1st Scientific Meeting of the Asia-Pacific Diabetes and Obesity Study Group

Program & Abstracts

Saturday, August 20 – Sunday, August 21, 2005

New Otani Kobe Harborland

Kobe, Japan

1st Scientific Meeting of the Asia-Pacific Diabetes and Obesity Study Group

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Welcome Message

It is both a pleasure and honor for us, as Organizers of this 1st Scientific Meeting of the Asia-Pacific Diabetes and Obesity Study Group, to extend a warm welcome to all participants to what is sure to be an extremely important and stimulating meeting. As you are all aware, the incidence of diabetes is growing into epidemic proportions throughout the world. Previously seen more as a disease of developed Western countries, this is no longer the case. It is gaining in prevalence throughout Asia and constitutes a huge health risk both at the individual, national and international level. It is therefore extremely timely that this 1st Scientific Meeting is being held. With a faculty including the most influential researchers and physicians at the forefront of diabetes and obesity research, we are all sure to update our knowledge in this field. Most importantly this meeting will we believe highlight the high quality of this emerging research discipline in our region. We begin with an Instructional Lecture detailing the role of insulin signaling and adipokines in diabetes, and then move to a wide range of summaries outlining groundbreaking research occurring at the forefront of the diabetes field. Researchers from Japan, Australia, USA, Korea, China, Hong Kong, Singapore and New Zealand will help illuminate this area, and help us plan necessary future directions for ongoing research aimed at preventing and treating this disease. We invite all participants to sit back, relax and take the opportunity of learning from these eminent specialists and to get to know researchers with like interests in the Asia Pacific region.

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
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
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Members

Takashi Kadowaki, MD, PhD
Professor
Dept. of Metabolic Diseases
Graduate School of Medicine
The University of Tokyo
Tokyo, Japan

Iichiro Shimomura, MD, PhD
Professor
Dept. of Internal Medicine & Molecular Science
Graduate School of Medicine
Osaka University
Osaka, Japan

● INVITED SPEAKER

Harvey F. Lodish
Member, Whitehead Institute of Biomedical Research
Professor of Biology, Massachusetts Institute of Technology

GENERAL INFORMATION

● DATES

Saturday, August 20 - Sunday, August 21, 2005

● VENUE

New Otani Kobe Harborland

1-3-5 Higashi Kawasaki-cho, Chuo-ku, Kobe 650-0044
Tel: +81-(0)78-360-1111 Fax: +81-(0)78-360-7799

● 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 14:00-16:00 on August 20 and dismantle it after the reception.
2. Remaining posters will be taken away by the secretariat.

PROGRAM

Saturday, August 20

17:00 ● **Opening Remarks**

David James, *Australia*

17:15-17:55 ● **Session 1: Instructional Lecture**

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

Insulin signaling, adipokines and diabetes mellitus
Masato Kasuga, *Japan*

17:55-18:00 *Break*

18:00-19:45 ● **Session 2: Oral Presentation**

co-chairs: **Jae Bum Kim,** *Korea*
Tao Xu, *China*

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

[Oral 2-1] GRK2 mediates endothelin-1-induced insulin resistance via the inhibition of both Gαq/11 and IRS-1 pathways in 3T3-L1 adipocytes
Isao Usui, *Japan*

[Oral 2-2] Overexpressed G6PD in adipocytes mediates lipid dysregulation and insulin resistance in obesity
Jae Bum Kim, *Korea*

[Oral 2-3] Cide proteins, metabolic network and obesity
Peng Li, *Hong Kong*

[Oral 2-4] Regulation of insulin signaling by protein tyrosine phosphatases
Tony Tiganis, *Australia*

[Oral 2-5] Insulin stimulated glucose transporter translocation and membrane lipid microdomains
Kan Liao, *China*

[Oral 2-6] P-Rex1 enhances insulin-mediated PI-3-kinase-dependent GLUT4 translocation and plasma membrane fusion in 3T3L1 adipocytes
Christina Mitchell, *Australia*

[Oral 2-7] TIRF imaging analysis of the dynamic motion of insulin granules by antidiabetic agents
Shinya Nagamatsu, *Japan*

20:00-23:00 ● Welcoming Reception and Poster Discussion

<Poster viewing with an open and free discussion will be held during the welcoming reception.>

[P - 1] Identification of resistin promoter SNP-420 as a type 2 diabetes susceptibility gene
Haruhiko Osawa, *Japan*

[P - 2] Therapeutic role of PPARγ in type II diabetes and diabetic nephropathy
Youfei Guan, *China*

[P - 3] A purine analogue kinase inhibitor, CK59, reveals a role for CaMKII in insulin-stimulated glucose transport
Lance Macaulay, *Australia*

[P - 4] MCP-1 links obesity to insulin resistance
Yoshikazu Tamori, *Japan*

[P - 5] Protein phosphatase 2A activation is essential for protein-tyrosine phosphatase 1B-induced sterol regulatory element-binding protein-1 gene expression
Hiroshi Maegawa, *Japan*

[P - 6] Glucose-stimulated upregulation of GLUT2 gene is mediated by SREBP-1c in the hepatocytes
Yong-Ho Ahn, *Korea*

[P - 7] Quantitatively investigating the agonist/antagonist ligand-binding regulation against PPARγ/RXRα heterodimerization
Xu Shen, *China*

[P - 8] Hyperglycaemia-driven pathogenic abnormality of copper homeostasis in diabetes and its reversibility by selective chelation: implications for the origins of the cardiovascular complications
Garth JS Cooper, *New Zealand*

[P - 9] Adipocyte/macrophage fatty acid binding proteins controlling metabolic syndrome
Kazuhisa Maeda, *Japan*

[P-10] Depletion of mtDNA and impaired glucose utilization
Wan Lee, *Korea*

[P-11] Ciliary neurotrophic factor reverses skeletal muscle insulin resistance associated with high fat fed diets by activating AMPK
Mark A Febbraio, *Australia*

[P-12] IA-2β is a key molecule for ghrelin's inhibitory effect on insulin secretion
Asako Doi, *Japan*

[P-13] AMP-activated protein kinase (AMPK) β2 subunit is required for stimulation of glucose uptake in skeletal muscle
Bruce E Kemp, *Australia*

[P-14] Genetic variation in PSARL is associated with plasma insulin concentration
Ken Walder, *Australia*

Sunday, August 21

07:30-08:00 ● **Morning Session**

sponsored by Takeda Pharmaceutical Company Limited

08:00-08:50 ● **Session 3: Invited Lecture**

chair: **David James,** Australia

Adiponectin and its paralogs: regulation, receptors and signal transduction
Harvey F. Lodish, *USA*

08:50-09:00 *Break*

09:00-10:30 ● **Session 4: Oral Presentation**

co-chairs: **Garth JS Cooper,** New Zealand
Takashi Kadowaki, Japan

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

[Oral 4-1] Pathophysiological roles of adiponection receptors AdipoRs
Toshimasa Yamauchi, *Japan*

[Oral 4-2] Angiopoietin-like protein 4: a novel adipokine with insulin-sensitizing and anti-proliferative actions
Karen SL Lam, *Hong Kong*

[Oral 4-3] Interleukin-18 enhances insulin sensitivity through PI3K/Akt pathway in 3T3-L1 adipocytes
Xiaoying Li, *China*

[Oral 4-4] Central resistance to leptin during pregnancy is associated with leptin receptor down regulation and suppression of leptin-induced phosphorylation of STAT3 in the ventromedial hypothalamus
David R. Grattan, *New Zealand*

[Oral 4-5] Melanocortin peptides, appetite and body weight regulation
Kathleen Mountjoy, *New Zealand*

[Oral 4-6] c-Cbl deficient mice are protected against dietary-induced obesity and insulin resistance
Gregory J Cooney, *Australia*

10:30-10:45 *Break*

10:45-11:45 ● Session 5: Oral Presentation

co-chairs: **Mark Febbraio,** *Australia*
Susumu Seino, *Japan*

[Oral 5-1] A-to-I editing of GluR-B RNA in mouse pancreatic islets under metabolic stress
Yong Liu, *China*

[Oral 5-2] Impaired insulin release and increased susceptibility to diabetes in the mice with β-cell-specific disruption of VEGF-A gene
Hirotaka Watada, *Japan*

[Oral 5-3] Molecular mechanisms underlying pancreatic β-cell loss in a murine model of wolfram syndrome
Hisamitsu Ishihara, *Japan*

[Oral 5-4] Genome-wide screening for genes that regulate cellular dynamics of lipid bodies in the budding yeast Saccharomyces cerevisiae
Hongyuan Yang, *Singapore*

11:45-12:45 *Lunch (photo session)*

12:45-14:30 ● Session 6: Oral Presentation

co-chairs: **Kan Liao,** *China*
Bruce Kemp, *Australia*

[Oral 6-1] AMP-activated protein kinase (AMPK) agonist 5'-phosphoribosyl-4-carboxamide-5-aminoimidazole (AICAR) reversed the glucolipotoxicity induced beta-cell dysfunction through suppression of PGC-1 (PPAR-gamma-coactivator-1) overexpression
Kun-Ho Yoon, *Korea*

[Oral 6-2] Granuphilin molecularly docks insulin granules to the fusion machinery
Tetsuro Izumi, *Japan*

[Oral 6-3] New insights into adipogenesis: Novel targets for treatment of obesity
Jon Whitehead, *Australia*

[Oral 6-4] The anti-obesity effect of adipose Gαs protein
Ying Hue Lee, *Taiwan*

[Oral 6-5] DOC2b regulates GLUT4 vesicle fusion in 3T3-L1 adipocytes
Masahiro Emoto, *Japan*

[Oral 6-6] Human evidence that genes involved in oxidative phosphorylation are up-regulated in type 2 diabetic liver
Toshinari Takamura, *Japan*

[Oral 6-7] Id2: A new player in glucose mediated gene regulation mechanisms
Peter Shepherd, *New Zealand*

[Oral 6-8] Dissecting the steps of GLUT4 trafficking and identifying the site of insulin action
Tao Xu, *China*

14:30 ● **Closing Remarks**

Takashi Kadowaki, *Japan*

14:40 ● **adjourned**

ABSTRACTS

Harvey F. Lodish, Ph.D.

Member, Whitehead Institute for Biomedical Research and Professor of Biology and Professor of Bioengineering, Massachusetts Institute of Technology, Cambridge, MA

Dr. Lodish received his A.B. degree Summa Cum Laude and with Highest Honors in Chemistry and Mathematics, from Kenyon College in 1962, and his Ph.D. degree in genetics with Dr. Norton Zinder from the Rockefeller University in 1966. Following two years of postdoctoral research at the M.R.C. Laboratory of Molecular Biology with Drs. Sydney Brenner and Francis Crick, he joined the faculty of the MIT Department of Biology. He was promoted to Professor in 1976, and in 1983 was appointed Member of the new Whitehead Institute for Biomedical Research.

Initially, his work focused on translational control of protein synthesis, and on regulation of gene expression during differentiation of the cellular slime mold. Beginning in 1973, his laboratory has concentrated on the biogenesis, structure, and function of several important secreted and plasma membrane glycoproteins. He defined the biosynthesis and maturation of the vesicular stomatitis virus glycoprotein, identified the intracellular organelles that mediate recycling of the asialoglycoprotein and transferrin receptors, and clarified the role of pH changes in delivery of iron to cells and recycling of the transferrin receptor. More recently, his group has elucidated steps in folding and oligomerization of several proteins within the endoplasmic reticulum, shown that exit of newly-made proteins from this organelle requires that they be properly folded, and developed probes for measurement of the redox state within the endoplasmic reticulum.

His group was the first to clone and sequence mRNAs encoding a mammalian glucose transport protein, an anion exchange protein, a transporter for free fatty acids, the hepatic asialoglycoprotein receptors, intestinal sucrose-isomaltase, the erythropoietin receptor, the calcitonin and endothelin receptors, two subunits of the TGFβ receptor, and several adipocyte- specific proteins including adiponectin (formerly Acrp30). These have been used to define the structure, biosynthesis, and cellular functions of these and related proteins and to identify and characterize related genes that encode proteins of related physiological functions.

Current efforts of his group focus on:

1) The erythropoietin receptor – understanding how it prevents apoptosis and controls proliferation and differentiation of erythroid progenitor cells.
2) Hematopoietic stem cells – characterizing new marker cell surface proteins and new growth factors for their expansion in culture
3) A family of fatty acid transport proteins – understanding their physiological functions and their involvement in energy homeostasis
4) Adiponectin, a new adipocyte – produced hormone that potently enhances glucose and fatty acid metabolism by muscle, and a family of adiponectin homologs.
5) Understanding the role of micro RNAs in regulating hematopoiesis and fat and muscle cell development and metabolism

Dr. Lodish was on the Editorial Board of the Proceedings of the National Academy of Sciences from 1995 to 1999 and on the Board of Reviewing Editors of Science from 1991 through 1999. He was Editor of Molecular and Cellular Biology from 1981 to 1987 and he has been on the editorial boards of a number of journals, including the Journal of Cell Biology, the Journal of Biological Chemistry, and Nucleic Acids Research. Dr. Lodish has served on advisory panels for the NIH, NSF, and American Cancer Society, and on the advisory boards of several institutions, including the Biozentrum of the University of Basle, the European Molecular Biology Laboratory in Heidelberg, the Center for Molecular Biology Heidelberg (ZMBH) in Germany, and the PEW Scholars Program in Biomedical Sciences. He is currently a member of the Board of Trustees of Kenyon College and of the Massachusetts Eye and Ear Infirmary. He was chair of the advisory board of the Division of Basic Sciences of the Fred Hutchinson Cancer Center and is currently Chair of the Advisory Board of Children's Hospital, Boston. He also serves on the advisory boards of the Cleveland Clinic Lerner Research Institute, the Life Sciences Institute of the University of Michigan, and the California Institute of Technology Division of Biology.

He is the lead author of the textbook *Molecular Cell Biology*. The fifth edition was published in 2003 and the book has been translated into six languages.

During the 2004 calendar year Dr. Lodish served as President of the American Society for Cell Biology.

Dr. Lodish is a Member of the National Academy of Sciences, a Fellow of the American Association for the Advancement of Science, a Fellow of the American Academy of Arts and Sciences, and a Fellow of the American Academy of Microbiology. He is also an Associate (Foreign) Member of the European Molecular Biology Organization. Dr. Lodish received a MERIT award from the National Institute of Diabetes and Digestive and Kidney Diseases. He is also a recipient of a Guggenheim Fellowship, an honorary D.Sc. from Kenyon, and the Stadie Award from the American Diabetes Association.

Dr. Lodish was a founder and scientific advisory board member of Genzyme, Inc., Arris Pharmaceuticals, Inc, and Millennium Pharmaceuticals, Inc. He serves on the Scientific Advisory Board for the Eisai Research Institute, and previously served on the Scientific Advisory Board of Astra and then AstraZeneca Pharmaceuticals.

Adiponectin and its paralogs: regulation, receptors and signal transduction

Harvey F. Lodish, Christopher Hug, Guang W. Wong
Whitehead Institute for Biomedical Research
Department of Biology, Massachusetts Institute of Technology

We used an expression cloning strategy to identify T- cadherin as a receptor for hexameric and high molecular weight forms of adiponectin. T-cadherin is highly expressed in the vasculature, where it is predominantly found in endothelial and smooth muscle cells in the blood vessel intima. T-cadherin is attached to the membrane via a GPI anchor at the C-terminus. Our recent studies indicate that it is the major adiponectin binding protein in the body, as deletion of T-cadherin results in a many-fold increase in the level of high molecular weight adiponectin in the circulation. T- cadherin is upregulated following vascular injury and we hypothesize that, by binding to adiponectin, it plays a role in atherosclerosis progression.

Last year we used multiple genomic approaches to identify a family of seven highly conserved human and mouse proteins homologous in sequence and presumed structure to adiponectin, designated as C1q/TNF-α related proteins (CTRP)-1 to 7. Expression of CTRP1, 2, and 7 mRNAs, like that of adiponectin, is higher in adipose tissue that in any other tissue tested. Like that of adiponectin, expression of CTRP1, 2, and 7 mRNAs in 3T3- L1 adipocytes is upregulated by treatment with a thiazolidinedione agonist of PPAR-γ. CTRP2 is the closest paralog of adiponectin; our data show that CTRP2 is structurally homologous to adiponectin in that both form higher order structures including trimers and hexamers. Moreover, CTRP1, 2, and 7 are functionally homologous to adiponectin in their ability to activate the key metabolic sensor AMP-activated protein kinase (AMPK) in muscle and lung cells. Similar to adiponectin, treatment of C2C12 myotubes with CTRP2 resulted in increased accumulation of glycogen and enhanced oxidation of long chain fatty acids, the latter due to phosphorylation of Acetyl CoA Carboxylase (ACC) by AMPK. Taken together, these results suggest significant metabolic functions for CTRP1, 2, and 7, but the natural target cells of these hormones and the functions they control are not known. However, this discovery of a family of adiponectin paralogs has implications for understanding the control of energy homeostasis and could provide new targets for pharmacologic intervention in metabolic diseases such as diabetes and obesity.

2-1 GRK2 mediates endothelin-1-induced insulin resistance via the inhibition of both Gαq/11 and IRS-1 pathways in 3T3-L1 adipocytes

Isao Usui, Takeshi Imamura, Ken Ishizuka, Yukiko Kanatani, Shiho Fujisaka, Masashi Kobayashi
First Department of Medicine, Toyama Medical and Pharmaceutical University

GRKs regulate seven transmembrane receptors (7TMRs) by phosphorylating agonist-activated 7TMRs. Recently, we have reported that GRK2 can function as a negative regulator of insulin action. We have also reported that chronic endothelin-1 (ET-1) treatment leads to heterologous desensitization of insulin signaling. In this study, we have investigated the role of GRK2 in chronic ET-1-induced insulin resistance in 3T3-L1 adipocytes. Insulin-induced GLUT4 translocation was inhibited by pretreatment with ET-1 for 24 h, and this inhibitory effect was rescued by microinjection of anti-GRK2 antibody or GRK2 siRNA. Adenovirus-mediated overexpression of either wild type (WT)- or kinase-deficient (KD)-GRK2 inhibited Gαq/11 signaling. ET-1 treatment caused serine phosphorylation and degradation of IRS-1. Overexpression of KD-GRK2, but not WT-GRK2, inhibited ET-1-induced serine 612 phosphorylation of IRS-1 and restored activation of this pathway. Taken together, GRK2 mediates ET-1-induced insulin resistance by both inhibiting Gαq/11 activation in its kinase activity-independent manner and inducing IRS-1 serine phosphorylation and degradation.

2-2 Overexpressed G6PD in Adipocytes Mediates Lipid Dysregulation and Insulin Resistance in Obesity

Jiyoung Park, Ho Kyung Rho, Kang Ho Kim, Sung Sik Choe, Yun Sok Lee, Jae Bum Kim
Department of Biological Sciences, Seoul National University

Glucose-6-phosphate dehydrogenase (G6PD) produces cellular NADPH, which is required for the biosynthesis of fatty acids and cholesterol. Although G6PD is required for lipogenesis, it is poorly understood whether G6PD in adipocytes is involved in energy homeostasis such as lipid and glucose metabolism. Here, we report that G6PD plays a role in adipogenesis and its increase is tightly associated with the dysregulation of lipid metabolism and insulin resistance in obesity. We observed that the enzymatic activity and expression levels of G6PD were significantly elevated in white adipose tissues of obese models including *db/db*, *ob/ob* and diet induced obesity (DIO) mice. In 3T3-L1 cells, G6PD overexpression stimulated the expression of most adipocyte marker genes and elevated the levels of cellular free fatty acids (FFAs), triglyceride (TG) and FFA release. Consistently, G6PD knockdown via small interfering RNA (siRNA) attenuated adipocyte differentiation with less lipid droplet accumulation. Surprisingly, the expression of certain adipocytokines such as TNFα and resistin was increased while that of adiponectin was decreased in G6PD overexpressed adipocytes. In accordance with these results, overexpression of G6PD impaired insulin signaling and suppressed insulin dependent glucose uptake in adipocytes. Taken together, these data strongly suggest that aberrant increase of G6PD in obese and/or diabetic subjects would alter lipid metabolism and adipocytokine expression, thereby resulting in failure of lipid homeostasis and insulin resistance in adipocytes.

2-3 Cide proteins, metabolic network and obesity

Peng Li
Department of Biology, Hong Kong University of Science and Technology

Obesity has become the most prevalent chronic disorder that affects large population in the world. Obesity is due largely to the imbalance between energy intake and expenditure. Adipose tissues including brown adipose tissue (BAT) and white adipose tissue (WAT) play crucial roles in maintaining energy homeostasis. While WAT stores energy in the form of triglycerides; BAT increases energy expenditure through thermogenesis. Cide proteins including Cidea, Cideb and Fsp27, are expressed at high levels in BAT, liver and WAT, respectively. Cidea$^{-/-}$ mice exhibit increased lipolysis in BAT and are resistant to high fat diet induced obesity and diabetes. Our recent data suggest that Cideb and Fsp27 also play important roles in the development of obesity by regulating various metabolic pathways in liver and WAT. The molecular mechanism of Cide proteins in regulating metabolic network and obesity will be discussed.

2-4 Regulation of insulin signaling by protein tyrosine phosphatases

Tony Tiganis
Department of Biochemistry and Molecular Biology, Monash University, Victoria 3800, Australia

Type 2 diabetes mellitus has reached epidemic proportions afflicting roughly 6% of the adult population in Western society. Although the underlying genetic causes and the associated pathological symptoms are heterogenous, a common feature is high blood glucose due to peripheral insulin resistance. The molecular basis of insulin resistance is thought to be attributable to defects in insulin receptor (IR) signalling. The IR is a protein tyrosine kinase that phosphorylates itself and downstream protein substrates on tyrosine in response to insulin. Protein tyrosine phosphatases (PTPs) that dephosphorylate the IR and its substrates might be important targets for therapeutic intervention in type 2 diabetes; inhibition of specific PTPs may allow for enhanced insulin-induced signalling to alleviate insulin resistance. For such a strategy to succeed it is important to understand which PTPs are the relevant regulators of insulin signalling. PTP1B is a physiological regulator of IR activation and glucose homeostasis and a validated therapeutic target for the treatment of type 2 diabetes. Recently we identified the phosphatase TCPTP as another important negative regulator of insulin signalling. We now show that PTP1B and TCPTP can act in the same cell to regulate IR phosphorylation. We demonstrate that PTP1B and TCPTP can act in unison to control the intensity and duration of IR activation to regulate both common and distinct insulin signaling pathways. These studies and others addressing the spatiotemporal control of IR activation and signalling will be presented.

2-5 Insulin stimulated glucose transporter translocation and membrane lipid microdomains

Kan Liao, Taichang Yuan, Shangyu Hong
Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences

Caveolae and lipid rafts, as two subtypes of plasma membrane lipid microdomains, are morphologically distinct membrane structures, but share the similar lipid composition of enriched cholesterol and sphingolipids. They play important role in many cellular functions, such as transmembrane signal transduction, membrane translocation, etc. Insulin stimulated glucose uptake in adipocytes involves two important steps: the receptor transmembrane signaling and glucose transporter (Glut-4) translocation. It has been postulated that plasma membrane lipid microdomains play an important role in insulin stimulated glucose uptake. However, it is not clear whether caveolae and non-caveolar lipid rafts play the similar function or not. Using RNAi interference, 3T3-L1 adipocytes without caveolae were generated. Thus, non-caveolar lipid rafts were the only membrane lipid microdomain structure in these cells. When stimulated with insulin, the glucose uptake in these RNAi adipocytes were stimulated to the same extend as the wild type adipocytes. Without caveolae, the glucose transporter could still be translocated into plasma membrane and into the lipid microdomains. Further analysis suggested that caveolae were involved in the recycling of glucose transporter from the plasma membrane.

2-6 P-Rex1 enhances insulin-mediated PI-3-kinase-dependent GLUT4 translocation and plasma membrane fusion in 3T3L1 adipocytes

Christina A. Mitchell, Demis Balamatsias, Absorn Sriritana, Joanne E. Waters, Anne Kong
Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, 3800, Australia

Actin cytoskeletal remodeling is required for insulin-stimulated trafficking of the glucose transporter GLUT4 to the plasma membrane, although the molecular mechanisms by which this occurs remain incompletely defined. Both phosphoinositide 3-kinase (PI 3-kinase) dependent and independent mechanisms have been suggested to play a role. The novel RacGEF, P-Rex1, is synergistically activated by the Gβγ subunit of heterotrimeric G-proteins and the phosphoinositide 3-kinase signalling molecule phosphatidylinositol-(3,4,5) trisphosphate (PtdIns(3,4,5)P3) promoting Rac activation, in neutrophils. P-Rex1 contains N-terminal Dbl-homology (DH) and pleckstrin-homology (PH) domains which are found in the majority of RacGEFs and a C-terminal domain homologous to Type I inositol 4-phosphatase, however, P-Rex1 does not exhibit polyphosphoinositide phosphatase activity. Immunoblot analysis using affinity-purified anti-P-Rex1 antibodies demonstrated P-Rex1 is expressed in the cytosol differentiated 3T3-L1 adipocytes. Overexpression of HA-P-Rex1 induced extensive membrane ruffling and actin rearrangement, in both quiescent and insulin-stimulated 3T3-L1 adipocytes and increased insulin-dependent GFP-GLUT4 translocation to the plasma membrane in a dose-dependent manner (>2.0 fold increase at 1 nM and 10 nM, and 1.5 fold at 100 nM insulin $p<0.01$) dependent on PI3-kinase activity. Cytochalasin D and Latrunculin A treatment also significantly reduced P-Rex1-mediated GFP-GLUT4 translocation, indicating that the P-Rex1-induced phenotype was dependent on rearrangement of the actin cytoskeleton. P-Rex1 ectopic expression promoted a >2.5 fold ($p<0.01$) increase in insulin-stimulated fusion of exofacial myc-GLUT4-GFP with the plasma membrane, while P-Rex1 mutants lacking the DH domain acted as a dominant negative mutant inhibiting insulin-stimulated GFP-GLUT4 translocation (>50% inhibition at 1 nM and 100 nM insulin, $p<0.05$). By contrast, C-terminal 4-phosphatase domain deletion mutants had no effect on GFP-GLUT4 plasma membrane translocation. Interestingly, expression of mutant P-Rex1 which contained the C-terminal 4-phosphatase domain and central domain, but lacked the N-terminal DH/PH/DEP/DEP/PDZ/PDZ domains, also inhibited insulin-stimulated GFP-GLUT4 translocation (>50% inhibition at 1nM and 100 nM insulin, $p<0.05$). Collectively, this study has identified P-Rex1 as a novel PI-3-kinase effector that promotes GLUT4 translocation and fusion with the plasma membrane via induction of actin remodeling necessary to facilitate GLUT4 vesicle exocytosis.

2-7 TIRF imaging analysis of the dynamic motion of insulin granules by antidiabetic agents

Shinya Nagamatsu, M. Ohara-Imaizumi
Department of Biochemistry, Kyorin University School of Medicine, Tokyo 181-8611, Japan

Glinide and sulphonylurea evoke the rapid insulin release, however, it is still incompletely understood how these antidiabetic agents act on the insulin granule motion. Total internal reflection fluorescence (TIRF) microscopy imaging analysis is a powerful tool to determine the dynamic motion of insulin granules near the plasma membrane within 100 nm. In the present study, we utilized TIRFM to analyze the action of these agents on real-time insulin granule motion in live primary beta cells. The fusion in response to these agents originated from newcomer granules, on the other hand, the total number of insulin granules docked on the plasma membrane was nearly constant. In order to examine the relationship between mitiglinide action and K_{ATP} channel activity, rat beta cells were exposed to 50μM diazoxide, then stimulated by 500nM mitiglinde. TIRF data showed that mitiglinide caused many fusion events, but 22 mM glucose did not (fusion events: 27.6 ± 4.30 vs. 2.04 ± 1.00 in 0-4min, $p<0.0001$). Diabetic GK (Goto-Kakizaki rat) betacells showed a marked reduction of fusion from previously docked granules stimulated by 22 mM glucose during the first phase, however, these agents evoked the rapid fusion from newcomers (fusion events: 41.9 ± 7.00 (500 nM mitiglinide) vs. 10.4 ± 1.54 (22mM glucose) in 0-4min, $p<0.001$). Thus, these antidiabetic agents primary activate the prompt fusion from newcomer granules, in particular, mitiglinide may act on molecules other than sulphonylurea receptor.

4-1 Pathophysiological roles of adiponectin receptors AdipoRs

Toshimasa Yamauchi, Yusuke Hada, Naoto Kubota, Kazuo Hara, Kohjiro Ueki, Kazuyuki Tobe, Takashi Kadowaki
Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo

Adiponectin/Acrp30 is a hormone secreted by adipocytes that acts as an antidiabetic and anti-atherogenic adipokine. We reported that AdipoR1/R2 serve as receptors for adiponectin and mediate increased fatty-acid oxidation and glucose uptake by adiponectin. Moreover, obesity was associated with decreased plasma adiponectin levels as well as decreased expression levels of AdipoR1/R2, the latter reduced adiponectin sensitivity, both of which finally lead to insulin resistance. In this study, to clarify the physiological and pathophysiological roles of AdipoRs in vivo, we studied the effects of adenovirus-mediated upregualtion or downregulation of AdipoR1 in the mice liver. Here we show that adenovirus-mediated expression of AdipoR1 in the liver of db/db mice resulted in decreased blood glucose levels, improved glucose tolerance, increased adiponectin sensitivity such as increased activation of AMP kinase by adiponectin, decreased molecules involved in gluconeogenesis and increased fatty-acid oxidation. On the contrary to the upregulation of AdipoR1 in the liver, adenovirus-mediated suppression of AdipoR1 by 40% resulted in increased expression levels of molecules involved in gluconeogenesis and increased fasted plasma glucose levels. Interestingly, suppression of AdipoR1 by approximately 40% resulted in upregulation of AdipoR2 by approximately 2-fold in the liver, which was associated with increased expression of PPARα target genes such as ACO. These data raised the possibility that AdipoR1 may be more tightly linked to activation of AMP kinase pathway, while AdipoR2 may be more tightly linked to activation of PPARα pathway.

4-2 Angiopoietin-like protein 4: a novel adipokine with insulin-sensitizing and anti-proliferative actions

Karen SL Lam, Aimin Xu, MC Lam, JY Xu, KW Chen, YH Yan, AWK Tso, WS Chow
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Angiopoietin-like protein 4 (ANGPTL4) is a circulating protein predominantly produced in adipose tissue and the liver. Its expression in humans and rodents is increased by PPAR agonists used in the treatment of diabetes and dyslipidemia. Our recent studies have provided evidence that it is a blood-borne hormone with insulin-sensitizing and anti-proliferative properties. We have shown that adenovirus-mediated increased expression of ANGPTL4 can reduce blood glucose and improve glucose tolerance in C57 mice, but induces transient hyperlipidemia, fatty liver and hepatomegaly. In db/db diabetic mice, ANGPTL4 treatment normalizes hyperglycemia and reduces hyperinsulinemia. Ex vivo studies on primary rat liver cells show that it reduces hepatic glucose production and enhances insulin-mediated inhibition of gluconeogenesis. Using an in-house human ELISA assay, we have found that ANGPTL4 levels are reduced in type 2 diabetic patients and increased by rosiglitazone. Serum ANGPTL4 levels are inversely related to fasting blood glucose and HOMA-IR, suggesting that this hormone is also involved in glucose metabolism in humans. On the other hand, no significant correlation is found between serum ANGPTL4 and circulating lipid levels. ANGPTL4 is cleaved into C-terminal and N-terminal fragments, probably prior to its secretion into the circulation. Both N-terminus and C-terminus of ANGPTL4 form oligomers. Functional studies on human umbilical vascular endothelial cells demonstrate that the C-terminus of ANGPTL4, but not its N-terminus, inhibits bFGF-induced cell proliferation, as judged by the thymidine incorporation assay. Genetic analysis of the ANGPTL4 gene has revealed four novel polymorphisms in Chinese. Association studies using plasma and DNA samples and clinical database from cross-sectional and prospective population-based studies are in progress to investigate the role of ANGPTL4 in the predisposition to diabetes and carotid atherosclerosis.

4-3 Interleukin-18 enhances insulin sensitivity through PI3K/Akt pathway in 3T3-L1 adipocytes

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A number of studies showed that plasma interleukin-18 (IL-18) concentration is elevated in patients with obesity, type 2 diabetes and polycystic ovary syndrome (PCOS). However it has not been elucidated that this proinflammatory cytokine involves in insulin resistance. We treated 3T3-L1 adipocytes and further insulin resistant 3T3-L1 cells induced by TNF-α with recombinant mouse IL-18 at 0.1–100 ng/ml concentration and insulin. ^{3}H incorporated glucose uptake test was performed and the insulin signaling pathway was dissected in IL-18 treated 3T3-L1 cells. Our results showed that IL-18 at the concentration of 100ng/ml significantly enhanced insulin mediated glucose uptake in both 3T3-L1 and insulin resistant 3T3-L1 adipocytes. Phospho-Akt was increased in IL-18 treated 3T3 adipocytes. We concluded that IL-18 could significantly enhance insulin sensitivity in dose-dependent manner through PI3K/Akt pathway in 3T3-L1 adipocytes.

4-4 Central resistance to leptin during pregnancy is associated with leptin receptor down regulation and suppression of leptin-induced phosphorylation of STAT3 in the ventromedial hypothalamus

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Despite elevated plasma leptin concentrations, food intake and fat deposition is increased during pregnancy. We have demonstrated that intracerebroventricular (i.c.v.) leptin administration is unable to suppress food intake in pregnant rats, as it does in non-pregnant animals. Hence, a state of leptin resistance develops during pregnancy. To investigate the mechanism underlying this central leptin resistance, we have measured levels of mRNA for the leptin receptor in the hypothalamus, and examined leptin-induced phosphorylation of STAT3 (pSTAT3) by Western blot and immunohistochemistry. Groups of animals were sacrificed at various timepoints during pregnancy and lactation. Real time RT-PCR was used to determine levels of mRNA for the two major leptin receptor isoforms (ObRa and ObRb) in microdissected hypothalamic nuclei and the choroid plexus. Further groups of non-pregnant and day 14 pregnant rats were treated with leptin (4 μg, i.c.v.) or vehicle. Some of these animals were sacrificed by decapitation and brains collected for measurement of pSTAT3 using Western blot analysis. The remaining animals were perfused with 4% paraformaldehyde and the brains processed for pSTAT3 immunohistochemistry. A significant reduction of Ob-Rb mRNA levels was observed in the ventromedial hypothalamic nucleus (VMH) during pregnancy compared to non-pregnant animals, with no changes detected in other hypothalamic nuclei. There were also reduced levels of mRNA for Ob-Ra, a proposed leptin transporter molecule, in the choroid plexus on days 7 and 21 of pregnancy. Levels of leptin-induced pSTAT3 were specifically suppressed in the VMH and arcuate nucleus of pregnant rats compared to non-pregnant rats. The number of cells positive for leptin-induced pSTAT3 in the VMH was greatly reduced during pregnancy compared to non-pregnant rats, whereas no differences were observed in the arcuate nucleus. These data demonstrate that pregnancy-induced leptin resistance is associated with suppression of leptin receptor expression in the VMH, together with changes in leptin-induced activation of STAT3. The data implicate the VMH as a key hypothalamic site involved in hormone-induced leptin resistance during pregnancy. In addition, suppression of ObRa mRNA levels in the choroid plexus is consistent with the idea that diminished transport of leptin into the brain may also contribute to leptin resistance during pregnancy.

4-5 Melanocortin peptides, appetite and body weight regulation

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The pivotal role of the melanocortin system in regulation of appetite, metabolism and body weight is demonstrated clearly by human melanocortin 4 receptor (MC4R) and pro-opiomelanocortin (POMC) variants, two knockout mouse models (MC3R and MC4R) 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 on the hypothalamus. Signals acting through neural MC3R and MC4R are critical for restricting food intake and regulating energy homeostasis. Still unresolved though, are the central melanocortin signalling pathways and the role of each POMC-derived peptide. Of particular interest is the existence of acetylated and non-acetylated forms of $ACTH_{1-13}$, a major POMC-derived peptide. N-terminal acetylation of desacetyl-α-MSH ($ACTH_{1-13}$ NH_2) to form α-MSH (acetyl- $ACTH_{1-13}$ NH_2) occurs in secretory vesicles just prior to exocytosis but not all desacetyl-α-MSH is acetylated since desacetyl-α-MSH is present in the brain and in the circulation. We investigated biological activity and function/s of desacetyl-α-MSH compared with α-MSH in the hypothalamus by (1) administering 10μg each peptide *icv* into fasted adult rats and after 3h compared effects on food intake and hypothalamic protein changes and (2) administering the peptides subcutaneously daily for postnatal days 0-14 and monitoring postnatal body growth and hypothalamic protein changes at day 14. Using a 2DE-gel based proteomic approach to analyse hypothalamic protein changes we found different protein expression patterns induced by each melanocortin peptide but only α-MSH significantly inhibited food intake and only desacetyl-α-MSH significantly slowed neonatal body growth over 10 days. On postnatal day 14 both a-MSH and desacetyl-α-MSH treated pups were significantly heavier ($p<0.05$) than the vehicle treated control animals. The proteomic data suggests that modification for cytoskeleton, more efficient energy supply and protective stress response are all coordinated cellular responses to *icv* administration of α-MSH. In contrast, central administration of descaetyl-α-MSH was found to decrease levels of several key enzymes in metabolic pathways, which may reflect a general downturn in cellular metabolic state. Subcutaneous administration of each peptide significantly changed expression of a number of the same proteins, specifically metabolic enzymes, cytoskeleton, signalling and stress response proteins. We also found some opposing actions of α-MSH and desacetyl-α-MSH on cytoskeleton protein changes. Our data therefore supports the hypothesis that desacetyl-α-MSH is biologically active *in vivo* and further suggests that biological functions for desacetyl-α-MSH *in vivo* can differ from those for α-MSH. Therefore the relative abundance of endogenous POMC-derived peptides is likely to be important in the regulation of energy homeostasis and metabolism.

4-6 c-Cbl deficient mice are protected against dietary-induced obesity and insulin resistance

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c-Cbl is an E3-ubiquitin ligase involved in the in regulating the degradation of multiple receptor tyrosine kinase enzymes which has also been implicated as an adaptor protein in a novel pathway of insulin-stimulated glucose transport. We recently reported the novel finding that c-Cbl-/- mice have increased insulin sensitivity and a lean phenotype most likely due to an increase in whole body energy expenditure. To examine whether this phenotype would protect c-Cbl-/- mice from dietary induced obesity and insulin resistance we fed wild type and c-Cbl-/- mice a diet high in saturated fat for 4 weeks and then assessed glucose tolerance, insulin action and energy expenditure. Knockout mice fed a high-fat diet maintained higher energy expenditure (27%) and greater activity compared to wild type mice fed the same diet. Fat-fed c-Cbl-/- mice also had less adipose tissue and exhibited improved glucose tolerance and insulin action compared to fat-fed wild type mice. The increased phosphorylation of acetyl CoA carboxylase observed in muscle of fat-fed c-Cbl-/- mice suggests that an increased capacity for fatty acid oxidation in this tissue underlies the reduced fat content and increased energy expenditure in the knockout animals that results in a protection against dietary-induced obesity and insulin resistance. More recent studies show that this role of c-Cbl in energy homeostasis is mediated through its function as an E3-ubiquitin ligase suggesting that this function of c-Cbl provides a novel target for manipulation of energy expenditure and body fat.

5-1 A-to-I editing of GluR-B RNA in mouse pancreatic islets under metabolic stress

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RNA editing through the conversion of adenosine (A) to inosine (I) within pre-mRNA molecules is a widespread phenomenon found as a post-transcriptional regulatory mechanism in a variety of organisms, including mammals. It is catalyzed by two major families of adenosine deaminase acting on RNA (ADAR), denoted ADAR1 and ADAR2. Previously reported studies have demonstrated that A-to-I RNA editing plays critical roles in the function and development of the central nervous system (CNS), largely due to the regulation of the functional properties and activities of the edited neurotransmitter receptors and ion channels. Little is known, however, about the possible functions of A-to-I RNA editing in the homeostatic control of glucose metabolism. To this end, we have found that both ADAR1 and ADAR2 deaminases are predominately expressed in mouse pancreatic islets, with the level of ADAR1 similar to that observed in the brain. When mice are fed on a high-fat diet at 16 weeks with exhibition of obesity-induced insulin resistance and hyperinsulinemia, the expression of ADAR2, in contrast to ADAR1, is increased nearly two-fold in the islets. Interestingly, the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor subunit GluR-B, one of the known editing substrates of ADARs in the brain, is also abundantly expressed and efficiently edited in the islets. Moreover, the editing efficiency in the islets, as compared to that in the brain, is dramatically increased at all of the three edited sites, the Q/R site, R/G site and intronic hotspot +60 site, of GluR-B RNA transcripts under the high-fat diet-induced metabolic stress. In the mouse pancreatic β-cell line MIN6, not only the transcription of ADAR2 and GluR-B, but the editing of GluR-B RNA, is enhanced in response to the treatment by high concentration of glucose. Our results suggest a very likely important role for the A-to-I RNA editing in the exquisite regulation of pancreatic β-cell function.

5-2 Impaired insulin release and increased susceptibility to diabetes in the mice with β-cell-specific disruption of VEGF-A gene

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Altered regulation of insulin secretion and islet mass is an important characteristic of individuals with type 2 diabetes. In the present study, using the mice with disrupted VEGF-A gene specifically in β cells (RIP-Cre:$Vegf^{fl/fl}$), we elucidated the relation between islet vascular structure and β cell function. These mice showed reduced islet vascular density with impaired formation of endothelial fenestration. While fasting glucose and body weight of RIP-Cre:$Vegf^{fl/fl}$ mice were comparable to control RIP-Cre and $Vegf^{fl/fl}$ mice, RIP-Cre:$Vegf^{fl/fl}$ mice exhibited impaired glucose tolerance with impaired rapid insulin release. On the other hand, the glucose responsive insulin release from isolated islets was enhanced. High fat diet for 12 weeks markedly deteriorated glucose tolerance and increased fasting glucose levels in RIP-Cre:$Vegf^{fl/fl}$ mice compared with control mice. Isolated islets from high-fat-fed RIP-Cre:$Vegf^{fl/fl}$ mice exhibited increased glucose sensitivity of insulin secretion. In addition, a significant increase in islet β cell mass was observed in RIP-Cre:$Vegf^{fl/fl}$ mice compared with high-fat-fed control mice. Our results indicate that the impaired rapid insulin release into blood stream observed in RIP-Cre:$Vegf^{fl/fl}$ mice was due to abnormal quality and quantity of blood vessels in the islets, rather than a defect in β cells. With the existence of insulin resistance, these mice developed diabetes without apparent defects in adaptive reaction of β cells. Especially about the regulation of islet mass, even though insulin release to blood stream was disturbed in these mice, autocrine effect of insulin was not theoretically impaired. This might be possible reason that the increase in islet mass was normally observed in these mice.

5-3 Molecular mechanisms underlying pancreatic β-cell loss in a murine model of wolfram syndrome

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Wolfram syndrome, a rare autosomal recessive disorder characterized by juvenile onset diabetes mellitus, optic atrophy, diabetes insipidus and sensorineural deafness, is caused by mutations in the *WFS1* gene, which encodes an ER resident membrane protein. We have recently established mutant mice with disrupted *wfs1* gene and found that mutant mice exhibited impaired glucose homeostasis due to progressive β-cell loss and impaired stimulus-secretion coupling in insulin secretion. To further investigate mechanisms of β-cell dysfunction, we have conducted studies of cellular physiology and gene expression, especially focusing on ER functions and ER-stress responses, in *wfs1*-deficient cells. ER calcium concentrations, measured using ER-targeted aequorin, was lower in the *wfs1*-deficient cells, which seemed to be attributable to reduced capacitative calcium entry. We also found that increased PERK phosphorylation and XBP-1 expression, indicating that the ER stress responses were enhanced in *wfs1*-deficient islets. The enhanced ER stress responses were accompanied by increased expression of proapoptotic proteins CHOP and caspase-3. These data suggest that WFS1-deficiency causes impaired ER calcium homeostasis in β-cells, leading to chronic activation of the ER-stress response, which then eventually apoptosis. Our data thus establish Wolfram syndrome as one of the diseases, besides Wolcott-Rallison syndrome, in which the ER-stress is the pathological basis for human diabetes.

5-4 Genome-wide screening for genes that regulate cellular dynamics of lipid bodies in the budding yeast Saccharomyces cerevisiae

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Lipid bodies are intracellular lipid droplets comprised of triglycerides (TAG) and sterol esters (SE), and are bounded by a monolayer of phospholipids and proteins. Varying in size and composition, lipid bodies can be found in most eukaryotic cells and serve as a storage form of energy and bioactive lipids. Changes in cellular dynamics of lipid bodies are associated with many devastating human diseases, including obesity and atherosclerosis. The yeast *Saccharomyces cerevisiae* is a powerful model genetic system and has proven to be invaluable to the understanding of cellular lipid metabolism and homeostasis. Biochemical pathways leading to the synthesis of SE and TAG are largely defined in yeast. Many resident lipid-body proteins have been identified. As in animal cells, lipid bodies in yeast are also believed to originate from microdomains of the endoplasmic reticulum (ER), where most enzymes for lipid synthesis reside.

Here, we identified gene products that effect lipid-body dynamics (biogenesis, maturation and degradation) in yeast with a "reverse genetic" approach. The entire collection of yeast deletion mutants were screened for changes in the quantity and morphology of lipid bodies. The 5000~ viable mutants were visually screened using the lipid-body specific vital dye, Nile Red and we have identified ~100 yeast mutants with severely reduced numbers of lipid bodies. The rate of TAG and SE synthesis and their mass have been measured. Our results offers novel insights into the cellular dynamics of lipid bodies.

6-1 AMP-activated protein kinase (AMPK) agonist 5'-phosphoribosyl-4-carboxamide-5-aminoimidazole (AICAR) reversed the glucolipotoxicity induced beta-cell dysfunction through suppression of PGC-1 (PPAR-gamma-coactivator-1) overexpression

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Glucolipotoxicity plays an important role in the development and progression of type 2 diabetes. We elucidated the role of AMP-activated protein kinase (AMPK) which is a key regulator of intracellular energy metabolism in glucolipotoxicity of pancreas β-cells. Administration of AMPK agonist AICAR in 90% partial pancreatectomized mouse improved glucose tolerance and insulin secretion and preserved insulin and BETA2/NeuroD mRNA expression in isolated islets from remnant pancreas. Exposure of isolated rat and human islets to glucolipotoxicity condition for 3 days induced suppression of insulin and BETA2/NeuroD mRNA expressions, which were also normalized by AICAR co-treatment, while Pdx-1 mRNA and protein levels were not changed during the experiment. However, expression of Pdx-1 mRNA was repressed in glucolipotoxicity for 8 days. Glucolipotoxicity induced blunting of glucose-stimulated insulin secretion was also rescued by AICAR in vitro. The expression of transcriptional coactivator PGC-1 (PPARσ coactivator-1) which might be one of the mediators of glucolipotoxicity in beta-cell was gradually increased by glucolipotoxicity and suppressed by AICAR over time. Overexpressions of PGC-1 using adenoviral vector in fresh isolated rat islets also suppressed insulin, BETA2/NeuroD and PDX-1 gene expressions and were not reversed by AICAR. However suppression of overexpressed PGC-1 by PGC-1 SiRNA was normalized those gene expressions. We propose that AMPK might function as a key molecule, offering protection to the glucolipotoxicity-induced β-cell dysfunction, through the suppression of PGC-1 overexpression induced by glucolipotoxicity.

6-2 Granuphilin molecularly docks insulin granules to the fusion machinery

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Monomeric small GTPase Rab27a and its effector granuphilin are specifically localized on insulin granules in pancreatic beta cells and involved in their exocytosis, although the precise mechanism for the plasma membrane-anchored SNARE, syntaxin 1a, and active Rab27a enhances the complex formation. We previously suggested that this interaction, at least in part, mediates the docking of insulin granules to the plasma membrane. Here we present biochemical, physiological, and genetic evidence to support this notion, particularly using the pancreatic beta cells derived from Rab27a-mutated *ashen* mice and newly established granuphilin-deficient mice. The results indicate that granuphilin is not only essential for the docking of insulin granules but also regulates subsequent fusion of docked granules through the interaction with the fusion machinery syntaxin-1a, which provides a novel paradigm for the docking machinery in regulated exocytosis.

6-3 New insights into adipogenesis: Novel targets for treatment of obesity

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Adipogenesis, the process of preadipocyte proliferation and differentiation, involves a coordinated series of events and underpins the increase in fat mass associated with obesity. We have recently identified two factors that appear to be required for adipogenesis.

(1) Inosine-5' Monophosphate Dehydrogenase (IMPDH), the key enzyme in de novo biosynthesis of GMP/GTP, which is implicated in lipid body dynamics and lipid accumulation (Whitehead et al, Traffic, 2004, 5, 739). We have investigated a putative role for IMPDH during lipid accumulation associated with adipogenesis of 3T3-L1 cells and primary human preadipocytes. IMPDH expression increased transiently (5-10 fold) during differentiation and inhibition of IMPDH activity, using mycophenolic acid (MPA), blocked lipid accumulation and differentiation in both cell types. In 3T3-L1 cells, treatment with MPA for the first 3 days of differentiation blocked induction of the adipogenic transcription factor CEBPα, but not CEBPβ or PPARγ, and delayed mitotic clonal expansion. Lipid accumulation and differentiation were reduced by 20%. Inhibition of IMPDH during days 3-6 reduced differentiation by 40%. Co-treatment with low-dose guanosine, which serves as substrate in the salvage pathway, reversed the effects of MPA. Surprisingly treatment with higher-dose guanosine alone inhibited differentiation. Collectively these observations suggest a requirement for strict regulation of guanylate metabolism during adipogenesis, which is not seen in other models of differentiation, and are consistent with a previously unrecognised role for IMPDH activity in the process of adipogenesis.

(2) Fibroblast Growth Factor-1 (FGF-1). We have recently shown that FGF-1 is a potent adipogenic factor, which facilitates proliferation and differentiation of primary human preadipocytes (Hutley et al, Diabetes, 2004, 53, 3097). Inhibition of FGF-1 using a variety of strategies (including neutralizing antibodies, suramin and peptides) inhibits proliferation and differentiation. Elucidating the mechanisms by which FGF-1 mediates these adipogenic effects is the focus of current work involving a variety of biochemical, pharmacological and genetic approaches. FGF-1 may act through a combination of the FGF receptors (FGFR1/2/3/4), members of the receptor tyrosine kinase superfamily, all of which are expressed in human preadipocytes and adipocytes. To facilitate investigations we have generated four stable cell lines, each of which expresses one of the FGFRs in an inducible fashion. This approach circumvents potential problems associated with constitutive high level expression which may lead to autophosphorylation and activation in the absence of ligand. Induction of the receptors results in increased FGF-1 stimulation of p38MAPK and ERK1/2, and the latter appears to be required for FGF's adipogenic effects. We are currently using this platform to interrogate the specificity and efficacy of established inhibitors and a suite of custom made inhibitors.

Collectively these studies identify IMPDH and FGF-1 as factors required for adipogenesis and potential therapeutic targets for the treatment of obesity.

6-4 The anti-obesity effect of adipose Gαs protein

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G proteins (guanine nucleotide-binding proteins) are crucial in mediating the regulatory action of growth factors and neurotransmitters during growth and development. The G protein, when coupled to plasma membrane receptors, is heterotrimer, consisting of α, β and γ subunits, each of which has numerous isoforms. When signaling after the binding of receptor to its ligand, G protein subunits function as monomer and dimmer, as Gα and Gβγ complex, respectively. The G protein signaling has been extensively studied and well documented. For example, in adipose tissues, G protein transmits the thermogenic signal of β-adrenoceptors by stimulating cAMP-PKA pathway that activates lipolysis and increase thermogenesis. Interestingly, in the adipocytes of white adipose tissue (WAT) in our C/EBP gene replacement mice (namely β/β mice in which the C/EBPα coding region was replaced with that of C/EBPβ in the genome; the modified C/EBPα gene allele was referred as β allele), we have found that the β/β allele exerted its effect through the elevated expression of the G protein stimulatory α subunit (Gαs) in WAT. Furthermore, over-expression alone of Gαs effectively increased mitochondrial biogenesis and prevented fat accumulation in lipid-rich cells, suggesting that Gαs might play an active role in programming the lipid-rich cells to be efficient in energy oxidation. Accordingly, Gαs may be used to program the lipid-rich cell to be an efficient energy oxidizer for anti-obesity purpose. To test the direct effect of adipose Gαs in regulating body weight and metabolism in mice, we have further generated a transgenic mouse line in which human Gαs is expressed specifically in fat cells under the control of aP2 promoter. We have found that human Gαs increased energy oxidation and reduced fat accumulation in WAT of mice. The phenotypic characterization of the Gαs transgenic mice and the therapeutic effect of Gαs in preventing weight-gain will be discussed in details.

6-5 DOC2b regulates GLUT4 vesicle fusion in 3T3-L1 adipocytes

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Calcium regulates membrane fusion of the glucose transporter GLUT4 vesicles to the plasma membrane by a mechanism that requires VAMP2, Syntaxin4, and SNAP23. However, little is known about the proteins sensing calcium signals. We focused on the SNARE related proteins containing the double C2 domain and identified DOC2b that translocated to the plasma membrane upon insulin stimulation. Immunofluorescent microscopy analysis showed that the translocation of DOC2b was blocked by BAPTA-AM, a cell permeable Ca^{2+}-chelating agent, but not by wortmannin. These data are consistent with the idea that adipocytes have calcium dependent insulin signals distinct from the classical PI3 kinase pathway. Expression of DOC2b in 3T3-L1 adipocytes increased insulin-stimulated glucose uptake by 50%, whereas reduction of DOC2b expression by siRNA decreased insulin-stimulated glucose uptake by 60%. Co-expression of DOC2b and myc-tagged GLUT4, which has epitope-tag in the first extracellular loop of GLUT4, demonstrated that cell surface myc-label markedly increased in 3T3-L1 adipocytes expressing DOC2b, compared with control adipocytes expressing only myc-tagged GLUT4. On the other hand, total GLUT4 docked to the plasma membrane did not differ between the cells with/without DOC2b overexpression.

These data indicate that DOC2b is important for the membrane fusion step, but not in the translocation or docking of GLUT4 vesicles. Taken together, our results suggest that DOC2b functions in the membrane fusion process of GLUT4 vesicles and activates glucose transport in a calcium-dependent manner.

6-6 Human evidence that genes involved in oxidative phosphorylation are up-regulated in type 2 diabetic liver

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Background: Mitochondrial oxidative phosphorylation (OXPHOS) plays an important role in the pathophysiology of type 2 diabetes. Genes for OXPHOS have reported to be coordinately down-regulated in skeletal muscle from type 2 diabetic patients, however, the hepatic regulation still remains unclear. We comprehensively analyzed expression of OXPHOS genes in the liver of type 2 diabetic patients using serial analysis of gene expression (SAGE).
Subjects and methods: We constructed SAGE libraries from liver tissues of type 2 diabetic patients (DM, n=5, Male 3, Age 60±11 yrs, BMI 25.2±5.2 kg/m^2, FPG 171±54 mg/dl, HbA_{1c} 8.1±2.0%, Values are mean±SD) and normal control subjects (NGT, n=5).
Results: (1) A total of 144,901 tags were obtained (DM 44,280 tags, NGT 100,621 tags). (2) Of 53 nuclear transcripts involved in OXPHOS, 36 (70%) were up-regulated in DM library, and the corresponding tag counts was 1.7 times higher in DM library compared with in NGT library (2355 vs. 1385 tags $p<0.00001$). (3) Tag count comparison of mitochondrial transcripts showed that mitochondrial rRNAs were 3-fold over-expressed, and mRNAs were 1.3-fold over-expressed in DM library (64905 vs. 18485 tags, 74505 vs. 61040 tags, respectively, $p<0.00001$). (4) Real-time PCR analysis confirmed that genes involved in gluconeogenesis such as PGC-1α, PEPCK1, PEPCK2, and GLUT2 were also up-regulated in DM library.
Conclusion: Genes involved in OXPHOS are up-regulated in the liver of type 2 diabetic patients, which look like a mirror image of those in skeletal muscle. Searching for the upstream master gene that regulates tissue-specific expression of OXPHOS genes may become an important issue to elucidate the mechanism of glucose homeostasis.

6-7 Id2: A new player in glucose mediated gene regulation mechanisms

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Glucose mediates a range of important changes in gene expression in cells but the mechanisms involved are poorly understood. We used DNA microarrays to identify genes regulated by glucose with the aim of identifier new mechanisms involved in glucose mediated gene regulation. Of the genes identified Id2 was the best candidate as it acts as a master switch for regulation of basic-helix-loop-helix (bHLH) transcription factors by binding class-A bHLH transcription factors and the sterol regulatory element binding protein 1c (SREBP-1c). We find that levels of Id2 protein are rapidly increased by glucose in J774.2 cells and this upregulation is maintained for at least 24 hours. Similar results were observed in HepG2 cells and in 3T3-L1 adipocytes. The upregulation is not mediated by osmolarity, Protein kinase-C or glucose-6-phosphate. However, the effect requires glutamine, is mimicked by glucosamine and is inhibited by azaserine, an inhibitor of glutamine:fructose-6-phosphate amidotransferase (GFAT). Further, adenoviral mediated overexpression of GFAT induces Id2 expression. Further we find that expression of Id2 in CHO cells blocks SREBP-1 induced induction of HSL gene promoter activity demonstrating changes in Id2 levels can have functional effects on regulation of metabolic genes. Therefore, these studies identify a mechanism by which cells are able to regulate gene expression in response to changes in glucose concentration by using flux through the hexosamine biosynthetic pathway (HBP) to rapidly regulate a transcriptional modifier that can subsequently modulate expression of metabolic genes.

6-8 Dissecting the steps of GLUT4 trafficking and identifying the site of insulin action

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Insulin-stimulated GLUT4 translocation to the plasma membrane (PM) constitutes the basis for blood glucose control. Yet functional assays to distinguish multiple steps along the GLUT4 vesicle trafficking cascade are lacking, which hinders current progress towards the elucidation of the underlying molecular events and the identification of the key step(s) that is/are regulated by insulin. In the current study, we have employed time-resolved total internal reflection fluorescence microscopy (TIRFM) to monitor the motion of GLUT4 storage vesicles (GSVs) in living 3T3-L1 cells. We have developed method for dissecting and systematic analyzing the docking, priming and fusion steps of GSV in vivo. With this approach, we have been able to unequivocally identify the key step regulated by insulin, which is the preparing of GSV for fusion competence after docking at the PM.

P-1 Identification of resistin promoter SNP-420 as a type 2 diabetes susceptibility gene

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Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance in insulin target tissues, namely, liver, skeletal muscle, and adipose tissues. Resistin is secreted from adipocytes and antagonizes insulin. Transgenic mice overexpressing resistin gene (Retn) in adipose tissue are insulin-resistant, whereas Retn (-/-) mice show lower fasting blood glucose, suggesting that altered Retn promoter function could cause diabetes. To determine the role of RETN in human T2DM, we analyzed single nucleotide polymorphisms (SNPs) in this gene. No SNPs were found in the coding region, and three SNPs identified in introns were not associated with T2DM. Of seven SNPs identified in the 5'flanking region, the G/G genotype of SNP-420 was associated with T2DM susceptibility. Meta-analysis including 1888 cases and 1648 controls typed confirmed this association. Linkage disequilibrium (LD) analysis including ~70kb region revealed that the -420G/G genotype itself was a primary variant determining T2DM susceptibility. Functionally, Sp1 and Sp3 transcription factors specifically bound to the susceptible DNA element including -420G. Overexpression of Sp1 or Sp3 enhanced RETN promoter activity with -420G in Drosophila SL2 cells lacking endogenous Sp family members. Consistent with these findings, fasting serum resistin levels were higher in T2DM subjects with -420G/G genotype. Therefore, the specific recognition of -420G by Sp1/3 increases RETN promoter activity, leading to enhanced serum resistin levels, thereby inducing human T2DM.

P-2 Therapeutic role of PPARγ in type II diabetes and diabetic nephropathy

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Peroxisome proliferators-activated receptor gamma (PPARγ) belongs to the superfamily of nuclear receptor transcription factors. This receptor has attracted enormous attention as a result of the key role it plays in controlling many biological processes involved in the pathogenesis of the metabolic syndrome, type II diabetes, and diabetic nephropathy. The intense research interest in PPARγ also stems largely from the therapeutic actions of its synthetic agonists in alleviating insulin resistance and improving glycemic control in type II diabetes. As a nuclear receptor transcription factor, PPARγ dimerizes with the retinoic acid receptor RXRα and then modulates the transcription of its target genes. Most of the PPARγ target genes thus far identified play important roles in insulin sensitivity, adipogenesis, and lipid metabolism. Activation of PPARγ by synthetic thiazolidinediones (TZDs) including pioglitazone and rosiglitazone exhibits potent insulin-sensitizing and hypoglycemic effect in type II diabetes via multiple mechanisms. Moreover, TZD PPARγ agonists are also effective in lowering albuminuria in type II diabetic subjects. Both metabolic changes and direct renal action are thought to mediate the favorable renal effect of TZD PPARγ agonists. In addition, recent studies have demonstrated that activation of PPARγ in renal collecting duct may be responsible for TZD-induced fluid accumulation and edema in patients taking TZDs. Collectively, PPARγ may serve as a therapeutic target for treating type II diabetes, diabetic nephropathy and TZD-activated fluid retention.

P-3 A purine analogue kinase inhibitor, CK59, reveals a role for CaMKII in insulin-stimulated glucose transport

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Olomoucine is known as a cyclin-dependent kinase (CDK) inhibitor. We found that olomoucine blocked the ability of insulin to stimulate glucose transport. It did so without affecting the activity of known insulin signalling proteins. Since CDKs are either inactive, or of very low activity, in differentiated cells, these data suggested olomoucine inhibited a novel kinase affecting glucose transport downstream of known insulin-sensitive kinase. To identify the olomoucine-sensitive kinase(s), we prepared analogues that could be immobilised to an affinity resin to isolate binding proteins. One of the analogues generated inhibited insulin-stimulated glucose uptake with increased sensitivity compared to olomoucine.. The IC_{50} for inhibition of insulin-stimulated glucose uptake occurred at analogue concentrations as low as 0.1 μM. To identify proteins binding to the analogue, [^{35}S]-labelled cell lysates prepared from 3T3-L1 adipocytes were incubated with analogue chemically cross-linked to a resin support. Non-binding proteins were washed from the resin and binding proteins analysed by SDS-PAGE. The major binding species was a doublet at 50-60kDa. This doublet was identified as CaMKII by N-terminal peptide analysis, and confirmed by MALDI-MS as the δ isoform of CaMKII. To investigate CaMKII involvement in insulin-stimulated glucose uptake, 3T3-L1 adipocytes were infected with retrovirus encoding GFP-HA tagged CaMKII wild-type or the ATP binding mutant, K42M.. GFP-HA-CaMKII K42M cells had less kinase activity than cells expressing wild-type GFP-HA-CaMKII. Insulin-stimulated glucose transport was significantly decreased in GFP-HA-CaMKII K42M cells compared to non-transfected cells and cells expressing either GFP-HA-CaMKII or GFP-HA. There was not a concomitant decrease in insulin-stimulated GLUT4 translocation in GFP-HA-CaMKII K42M cells when compared to GFP-HA alone. However, insulin-stimulated GLUT4 translocation in GFP-HA-CaMKII cells was significantly higher compared to either GFP-HA or GFP-HA-CaMKII K42M cells. Our results implicate the involvement of CaMKII in glucose transport. Delineation of the pathway for insulin stimulation of glucose transport may provide new targets for the treatment of type 2 diabetes and obesity.

P-4 MCP-1 links obesity to insulin resistance

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Adipose tissues secrete a variety of bioactive molecules and affect the insulin sensitivity of other insulin responsive tissues of whole bodies. In our trial of identifying novel secretory factors from adipocytes, expression of monocyte chemoattractant protein-1 (MCP-1) was found to be remarkably increased not only in adipose tissues but also in plasma of genetically obese diabetic db/db mice and high fat diet (HFD)-induced obese mice. aP2 promoter/enhancer-driven MCP-1 transgenic mice exhibited insulin resistance along with macrophage infiltration into adipose tissues and increased hepatic triglyceride contents. Furthermore, HFD-induced insulin resistance and hepatic steatosis were ameliorated in MCP-1 homozygous knockout mice. Macrophage infiltrations into adipose tissues observed in HFD feeding were also inhibited in MCP-1 homozygous knockout mice. In addition, expression of dominant-negative mutant of MCP-1 improves insulin resistance of db/db mice and HFD-induced obese mice by reducing hepatic glucose production during clamp period. These findings suggest that increased MCP-1 expression in adipose tissues results in macrophage infiltration into adipose tissues and hepatic insulin resistance observed in obese mice.

P-5 Protein phosphatase 2A activation is essential for protein-tyrosine phosphatase 1B-induced sterol regulatory element-binding protein-1 gene expression

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Sterol regulatory element-binding protein-1 (SREBP-1) is a key transcription factor in stimulating lipogenesis in the liver. We reported that protein-tyrosine phosphatase 1B (PTP1B) induces SREBP-1 gene expression. PTP1B is reported to be anchored on the endoplasmic reticulum (ER) via its C-terminal tail, and we recently reported that change in intracellular localization of PTP1B by C-terminal-truncation did not alter its inhibitory effects on insulin signaling in 3T3-L1 adipocytes. In this study, we found that overexpression of C-terminal truncated PTP1B (ΔCT) did not induce SREBP-1 gene expression. Furthermore, ΔCT failed to dephosphorylate PP2A, which is critical for activation of PP2A. On the other hand, overexpression of wild-type PTP1B dephosphorylated and activated PP2A. Finally, transient liver-specific overexpression of wild-type PTP1B led to increased serum triglyceride levels with enhanced SREBP-1 gene expression in mice. However, overexpression of ΔCT failed to increase SREBP-1 mRNA expression as well as serum triglyceride levels, despite causing insulin resistance. In conclusion, anchoring of PTP1B on the ER is essential for PP2A activation, and PP2A activation is crucial for PTP1B-induced SREBP-1 gene expression both *in vivo* and *in vitro*.

P-6 Glucose-stimulated upregulation of GLUT2 gene is mediated by SREBP-1c in the hepatocytes

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Glucose transporter type 2 isoform (GLUT2) is mainly expressed in the liver, β-cells of the pancreas, and the basolateral membrane of kidney proximal tubules and plays an important role in glucose homeostasis in living organisms. The transcription of the GLUT2 gene is known to be upregulated in the liver during postprandial hyperglycemic states or in type 2 diabetes. However, a molecular mechanism by which glucose activates GLUT2 gene expression is not known.

In this study, we report evidence that sterol response element binding protein (SREBP)-1c plays a key role in glucose-stimulated GLUT2 gene expression. The GLUT2 promoter reporter is activated by SREBP-1c and the activation is inhibited by a dominant-negative form of SREBP-1c (SREBP-1c DN). Adenoviral expression of SREBP-1c DN suppressed glucose-stimulated GLUT2 mRNA level in primary hepatocytes. An electrophoretic mobility shift assay and mutational analysis of the GLUT2 promoter revealed that SREBP-1c binds to the –84/-76 region of the GLUT2 promoter. Chromatin immunoprecipitation revealed that the binding of SREBP-1c to the -84/-76 region was increased by glucose concentration in a dose-dependent fashion. These results indicate that SREBP-1c mediates glucose-stimulated GLUT2 gene expression in hepatocytes.

P-7 Quantitatively investigating the agonist/antagonist ligand-binding regulation against PPARγ/RXRα heterodimerization

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Peroxisome proliferator-activated receptor γ (PPARγ) is an important therapeutic drug target against several diseases such as diabetes, inflammation, dyslipidemia, hypertension and cancer. It requires multiple protein-protein interaction for modulating target gene expression. As such, PPARγ is believed to function by forming a heterodimer with another nuclear receptor, the 9-*cis*-retinoic X receptor (RXR) *in vivo*. In this work, the agonist and antagonist ligand-binding regulation of the PPARγ/RXRα heterodimerization was totally analyzed. By use of native-PAGE, we firstly investigated the association of PPARγ ligand binding domain (LBD) (PPARγ-LBD) with RXRα-LBD *in vitro*. The results from the SPR technology based Biacore 3000 showed that both PPARγ agonists (GI262570, *cis*-parinaric acid, Indomethacin, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ$_2$) and Troglitazone) and RXR ligand (9-*cis*-retinoic acid) could stimulate the heterodimer formation by 10-100 orders of magnitude, while the PPARγ antagonist GW9662 decreases the PPARγ/RXRα interaction in comparison with the agonist binding case. In addition, two simulation models were constructed based on the crystal structure of the nuclear receptor PPARγ/RXRα heterodimer (PDB entry code 1FM9) to investigate the interactions between the ligand binding domains of PPARγ and RXRα. One is composed of PPARγ and RXRα ligand binding domains respectively bound with GI262570 and 9-cis-retinoic acid (9cRA). The second model only includes the ligand binding domains of PPARγ and RXRα. It is suggested that the receptor's conformation change caused by the ligand binding plays a decisive role in affecting PPARγ/RXRα interaction. Hopefully, such a ligand-binding modulation of PPARγ/RXRα interaction *in vitro* might be used as a potential approach in the discovery of the agonist and antagonist of PPARγ or RXRα at the early stage.

P-8 Hyperglycaemia-driven pathogenic abnormality of copper homeostasis in diabetes and its reversibility by selective chelation: Implications for the origins of the cardiovascular complications

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We recently showed that treatment with the Cu^{II}-selective chelator, trientine, alleviated heart failure in diabetic rats, improved left ventricular hypertrophy in humans with type-2 diabetes, and increased urinary Cu excretion in both groups compared with non-diabetic controls (1). We have now characterized Cu homeostasis in human subjects with type 2 diabetes and in age-matched controls, in whom we probed elemental balance with oral trientine in a parallel-group placebo-controlled study (2). Mean extracellular superoxide dismutase (EC-SOD) activity was elevated in diabetic subjects and its activity correlated strongly with the interaction between $[Cu]_{serum}$ and hemoglobin A_{1c}. Trientine caused Cu balance to become negative in diabetic subjects through elevated urinary Cu losses and suppressed elevated EC-SOD. Basal urinary Cu predicted urinary Cu losses during treatment, which caused extraction of systemic Cu^{II}. We suggest that cardiovascular complications in diabetes might be better controlled by therapeutic strategies that focus on lowering both plasma glucose and loosely-bound systemic Cu^{II}.

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P-9 Adipocyte/macrophage fatty acid binding proteins controlling metabolic syndrome

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Fatty acid binding proteins (FABPs) are cytosolic fatty acid chaperones whose biological role and mechanisms of action are not well understood. Here, we developed mice with targeted mutations in two related adipocyte FABPs, aP2 and mal1, to resolve their role in systemic lipid, glucose, and energy metabolism. Mice lacking aP2 and mal1 exhibited a striking phenotype with strong protection from diet-induced obesity, insulin resistance, type 2 diabetes, and fatty liver disease. These mice have altered cellular and systemic lipid transport and composition, leading to enhanced insulin receptor signaling, enhanced muscle AMP-activated kinase (AMP-K) activity, and dramatically reduced liver stearoyl-CoA desaturase-1 (SCD-1) activity underlying their phenotype. Taken together with the previously reported strong protection against atherosclerosis, these results demonstrate that adipocyte/macrophage FABPs have a robust impact on multiple components of metabolic syndrome, integrating metabolic and inflammatory responses in mice and constituting a powerful target for the treatment of these diseases.

P-10 Depletion of mtDNA and impaired glucose utilization

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Mitochondrial dysfunction contributes to a number of human diseases, such as hyperlipidemia, obesity, and diabetes. The mutation and reduction of mitochondrial DNA (mtDNA) have been suggested as factors in the pathogenesis of diabetes. To elucidate the association of cellular mtDNA content and insulin resistance, we produced L6 GLUT4myc myocytes depleted of mtDNA by long-term treatment with ethidium bromide (EtBr). L6 GLUT4myc cells cultured with 0.2μg/ml EtBr (termed depleted cells) revealed a marked decrease in cellular mtDNA and ATP content, concomitant with a lack of mRNAs encoded by mtDNA. Interestingly, the mtDNA-depleted cells showed a drastic decrease in basal and insulin-stimulated glucose uptake, indicating that L6 GLUT4myc cells develop impaired glucose utilization and insulin resistance. The repletion of mtDNA normalized basal and insulin-stimulated glucose uptake. The mRNA level and expression of IRS-1 associated with insulin signaling were decreased by 76 and 90% in the depleted cells, respectively. The plasma membrane (PM) GLUT4 in the basal state was decreased, and the insulin-stimulated GLUT4 translocation to the PM was drastically reduced by mtDNA depletion. Moreover, insulin-stimulated phosphorylation of IRS-1 and Akt2/PKB were drastically reduced in the depleted cells. Those changes returned to control levels after mtDNA repletion. Taken together, our data suggest that PM GLUT4 content and insulin signal pathway intermediates are modulated by the alteration of cellular mtDNA content, and the reduction in the expression of IRS-1 and insulin-stimulated phosphorylation of IRS-1 and Akt2/PKB are associated with insulin resistance in the mtDNA-depleted L6 GLUT4myc myocytes.

P-11 Ciliary neurotrophic factor reverses skeletal muscle insulin resistance associated with high fat fed diets by activating AMPK

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Ciliary neurotrophic factor (CNTF) induces weight loss in both rodents and humans through central effects on food intake. Here, we demonstrate that CNTF also increases fatty acid oxidation in isolated skeletal muscle, effects that are associated with activation of AMP activated protein kinase (AMPK) and are mediated by signaling via CNTFRα or the IL-6R and inhibited by the expression of a dominant negative AMPK. Moreover, we show that unlike leptin, the effects of CNTF are maintained in diet-induced obesity. We show that the metabolic effects of CNTF override induction of suppressors of cytokine signalling (SOCS) 3 since, in cultured muscle cells, the over-expression of SOCS3 inhibited leptin but not CNTF activation of AMPK and phosphorylation of acetyl-CoA carboxylase . Chronically, CNTF treatment increases fatty acid oxidation reduces body mass and improves insulin stimulated glucose uptake to a greater degree then calorie restriction alone. We therefore have identified a compound with "leptin like" effects but one that is resistant to the negative effects of diet induced obesity and SOCS3 induction, suggesting a potential peripheral tissue drug target for the treatment of obesity related diseases.

P-12 IA-2β is a key molecule for ghrelin's inhibitory effect on insulin secretion

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Ghrelin is a newly discovered endogenous ligand for growth hormone secretagogue receptor (GHSR). However, ghrelin has been shown to possess various central and peripheral effects other than GH secretion, which include orexigenic, gastric and cardiac effects. Both ghrelin and GHSR are expressed in pancreatic islets. We have identified several ghrelin-induced genes by PCR-select cDNA subtraction method. One of these is IA-2β, a β cell autoantigen for type 1 DM. Since IA-2β locates in insulin secretary granules of pancreaticβcells and ghrelin has been reported to inhibit insulin secretion, we have investigated a possible link among ghrelin, IA-2β, and insulin secretion. Intraperitoneal administration of ghrelin, increased IA-2β mRNA both in mouse brain and pancreas in Northern analysis. Ghrelin inhibited glucose-stimulated insulin secretion in MIN6 insulinoma cells dose-dependently. Ghrelin also increased IA-2β mRNA but did not affect IA-2 mRNA, another structurally related type 1 DM autoantigen. Overexpression of IA-2β but not IA-2 inhibited glucose-stimulated insulin secretion in MIN6 cells. Moreover, transfection of siRNA for IA-2β ameliorated ghrelin's inhibitory effect on insulin secretion. These findings strongly suggest that IA-2β is a key molecule in ghrelin's inhibitory effect on insulin secretion.

P-13 AMP-activated protein kinase (AMPK) β2 subunit is required for stimulation of glucose uptake in skeletal muscle

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AMPK is a major metabolic regulatory enzyme that stimulates glucose uptake by skeletal muscle, and is therefore a target for the treatment of type-2 diabetes and obesity. AMPK is an αβγ heterotrimer with seven genes encoding the AMPK subunits (α1, α2, β1, β2, γ1, γ2, γ3). The β subunit functions as a targeting scaffold for the α and γ subunits facilitating AMPK binding to membranes and glycogen via its N-terminal myristoyl group and glycogen binding domain respectively. We generated AMPK β2 subunit null mice to examine the role of the β2 subunit in glucose uptake in skeletal muscle. Real-time RT-PCR and Western blot analyses were used to examine the effect of β2 null on AMPK subunit expression in skeletal muscle. Glucose uptake was measured using [^{14}C]2-deoxy-glucose in isolated extensor digitorum longus (EDL) muscle from wildtype and β2 null mice in the presence and absence of the pharmacological AMPK activator 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR). RT-PCR and Western blot analysis showed no upregulation of β1 mRNA or protein expression in skeletal muscle of β2 null mice, but marked suppression of AMPK α2 protein levels. AICAR caused a 2-fold increase in glucose uptake in EDL of wildtype mice, but no increase in β2 null mice. These results support idea that the β2 subunit is essential for AMPK-stimulated glucose uptake in skeletal muscle, and the β1 subunit is unable to compensate for this loss of function.

P-14 Genetic variation in PSARL is associated with plasma insulin concentration

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We recently identified a gene encoding a mitochondrial intramembrane protease known as PSARL (presenilins-associated rhomboid-like protein) that is located on chromosome 3q27 and is associated with insulin resistance. Studies in our laboratory and others have shown that PSARL is a key regulator of mitochondrial function, and strongly suggest a role for PSARL in the development of insulin resistance. *PSARL* gene expression in skeletal muscle was correlated with insulin sensitivity in both animal models and human subjects. Using the family material from the MRCOB study in which the original 3q27 QTL for plasma insulin and other metabolic syndrome phenotypes was first discovered, we resequenced 51 individuals and identified 15 polymorphisms in *PSARL* (minor allele frequency 0.003-0.497, average LD=0.44). We performed robust Bayesian quantitative trait nucleotide analysis to examine the relationship between SNP genotypes and plasma insulin levels in 1031 subjects from 169 families of predominantly northern European ancestry and residing in the US (759 females, 272 males, mean age 47.2, plasma insulin 87±38 pmol/L, BMI 30±7 kg/m^2). This statistical analysis explicitly allowed for the interaction of SNP genotype with age due to the well known effect of aging on mitochondrial function. After correction for multiple testing, 3 SNPs that were in very high LD (rho>0.9) showed strong evidence of association with plasma insulin including genotype-by-age interaction ($p < 0.0024$). Bayesian analysis revealed that this set of SNPs had a very high posterior probability (PP = 0.96) of having a direct effect on plasma insulin levels. We are currently completing functional analysis of two of these SNPs, a promoter variant and a coding variant in exon 7. Overall, these data strongly support the hypothesis that genetic variation in *PSARL* represents an important new risk factor for insulin resistance and type 2 diabetes.