energy homeostasis. Still unresolved though are the roles of each melanocortin peptide, the intracellular signaling pathways melanocortin peptides use, and the contributions of peripherally expressed melanocortin receptors. We have examined functional roles for α-MSH and desacetyl-α-MSH centrally and peripherally by injecting the peptides centrally into adult rats and measuring food intake, by injecting the peptides peripherally into neonatal rats and measuring body growth, and by treating 3T3L1 adipocyte cell cultures with melanocortin peptides and measuring activation of the protein kinase A pathway. We have evidence that both peptides are biologically active and both are likely to be contributing to energy homeostasis acting centrally and peripherally.

3-1

Forkhead transcription factor FoxO1 in brown adipose tissue regulates energy expenditure through regulation of Pgc-1α and 4E-BP1 expression

Jun Nakae, Yongheng Cao, Miyo Oki, Yasuko Ohba, Hirofumi Sawa
21st Century Center of Excellence Program, Kobe University Graduate School of Medicine

The balance between food intake and energy expenditure determines energy homeostasis in vivo. Brown adipose tissue owns an important role in energy expenditure. Forkhead transcription factor FoxO1 is regulated by insulin/Igf-1 through PI3K/Akt signaling pathway and determines insulin sensitivity by regulating several gene expressions in insulin-responsive tissues. FoxO1 has been reported to be involved in adipocyte differentiation of 3T3-F442A cells. However, there are no reports about roles of FoxO1 in brown adipose tissue. Here, we constructed brown adipose tissue-specific FoxO1 transgenic mice in which a dominant negative FoxO1 (*Flag-Δ256*) is expressed in brown adipose tissue and analyzed their glucose metabolism and energy expenditure. These mice show improved glucose tolerance and increased energy expenditure and altered Pgc-1α and 4E-BP1 genes and protein expression under high fat dieted condition. In conclusion, FoxO1 in brown adipose tissue has an important role in glucose metabolism and energy expenditure by regulating expression of Pgc-1α and 4E-BP1.

3-2

Involvement of apolipoprotein E in excess fat accumulation and insulin resistance

Yasushi Ishigaki[1], Hideki Katagiri[2], Yoshitomo Oka[1]
[1] Division of Molecular Metabolism and Diabetes, [2] Division of Advanced Therapeutics for Metabolic Diseases, Center for Translational and Advanced Animal Research, Tohoku University Graduate School of Medicine

Although apolipoprotein E (apoE) is well known to play major roles in lipid metabolism, its roles in glucose and energy homeostasis remain unclear. Herein, we established apoE-deficient genetically obese Ay (apoE$^{-/-}$;Ay/+) mice. ApoE deficiency in Ay mice prevented development of obesity, with decreased fat accumulation in the liver and adipose tissues. ApoE$^{-/-}$;Ay/+ mice exhibited better glucose tolerance than apoE$^{+/+}$;Ay/+ mice. Insulin tolerance testing and hyperinsulinemic euglycemic clamp study revealed marked improvement of insulin sensitivity, despite increased plasma free fatty acid levels. These metabolic phenotypes were reversed by adenoviral replenishment of apoE protein, indicating circulating apoE to be involved in increased adiposity and obesity-related metabolic disorders. Uptake of apoE-lacking very low-density lipoprotein (VLDL) into the liver and adipocytes was markedly inhibited, but adipocytes in apoE$^{-/-}$;Ay/+ mice exhibited normal differentiation, suggesting that apoE-dependent VLDL transport is involved in development of obesity, i.e. surplus fat accumulation, but does not play a major role in normal adipocyte function. Interestingly, apoE$^{-/-}$;Ay/+ mice exhibited decreased food intake and increased energy expenditure. Pair-feeding experiments indicate both these phenomena to contribute to the obesity-resistant phenotypes associated with apoE deficiency. Thus, apoE is involved in maintaining energy homeostasis. ApoE-dependent excess fat accumulation is a promising therapeutic target for the metabolic syndrome.

3-3

Skp2 plays an essential role in adipocyte proliferation during the development of obesity

Tamon Sakai[1], Hiroshi Sakaue[1], Takehiro Nakamura[1], Mitsuru Okada[1], Yasushi Matsuki[2], Eijiro Watanabe[2], Ryuji Hiramatsu[2], Keiko Nakayama[3], Kei-ichi Nakayama[4], Masato Kasuga[1]

[1] Department of Clinical Molecular Medicine, Division of Diabetes and Digestive and Kidney Diseases, Kobe University Graduate School of Medicine; [2] Genomics Science Laboratories, Sumitomo Pharmaceuticals Co. Ltd.; [3] Department of Functional Genomics, Division of Developmental Genetics; [4] Department of Molecular and Cellular Biology, Division of Cell Biology, Medical Institute of Bioregulation, Kyushu University

In obesity, an increase in adipose tissue mass can arise through increases in cell size, cell number, or both. Here we show that long-term High Fat diet (HFD) for ~ 25 weeks induces the increase in adipocyte number as well as in adipocyte size in *C57BL/6* mice. In white adipose tissue (WAT) of mice fed with long-term HFD, the expression of F box protein Skp2 is induced, contrary to the significantly decrease in the protein of cyclin kinase inhibitor $p27^{Kip1}$, a principal downstream effector of SCF^{Skp2} ubiquitin ligase. Lack of Skp2 in mice protected the development of obesity induced by HFD and *lethal yellow agouti* (A^y) mutation due to the reduction of number, but not size, of adipocyte, although the reduction of number of β-cell in *Skp2–/–* mice caused glucose intolerance. Finally, lack of Skp2 in primary mouse embryo fibroblasts (MEFs) results in the inhibition in adipocyte differentiation with an accumulation of $p27^{Kip1}$. Our observations thus demonstrate that Skp2 plays an essential role in adipocyte proliferation during the development of obesity.

3-4

Down-regulation of HDACs stimulates adipocyte differentiation

Eung Jae Yoo, Jun-Jae Chung, Hyun Woo Lee, Sung Sik Choe, Kang Ho Kim, Jae Bum Kim

Department of Biological Sciences, Seoul National University

Specific cell type differentiation is driven by programmed regulation of gene expression which is the result of coordinated modulation of the transcription machinery and chromatin remodeling factors. We present evidence here that the down-regulation of histone deacetylases is an important process during adipocyte differentiation. In 3T3-L1 cells, histone hyperacetylation was selectively induced at the promoter regions of adipogenic genes during adipocyte differentiation. Interestingly, this was accompanied by a dramatic decrease in the expression level of several histone deacetylases including HDAC1, -2, and -5 and a reduction in overall histone deacetylase enzyme activity. Inhibition of histone deacetylase activity using sodium butyrate resulted in stimulation of adipogenic gene expression and adipocyte differentiation. Consistently, HDAC1 knock-down promoted adipogenesis while HDAC1 overexpression attenuated adipocyte differentiation in 3T3-L1 cells. Together, these results suggest that the regulation of not only adipogenic transcription factors, but also chromatin modifying enzymes is crucial for the execution of *bona fide* adipogenesis.

3-5

Role of IRS-1 serine phosphorylation in the regulation of insulin signaling and glucose transport

Young-Bum Kim

Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center and Harvard Medical School

It is clear that serine phosphorylation of IRS-1 protein in response to insulin has a dual role either to enhance or inhibit insulin signaling. Identifying the serine kinase that phosphorylates IRS-1 specific serine residues is important in understanding the mechanism behind the dysregulation of insulin action in altered metabolic states. We have previously shown that Rho-kinase directly phosphorylates serine 632/635 on IRS-1 *in vitro*. To establish the effects of serine 632/635 on IRS-1 function, we generated an IRS-1 with a point mutation that replaced serine 632/635 with alanine. In control cells transfected with WT-IRS-1, insulin increased total tyrosine phosphorylation of IRS-1 in CHO_{IR} cells. However, the ability of insulin to activate total tyrosine phosphorylation of IRS-1 was markedly decreased in these cells expressing IRS-1 mutants. In addition, tyrosine phosphorylation of p85 binding YXXM domain in IRS-1 was also reduced in IRS-1 mutants expressing cells. Concurrently, expression of IRS-1 serine 632/635 to the alanine mutant significantly decreased insulin-stimulated IRS-1 binding to the p85 subunit of PI 3-kinase, without changes in IR tyrosine phosphorylation. These data suggest that IRS-1 serine 632/635 residues play an important role in regulating IRS-1 function during insulin stimulation. To determine whether the modulation of IRS-1 serine 632/635 phosphorylation can alter insulin-stimulated glucose transport and PI 3-kinase activity, we transduced the recombinant adenovirus encoding the mutant of S632/635A-IRS-1 or S632/635E-IRS-1 in 3T3-L1 adipocytes. Expression of S632/635E-IRS-1 active mutant significantly increased insulin-stimulated glucose transport in 3T3-L1 adipocytes whereas expression of the S632/635A-IRS-1 inactive mutant decreased this. Expression of S632/635A-IRS-1 inactive mutants resulted in a ~40% decrease in PI 3-kinase activity compared with WT-IRS-1 expressing cells. Importantly, insulin-induced IRS-1 serine 632/635 phosphorylation was impaired in skeletal muscle of insulin-resistant mice with obesity and diabetes. Moreover, infusion of lipid resulted in a significant inhibition of IRS-1 serine 632/635 phosphorylation in skeletal muscle *in vivo*. Together, these data suggest that IRS-1 serine 632/635 phosphorylation positively regulates insulin-stimulated insulin signaling and glucose transport, and further implicate that a reduction in these phosphorylations could be involved in insulin-resistant state.

Session 6
Oral Presentation

6-1

From QTL to candidate gene, a story told by a diabetes mouse model

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New Zealand Obese (NZO) mice develop juvenile-onset obesity and maturity-onset hyperinsulinemia, hyperleptinemia, hyperglycemia, and hypertension. 50% of NZO males develop obesity-associated diabetes (diabesity). Genetic and biochemical analysis indicates a dysregulated hepatic phosphatidylcholine (PC) metabolism in these mice. Segregation analysis between NZO/HILt and NON/Lt identified a recessive NZO-derived diabesity QTL designated *Nidd3* on Chromosome 11. A null mutation in the NZO *Pctp* allele encoding the PC transfer protein represents an excellent *Nidd3* candidate gene. *Pctp*, phosphatidylcholine transfer protein, is one candidate gene in *Nidd3* participating in PC metabolism. PCTP is a highly specific PC transporter between membranes in the cytosol. It belongs to the steroidogenic acute regulatory protein related transfer (START) domain superfamily of hydrophobic ligand-binding proteins, and its physiological importance is currently unknown. Sequence analysis in the NZO 5'-promoter region revealed a 12 bp deletion and a polymorphism in the CAAT-box. In the *Pctp* coding region we found the NZO allele contained a point mutation, R120H, that would alter steric interactions between R120 and two conserved aspartic acid residues (D70, D122). Although qPCR showed a near 20-fold lower *Pctp* transcript expression in NZO liver relative to NON, the strain carrying a diabesity resistance allele at *Nidd3*, protein content was comparable. Functional studies in vitro comparing recombinant PCTP proteins confirmed the null-functional consequences produced by the R120H mutation on PC transport. Thus, defects in the NZO *Pctp* gene combines with other deficiencies in PC metabolism in this mouse model of polygenic diabesity.

6-2

Diabetes protein signature in human serum revealed by label-free and pathway-associated differential proteomics

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Diabetes remains one of the most frequent progressing diseases in the world. Recent advances in the proteomics has open the way to wider and deeper understanding of pathogenesis of diabetes aiming at the development of precise and targeted prediction and intervention. As a metabolic disease, diabetes-related proteins tend to enter the circulation system, thus the biomarker discovery in plasma has the potential to play an important role in the early detection and treatment of diabetes. Protein analysis of plasma is a formidable challenge, due to its huge complexity and dynamic range. In this work, human serum from normal and diabetes were separated and identified by gel enhanced liquid chromatography tandem mass spectrometry (GeLC-MS/MS). A total of 4787 proteins and 4885 proteins were identified from the two cohorts. Label-free analysis based on peptide spectral counts offer a fast and sensitive approach to detect the diabetes-associated protein patterns. This qualitative and quantitative analysis of serum-proteins provide a snapshot of the protein expression state in normal and diabetes serum and should be very helpful to identify the biomarker and understand the mechanism of diabetes.

6-3

Berberine activates AMPK and has beneficial metabolic actions in diabetic and insulin resistant states

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Berberine (BBR), a natural plant product used in traditional Chinese medicine, has been reported to have an antidiabetic action, although this is not well documented and the mechanisms involved are unknown. Our aim was to identify beneficial metabolic effects of berberine and to clarify possible mechanisms of action. Gavage of BBR (560 mg/day for 14 days) to db/db mice significantly lowered basal blood glucose compared with vehicle-treated controls (16±2 vs 27±2 mM, $p<0.01$)) and improved glucose tolerance after intraperitoneal glucose loading. In addition rats made insulin resistant by 4 weeks high fat feeding and gavaged with BBR (380 mg/kg/day) for the last 2 weeks had improved insulin sensitivity compared with vehicle controls as assessed by euglycemic clamp (glucose infusion rate 28 1 vs 22 1 mg/kg/min, $p<0.01$)); this was associated with lower circulating triglyceride levels (0.8±0.1 vs 1.1±0.2 mM, $p<0.01$) and liver triglyceride content(reduced by 50%, $p<0.01$). Food intake was not affected by BBR in either study, nor did BBR alter parameters in normal rodents. To provide a mechanism, we assessed acute BBR action in L6 cells; here BBR increased AMPK activation (phosphorylated AMPK increased twofold) and potently and rapidly (within 2 min) enhanced GLUT4 translocation (fraction of GLUT4 at plasma membrane increased fourfold using a reporter fluorescence assay), similar to that observed for insulin. In contrast to insulin however, BBR stimulation was not blocked by Wortmannin, indicating that this occurred in a PI3 kinase independent manner, and was similar to GLUT4 translocation by AICAR, a known AMPK agonist. We conclude that BBR has beneficial metabolic actions in diabetic and insulin resistant states, likely to be at least partly related to activation of the AMPK pathway.

6-4

Dissecting the role of individual isoforms of PI 3-kinase in insulin signalling

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A large body of evidence indicates that PI 3-kinase plays a crucial role in mediating insulins effects on many downstream signalling pathways although currently very little is known about which isoforms of PI 3-kinase are involved in this process. We have used novel isoform selective inhibitors of PI 3-kinase to dissect insulin signalling pathways in a range of cell types. The compounds used are the p110α inhibitor PIK75 and the p110β specific inhibitor TGX221. We find that TGX221 has no effect on insulin signalling to PKB, GSK3 or p70S6kinase in a range of cell lines including 3T3-L1 fibroblasts, 3T3-L1 adipocytes, HepG2 cells and CHO-IR cells at concentrations up to a 100x the IC_{50} for p110β. This provides evidence that p110β is not a major player in insulin signalling in these cells. In contrast, PIK75 blocks insulin stimulation of PKB in 3T3-L1 fibroblasts, 3T3-L1 adipocytes and CHO-IR at concentrations only slightly above the IC_{50} for p110α. Surprisingly though it had no effect on insulins stimulation of PKB in HepG2 cells. This indicates that p110α plays a major role in insulin signalling in some, but not all cell types. PIK75 also blocked insulin induced phosphorylation of GSK3b in CHO-IR cells consistent with the canonical link between PKB and GSK3. However, in 3T3-L1 cells this was not observed, despite the fact that PIK75 blocked activation of PKB in these cells. This provides clear evidence that in these cells that the phosphorylation of GSK3β does not require the activation of PKB and that an alternative kinase must be responsible for phosphorylating GSK3β in these cells. In conclusion, the availability of isoform specific inhibitors has allowed us to reveal novel linkages in insulin signalling pathways. Ongoing studies will be described using these inhibitors to more fully probe intracellular signalling pathways.

Session 6
Oral Presentation

6-5

Physiological function of the unfolded protein response using single target gene analysis

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Obesity, a contributor of type 2 diabetes, activates the cellular unfolded protein response (UPR). The UPR is a stress inducible pathway found in all eukaryotes that monitors the health of the endoplasmic reticulum (ER). If an imbalance is detected, signals are transmitted to regulate the expression of specific target genes and, in the case of metazoans, modulate protein synthesis. Once homeostasis is restored, cells are returned to a resting state. Genome wide expression studies have shown that multiple pathways including protein folding, protein quality control/degradation, and membrane trafficking are regulated by the UPR. How the activation of individual target genes contributes to stress tolerance is, however, unknown. To address the question directly, we uncoupled transcriptional regulation of the molecular chaperone BiP from the UPR in budding yeast. In these cells, the UPR is normal except in its activation of BiP. As expected, unstressed cells grow normally but exhibit stress tolerance defects. We demonstrate that the basis for the defect is in BiP's inability to process misfolded proteins for degradation when limiting. As a consequence, cells accumulate aberrant proteins in the ER that leads to cell death.

6-6

Inhibition of PKC epsilon as a novel strategy for treatment of type 2 diabetes, by reconstitution of glucose-induced insulin secretion and diminution of hepatic insulin clearance

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Type 2 diabetes (T2D) is defined by a failure of the pancreatic β-cell to secrete sufficient insulin to compensate for peripheral insulin resistance. Here we address a role for the lipid-regulated, protein kinase C(PKC) isoform, PKCε, in these processes. We show that, following a high-fat diet, glucose tolerance is profoundly improved in mice deleted in the PKCε gene, compared to wildtype mice. This was not associated with improved muscle insulin action, but rather an enhanced availability of insulin due to two independent effects. Firstly, we found that PKCε deletion decreased whole-body insulin clearance, independent of diet, which was confirmed as a diminished uptake of insulin by PKCε$^{-/-}$ hepatocytes *ex vivo*. PKCε deletion was without effect on insulin secretion *per se*, but specifically restored secretory responsiveness to glucose in fat-fed animals, or in isolated islets chronically exposed to fatty acids *in vitro*. Intraperitoneal administration of a cell-permeant PKC-inhibitory peptide also improved both insulin availability and glucose tolerance in *db/db* mice with pre-existing diabetes. In addition, the inhibitory peptide reconstituted glucose-induced insulin secretion *in vitro* using islets isolated from *db/db* mice, but had no effect on secretion with islet from non-diabetic animals. This strongly suggests that the PKCε inhibitors actually target the underlying cause of secretory dysfunction, rather than over-riding, or bypassing it, as all current T2D therapeutics do. This was confirmed with the demonstration that PKCε was activated in islets from diabetic, but not control, mice. Our findings provide proof-of-principle that inhibition of previously unknown functions of PKCε in liver and pancreatic islets might constitute a completely novel, and highly specific, strategy for the treatment of Type 2 diabetes.

7-1

Role of cyclophilin B in ER stress and insulin processing

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We here describe the possible role (s) of cyclophilin B (CypB) as a novel defense against ER stress and misfolding of insulin during synthesis. Expression of the CypB gene is rapidly up-regulated through a novel ERSE in cells exposed to thapsigargin (Tg) and tunicamycin (Tm). Overexpression of CypB attenuates ER stress-induced cell death, whereas that of a mutant, CypB/R62A defective in foldase activity, not only increases calcium leakage from the ER and ROS generation, but also decreases mitochondrial membrane potential leading to cell death after exposure to ER stress inducing reagents. siRNA-mediated inhibition of CypB expression renders cells more vulnerable to ER stress. Also, CypB shows physical interactions with ER stress-related chaperones, Bip/Grp78 and Grp94. Interestingly, all insulins of human, rat, pig and bovine have CypB binding site. GST-pulldown assays also show physical association of insulin with CypB. Taken together, we concluded that CypB plays an essential role in guarding cells against ER stress and correct folding of insulin during insulin synthesis.

7-2

Upregulation of islet beta-cell fructose 1,6 bisphosphatase results in insulin secretory dysfunction

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Session 7
Oral Presentation

Fructose-1,6-bisphosphatase (FBPase). is a key enzyme in the gluconeogenic pathway and is abundant in the liver and kidneys, but is poorly expressed in pancreatic β -cells. Interestingly, FBPase has been shown to be unregulated in islets or pancreatic β-cell lines exposed to high fatty acid environments, conditions known to cause defects in insulin secretion. Furthermore we have preliminary data showing increased FBPase expression in islets of patients with Type 2 diabetes compared to control subjects. We propose that upregulation of FBPase in pancreatic β–cells contributes to defects in insulin secretion either by activating the hexosamine biosynthesis pathway or by futile cycling with phosphofructokinase, an enzyme that opposes the action of FBPase. To test our hypothesis we have generated: (a). a pancreatic β -cell line MIN6. Overexpressing the human FBPase gene, and (b). transgenic mice overexpressing the human FBPase gene specifically in the pancreatic β -cells. Compared to the un-transfected parental cells,

FBPase overexpressing MIN6 cells have an increase in both FBPase protein level and activity, a decrease in the rate of cell proliferation, significantly depressed glucose and non-glucose induced insulin secretion, associated with a similar flux through the hexosamine biosynthesis pathway but a decrease in the rate of glucose utilisation and ATP levels. Consistent with the overexpression of FBPase, its substrate fructose-1,6-bisphosphate level was lower and its product fructose-6-phosphate level was higher in the transfected compared with the parental cells. In support of the *in vitro* studies, FBPase transgenic mice showed a reduced first phase insulin secretion during an intravenous glucose (5.2 ± 1.1 vs 12.9 ± 2.2 ng/mlx5min, $P<0.05$) or arginine (8.1 ± 0.4 vs 18.6 ± 1.5 ng/mlx5min, $P<0.05$) bolus compared to their negative littermates. Taken together these results suggest that upregulation of FBPase in pancreatic β -cells can contribute to insulin secretory dysfunction similar to that caused by excess circulating fatty acids and adiposity present in type 2 diabetes.

7-3

RNA editing by ADAR2 is metabolically regulated in pancreatic islets and β-cells

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RNA editing via the conversion of adenosine (A) to inosine (I) is catalyzed by two major families of adenosine deaminases acting on RNA (ADARs), ADAR1 and ADAR2. This genetic recoding process is known to play essential roles in the brain, due in part to the changes in functional activities of edited neurotransmitter receptors and ion channels. Little is known, however, about the physiological regulation and function of A-to-I RNA editing in peripheral tissues and other biological processes. Here we report that both ADAR1 and ADAR2 were expressed in the murine pancreatic islets, and ADAR2 was primarily localized in the islet endocrine cells. In contrast to ADAR1, ADAR2 transcription in the pancreatic islets increased by nearly two-fold in insulin-resistant mice chronically fed a high-fat diet. Concurrent with this diet-induced metabolic stress, RNA editing in the islets was dramatically enhanced for the RNA transcripts encoding the ionotropic glutamate receptor GluR-B. Moreover, ADAR2 protein expression was repressed in the islets under fuel deficiency condition during fasting, and this repression could be reversed by refeeding. We also found that, specifically in pancreatic β-cell lines, not only the expression of ADAR2, but the GluR-B editing and ADAR2 self-editing were markedly augmented in response to glucose stimulation at the physiological concentration for insulin secretion stimulation. Hence RNA editing by ADAR2 in pancreatic islets and β-cells is metabolically regulated by nutritional and energy status, suggesting that A-to-I RNA editing is most likely involved in the modulation of pancreatic islet and β-cell function. Currently, the functional impact of A-to-I editing by ADAR2 upon the endocrine modulation of pancreatic islets and β-cells is under further investigations in our attempt to delineate the mechanistic function of RNA editing in the homeostatic control of energy metabolism.

7-4

Crucial role for an evolutionarily conserved *cis*-regulatory region of *pdx1* in pancreas organogenesis and islet function

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Pdx1 (*IPF-1* in humans, which is altered in *MODY-4*) is essential for pancreas development and mature β-cell function. Pdx1 is expressed dynamically within the developing foregut, but how its expression characteristics are controlled *in vivo* is poorly understood. We previously identified an evolutionarily conserved enhancer region (Area I-II-III) in the 5' upstream region of *Pdx1* gene. Deletion of the conserved enhancer region from *Pdx1* produced a hypomorphic allele. Homozygous Area I-II-III deletion mutants ($Pdx1^{\Delta I\text{-}II\text{-}III/\Delta I\text{-}II\text{-}III}$) revealed lack of ventral pancreatic bud outgrowth and early-onset hypoplasia in the dorsal bud. Acinar tissue formed in the hypoplastic dorsal bud, but endocrine maturation was greatly impaired. Heterozygous ($Pdx1^{\Delta I\text{-}II\text{-}III/+}$) mice had severely impaired glucose tolerance with abnormal islet structures. These findings provide *in vivo* evidence for a crucial role for the enhancer element in pancreas organogenesis and islet function.

Session 7 Oral Presentation

7-5

Inducible nitric oxide synthase (iNOS) plays an important role in hypoxic injury to pancreatic beta cells

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Background: Islet transplantation is an alternative potential strategy to cure type 1 diabetes mellitus. However, two or more donors are usually needed for one recipient because a substantial part of the graft becomes nonfunctional due to several factors including hypoxia. Pancreatic beta cells are susceptible to various cytokines. Among them, nitric oxide (NO) is one of the important cytokines. In type I diabetes, NO is known as an important cytokine, involved in the pathogenesis of beta cell dysfunction. In addition, hypoxia is known to induce inducible nitric oxide synthase (iNOS)-derived nitric oxide (NO) production in many types of cells. The aim of the study was to investigate iNOS-NO signalling and its role in pancreatic β cells damage under hypoxic conditions.

Methods: Mouse insulinoma cells (MIN6) and primary isolated islets from Sprague-Dawley rats were incubated in an anaerobic chamber (85% N_2/5% H_2/10% CO_2) for up to 12 hours. Cell viability was measured by acridine orange/propidium iodide staining. Caspase-3 activation was also determined using Western blof analysis. NO release into culture medium was measured using a Griess reagent. The expression of iNOS mRNA and protein was examined using real time RT-PCR and Western blot analysis. The PDX-1 promoter activity was performed by luciferase assay.

Results: Marked cell death (~50%) was observed within 6 hour after hypoxic exposure to MIN6 cells and rat islets. Immunoreactivity to activated caspase-3 was observed. NO production measured by Griess reagent method was increased in a time dependent manner. Expression of iNOS mRNA and protein was significantly increased at 4 and 6 hour after hypoxia, and iNOS specific inhibitor (1400W) lowered cell death rate effectively. iNOS expression was confirmed by immunostaining. Of note, Pdx-1 mRNA expression was markedly attenuated by hypoxia. The Pdx-1 gene promoter activity in MIN6 was negatively regulated by hypoxia or a NO donor (DETA-NO). Pretreatment with a selective iNOS inhibitor, 1400W, significantly prevented beta cell death induced by hypoxic injury.

Conclusion: Our data suggest that iNOS-NO play an important role in hypoxic injury to pancreatic β cells and PDX-1 regulation. Therefore, iNOS-NO might be a potential therapeutic target for improving engraftment of transplanted islets and helpful for prevention of beta cells damage to hypoxic injury.

9-1

Adiponectin inversely correlates with triglyceride and high-sensitivity C-reactive protein but not with insulin or insulin resistance index in Japanese men

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Adiponectin (AD) is an adipokine that ameliorate insulin resistance and that possess anti-atherosclerotic effect. Hypoadiponectinemia due to increased visceral fat is considered to convey obesity to metabolic syndrome (MetS). However, pathophysiological significance of AD is not established in Japanese general population where the degree of obesity and insulin resistance is mild. To this end, we analyzed various parameters in 166 consecutive Japanese male adult health examinees. Established diabetic subjects were excluded. The various parameters including age, body mass index (24.5±2.8), %body fat, abdominal circumference, blood pressure, fasting plasma glucose, fasting insulin, LDL-cholesterol, HDL-cholesterol, triglyceride (TG), serum AD concentration, high sensitive CRP (hsCRP) were determined. Ten subjects were excluded from the analysis because of abnormally high of CRP indicating acute inflammatory state.

Results:

1) Lower AD was independently correlated with higher TG (ß=-0.254, *p*=0.003) and higher hsCRP (ß=-0.252, *p*=0.001). None of other variables including fasting IRI and an index of insulin resistance such as HOMA-R was independently correlated with AD.
2) Higher TG, but not all other variables, was significantly (RR=13.03, *p*=0.021) related to hyoadiponectinemia (<4 µg/ml).
3) When hsCRP was taken as a dependent variable, only AD was significantly correlated (ß=-0.267, *p*=0.002).
4) In subjects with MS, AD was lower and hsCRP higher than in those without it (AD, 5.4±2.8 *vs* 7.5±4.2 µg/ml, *p*=0.002; hsCRP, 832±605 *vs* 470±524 ng/ml, *p*=0.0004). Other measurements were not siginficantly differente between the two groups.

Conclusion: In Japanese men, correlation between AD and TG, and AD and hsCRP were unequivocal. In contrast, independent correlation between AD and insulin or HOMA-R did not exist. In a population where both obesity and insulin resistance are mild, AD may not be a dominant determinant of insulin sensitivity.

9-2

The role of the macrophage in obesity induced insulin resistance

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Although the pathogenesis of type 2 diabetes is not fully understood, growing evidence links this disease to a state of chronic inflammation in several tissues such as the pancreas, liver, adipose tissue and skeletal muscle. Recent studies have identified that obesity is associated with macrophage accumulation in adipose tissue which has prompted the scientific community to consider new models to include a major role for macrophages in the molecular changes that occur in adipose tissue in obesity. It is now thought that with weight gain, an increased expression of chemoattractant proteins, such as MCP-1, results in recruitment of macrophages into tissue beds such as adipocytes. Recent work from our group has uncovered that hyperlipidemia is the stimulus for macrophage inflammation. Our data show that saturated fatty acids result in activation of several inflammatory pathways in macrophages. We have shown that fatty acids activate the 'Toll-like' receptor signaling pathway which leads to the activation of IKK and subsequently the up-regulation of NFκB-dependent genes such as TNF-α. This leads to newly synthesized TNF-α protein which is ultimately released to impaired insulin signal transduction pathways in adipocytes. We have also shown that fatty acids activate the endoplasmic reticulum (ER) stress pathway, and blocking ER stress can ameliorate the negative effects of hyperlipidemia. Studies *in vivo* indicate that disruption of anti-inflammatory processes leads to local and systemic insulin resistance, while disrupting fatty acid uptake by the macrophage may convey protection against diet-induced obesity. This presentation will review this recent work which indicates that the macrophage is an important therapeutic target for the treatment of obesity related diseases.

9-3

Overexpression of Monocyte Chemoattractant Protein 1 (MCP-1) in adipose tissues causes systemic insulin resistance

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Adipose tissue expression and circulating concentrations of monocyte chemoattractant protein-1 (MCP-1) correlate positively with adiposity, but the role of elevated MCP-1, which may work locally or systemically, in insulin resistance, has not been directly addressed. To ascertain the roles of MCP-1 overexpression in adipose, we generated transgenic mice by utilizing the aP2 promoter (aP2-MCP-1 mice). These mice had higher plasma MCP-1 concentrations and increased macrophage accumulation in adipose tissues, as confirmed by immunochemical, flow cytometric and gene expression analyses. TNF-α and IL-6 mRNA levels in white adipose tissue and plasma NEFA levels were increased in transgenic mice. aP2-MCP-1 mice showed insulin resistance, suggesting that inflammatory changes in adipose tissues may be involved in the development of insulin resistance. Insulin resistance in aP2-MCP-1 mice was confirmed by hyperinsulinemic euglycemic clamp studies showing that transgenic mice had lower rate of glucose disappearance and higher endogenous glucose production than wild-type mice. Consistent with this, insulin-induced phosphorylations of insulin receptors, insulin receptor substrates and Akt were significantly decreased in both skeletal muscles and livers of aP2-MCP-1 mice. In vitro MCP-1 treatment of myotube cells attenuated insulin-induced phosphorylation of Akt. In addition, MCP-1 pretreatment of isolated skeletal muscle blunted insulin-stimulated glucose uptake, which was partially restored by treatment with the MEK inhibitor U0126, suggesting that circulating MCP-1 may contribute to insulin resistance in aP2-MCP-1 mice. We concluded that both paracrine and endocrine effects of MCP-1 contribute to the development of insulin resistance in aP2-MCP-1 mice.

10-1

Adipocyte-FABP as a novel player in the metabolic syndrome: clinical and functional studies

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Adipocyte-FABP (A-FABP) is known as a cytosolic fatty acid chaperone expressed in adipocytes and macrophages. Mice with A-FABP were strongly protected from hyperglycemia and insulin resistance in dietary and genetic obesity and atherosclerosis, suggesting that A-FABP plays a critical role in regulating systemic insulin sensitivity, lipid metabolism and inflammation. Recent data from our laboratory suggest that A-FABP can act as a circulating hormone to regulate lipid metabolism and insulin sensitivity. Murine A-FABP was confirmed to constitute >1% of the total protein secreted from 3T3-L1 adipocytes, separated by two-dimensional gel electrophoresis and identified by Edam degradation sequencing and MALDI TOF MS/MS mass spectrometry. Western blot analysis showed that A-FABP was abundantly present inside the cells as well as in the extracellular medium while β-tubulin, a cytoskeleton protein, was present only in the cell lysate. Using similar proteomics-based strategy, we also confirmed the presence of A-FABP in the human plasma. Using a sandwich ELISA assay for human A-FABP, we were able to show that the circulating levels of A-FABP correlated highly with BMI, plasma triglyceride, fasting insulin and 2-hour post-challenge plasma glucose levels. Its role as a novel marker of the metabolic syndrome was suggested by the finding that increasing A-FABP level was associated with increasing number of components of the metabolic syndrome in a cross-sectional study. This was confirmed by the subsequent finding of a population-based prospective follow-up study which showed that basal A-FABP level was predictive of the development of the metabolic syndrome. Daily intra-peritoneal injection of 200 μg AFABP administered to lean C57 mice twice a day for 2 weeks significantly increased circulating triglyceride levels and caused a reduction in the weight of the epididymal fat pads. In vitro treatment of A-FABP was found to decrease the insulin-stimulated glucose uptake in L6 muscle cells, in part via a reduction of Akt phosphorylation. Collectively, these data suggest that A-FABP is released from the adipocytes into the blood stream. Furthermore, in addition to its intracellular action in the adipocytes, it appears to have actions on insulin sensitivity at the level of the muscle cells, which may also contribute to its effect on glucose and lipid metabolism, and the development of the metabolic syndrome. More recently, data from population-based genetic studies have demonstrated a relationship between functional promoter variants of the human A-FABP gene and the risk of hypertriglyceridaemia, type 2 diabetes and cardiovascular disease. In addition to important insight into the genetic regulation of A-FABP expression, these data have provided further support for the importance of A-FABP in obesity-related metabolic and cardiovascular disorders.

10-2

Elevated circulating concentrations of lipocalin 2 may contribute to obesity-associated ectopic lipid accumulation and insulin resistance in mice

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The PPARγ agonists thiazolidinediones (TZDs) are now widely used for the treatment of obesity-related insulin resistance and Type 2 diabetes. To get more insight into the molecular events that underlie the therapeutic actions of TZDs, we have used DNA array analysis to identify genes differentially expressed in response to rosiglitazone treatment in *db/db* diabetic/obese mice. We found that the expression of the gene encoding lipocalin 2, a 25 kDa secreted glycoprotein, was selectively increased in adipose tissue and liver of *db/db* mice. Treatment of rosiglitazone decreased the mRNA expression of lipocalin 2 and also reduced its serum protein concentrations. Clinical studies on 229 subjects demonstrated that serum lipocalin 2 levels in obese subjects were significantly higher than those in the age- and sex- matched lean individuals. In addition, serum lipocalin 2 was positively correlated with body weight index, fat percentage, dyslipidemia, hyperglycemia and the index of insulin resistance. Chronic treatment of C57 mice with recombinant lipocalin 2 decreased glucose tolerance and insulin sensitivity, and increased triglyceride accumulation in liver and skeletal muscle. In addition, lipocalin 2 enhanced fatty acid uptake and suppressed fatty acid oxidation in myotubes, possibly by modulating the expression of several key genes involved in these events. Taken together, our results suggest that elevated lipocalin 2 in obesity play a causative role in the development of insulin resistance and diabetes, and that the insulin-sensitizing effects of the PPARγ agonists might be partly attributable to their suppressive effects on lipocalin 2 production.

10-3

CXS741, a novel secreted protein involved in the pathogenesis of obesity and type 2 diabetes

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Background: Secreted and membrane bound proteins play an important role in intercellular communication and are targets for the development of therapeutic agents. CXS741 was recently identified as a differentially expressed secreted protein using signal sequence trap methodology in conjunction with a cDNA microarray in red gastrocnemius tissue from *Psammomys obesus*, a polygenic model of obesity and insulin resistance.
Methods: CXS741 gene expression was measured in *P. obesus* using RT-PCR and an ELISA was developed to measure CXS741 levels in human plasma from 145 NGT and 142 T2D subjects.
Results: Analysis of CXS741 gene expression by RT-PCR in *P. obesus* revealed markedly higher gene expression in adipose tissue compared to all other tissues investigated, and expression was higher in visceral adipose tissue in comparison to subcutaneous fat. Plasma CXS741 measured by ELISA was found to be significantly elevated by 14% in T2D subjects ($p=0.043$). Furthermore, in the male subjects plasma CXS741 levels correlated with waist circumference ($p=0.007$), and both fasting and 2-hour glucose levels in an OGTT ($p=0.024$ and $p=0.001$, respectively).
Conclusions: CXS741 represents a novel candidate secreted protein for further characterisation in the pathogenesis of obesity and type 2 diabetes.

10-4

Pathophysiological roles of adipokine network in metabolic syndrome

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Adiponectin/Acrp30 is a hormone secreted by adipocytes, which acts as an antidiabetic adipokine via inhibition of gluconeogenesis and stimulation of fatty acid oxidation. We reported previously that AdipoR1 and -R2 serve as receptors for adiponectin in vitro, and that their expression levels are reduced in obesity, which appeared to be correlated with reduced adiponectin sensitivity.

In this study, we showed that adenovirus-mediated expression of AdipoR1 in liver of db/db mice increased adiponectin effect such as increased activation of AMP kinase by adiponectin, decreased molecules involved in gluconeogenesis and increased fatty-acid oxidation, thereby ameliorating diabetes. Moreover, adenovirus-mediated expression of AdipoR2 in liver of db/db mice increased adiponectin effect such as increased PPARα target genes including molecules involved in fatty acid oxidation and energy dissipation, thereby ameliorating diabetes.

In contrast to overexpression, disruption of AdipoR1 or R2 resulted in glucose intolerance via decreased activation of primarily AMP kinase or PPARα pathway, respectively. Importantly, simultaneous disruption of AdipoR1 and R2 almost abolished adiponectin specific binding and glucose-lowering effect by adiponectin, indicating that AdipoR1 and R2 serve as receptors for adiponectin in vivo. These data suggested that AdipoR1 may be tightly linked to activation of AMP kinase pathway, whereas AdipoR2 may be tightly linked to activation of PPARα pathway.

Recently, it has been reported that chronic inflammation in white adipose tissue (WAT) by macrophage infiltration may result in whole-body insulin resistance in obese diabetic animals. We showed that simultaneous activation of PPARγ and PPARα increased adiponectin and AdipoRs, respectively, and at the same time reduced MCP-1 and macrophage infiltration, leading to amelioration of obesity-induced inflammation and insulin resistance.

Taken together, adiponectin receptor agonists and adiponectin sensitizers should serve as versatile treatment strategies for obesity-linked diseases such as diabetes and metabolic syndrome.

P-1

Glucose and fat metabolism in adipose tissue of acetyl-CoA carboxylase 2 knockout mice

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Acc2$^{-/-}$ mutant mice, when fed a high-fat/high-carbohydrate (HF/HC) diet, were protected against diet-induced obesity and diabetes. To investigate the role of ACC2 in the regulation of energy metabolism in adipose tissues, we studied fatty acid and glucose oxidation in the primary cultures of adipocytes isolated from the wild type and *Acc2*$^{-/-}$ mutant mice fed either a normal chow or a HF/HC diet. When fed a normal chow, the oxidation of [^{14}C]-palmitate in the adipocytes of *Acc2*$^{-/-}$ mutant mice was about 80% higher than that of WT, and it remained significantly higher in the presence of insulin. Interestingly, in addition to the increased fatty acid oxidation, we also observed increased glucose oxidation in the adipocytes of *Acc2*$^{-/-}$ mutant mice compared to the WT mice. When fed a HF/HC diet for 4 to 5 months, the adipocytes of *Acc2*$^{-/-}$ mutant mice maintained a 25% higher palmitate oxidation and 2-folds higher glucose oxidation than those of WT mice. The mRNA level of glucose transporter 4 (GLUT4) decreased several folds in the adipose tissue of WT mice fed a HF/HC diet; however, in the adipose tissue of *Acc2*$^{-/-}$ mutant mice, the mRNA level of GLUT4 was 7 folds higher than in WT mice. In addition, the lipolysis activity was higher in adipocytes of *Acc2*$^{-/-}$ mutant mice compared to that of WT mice. These findings suggest that continuous fatty acid oxidation in the adipocytes of *Acc2*$^{-/-}$ mutant mice, combined with a higher level of glucose oxidation and a higher rate of lipolysis, are major factors leading to efficient maintenance of insulin sensitivity and leaner *Acc2*$^{-/-}$ mutant mice.

P-2

The role of adipose glycerol channel; aquaporin adipose/7

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Triglycerides in adipocytes are hydrolyzed to fatty acids and glycerol and both are released into the circulation. The molecular mechanism involved in the transport of glycerol from adipocytes remained unclear. We previously cloned a cDNA belonging to aquaporin family from a human adipose tissue cDNA library, designated as aquaporin adipose (AQPap). Subsequently, other groups demonstrated that AQPap was a human homologue of AQP7, which was cloned from the rat testis. AQPap/7 is mainly expressed in adipose tissue, testis, heart, and kidney, and subcategorized to aquaglyceroporin that permeate glycerol as well as water. AQPap/7 is involved in glucose and glycerol metabolism based on marked modulation of its expression by dietary conditions.

We recently generated and analyzed the mice lacking AQPap/7. Although there was no difference in body weight between wild-type (WT) and AQPap/7 knockout (KO) mice until 10 weeks of age, here we found that KO mice developed adult-onset obesity. Adipocytes of KO mice were large and exhibited accumulation of triglycerides compared with WT mice. The KO mice developed obesity and insulin resistance even at a young age after consumption of high-fat/high-sucrose diet. To clarify the underlying mechanism for obesity in KO mice, we further analyzed these mice at 6-10 weeks of age. We found the increased glycerol contents and the elevated activity of glycerol kinase (Gyk) in adipose tissue of KO mice. Gyk is a key enzyme that converts glycerol to glycerol-3-phosphate. To confirm the effect of AQPap/7 deficiency on adipocytes, we knocked down AQPap/7 in 3T3-L1 adipocytes by using RNAi. Knockdown of AQPap/7 in adipocytes was associated with a significant reduction of glycerol in media and elevation of cellular glycerol content. Enzymatic activation of Gyk was observed in AQPap/7-knockdown adipocytes. Finally, the uptake of oleic acid significantly increased in AQPap/7-knockdown adipocytes. In summary, KO mice developed obesity following severe insulin resistance, and that the lack of AQPap/7 induced glycerol kinase activity and accumulation of triglycerides in adipocytes.

P-3

Pioglitazone improves insulin sensitivity by increasing adiponectin secretion from fat tissue via reduced expression of SOCS3

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Pioglitazone is widely used for the treatment of diabetic patients with insulin resistance. However, its precise mechanism to improve insulin sensitivity is not fully understood. Recent studies have indicated that SOCS3 may be involved in the development of insulin resistance. In this study, we examined the involvement of SOCS3 in the mechanism for insulin-sensitizing effect of pioglitazone. In *db/db* mice, expression levels of SOCS3 in fat, liver, and skeletal muscle were increased compared to the lean control mice, and pioglitazone suppressed them. In 3T3-L1 adipocytes, various mediators that induce insulin resistance, i.e., TNFα, IL-6, growth hormone and insulin, increased expression of SOCS3, which was partially inhibited by pioglitazone. PPARγ overexpression enhanced the effects of pioglitazone to inhibit SOCS3 induction by TNFα, demonstrating an important role of PPARγ activation in the mechanism. Overexpression of SOCS3 in 3T3-L1 adipocytes partially inhibited adiponectin expression, which was accompanied by decreased STAT3 phosphorylation. Treatment of the cells with tyrphostin AG490, a JAK2 inhibitor, or the expression of dominant negative STAT3 suppressed adiponectin expression, in parallel with decreased STAT3 phosphorylation. Furthermore, pioglitazone administration to *db/db* mice increased STAT3 phosphorylation in fat tissue. Taken together, pioglitazone suppresses SOCS3 expression in fat tissue, with the enhancement of STAT3 activation and adiponectin secretion. This may be at least a part of the mechanisms for the improved whole body insulin sensitivity by pioglitazone treatment.

Session 4
Poster

P-4

Uncoupling protein 2 promoter polymorphism -866G/A affects peripheral nerve dysfunction in Japanese type 2 diabetic patients

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OBJECTIVE

To determine genetic predispositions for diabetic polyneuropathy, we investigated the relationship between -866G/A polymorphism of uncoupling protein 2 (UCP2) and neurological manifestations in 197 type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

We first examined whether UCP2 mRNA had been expressed in the dorsal root ganglion (DRG) in 4 Long Evans Tokushima Otsuka (LETO) rats using reverse transcription-polymerase chain reaction (RT-PCR) and electrophoresis. Genotyping of UCP2 promoter polymorphism -866G/A was then performed in 197 unrelated Japanese type 2 diabetic patients, who were subjected to nerve conduction, quantitative vibratory perception, head-up tilt and heart rate variability tests, by PCR-restriction fragment-length polymorphism. The relationships between UCP2 genotype and various nerve functions were analyzed by uni- and multi-variable analysis.

RESULTS

Expression of UCP2 mRNA was confirmed in rat DRG. Multiple regression analysis clarified that the G/A+A/A genotype was significantly related with decreased motor nerve conduction velocity and impaired blood pressure maintenance on head-up tilt test. Multiple logistic regression analysis revealed that the G/A+A/A genotypes are a significant risk factor for sensory nerve conduction slowing and orthostatic hypotension.

CONCLUSIONS

UCP2 promoter gene polymorphism -866 G/A was significantly associated with nerve conduction slowing and vasomotor sympathetic functions. These findings suggest that the higher UCP2 activity related to the A allele has an energy depleting effect on peripheral nerve function in type 2 diabetic patients.

Session 4 Poster

P-5

Obesity and late-onset diabetes in the mice lacking *Sqstm1*/p62 associate with mitochondrial dysfunction

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Decline in mitochondrial function with age appears to be responsible for the high prevalence of diabetes and obesity among the elderly. Mitochondrially-localized p62 stabilizes the electron transport system (ETS) and delays the mammalian aging process. Here, we show that $p62^{-/-}$ mice also exhibit obesity and late-onset hyperglycemia, which were attributed to mild hyperphagia, reduction in the basal metabolic rate, and insulin resistance acquired during their adult life. However, heterozygous mutant ($p62^{+/-}$) mice kept approximately at the same levels of food intake, body weight, and serum glucose as those observed in wild-type littermates. Due to preferential mitochondrial targeting of p62, the $p62^{+/-}$mitochondria maintained normal p62 level, ETS function, and NADH re-oxidation capacity. These results suggest that accelerated dysfunction of $p62^{-/-}$ mitochondria with age contributes, at least in part, to the development of obesity and diabetes in the $p62^{-/-}$ mice.

P-6

Expression of glutathione peroxidae 3 gene is regulated by peroxisome proliferator-activated receptor-γ

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Glutathione peroxidase 3 (GPx3) is a member of the family of selenium dependent antioxidant enzymes, GPx, and found in the extracellular fluid and plasma. GPx3 scavenges hydrogen peroxide, organic or lipid hydroperoxides with the reduced glutathione. Thiazolidinediones (TZDs) are potent agonists of peroxisome proliferator-activated receptor-γ (PPARγ) and increase insulin sensitivity in cells and animals. During investigating genes regulated by overexpression or activation of PPARγ in human muscle cells, we found that the mRNA level of GPx3 was significantly increased by activation of PPARγ. This effect was specific to GPx3 and expression of other GPx family member, GPx1, was not affected. Other TZDs such as rosiglitazone and pioglitazone, also increased the GPx3 expression and GPx3 mRNA level was increased 6 h after TZD treatment, suggesting that these effects were induced by PPARγ mediated transcription regulation. A PPAR-response element (PPRE) in the promoter of the mouse GPx3 gene was identified by transient transfection and reporter assays. Binding of PPARγ and RXR to the PPRE was confirmed by electro-mobility shift assays. TZDs also reduced the level of extracellular hydrogen peroxides induced by glucose oxidase and overexpression of GPx3 showed the similar effect. These results indicated that PPARγ could protect cells from the oxidative stress by stimulating the expression of GPx3.

P-7

Overall and early stage hypertrophy of β-cells in humans with type 2 diabetes

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Background. β-cell hypertrophy can be a compensatory mechanism along with β-cell proliferation in response to increased insulin demand. But, hypertrophy also may cause cells to be more susceptible to apoptosis. β-cell deficit or β-cell loss is known as a cause of development of type 2 diabetes mellitus. So we undertook this study to evaluate extent of β-cell hypertrophy and morphologic alterations of pancreatic islets in humans with type 2 diabetes mellitus.

Method. We measured islet size and β-cell area and calculated β-cell fraction in islet to examine characteristics of islet morphology from 2,859 islets in 7 normal subjects (control group) and 5,420 islets in 7 diabetic patients (DM group). We measured beta cell size, cytoplasm size and nucleus size to examine extent of beta cell hypertrophy from 5,982 and 3,971 beta cells in randomly selected islets in control and DM group, respectively by means of morphometric analysis using image analyzer. We calculated C/N ratio (ratio of cytoplasm area to nucleus area).

Result. Average cell size of total β-cells of control and DM group was 121.8 ± 17.2μm^2 and 144.9 ± 24.4μm^2 ($p<0.001$). Moreover C/N ratio of DM group was significantly higher than that of control group (4.2 ± 1.5 vs 3.0 ± 0.7, $P<0.001$). The results on β-cell size, nucleus size and C/N ratio according to β-cell size including single β-cell units are shown in the table below. Moreover, The cell size and C/N ratio of transdifferentiated ductal cells (insulin positive cells) as well as scattered β-cells (single β-cell units) was also bigger in subjects with diabetes than those in normal subjects. In morphologic classification of islets, the relative contribution rate of 'scattered β-cells' was 5.6% vs. 8.6% in control and DM group, respectively. The contribution rate 'large and healthy type' was 32.7% vs. 21.6%, while that of 'large and β-cell depleted type' was 23.5% and 29.2%.

Conclusion. The relative contribution rate of 'large and β-cell depleted type' islets was higher in DM group, while that of scattered β-cells was also higher in DM group. We found β-cell hypertrophy DM group in both small islets and large islets. Furthermore, it was found even at scattered β-cells. We need to further investigate the effects of such a hypertrophy on morphologic alterations of pancreatic islets.

Session 4
Poster

P-8

AMPK action; cell cycle progression and cell death in glucolipotoxicity

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Bacground: Because the expressions of various beta-cell specific transcription factors including PDX-1 and insulin gene might be regulated by AMP-activated protein kinase (AMPK) recognized as a key molecule of energy metabolism, glucolipotoxicity induced beta-cell death might be protected by the activation of AMPK.

Aim: We investigated the effect of AICAR, an AMPK activator, on cell proliferation and cell death in isolated pancreatic islets and beta cell line.

Methods: For cell death study; glucolipotoxicity-induced cell apoptosis after treatment with 400 μM AICAR were analyzed by caspase3 activity, TUNEL and MTT assay in MIN cells. For cell cycle progression study, we performed the western blot using an antibodies-p27, p53, cdk4, cyclinD and Rb.

Results: Glucolipotoxicity-induced caspase3 activity and apoptosis analyzed by TUNEL assay were protected by AICAR treatment for 6 days and decreased cell survival was increased by AICAR treatment.

AMPK decreased the expression of glucolipotoxicity-induced p27 and also decreased the p53 protein expression. And increased p53 protein expression was accompanied with the decreased Rb phosphorylation in AICAR treated glucolipotoxicity-induced MIN cells.

Conclusion: It is concluded that chronic glucolipotoxicity-induced beta cell death and cell cycle progression protected by AICAR treatment. Therefore, we suggesting that AMPK activation could be target for the prevention of development and disease progression of type2 diabetes.

P-9

The participation of PKCζ in insulin-medicated glucose transport in rat skeletal muscle L6 cells

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Background - PKCζ has been suggested to play an important role in mediating insulin-induced glucose transporter isoform-4 (GLUT4) translocation and glucose uptake in skeletal muscle. We have previously shown that actin remodeling following insulin stimulation is required for translocation of GLUT4 vesicles. In this study, we investigated the effect of PKCζ on actin remodeling and glucose transport in rat skeletal muscle cells (L6).

Methods –L6 muscle cells expressing c-myc epitope tagged GLUT4 (GLUT4myc) were differentiated into myotubes. Stable transfection was carried out by inserting the PKCζ gene into pEGFP-N1 vector through PCR. Spatial and temporal distribution of PKCζ, filamentous actin and GLUT4myc were examined by immunofluorescence microscopy and subcellular fractionation.

Results - On insulin stimulation, PKCζ translocated from low-density microsomes (LDM) to plasma membrane (PM) accompanied by increase in GLUT4 translocation and glucose uptake. Preliminary data indicated that there was a rapid insulin-mediated circulation of PKCζ between the LDM and the PM. The expression and phosphorylation of PKCζ increased significantly in the PM fraction, accompanied with a corresponding reduction in the low density microsome (LDM) after 1 min of insulin stimulation. By 3 min, the levels of PKCζ in the PM and LDM fractions returned to the pre-stimulation state. Intriguingly, PKCζ expression and phosphorylation appeared to be increased in the PM after 5 min of insulin treatment. In contrast, the translocation of GLUT4myc was not evident until 3 min after insulin stimulation. Z-scan confocal microscopy revealed a spatial colocalization of relocated PKCζ with the small GTPase Rac-1, actin, and GLUT4 after insulin stimulation. The insulin-mediated colocalization, PKCζ distribution, GLUT4myc translocation, and glucose uptake were inhibited by wortmannin and cell-permeable PKCζ pseudosubstrate peptide. In stable transfected cells, overexpression of PKCζ caused an insulin-like effect on actin remodeling accompanied by a 2.1-fold increase in GLUT4 translocation and 1.7-fold increase in glucose uptake in the absence of insulin. The effects of PKCζ overexpression were abolished by cell-permeable PKC_pseudosubstrate peptide, but not wortmannin. Transient transfection of constitutively active Rac-1 recruited PKCζ to new structures resembling actin remodeling, whereas dominant negative Rac-1 prevented the insulin-mediated PKCζ translocation.

Conclusion – Our results support the notion that PKCζ may facilitate insulin-induced glucose transport by actin remodeling in rat skeletal muscle cells. The presence and the potential implication of a dynamic circulation of PKCζ between different intracellular compartments following insulin stimulation remain to be confirmed.