# APDO SYMPOSIUM 2013

*Asia-Pacific Diabetes and Obesity Study Group*
*Joint with the 34th Annual Meeting of Japan Society for the Study of Obesity*

## Program & Abstracts

**October 12(Sat) to 13(Sun), 2013**

Tokyo International Forum

President : Takashi Kadowaki

Department of Diabetes and Metabolic Diseases
Graduate School of Medicine, The University of Tokyo

**Organizer**
Department of Diabetes and Metabolic Diseases
Graduate School of Medicine
The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-8655, JAPAN

# Greetings

Takashi Kadowaki MD, PhD

Chairman, Asia-Pacific Diabetes and Obesity Study Group symposium 2013
Professor, Department of Diabetes and Metabolic Diseases, Graduate School of Medicine,
The University of Tokyo, Japan

Dear participants,

It is a great pleasure and honor to host the Asia-Pacific Diabetes and Obesity Study Group symposium 2013 (APDO symposium 2013) at the Tokyo International Forum, Tokyo, Japan, from October 12-13, 2013.

The prevalence of diabetes and obesity are rising to epidemic proportions, with the financial cost associated with these problems inflicting a huge burden throughout the Asia-Pacific region. We desperately need remedies for these conditions. There is a growing recognition that diabetes and obesity develop in a different fashion in Asian countries compared to Western countries : Asians tend to develop diabetes with relatively mild obesity while Westerners tend to develop severe obesity, presumably due to their different genetic background and environmental factors. Therefore, it is particularly important for Asian-Pacific practitioners and researchers to share ideas and knowledge in order to elucidate the mechanisms behind the region-specific disease development and to establish and disseminate an evidence-based standardized treatment optimized for the region.

The Asia-Pacific Diabetes and Obesity (APDO) Study Group was established with an aim of promoting medical research by clinical and basic researchers in the fields of obesity and diabetes in the Asia-Pacific region. The organizing committee is comprised of representatives from Japan, Korea, China, Hong Kong, Singapore, Taiwan, Australia and New Zealand. The group has held symposiums every year since 2005. Over these years, the symposium has not only served as a forum where participants join to share the latest advances and achievements in the field, but also has played a significant role in nurturing the next generation of experts. The APDO symposium 2013 will be held as an international symposium, in conjunction with the 34th Annual Meeting of Japan Society for the Study of Obesity (October 11-12, 2013), with the two meetings working mutually to encourage participation and promote successful interaction.

Tokyo is the center of various activities in Japan including politics, economy, culture and entertainment. Fall is one of the best seasons to enjoy Tokyo. We look forward to seeing you this October, when we will welcome you with heartfelt hospitality.

# Program

## [Day 1] October 12 (Sat.)

### Luncheon Seminar

Co-sponsored by Takeda Pharmaceutical Company Limited

Date and Time: October 12 (Saturday) 12:30~13:20
Session Room: Hall D7
Chair: Kenji Kangawa
(National Cerebral and Cardiovascular Center, Japan)

LS **Gut hormones regulating energy homeostasis**
Masamitsu Nakazato
(Division of Neurology, Respirology, Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan)

### Main Symposium 1
### Islet Biology

Co-sponsored by Novartis Pharma K.K.

Date and Time: October 12 (Saturday) 13:30~14:45
Session Room: Hall D7
Chairs: Noriko Takahashi
(Structural Physiology, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, Japan)
Weiping Han
(Laboratory of Metabolic Medicine, Singapore Bioimaging Consortium, A*STAR, Singapore)

OS-01 **Role of insulin signaling in the regulation of pancreatic β cell function and mass**
Kohjiro Ueki
(Department of Diabetes and Metabolic Diseases, Graduate School of Medicine, the University of Tokyo, Japan)

OR-01 **Molecular regulation of insulin secretion and diabetes**
Weiping Han
(Laboratory of Metabolic Medicine, Singapore Bioimaging Consortium, A*STAR, Singapore)

OR-02 **Rescuing the Deficient Insulin Secretion in Type 2 Diabetes with Newcomer Insulin Granules**
Herbert Y. Gaisano
(University of Toronto, Toronto, Ontario, Canada)

OR-03 SNAREs in Membrane Traffic and Phsyiology

Wanjin Hong

(Institute of Molecular & Cell Biology, A*STAR, Singapore)

OR-04 Polarisation of insulin secretion

Peter Thorn

(School of Biomedical Sciences, University of Queensland, Australia)

OR-05 PREADIPOCYTE FACTOR-1/DLK1 PROMOTES HUMAN DUCTAL CELL TRANSDIFFERENTIATION INTO β-CELLS

Kun-Ho Yoon

(Department of Endocrinology and Metabolism, The Catholic University of Korea, Seoul, Korea)

## Main Symposium 2
## Insulin Action

Co-sponsored by Takeda Pharmaceutical Company Limited

Date and Time: October 12 (Saturday) 14:45~16:40

Session Room: Hall D7

Chairs: Masato Kasuga

(National Center for Global Health and Medicine, Japan)

David James

(Diabetes & Obesity Research Program, Garvan Institute of Medical Research, Australia)

## Special Lecture

SL-01 Molecular regulation of brown fat cell fate

Patrick Seale

(Cell and Developmental Biology, School of Medicine, University of Pennsylvania, USA)

OR-06 REGULATION OF INSULIN SENSITIVITY AND INFLAMMATORY RESPONSES IN ADIPOCYTES

Jae Bum Kim

(Department of Biophysics and Chemical Biology, School of Biological Sciences, Institute of Molecular Biology & Genetics, Seoul National University, Seoul, Korea)

OR-07 **Rats fed a high-fat diet have decreased glucose uptake in muscle during feeding, without alteration in insulin signalling**

Gregory J. Cooney

(Diabetes and Obesity Research Program, Garvan Institute, Sydney, NSW, Australia)

OR-08 **Regulation of sphingolipid metabolism by endoplasmic reticulum stress**

Tae-Sik Park

(Department of Life Science, Gachon University, Sungnam, Korea)

OR-09 **Role of arginine methylation on CRTC-dependent glucose metabolism**

Seung-Hoi Koo

(Department of Life Sciences, Korea University, Seoul, Korea)

OR-10 **Central Role of Fasting Inducible CITED2-GCN5 Interaction in Hepatic Gluconeogenesis**

Michihiro Matsumoto

(Department of Molecular Metabolic Regulation, Diabetes Research Center, Research Institute, Japan / National Center for Global Health and Medicine, Tokyo, Japan)

OR-11 **FAM3A activates p110α/Akt signaling to ameliorate hepatic gluconeogenesis and lipogenesis**

Youfei Guan

(Department of Physiology and Pathophysiology, Peking University Health Science Center, China / Shenzhen University Diabetes Center, Shenzhen University Health Science Center, China)

OS-02 **Pioglitazone ameliorates insulin resistance, diabetes and atherosclerosis by both adiponectin dependent and independent pathway**

Takashi Kadowaki

(Department of Diabetes and Metabolic Diseases, Graduate School of Medicine, The University of Tokyo, Japan)

## Main Symposium 3
## Incretin & Hot Topics (1)

Co-sponsored by Mitsubishi Tanabe Pharma Corporation/DAIICHI SANKYO COMPANY, LIMITED

Date and Time: October 12 (Saturday) 16:50~18:05
Session Room: Hall D7
Chairs: Iichiro Shimomura
(Department of Metabolic Medicine, Osaka University Graduate School of Medicine, Japan)
Lee-Ming Chuang
(Department of Internal Medicine National, Taiwan University Hospital, Taiwan)

OS-03 **Expectation for incretin: diabetes and cognitive impairment**
Ryo Suzuki
(Department of Diabetes and Metabolic Diseases, Graduate School of Medicine, The University of Tokyo, Japan)

OR-12 **STUDY THE ROLE OF GLUCAGON-LIKE PEPTIDE 1 IN ACTIN CYTOSLELETON REMODELING OF β-CELLS AND ITS RELEVEVANCE TO POTENTIATION OF INSULIN SECRETION DURING GLUCOTOXICITY**
Xiaosong Ma
(Shenzhen University Diabetes Center, Shenzhen, China)

OR-13 **Systemic NAD Biology Governs Circadian Control of Metabolic Behaviors**
Min-Seon Kim
(Appetite Regulation Laboratory, Asan Institute for Life Science, University of Ulsan College of Medicine, Seoul, Korea / Division of Endocrinology and Metabolism, Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea)

OR-14 **Deficiency of NPGPx, an Oxidative Stress Sensor, Leads to Obesity in Mice and Human**
Yi- Cheng Chang
(Genomics Research Center, Academia Sinica, Taipei, Taiwan / Graduate Program of Translational Medicine, National Taiwan University, Taipei, Taiwan / Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan)

OR-15 **Role of Novel Variants of PGC-1α in the Regulation of Energy Metabolism**
Kazuhiro Nomura
(Division of Diabetes and Endocrinology, Kobe University Graduate School of Medicine, Japan)

OR-16 **The Nuclear Receptor, Nor-1. Regulates oxidative metabolism and resistance to diet induced obesity**

George E.O. Muscat

(The University of Queensland, Institute for Molecular Bioscience, Obesity Research Centre, Australia)

OR-17 **Prostaglandin Reductase-3 Negatively Modulates Adipogenesis Throuht Regulation of Peroxisome Proliferator-Activated Receptor γ Activity**

Yu-Hsiang Yu

(Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan)

## Evening Seminar

Co-sponsored by MSD K.K.

Date and Time: October 12 (Saturday) 18:10~19:00
Session Room: Hall D7
Chair: Masato Kasuga
(National Center for Global Health and Medicine, Japan)

ES **Toward understanding the mechanisms of incretin secretion and action**

Nobuya Inagaki

(Department of Diabetes, Endocrinology, and Nutrition Graduate School of Medicine, Kyoto University, Japan)

## [Day 2] October 13 (Sun.)

### Morning Seminar

Co-sponsored by Novo Nordisk Pharma Ltd.

Date and Time: October 13 (Sunday) 8:00~8:50
Session Room: Hall D7
Chair: Toshimasa Yamauchi
(Department of Diabetes and Metabolic Diseases, Graduate School of Medicine, the University of Tokyo, Japan)

MS New long-acting insulin analogue; clinical efficacy and safety
Masato Odawara
(Third Department of Internal Medicine, Tokyo Medical University, Japan)

### Main Symposium 4
### Incretin & Hot Topics (2)

Co-sponsored by Nippon Boehringer Ingelheim Co., Ltd./Eli Lilly Japan K.K.

Date and Time: October 13 (Sunday) 9:00~10:15
Session Room: Hall D7
Chairs: Masato Kasuga
(National Center for Global Health and Medicine, Japan)
Iris Shai
(Ben-Gurion University of the Negev-Faculty of Health Sciences, Israel)

OS-04 Up-to-date information of incretin-based medicine
Jun-ichiro Miyagawa
(Division of Diabetes, Endocrinology and Metabolism, Department of Internal Medicine, Hyogo College of Medicine, Japan)

OR-18 Ceramide and Metabolic Disease: A Survey of Therapeutic Targets
Scott A. Summers
(Duke-NUS Graduate Medical School Singapore, Singapore/Duke University, USA)

OR-19 NPY: Central and peripheral control of energy homeostasis
Herbert Herzog
(Neuroscience Program, Garvan Institute of Medical Research, Sydney, Australia)

OR-20 **Role of Soluble Epoxide Hydrolase in Non-Alcoholic Fatty Liver Disease**

Yi Zhu

(Department of Physiology, Tianjin Medical University, China)

OR-21 **Central Activating Transcription Factor (ATF4) Regulates Hepatic Insulin Resistance in Mice Via S6K1 Signaling and the Vagus Nerve**

Feifan Guo

(Institute for Nutritional Sciences, SIBS, CAS, China)

OR-22 **The Suppression of Erythropoietin Production by Long-Chain Saturated Fatty Acids: Lipid Nephrotoxicity and ER Stress**

Anusornvongchai Thitinun

(Division of Nephrology and Endocrinology, The University of Tokyo School of Medicine, Tokyo, Japan / Division of Endocrinology, Department of Internal Medicine, Lerdsin General Hospital, Department of Medical Services, Bangkok, Thailand)

OR-23 **Pro-atherogenic Effects of Retinol-binding Protein 4 on Vascular Smooth Muscle Cells**

Nanping Wang

(Institute of Cardiovascular Science, Peking University, Beijing, China / Cardiovascular Research Center, Xi'an Jiaotong University, Xi'an, China)



## Special Lecture

Date and Time: October 13 (Sunday) 10:15~10:55
Session Room: Hall D7
Chair: Takashi Kadowaki
(Department of Diabetes and Metabolic Diseases, Graduate School of Medicine, the University of Tokyo, Japan)

SL-02 **Everlasting Challenge for Adiposcience and Adipomedicine in East Asia**

Kazuwa Nakao

(Kyoto University Medical Innovation Center, Japan)

## Main Symposium 5
## Obesity, Prevention and Therapy

Co-sponsored by AstraZeneca K.K.

Date and Time: October 13 (Sunday) 11:00~12:15
Session Room: Hall D7
Chairs: Wataru Ogawa
(Division of Diabetes and Endocrinology, Kobe University Graduate School of Medicine, Japan)
Jae-Bum Kim
(Department of Biophysics and Chemical Biology, School of Biological Sciences, Institute of Molecular Biology & Genetics, Seoul National University, Seoul, Korea)

OS-05 **Characteristics of Adiponectin as abundant defense protein**
Iichiro Shimomura
(Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, Japan)

OR-24 **FIBROBLAST GROWTH FACTOR 19 IN RELATION TO OBESITY AND LIPID METABOLISM IN MAN**
Elaine Hui
(Department of Medicine, The University of Hong Kong, Hong Kong / Research Centre of Heart, Brain, Hormone and Healthy Aging, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong)

OR-25 **CALORIE RESTRICTION VERSUS PERIODIC FASTING TO REDUCE RISK MARKERS FOR DIABETES AND CARDIOVASCULAR DISEASE IN HUMANS**
Leonie K. Heilbronn
(The University of Adelaide, Australia)

OR-26 **ADIPONECTIN GENETIC VARIANTS IN THE PREDICTION OF CORONARY ARTERY DISEASE IN CHINESE: A 16-YEAR PROSPECTIVE STUDY**
Chloe YY Cheung
(Department of Medicine, The University of Hong Kong, Hong Kong)

OR-27 **Targeting gp130 to prevent inflammation and promote insulin action**
Mark A. Febbraio
(Cellular and Molecular Metabolism Laboratory, BakerIDI Heart and Diabetes Institute, Melbourne, VIC, Australia)

OR-28 Exploring the Foregut Hypothesis Using Continuous Subcutaneous Glucose Monitoring, Metabolomics and Proteomics

Rinki Murphy
(Faculty of Medical and Health Sciences, The University of Auckland, New Zealand)

OR-29 UNDERSTANDING METABOLIC REMODELING OF SKELETAL MUSCLE IN TYPE 2 DIABETES

Sean L. McGee
(Metabolic Remodeling Laboratory, Metabolic Research Unit, School of Medicine, Deakin University, Geelong, Australia)

## Poster Session

Q&A Date & Time: October 12 (Sat.) 19:00~19:30
Room: Hall D7 Lobby

P-01 **Analysis of GLP-1 signaling and class IA phosphatidylinositol 3-kinase in pancreatic β cells**
Hirotsugu Suwanai
(The University of Tokyo Hospital, Department of Diabetes and Metabolic Diseases, Japan)

P-02 **PLASMA SOLUBLE LEPTIN RECEPTOR LEVELS ARE ASSOCIATED WITH β-CELL FUNCTION IN PATIENTS WITH TYPE 2 DIABETES**
Tomoaki Morioka
(Metabolism, Endocrinology, and Molecular Medicine, Osaka City University Graduate School of Medicine, Japan)

P-03 **EFFECT OF ALLOGLIPTIN AND METFORMIN COMBINATION THERAPY: A PILOT STUDY**
Miyako Kishimoto
(Department of Endocrinology Diabetes Metabolism, National Center for Global Health and Medicine, Japan / Diabetes and Metabolism Information Center, Diabetes Research Center, Research Institute, National Center for Global Health and Medicine, Japan)

P-04 **Adipocyte Dysfunction and Metabolic Disease**
Tetsuya Hosooka
(Division of Diabetes and Endocrinology, Kobe University Graduate School of Medicine, Japan)

P-05 **FEEDING BEHAVIOR IN MICE WITH HEREDITARY PREDISPOSITION TO HIGH FAT-DIET INDUCED GLUCOSE INTOLERANCE**
Mototsugu Nagao
(Department of Endocrinology, Diabetes and Metabolism, Graduate School of Medicine, Nippon Medical School, Japan)

P-06 **LIPIN-1 IN 3T3-L1 ADIPOCYTES: ITS REGULATION AND ROLE IN OBESITY**
Nobuhiko Takahashi
(Department of Internal Medicine, Health Science University of Hokkaido School of Dentistry, Japan / Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical University, Japan)

P-07 **Role of DNA Methylation for Leptin Gene Promoter in 3T3-L1 Adipocytes**
Masashi Kuroda
(Department of Nutrition and Metabolism, Institute of Health Biosciences, the University of Tokushima Graduate School, Japan)

P-08 Autophagic flux is suppressed via ROS in differentiated and hypertrophic adipocytes

Yuhei Mizunoe

(Molecular Pathology and Metabolic Disease, Department of Medical and Life Sciences, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Japan)

P-09 Effects of *trans*-Tiliroside Isolated from Rosehip on Glucose Tolerant and Lipid Metabolism through the Liver Functions

Kiyofumi Ninomiya

(Pharmaceutical Research and Technology Institute, Kinki University, Osaka, Japan)

P-10 SREBP-1c IS REQUIRED FOR LIFE-LONG CALORIC RESTRICTION-INDUCED MITOCHONDRIAL BIOGENESIS IN WHITE ADIPOSE TISSUE OF MICE

Yoshikazu Higami

(Molecular Pathology and Metabolic Disease, Department of Medical and Life Sciences, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Japan)

P-11 Serum Ketone Body Level Correlates with Daily Carbohydrate Intake in Low Carbohydrate Diet (LCD)

Hiroshi Bando

(Tokushima University, Japan)

P-12 EFFECT OF LONGITUDINAL CHANGES IN VISCERAL FAT AREA ON INCIDENCE OF METABOLIC RISK FACTORS

Yumi Matsushita

(Department of Clinical Research, National Center for Global Health and Medicine, Japan)

P-13 Effects of Physical Activity on Insulin Sensitivity and Pancreatic β-Cell Function Among Japanese Obese Adults: The Saku Control Obesity Program (SCOP)

Atsushi Goto

(Department of Diabetes Research, Diabetes Research Center, National Center for Global Health and Medicine, Tokyo, Japan)

P-14 PREVALENCE OF OBESITY FROM BIRTH TO INFANCY: A LONGITUDINAL STUDY AT AN ARBAN CITY, NARA, JAPAN

Rena Kato

(Department of Human Life and Environment, Nara Women's University, Japan)

P-15 Association between BMI and High-Sensitivity C-Reactive Protein

Naoko Nishitani

(Sugiyama Jogakuen University Department of Nursing, Japan)

P-16 TRAJECTORY OF BODY MASS INDEX BEFORE THE DEVELOPMENT OF DIABETES IN JAPANESE MEN: TORANOMON HOSPITAL HEALTH MANAGEMENT CENTER STUDY (TOPICS)

Yoriko Heianza
(Department of Internal Medicine, Niigata University Faculty of Medicine, Niigata, Japan / Health Management Center, Toranomon Hospital, Tokyo, Japan / Department of Internal Medicine, University of Tsukuba Institute of Clinical Medicine, Ibaraki, Japan)

P-17 Prediction of the Development of Hypertension by Body Mass Index History in Japan: Toranomon Hospital Health Management Center Study (Topics)

Yoriko Heianza
(Department of Internal Medicine, Niigata University Faculty of Medicine, Niigata, Japan / Health Management Center, Toranomon Hospital, Tokyo, Japan / Department of Internal Medicine, University of Tsukuba Institute of Clinical Medicine, Ibaraki, Japan)

P-18 Comparison of Various Body Mass Index Histories to Identify Undiagnosed Diabetes in Japanese Men and Women: Toranomon Hospital Health Management Center Study

Sakiko Yoshizawa
(Department of Internal Medicine, Niigata University Faculty of Medicine, Niigata, Japan)

P-19 Evaluation of Food Preference in Childhood Using A Picture Choice Method: A Relationship with Body Habitus

Shinji Kimura
(Department of Clinical Nursing, Faculty of Medicine, Shimane University, Japan)

P-20 Factors influencing the body image of Japanese college students

Tomoko Imai
(Department of Food Science and Nutrition, Faculty of Human Life and Science, Doshisha Women's College of Liberal Arts, Japan)

P-21 Aging-like skin changes occurred through mineralocorticoid receptor signaling pathway in a mouse model for metabolic syndrome

Tomoko Akase
(Department of Biological Sciences and Nursing, Graduate School of Medicine, Yokohama City University, Japan)

P-22 Diabetes Disrupts Homeostasis against Alzheimer's Disease

Naoyuki Sato
(Department of Clinical Gene Therapy, Graduate School of Medicine, Osaka University, Japan / Department of Geriatric Medicine, Graduate School of Medicine, Osaka University, Japan)

P-23 Chewing Betel Quid (Areca Nut) and Risk of Metabolic Disease, Cardiovascular Disease, and All-Cause Mortality: A Meta-Analysis

Tomohide Yamada
(Department of Diabetes and Metabolic Diseases, Graduate school of Medicine, University of Tokyo, Japan)

# Abstracts

# SL-01 Molecular regulation of brown fat cell fate

Patrick Seale

Cell and Developmental Biology, School of Medicine, University of Pennsylvania, USA

Brown adipocytes in dedicated depots of brown adipose tissue (BAT) arise from cellular precursors that also give rise to skeletal muscle cells. We found that *Early B cell factor-2* (*Ebf2*) expression in the mesoderm of mouse embryos marks brown fat precursors that are destined to activate Ppar$\gamma$ expression and differentiate into UCP1+ brown adipocytes. Loss of EBF2 in mice causes a very severe defect in BAT development. EBF2 cooperates with PPARγ to activate expression of many brown fat-selective target genes, including *Prdm16*, a key transcriptional co-activator in brown adipocytes. Surprisingly, genetic loss of *Prdm 16* specifically in the brown fat lineage does not disrupt embryonic BAT formation or the expression of brown fat-specific genes. Interestingly however, *Prdm16*-deficiency caused ectopic expression of white fat-selective genes and a severe aging-associated decline in the thermogenic characteristics of BAT. *Prdm16*-deficient BAT from older mice had reduced mitochondrial content and function. Moreover, animals lacking PRDM16 in BAT had a severely depressed capacity to increase their energy expenditure in response to β-adrenergic activators or cold. Importantly, BAT-selective deletion of *Prdm16* impaired BAT function but did not affect the thermogenic 'beige' adipocyte population in WAT; this serves as a model to distinguish the effects of brown and beige adipocytes in systemic metabolism. Ongoing studies are assessing the role of PRDM16 and BAT in high fat diet-induced obesity and metabolic disease.

# SL-02 Everlasting Challenge for Adiposcience and Adipomedicine in East Asia

Kazuwa Nakao

Kyoto University Medical Innovation Center, Japan

Adiposcience and adipomedicine were coined by members of Japan Society for the Study of Obesity (JASSO) to mean adipose tissue-related science and medicine, respectively. Together with annual meeting of JASSO, the annual conference named "Adiposcience Symposium" have attracted young scientists to the new field for almost 20 years in Japan and starting this year, the symposium has been held as the official meeting of JASSO. Target diseases of adipomedicine consist of obesity, metabolic syndrome and lipodystrophy.

Everlasting challenge of JASSO for adiposcience and adipomedicine in East Asia will be reported and discussed.

# LS　Gut hormones regulating energy homeostasis

Masamitsu Nakazato

Division of Neurology, Respirology, Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan



The concept that the gut and the brain are closely connected, and that this interaction plays an important part not only in gastrointestinal function but also in energy homeostasis is deeply rooted in our language. The molecular mechanisms of energy balance are coming to light by the recent robust progresses in the molecular biology and neuroscience. The gastrointestinal tract produces a large array of peptides to regulate feeding. The investigation to uncover the workings of the great wanderer, the vagus has resumed. We have clarified that the vagal afferent is the major pathway conveying feeding-regulatory signals of orexigenic and anorectic gut hormones to the hypothalamus, a center of energy homeostasis. Incretins, GLP-1 and GIP, attract a great attention of interest because of their physiological roles to stimulate insulin secretion. GIP is produced in the proximal intestine and GLP-1 is produced in the distal intestine. This different localization gives different mechanisms by which GIP serves as a hormone and GLP-1 as a neuropeptide. This understanding is important why DPP-4 inhibitors work well to improve glucose metabolism in diabetes. A high-fat diet causes resistance to leptin and ghrelin, but not to gut-derived anorectic peptides, GLP-1, CCK, or PYY. I will talk about functional roles and pathophysiological significance of gut hormones in the regulation of energy homeostasis.

# ES Toward understanding the mechanisms of incretin secretion and action

Nobuya Inagaki

Department of Diabetes, Endocrinology, and Nutrition Graduate School of Medicine, Kyoto University, Japan



Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are the incretins = released from enteroendocrine K-cells and L-cells, respectively, in response to nutrient ingestion to potentiate glucose-stimulated insulin secretion. We previously reported that GIP is involved in compensatory insulin hypersecretion in high fat diet (HFD)-induced obesity. To clarify the mechanism, we established GIP-GFP knock-in (GIP-GFP) mice to visualize K-cells by EGFP and identified regulatory factor X 6 (Rfx6), expressed exclusively in K-cells, by microarray analysis of isolated K-cells of these mice. Our *in vitro* and *in vivo* study suggests that Rfx6 increases GIP expression and content in K-cells and is involved in GIP hypersecretion in HFD-induced obesity.

As prepro-GIP is truncated in the GIP-GFP mice, GIP-GFP KI mice exhibited reduced GIP secretion in heterozygous state (GIP-reduced mice: $GIP^{gfp/+}$) and absence of GIP secretion in homozygous state (GIP-lacking mice: $GIP^{gfp/gfp}$). When fed standard chow, $GIP^{gfp/+}$ and $GIP^{gfp/gfp}$ mice had mild glucose intolerance with decreased insulin levels; bone volume was decreased in $GIP^{gfp/gfp}$ and preserved in $GIP^{gfp/+}$. Under high fat diet conditions, glucose levels during OGTT were not impaired in $GIP^{gfp/+}$ and $GIP^{gfp/gfp}$ mice, while insulin secretion remained lower. $GIP^{gfp/gfp}$ and $GIP^{gfp/+}$ mice showed reduced obesity and insulin resistance, accompanied with higher fat oxidation and energy expenditure. These results suggest that reduction of GIP has beneficial effects on the glucose profile and the insulin sensitivity in high fat diet-induced obesity without affecting profoundly the functions of GIP outside the entero-insular axis.

It is known that pancreatic β-cell mass is decreased in type 2 diabetes (T2DM) and may be involved in the progression of T2DM. We reported that GLP-1 restores glucose metabolism and glucose-induced insulin secretion in the β-cells of Goto-Kakizaki (GK) rats, a non-obese diabetic model with insulin failure. We also reported that GLP-1 protects pancreatic islet transplant by inhibiting apoptosis. These results suggest that GLP-1 may halt the progression of T2DM through preservation of β-cell mass. Non-invasive imaging of pancreatic β-cells is an unmet need required, and I will introduce the recent progress of our study.

# MS　New long-acting insulin analogue; clinical efficacy and safety

Masato Odawara

Third Department of Internal Medicine, Tokyo Medical University, Japan

The major goals of diabetes treatment are prevention of microvascular and macrovascular complications, and control of their progressions. To attain these goals, it is necessary to maintain good glycemic control for long periods of time. Insulin therapy is an effective means to achieve good glycemic control, and it is shown in the DCCT in type 1 diabetes and UKPDS and Kumamoto Study in type 2 diabetes that intensive glycemic control lowers the risk of complications. However, insulin therapy is associated with hypoglycaemia and weight gain. Particularly, hypoglycaemia impairs timely introduction of insulin treatment and is a barrier to achieve glycemic control target. In recent years, the results of analysis of ACCORD, ADVANCE and VADT suggest the possibility that serious hypoglycaemia with intensified insulin therapy may lead to an increase in cardiovascular event risk, and the importance of glycemic control intensification without inducing hypoglycaemia has been recognized.

Limitations of existing long-acting insulin analogues have been pointed out, including that the duration of action does not last for 24 hours in some patients, and intra-subject variation of insulin action exists.

Insulin Degludec is a new, long-acting insulin analogue, with action lasting over 24 hours, and low variability of insulin action leading to stable glycemic control. These characteristics enable improvement of glycemic control, without increasing hypoglycaemia which is shown in the clinical studies.

In this lecture, efficacy and safety of insulin Degludec will be presented.

# OS-01 Role of insulin signaling in the regulation of pancreatic β cell function and mass

Kohjiro Ueki, Hirotsugu Suwanai, Michinori Sakata, Takashi Kadowaki

Department of Diabetes and Metabolic Diseases, Graduate School of Medicine, the University of Tokyo, Japan

Studies using knockout animal models by ourselves and others have demonstrated that insulin signaling molecules play a pivotal role in the regulation of β cell functions and mass, although it still remains controversial whether autocrine insulin is the major input to activate the signaling pathway. Of these molecules, phosphoinositide-3 kinase (PI3K) appears to be the key player since it lies downstream of various signaling pathways that have been shown to affect the β cell functions and mass, such as insulin, growth hormone and incretins. Indeed, β cell specific deletion of the regulatory subunits of PI3K in mice (βDKO mice) results in glucose intolerance with a variety of defects in β cells. These mice show impaired glucose-stimulated insulin secretion, especially the first phase insulin secretion, associated with increased apoptosis, loss of synchronized insulin secretory response to glucose within the islet presumably due to the immature gap-junction development and decreased SNARE complex proteins. Expression of SNARE complex proteins and Cv36, a component of the gap-junction, is regulated by PI3K, presumably through FoxO1. Recently, we have also found that PI3K signaling controls cholesterol metabolism in β cells, which has been suggested to regulate the first phase insulin secretion. During the progress of diabetes, *db/db* mice exhibit progressive reductions in expression of insulin signaling molecules and decreased insulin secretion. These data may suggest that PI3K activated by autocrine insulin maintains β cell functions and mass. Thus, once insulin secretion is decreased, reduced insulin signaling activity further exacerbates impaired insulin secretion, making a vicious cycle. Furthermore, treatment of βDKO mice with Exendin-4 has very modest effect on insulin secretion compared to the control mice, suggesting that PI3K activity is essential for the effect of GLP-1 on insulin secretion as well. Taken together, activation of PI3K in β cells can be a promising therapeutic strategy for the treatment of type 2 diabetes.

## OR-01 Molecular regulation of insulin secretion and diabetes

Weiping Han

Laboratory of Metabolic Medicine, Singapore Bioimaging Consortium, A*STAR, Singapore

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

# OR-02 Rescuing the Deficient Insulin Secretion in Type 2 Diabetes with Newcomer Insulin Granules

Herbert Y. Gaisano

University of Toronto, Toronto, Ontario, Canada

In pancreatic islet beta-cells, only a small number of insulin secretory granules (SGs) are docked onto the plasma membrane (PM) and only a fraction of these docked SGs are competent or 'primed' for immediate release. Each of these sequential steps leading to secretion-release from a reserve pool within the cytoskeleton mesh just beneath the PM, insulin SG mobilization, loosely tethered then becoming fully docked onto the PM, where they undergo some time to get primed in order to become competent to undergo membrane fusion (exocytosis), are mediated by distinct sets of molecules. We recently found that majority of insulin SGs undergoing exocytosis actually arise from 'newcomer SGs' that require only minimal to no docking time on the PM before exocytosis; and that SGs could fuse with each other (SG-SG fusion) without having to all get to the PM to undergo exocytosis. These newcomer SGs account for almost all of second phase glucose stimulated insulin secretion (GSIS) and at least half of first phase GSIS. These two novel modes of exocytosis are very efficient and could compensate for the secretory deficiency in diabetes believe to be due to the loss of fusion-competence of the pool of docked SGs. In fact, both newcomer and SG-SG fusion could be amplified by GLP-1 signaling, the latter having become a new treatment for diabetes. In our recent work, we have found that newcomer SGs and SG-SG fusion share a similar exocytotic machinery consisting of Munc18b which organizes the assembly of Syntaxin 3 and VAMP8 and SNAP25. This is distinct from the SM/SNARE complex that mediates exocytosis of docked SGs, which consists of Munc18a, Syntaxin-1A and VAMP2. However, docked and newcomer SGs share a common priming protein Munc13-1, and common SG-tethering proteins RalA and Sec5. It is known that the islet protein levels of the entire exocytotic machinery for docked insulin SGs are severely reduced in type 2 diabetes rodents and patients. We have begun to express the exocytotic proteins mediating newcomer SGs, starting with Munc18b, into pancreatic islets of type 2 diabetic Goto-Kakizaki rats by viral gene transfer, which remarkably improved insulin secretion and glucose control alleviating the diabetic state. Thus, insights gained from our work may reveal novel sites within these alternate modes of exocytosis that can compensate for the deficiency of docked SGs in type 2 diabetes.

## OR-03 SNAREs in Membrane Traffic and Phsyiology

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The secretory and endocytic pathways of eukaryotic cells play fundamental role diverse cellular processes such as nutrient uptake, protein secretion, protein sorting, cell polarity and signaling and physiological processes such as hormone secretion and neurotransmission. Protein translocation between different membrane compartments is mediated by shuttling intermediates such as small vesicles that bud from a donor compartment and fuse with a target compartment. Vesicle docking and fusion are primarily governed by a family of proteins called SNAREs. Interaction of SNAREs on the vesicle (v-SNARE) with SNAREs on the target compartment (t-SNAREs) catalyzes the final fusion. SNAREs and their regulatory proteins are converging points for diverse signaling cascades to regulate physiological and metabolic processes. The overview of SNAREs, summary of our work in SNARE discovery and demonstration of a role of VAMP8 in diverse regulated secretions will be discussed.

## OR-04 Polarisation of insulin secretion

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Cellular polarisation and targeting of secretion is found in many cell types but is debated for insulin secretion in islets. If proven, this would change our view of the normal mechanisms of stimulus-secretion coupling in beta cells and would be important in our understanding of disease.

Here we use three-dimensional immunofluorescence of mouse islet slices to show that ELKS, and other proteins associated with the exocytic fusion machinery, are enriched on the beta cell membrane that faces the vasculature. To determine if this architectural polarisation of the beta cell is functionally significant we used live-cell 2-photon imaging experiments of intact islets. Here we measure the time and location of each insulin granule fusion event using an extracellular fluorescent dye. We show that within an optical slice taken through the islet there is an asymmetric, non-random, distribution of sites of insulin granule fusion. In separate experiments we image across multiple Z planes, and directly demonstrate focal targeting of insulin granule secretion to the beta cell membrane that faces the vasculature. Our results indicate that beta cells in situ within intact islets are polarised and target insulin secretion and have wide implications for our understanding of stimulus-secretion coupling in healthy islets and in disease.

# OR-05 PREADIPOCYTE FACTOR-1/DLK1 PROMOTES HUMAN DUCTAL CELL TRANSDIFFERENTIATION INTO β-CELLS

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Preadipocyte factor-1/Dlk1 (Pref-1/Dlk1) is involved in the proliferation and differentiation of various precursor cells. However, the intracellular signaling pathways controlling these processes are unclear. Here we show that Pref-1/Dlk1 increases Akt, ERK1/2, and Forkhead box protein O1 (FOXO1) phosphorylation in human pancreatic duct cells. Pref-1/Dlk1 induced FOXO1 activation is required for MAPK signaling, as evidenced by the fact that the ability of Pref-1/Dlk1 to increase FOXO1 phosphorylation is impaired when ERK1/2 is chemically inhibited. Concurrently, Pref-1/Dlk1 also increases PDX1 protein level that leads to an increase in the expression of the *insulin* gene, which is thought to be mediated by MAPK signaling. Importantly, proteomic analysis identifies Rab43 GTPase activating protein as a downstream target of the Akt signaling pathway. Inhibition of Rab43 suppresses Pref-1/Dlk1-induced elevation of synaptophysin and secretogranin and glucose-stimulated insulin secretion, suggesting that Rab43 is required for granule protein synthesis and insulin secretion. During Pref-1/Dlk1 stimulation, insulin content is regulated via the MAPK signaling pathway in pancreatic duct cells, whereas insulin secretion induced by glucose is regulated via the Akt signaling pathway. In 90%-pancreatectomize rats, treatment of Pref-1-mFc promotes pancreatic ductal cell and β-cell regeneration. In humans, the serum level of soluble Pref-1/Dlk1 showed significant decrease in the diabetic group compared with control group. Our data demonstrate that Pref-1/Dlk1 regulates pancreatic duct cell transdifferentiation into β-cells through stimulating Akt or MAPK signaling, establishing a new role for Pref-1/Dlk1 in insulin secretion. Pref-1/Dlk1 could also be a novel therapeutic candidate for type 2 diabetes.

## SL-01 Molecular regulation of brown fat cell fate

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Brown adipocytes in dedicated depots of brown adipose tissue (BAT) arise from cellular precursors that also give rise to skeletal muscle cells. We found that *Early B cell factor-2* (*Ebf2*) expression in the mesoderm of mouse embryos marks brown fat precursors that are destined to activate Ppar$\gamma$ expression and differentiate into UCP1+ brown adipocytes. Loss of EBF2 in mice causes a very severe defect in BAT development. EBF2 cooperates with PPARγ to activate expression of many brown fat-selective target genes, including *Prdm16*, a key transcriptional co-activator in brown adipocytes. Surprisingly, genetic loss of *Prdm 16* specifically in the brown fat lineage does not disrupt embryonic BAT formation or the expression of brown fat-specific genes. Interestingly however, *Prdm16*-deficiency caused ectopic expression of white fat-selective genes and a severe aging-associated decline in the thermogenic characteristics of BAT. *Prdm16*-deficient BAT from older mice had reduced mitochondrial content and function. Moreover, animals lacking PRDM16 in BAT had a severely depressed capacity to increase their energy expenditure in response to β-adrenergic activators or cold. Importantly, BAT-selective deletion of *Prdm16* impaired BAT function but did not affect the thermogenic 'beige' adipocyte population in WAT; this serves as a model to distinguish the effects of brown and beige adipocytes in systemic metabolism. Ongoing studies are assessing the role of PRDM16 and BAT in high fat diet-induced obesity and metabolic disease.

# OR-06 REGULATION OF INSULIN SENSITIVITY AND INFLAMMATORY RESPONSES IN ADIPOCYTES

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Adipose tissue plays key roles in whole body energy homeostasis by storing surplus energy in the form of triglycerides (TGs) and by releasing them to other tissues to fulfill energy needs. Furthermore, AT serves as an endocrine tissue by producing various signaling molecules such as leptin, resistin, and adiponectin. In obesity, increased adipose tissue is one of hallmarks which associate with metabolic dysregulation. Especially, in adult obesity, increased adiposity is primarily resulted from hypertrophic adipocytes. Recent findings have suggested that various immune cells are infiltrated into adipose tissue of obesity, accompanied with adipose tissue inflammation and insulin resistance. Despite of these, the correlation between adipocyte morphology and dysregulation of adipocyte function has not been clearly elucidated. In this study, we have investigated lipid-overloaded hypertrophic adipocytes in the aspects of insulin sensitivity and inflammation. Compared to normal adipocytes, lipid-overloaded hypertrophic adipocytes showed decreased insulin sensitivity. Similarly, ex vivo studies have revealed that hypertrophic adipocytes deteriorated insulin sensitivity in obesity. Although pro-inflammatory responses were also induced in lipid-overloaded adipocytes, pro-inflammatory responses were initiated earlier than insulin resistance in hypertrophic adipocytes. Moreover, certain hypertrophic adipocytes exhibited insulin resistance without pro-inflammatory responses. These results suggest that adipose tissue insulin resistance appears to be closely associated with hypertrophic adipocytes regardless of pro-inflammatory response in early obesity.

# OR-07 Rats fed a high-fat diet have decreased glucose uptake in muscle during feeding, without alteration in insulin signalling

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**Background:** Excess energy intake leads to lipid accumulation and decreased insulin-stimulated glucose metabolism in a variety of tissues. The mechanisms of lipid-induced insulin resistance in muscle are thought to involve lipid species (diaclyglycerides, ceramides) reducing phosphorylation of insulin signalling proteins. However most assessments of insulin resistance in rodent models are done in the middle of the sleeping (daylight) phase by imposing artificial conditions thought to represent the normal transition from fasting to feeding (hyperinsulinemic clamp, insulin injection). Because of the influence circadian rhythms have on energy metabolism, we investigated glucose uptake in muscle of chow and high-fat fed, insulin resistant rats over the normal fasting/feeding transition and examined potential mechanisms for any changes observed.

**Methods:** Male wistar rats (250g) were fed chow or high fat diet (HFD) (45% calories as fat) before surgery to implant a chronic jugular cannula. One week after surgery (4 weeks on HFD) rats were taken at 4pm near the end of the resting/inactive day, and 10pm after the beginning of the feeding/active night and the cannula used to infuse [$^{3}$H]-2-deoxyglucose tracer to assess glucose uptake into muscle. Blood was also sampled for assay of plasma glucose, fatty acids and insulin and after 45 mins rats were euthanazed and muscle collected, snap frozen and stored for determination of tracer accumulation and phosphorylation of signalling proteins by immunoblotting.

**Results:** Plasma glucose was similar in chow and HFD rats at 4pm (chow 8.4±0.2mM; HFD 8.4±0.4mM) and 10pm (chow 9.1±0.6mM; HFD 9.2±0.7mM). Plasma insulin increased significantly in both chow and HFD rats over the transition from fasting to feeding but there was no significant difference between chow and HFD rats (3pm chow 42.0±3.6μU/ml, HFD 46.0±3.5μU/ml; 10pm chow 94.0±5.0μU/ml, HFD 87.0±4.9μU/ml, $P<0.05$ for effect of time). In chow-fed rats glucose uptake in muscle increased 3-fold when the rats started to feed (chow 3pm 6.4±1.1μmol/min/100g; chow 10pm 20.1±2.5μmol/min/100g, $P<0.05$) however there was no increase in HFD rat muscle, even though insulin increased to a similar extent (HFD 3pm 5.3±0.6μmol/min/100g; HFD 10pm 6.5±0.7μmol/min/100g). Akt phosphorylation in muscle increased to a similar extent over the fasting/feeding transition in both chow and HFD rats but there was a significant difference in plasma fatty acid levels between chow and HFD rats during the dark/feeding phase (chow 0.21±0.02mM; HFD 0.56±0.08mM, $P<0.05$). There were also significantly higher levels of pyruvate dehydrogenase kinase 4 (PDK 4) in muscle of HFD rats.

**Conclusion:** Examining glucose uptake in muscle of chow and HFD rats over the normal fasting/feeding transition showed a significant increase in glucose uptake in chow but not HFD rats, despite similar increases in circulating insulin in both groups of rats. The reduced effect of insulin in HFD rats was associated with higher circulating fatty acid levels and increased PDK4 content in muscle, but there was no difference in the phosphorylation state of the signalling protein Akt in HFD muscle. These results indicate that factors such as substrate competition and PDK4 activity may be more relevant to HFD insulin resistance in muscle than alterations in insulin signalling.

## OR-08 Regulation of sphingolipid metabolism by endoplasmic reticulum stress

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The endoplasmic reticulum is the principal organelle in the cell for protein folding and trafficking, lipid synthesis and cellular calcium homeostasis. Perturbation of ER function results in activation of the unfolded protein response (UPR). Chronic ER stress is reported to have an important role in abnormal lipid biosynthesis and development of insulin resistance. The present study reports that transcription of sphingosine kinase 2 (Sphk2) is differentially regulated by ER stress-mediated UPR pathways. Expression of Sphk2, a major isotype of sphingosine kinase in the liver, was upregulated by tunicamycin and sphingosine 1-phosphate (S1P) was elevated in primary mouse hepatocytes. In contrast, chronic ER stress by high fat diet suppressed Sphk2 expression. Overexpression of the activating transcription factor 4 (ATF4) upregulated Sphk2 expression, whereas the spliced form of X-box binding protein 1 (sXBP1) downregulated Sphk2 as demonstrated by Sphk2 promoter assays and western blot analyses. Adenoviral Sphk2 overexpression activated pAKT with no alteration of IRS1 phosphorylation. In addition, cellular S1P levels were elevated by Sphk2 overexpression while ceramide and sphingomyelin were not altered. These results demonstrated that Sphk2 is differentially regulated by ER stress-induced UPR pathways would contribute to the amelioration of hepatic insulin resistance and steatosis via S1P production.

## OR-09 Role of arginine methylation on CRTC-dependent glucose metabolism

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Maintenance of fasting glucose homeostasis is achieved in part via transcriptional control of hepatic gluconeogenesis by cAMP response element binding protein CREB and its coactivator CRTC2. Previously, we have shown that PRMT1 is involved in the transcriptional control of hepatic glucose metabolism by regulation of FoxO1. Here we show that PRMT6, another type I PRMT family of proteins, is involved in the fasting-mediated activation of CRTC2. PRMT6 was found to be associated with CRTC2, which promoted asymmetric dimethylation of multiple arginine residues of this protein by mass spectrometric analysis. Methylation of CRTC2 triggers stronger association with CREB on the gluconeogenic promoters, resulting in the increased expression of PEPCK and G6Pase. Adenovirus-mediated overexpression of PRMT6 promotes higher blood glucose, while depletion of hepatic PRMT6 lowers fasting glycemia with improved pyruvate tolerance in mice. Interestingly, expression of hepatic PRMT6 is increased in mouse models of obesity and insulin resistance, and knockdown of PRMT6 restores euglycemia in these settings. We suggest that PRMT6 is a critical component of CRTC2-mediated hepatic glucose metabolism in mammals.

# OR-10 Central Role of Fasting Inducible CITED2-GCN5 Interaction in Hepatic Gluconeogenesis

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During fasting, induction of hepatic gluconeogenesis is important to ensure energy homeostasis in response to the energy demand. However, such induction is dysregulated in type 2 diabetes, resulting in the development of fasting hyperglycemia. Hormonal and nutrient regulation of metabolic adaptation during fasting is mediated predominantly by the transcriptional coactivator PGC-1α in concert with various other transcriptional regulators including the transcription factors FoxO1, HNF-4α and CREB as well as the histone acetyltransferse CBP. Although CITED2 (CBP/p300-interacting transactivator with glutamic acid- and aspartic acid-rich COOH-terminal domain 2) interacts with many of these molecules, the role of this protein in regulation of hepatic gluconeogenesis has been unknown. We investigated the role of CITED2 in hepatic gluconeogenesis by using gain- and loss-of-function approaches in vitro and in vivo. The abundance of CITED2 was found to be increased in the liver of mice by fasting as well as in cultured hepatocytes by glucagon and cAMP signaling. CITED2 inhibited the acetylation of gluconeogenic transcriptional coactivator PGC-1α by blocking its interaction with GCN5, a key acetyltransferase for PGC-1α. The consequent reduction in the level of PGC-1α acetylation resulted in an increase in its transcriptional coactivation activity and up-regulation of the expression of gluconeogenic genes. In addition, the interaction of CITED2 with GCN5 was found to be disrupted by insulin in a manner dependent on phosphoinositide 3-kinase signaling. These results thus reveal that CITED2 functions as a transducer of glucagon and insulin signaling in the regulation of PGC-1α activity associated with the transcriptional control of gluconeogenesis, and that this function is mediated through modulation of GCN5-dependent acetylation of PGC-1α. In addition, we recently unveiled the critical role of CITED2-GCN5 complex as a strong activator of gluconeogenesis. I will present our most recent findings suggesting that CITED2 upregulates gluconeogenesis via activation of both PGC-1α and GCN5.

# OR-11 FAM3A activates p110α/Akt signaling to ameliorate hepatic gluconeogenesis and lipogenesis

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FAM3A belongs to a novel cytokine-like gene family and its physiological role remains largely unknown. We found a marked reduction of FAM3A expression in the livers of db/db and high-fat diet (HFD)-induced diabetic mice. Hepatic overexpression of FAM3A markedly attenuated hyperglycemia, insulin resistance and fatty liver with increased Akt (pAkt) signaling and repressed gluconeogenesis and lipogenesis in the livers of those mice. In contrast, siRNA-mediated knockdown of hepatic FAM3A resulted in hyperglycemia with reduced pAkt levels and increased gluconeogenesis in the livers of C57BL/6 mice. In vitro study revealed that FAM3A was mainly localized in the mitochondria, where it increases ATP production and secretion in cultured hepatocytes. FAM3A activated Akt through p110α catalytic subunit of PI3K in an insulin-independent manner. Blockade of ATP receptor P2 receptor, or its downstream PLC and IP3R, and removal of medium calcium all significantly reduced FAM3A-induced increase in cytosolic free $Ca^{2+}$ levels and attenuated FAM3A-mediated PI3K/Akt activation. Moreover, FAM3A-induced Akt activation was completely abolished by the inhibition of calmodulin (CaM). In conclusion, FAM3A plays crucial roles in the regulation of glucose and lipid metabolism in the liver where it activates PI3K-Akt signaling pathway via a $Ca^{2+}$/CaM-dependent mechanism. FAM3A may represent an attractive target for the treatment of insulin resistance, type 2 diabetes and NAFLD.

# OS-02 Pioglitazone ameliorates insulin resistance, diabetes and atherosclerosis by both adiponectin dependent and independent pathway

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Thiazolidinediones (TZDs) have been shown to act as insulin sensitizers in animal models of obesity-linked insulin resistance and diabetes. TZDs have been widely used as therapeutic agents for the treatment of type 2 diabetes patients. In addition to its function in the increasing insulin sensitivity, TZDs have been implicated in direct actions at the vascular levels and prevent from atherosclerosis. Pioglitazone reduced the composite of all-cause mortality, non-fatal myocardial infarction, and stroke in patients with type 2 diabetes who have a high risk of macrovascular events by the PRO active Study.

Adiponectin is an adipose-tissue-derived secreted protein that circulates in plasma. We and others have also demonstrated that adiponectin-deficient (adipo$^{-/-}$) mice are insulin-resistant and glucose intolerant. Previous studies have shown that adiponectin stimulates fatty acid oxidation in skeletal muscle and inhibits glucose production in the liver by activating AMP-activated protein kinase (AMPK) through its specific receptors, AdipoR1 and AdipoR2. Moreover, we and others demonstrated the adipo$^{-/-}$ mice exhibited increased neointimal formation in response to cuff-injury or wire-injury. Adiponectin transgenic ApoE-deficient mice showed amelioration of atherosclerosis. As a result, adiponectin has come to be recognized as a major insulin sensitizing and anti-atherogenic hormone.

TZDs increase plasma adiponectin levels in animal models of obesity and diabetes, nondiabetic subjects, and patients with type 2 diabetes, and the improvement in insulin sensitivity and atherosclerosis in response to TZD administration is associated with an increase in circulating adiponectin. Thus, it is reasonable to speculate that the action whereby TZDs increase insulin sensitivity is mediated, at least in part, by increased adiponectin. However, whether the TZD-induced increase in plasma adiponectin is causally involved in TZD-mediated insulin sensitizing and anti-atherogenic effects has not been addressed. We investigated insulin resistance and atherosclerosis in the adipo$^{-/-}$ and adipo$^{-/-}$ob/ob mice by pioglitazone treatment.

After 10 mg/kg pioglitazone treatment, the insulin resistance and diabetes of ob/ob mice was significantly improved in association with significant upregulation of serum adiponectin levels. Amelioration of insulin resistance in ob/ob mice was attributed to decreased glucose production and increased AMP activated protein kinase (AMPK) in the liver, but not to increased glucose uptake in skeletal muscle. In contrast, insulin resistance and diabetes were not improved in adipo$^{-/-}$ob/ob mice. After 30 mg/kg pioglitazone treatment, insulin resistance and diabetes of ob/ob mice were again significantly ameliorated, which was attributed not only to decreased glucose production in the liver but also to increased glucose uptake in skeletal muscle. Interestingly, adipo$^{-/-}$ob/ob mice also displayed significant amelioration of insulin resistance and diabetes, which was attributed to increased glucose uptake in skeletal muscle, but not to decreased

glucose production in the liver. Thus, pioglitazone-induced amelioration of insulin resistance and diabetes may occur adiponectin dependently in the liver and adiponectin independently in skeletal muscle. Moreover, 3 weeks of pioglitazone significantly reduced the neointimal formation in the wild-type mice with upregulation of serum adiponectin levels, but 3 weeks of pioglitazone fail to reduce the neointimal formation in the adipo$^{-/-}$ mice. On the other hand, 8 weeks of pioglitazone exhibited similar significant reductions in neointimal formation in both wild-type and adipo$^{-/-}$ mice. Thus, pioglitazone-induced suppression of atherosclerosis occurs both dependently and independently of adiponectin.

Taken together, pioglitazone ameliorates insulin resistance, diabetes and atherosclerosis by both adiponectin dependent and independent pathway.

## OS-03 Expectation for incretin: diabetes and cognitive impairment

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Diabetes has reached an epidemic proportion globally. East Asian countries share rapid aging of the population as an important social issue, which also contributes to the rising incidence of diabetes in the region. This demographic change in diabetic patients enhances clinical importance of association between diabetes and cognitive impairment. People with diabetes are reported to have a greater rate of decline in cognitive function and an increased risk of Alzheimer's disease. Several studies have indicated that acute glucose fluctuations are associated with cognitive decline in diabetic patients. Furthermore, recent research revealed that cognitive deficits in humans are associated with insulin-resistant alterations in signaling pathway in the brain. In these years, in attempting to avoid hypoglycemia and postprandial glucose elevation, DPP-4 inhibitors are commonly prescribed for diabetes instead of sulfonylurea in Japan, where the patients often show impaired ability of glucose-stimulated insulin secretion. Intranasal insulin enhances some cerebral functions including attention, memory formation and cognition in humans. Recently, by using mouse models, we reported that insulin deficiency causes reduction of sterol synthesis in the brain (Cell Metabolism 12: 567, 2010), and that the disorder may affect cognitive function (PLoS Biology 11: 21001532, 2013). As for incretins, increasing number of evidence show that they have neuroprotective effects with anti-inflammatory properties. A paper indicated that exendin-4 prevents Aß oligomer-induced serine phosphorylation of IRS-1 in cultured neurons and improves cognition in mouse models of Alzheimer's disease. A randomized open label trial showed significant improvement in motor and cognitive measures in exenatide-treated patients who have Parkinson disease. In this talk, I will discuss potency of incretins and insulin for neuroprotection, which may have more significance in treatment of diabetes in the elderly.

# OR-12 STUDY THE ROLE OF GLUCAGON-LIKE PEPTIDE 1 IN ACTIN CYTOSLELETON REMODELING OF β-CELLS AND ITS RELEVEVANCE TO POTENTIATION OF INSULIN SECRETION DURING GLUCOTOXICITY

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Filamentous actin (F-actin) is implicated in granule trafficking and functions as a negative regulator of exocytosis of insulin-containing granules. Here, we show that INS-1 cells chronically exposure to 30 mM glucose have substantially increased amounts of F-actin stress fibers, and are refractory to glucose-induced actin remodeling and insulin secretion. By contrast, cells cultured in 5.5 mM glucose display short actin filaments and robust glucose-stimulated actin remodeling and insulin secretion. Interestingly, treatment with GLP-1 or actin depolymerizing agent latrunculin B markedly disrupts actin cytoskeleton and potentiates glucose-stimulated insulin secretion of cells cultured at 5.5 and 30 mM glucose. Moreover, treatment with forskolin and IBMX echo the effects of GLP-1 and latrunculin B. Notably, in the case of cells cultured at 30 mM glucose, the effect of GLP-1 is mimicked by the PKA agonist 6-Bnz-cAMP-AM whereas suppressed by the PKA inhibitor Rp-cAMPS, indicating that cAMP-PKA signaling pathway is responsible for GLP-1-stimulated actin depolymerization and GSIS at glucotoxicity.

# OR-13 Systemic NAD Biology Governs Circadian Control of Metabolic Behaviors

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Nicotinamide adenine dinucleotide ($NAD^+$) is an essential coenzyme of protein deacetylase sirtuins, which has been implicated multiple cellular functions. In mammals, intracellular $NAD^+$ levels largely depend on the nicotinamide phosphoribosyltransferase (Nampt) activity, a rate-limiting enzyme in $NAD^+$ salvage pathway. Nampt catalyzes a conversion of nicotinamide to nicotinamide mononucleotide (NMN), $NAD^+$ precursor in NAD biosynthetic pathway. Nampt can be secreted via Golgi-independent pathway and found in systemic circulation. Extracellular Nampt (eNampt) also has Nampt activity.

Interestingly, Nampt haploinsufficiency in mice causes a defective insulin secretion, which is completely recovered by NMN supply to islets. From these data, a hypothesis has been raised that systemic Nampt activity regulates insulin secretion by providing $NAD^+$ precursors to pancreatic islets, an organ with low intrinsic Nampt activity. On the other hand, intracellular Nampt and $NAD^+$ levels in hepatocytes display a remarkable circadian pattern. Cellular clock proteins Clock and Bmal stimulate Nampt transcription and Nampt inhibits transcriptional activity of these proteins through Sirt1 activation, thereby constituting a negative feedback loop. Therefore, Nampt is now regarded as an important component of cellular clockwork.

We found that plasma NAD, NMN, and eNampt levels had marked circadian rhythms with a midnight rise and a daytime fall in normal mice. Moreover, inhibition of systemic eNampt activity by continuous intraperitoneal (IP) infusion of FK866 increased food intake and decreased energy expenditure (EE). These effects were greater during a nighttime period when systemic Nampt activities were higher. Conversely, we artificially induced systemic Nampt activation by IP injection of Nampt peptide, NMN and NAD during the early daytime period in overnight-fasted mice. Acute IP administration of Nampt peptide, NMN and NAD suppressed fast-induced feeding and stimulated EE. These data support that increased systemic Nampt activity during the postprandial period may contribute to satiety formation and enhanced energy consumption. Indeed, IP injection of Nampt and NMN increased hypothalamic $NAD^+$ levels but not in hepatic $NAD^+$ levels. Moreover, we found that Nampt-mediated regulation of food intake and EE was mediated via hypothalamic Sirt2 activation and Sirt2-mediated Foxo1 inhibition. These findings demonstrate a critical role for systemic NAD biology in circadian regulation of feeding behavior and energy metabolism.

# OR-14 Deficiency of NPGPx, an Oxidative Stress Sensor, Leads to Obesity in Mice and Human

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Elevated oxidative stress is associated with obesity. Emerging evidence shows that instead of being a consequence of obesity, oxidative stress may also contribute to fat formation. NPGPx is a conserved oxidative stress sensor/transducer essential for reducing reactive oxygen species (ROS) with predominant expression in adipose tissue. Here we show that deletion of NPGPx enhances adipogenesis while re-expression or over-expression of NPGPx suppresses adipogenesis *in vitro*. NPGPx-deficient mice exhibit markedly increased fat mass and adipocyte hypertrophy. NPGPx deficiency promotes adipocyte differentiation via ROS-dependent dimerization of protein kinase A regulatory subunits and activation of CCAAT/enhancer-binding protein beta (C/EBPβ), which can be reversed by antioxidant N-acetylcysteine (NAC). Treatment with the NAC prevents white fat formation and adipocyte hypertrophy in NPGPx-deficient mice. Furthermore, single nucleotide polymorphism in the human NPGPx gene, which correlates with lower NPGPx expression level in adipose tissue, are associated with higher body mass index in several independent human populations. These results indicate NPGPx protects against fat accumulation in rodents and human via suppressing ROS, and highlight the importance of targeting redox homeostasis in obesity treatment.

# OR-15 Role of Novel Variants of PGC-1α in the Regulation of Energy Metabolism

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Peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α) is a transcriptional coactivator that regulates various metabolic processes, including mitochondrial biogenesis and thermogenesis. Given that mitochondrial dysfunction and impaired thermogenesis are often observed in individuals with insulin resistance and obesity and that the abundance of PGC-1α is reduced in skeletal muscle of such affected animals and humans, PGC-1α has been implicated in the pathogenesis of these global health problems. We and others have recently identified variants of PGC-1α generated by transcription from an alternative promoter. While canonical PGC-1α is relatively widely distributed, the novel variants are detected only in brown adipose tissue, skeletal muscle, and the heart. Moreover, the variants are robustly induced in skeletal muscle by acute exercise. Forced expression of the novel variants in cultured cells resulted in increased expression of genes known to be induced by canonical PGC-1α, and stimulated transactivation activity of PPARα, suggesting that the molecular function of the variants are similar to that of canonical PGC-1α. Mice specifically lacking the novel variants developed age-dependent obesity and insulin resistance. The number of mitochondria, fiber-type composition, and abundance of total PGC-1α in skeletal muscle were not altered in the mutant mice, likely because the canonical form is predominant under static conditions. However, increases in total PGC-1α abundance and energy expenditure in response to acute exercise were attenuated in the mutant mice, likely contributing to their obesity-prone phenotype. Whereas motor performance during a heavy exercise load was impaired, remodeling of skeletal muscle induced by chronic exercise was not affected in these mice. These results indicate that the acute induction of PGC-1α in response to exercise, for which the novel variants are largely responsible, plays an important role in the control of fat mass and insulin sensitivity through regulation of energy expenditure during exercise. Exercise mimetics are potential pharmacological treatments for obesity and type 2 diabetes mellitus. The novel variants of PGC-1α are promising targets for the development of such drugs.

# OR-16 The Nuclear Receptor, Nor-1. Regulates oxidative metabolism and resistance to diet induced obesity

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The nuclear hormone receptor superfamily of transcriptional regulators are ligand and hormone dependent DNA binding proteins that translate endocrine, metabolic and pathophysiological signals into organ specific gene regulation. The mRNA encoding the nuclear hormone receptor Nor-1, is strikingly and selectively induced by β2-adrenergic agonists in cell culture models, and glycolytic/oxidative skeletal muscle *in vivo*. In skeletal muscle cells, Nor-1 expression is necessary for oxidative metabolism, and Nor-1 siRNA expression leads to the anaerobic utilisation of glucose. Transgenic skeletal muscle specific expression of Nor-1 resulted in the acquisition of an endurance phenotype and an increase in type IIA/X oxidative muscle fibres (accompanied by a decrease in type I and Type IIB) (Mol. Endo. 2-12 (3) 372-). In addition, increased numbers of mitochondria, and myoglobin mRNA expression were observed. DXA and MRI analysis have revealed decreased adiposity in the Tg-Nor-1 mice, relative to wt littermates. Expression profiling revealed increased expression of genes involved in carbohydrate utilisation and oxidative phosphorylation. The Tg-Nor-1 mice were resistant to diet induced obesity, and maintained fasting glucose at normoglycemic levels, however, after 12 weeks of high fat diet feeding displayed insulin insensitivity. We examined the differential expression of the mRNAs encoding the PGC-1α1-4 splice variants by qRT-PCR, and identified very abundant and significantly increased expression of the PGC-1α splice variant associated with increased mitochondrial biogenesis and endurance. We are currently examining the expression of the mRNA α-actinin 3, a contractile component of type II muscle fibres. Decreased expression of this gene in mice and humans produces a similar phenotype to Nor-1 activation, including increased endurance, and increased mitochondrial enzymes Our current studies are directed toward elucidating the underlying Nor-1 dependent mechanism producing fatigue resistance, decreased adiposity, and resistance to diet induced obesity.

# OR-17 Prostaglandin Reductase-3 Negatively Modulates Adipogenesis Throuht Regulation of Peroxisome Proliferator-Activated Receptor γ Activity

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Adipocyte differentiation is a multistep program under regulation by several factors. Peroxisome proliferator-activated receptor γ (PPARγ) serves as a master regulator of adipogenesis. However, the endogenous ligand for PPARγ remained elusive until recently 15-keto-$PGE_2$ was identified as an endogenous PPARγ liagnd. In this study, we demonstrate Zn-containing alcohol dehydrogenase 2 (ZADH2) is a new member of prostaglandin reductase family (here termed as prostaglandin reductase 3, PTGR-3) that converts 15-keto-$PGE_2$ to 13, 14-dihydro-15-keto-$PGE_2$. Adipogenesis is accelerated when endogenous prostaglandin reductase 3 (PTGR-3) is silenced in 3T3-L1 preadipocytes, while forced expression of PTGR-3 significantly decreases adipogenesis. PTGR-3 expression decreased during adipocyte differentiation, accompanied with an increase level of 15-keto-$PGE_2$ level. 15-keto-$PGE_2$ exerts potent pro-adipogenic effect by enhancing PPARγ activity, while overexpression of PTGR-3 in 3T3-L1 preadipocytes markedly suppressed the pro-adipogenic effect of 15-keto-$PGE_2$ by repressing PPARγ activity. Taken together, these findings demonstrate for the first time that PTGR-3 is a novel 15-oxoprostaglandin-$\Delta^{13}$-reductase and plays a critical role in modulation of normal adipocyte differentiation via regulation of PPARγ activity. Thus, modulation of PTGR-3 might provide a novel avenue for treating obesity and related metabolic disorders.

# OS-04 Up-to-date information of incretin-based medicine

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GLP-1 (glucagon-like peptide-1) and GIP (glucose-dependent insulinotropic polypeptide) that belong to incretin (insulin secretion intestine) are intestinal hormones secreted by L cells and K cells, respectively, in the gut. They are considered to be the factors by which orally ingested nutrient and energy are effectively resorbed and accumulated in the proper tissues and organs. In addition to incretin effect, GLP-1 has multiple physiological functions including decrease of appetite and food intake, deceleration of gastric emptying, suppression of glucagon secretion of α cells, inhibition of apoptosis in β cells, cardiac muscle cells, and neuronal cells, increase of glucose uptake and glycogen synthesis in both adipocytes and skeletal muscle cells (indirect action?). Many of these actions are closely related glucose-lowering effects in diabetes mellitus, and recent development of incretin-based medicine has brought paradigm shift in the treatment of diabetes all over the world.

In the incretin-based diabetic medicine, two types of drugs, GLP-1 receptor agonists and DPP-4 (dipeptidyl peptodase-4) inhibitors have been widely used, and these drugs are unique in that they have multiple effects on the improvement of glucose intolerance, although their clinical history may not be enough for the establishment as an anti-diabetic drug. Furthermore, DPP-4 inhibitors are not simply an incretin enhancer, but may have GLP-1 signal-independent ancillary effects.

I would like to review the recent development of GLP-1 receptor agonists and DPP-4 inhibitors, and discuss on the extra-pancreatic or ancillary effects of these drugs.

## OR-18 Ceramide and Metabolic Disease: A Survey of Therapeutic Targets

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Metabolomic profiling reveals relationships between sphingolipids such as ceramide with various indices of metabolic disease (insulin resistance, hepatic steatosis, etc.). Moreover, genetic ablation or pharmacological inhibition of enzymes required for ceramide synthesis in rodents blocks or delays the development of diabetes, atherosclerosis, cardiomyopathy, steatosis, and hypertension in various rat or mouse models of these conditions. Collectively, these data suggest that ceramides may be amongst the most toxic lipid metabolites which accumulate in the obese and they identify enzymes controlling ceramide synthesis or metabolism as potential therapeutic targets in the treatment of metabolic disease. Herein we investigated the therapeutic potential of three ceramide synthesizing enzymes for treating diseases associated with obesity. The speaker will share results from these studies, which reveal new insight into the ceramide-mechanism of action.

## OR-19 NPY: Central and peripheral control of energy homeostasis

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Many factors and pathways have been implicated in the regulation of appetite and energy homeostasis, however, none of them are as central and essential as the NPY system. NPY is a complex system consisting of 3 ligand genes NPY, peptide YY (PYY) and pancreatic polypeptide (PP) and at least 5 different receptors (Y1, Y2, Y4, Y5 and Y6). Whereas central NPY is known to stimulate appetite and feeding behaviour, the mostly peripherally expressed family members PYY and PP have the opposite effect and have been identified as potent satiety factors. Negative energy balance, leads to increased hypothalamic NPY expression and the activation of appetite stimulatory pathways and other feeding related behaviours. However, NPY causes also neuroendocrine and metabolic changes which favour energy storage including decreased thermogenesis, hyperinsulinemia, insulin hyper-responsiveness in white adipose tissue, activation of the hypothalamo-pituitary-adrenal axis, and decreased activity of the hypothalamo-pituitary-thyrotropic, -somatotropic, and -gonadotropic axes. However, whole body homeostasis does not only involve the regulation of fat and lean mass but also that of bone mass and coordinating this to bodyweight, eg larger body mass requires stronger bones. Recently, we have identified the critical role of the NPY system in the regulation of bone formation with reduced central and peripheral Y-receptor signalling leading to elevation in bone formation and bone volume. Interestingly, mice lacking Y1 receptors solely in cells of the osteoblastic lineage not only show increased bone formation but also altered whole-body glucose metabolism. This is due to significantly decreased pancreatic insulin content, pancreas weight, and insulin secretion in these mice, leading to elevated glucose levels and reduced glucose tolerance, but with no effect on insulin induced glucose clearance. Furthermore, increased activity of Y1 signalling induced by adult onset over-expression of its ligand PYY, solely in osteoblastic cells also led to impaired glucose tolerance, elevated insulin secretion and impaired insulin sensitivity in mice. These data reveal a novel mechanism by which NPY signalling in bone tissue is involved in the control of energy homeostasis. New outcomes of the analysis of various transgenic models from the NPY family in regards to the regulation of energy and bone homeostasis with a particular focus on stress-induced changes will be presented.

# OR-20 Role of Soluble Epoxide Hydrolase in Non-Alcoholic Fatty Liver Disease

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Non-alcoholic fatty liver disease is associated with obesity and considered an inflammatory disease. Soluble epoxide hydrolase (sEH) is a major enzyme hydrolyzing epoxyeicosatrienoic acids, metabolites of arachidonic acid and attenuates their cardiovascular protective and anti-inflammatory effects, therefore manipulation of EET levels and sEH activity is a potentially useful pharmacological strategy. We examined whether sEH inhibition can protect against diet-induced fatty liver in mice and the underlying mechanism. Compared with wild-type littermates, sEH-null mice showed lower high-fat (HF)-diet-induced lipid accumulation in liver, as seen by Oil-red O staining and triglycerides levels. We studied the effect of sEH inhibition on diet-induced fatty liver by feeding C57BL/6 mice an HF diet for 8 weeks (short-term) or 16 weeks (long-term) and administering t-AUCB, a selective sEH inhibitor. sEH inhibition had no effect on the HF-diet-increased body and adipose tissue weight or impaired glucose tolerance but alleviated the diet-induced hepatic steatosis. Adenovirus-mediated overexpression of sEH in liver increased the level of triglycerides in liver and the hepatic inflammatory response. Surprisingly, the induced expression of sEH in liver occurred only with the long-term but not short-term HF diet, which suggests a secondary effect of HF diet on regulating sEH expression. Furthermore, sEH inhibition attenuated the HF-diet-induced increase in plasma levels of proinflammatory cytokines and their mRNA upregulation in adipose tissue, which was accompanied by increased macrophage infiltration. Therefore, sEH inhibition could alleviate HF-diet-induced hepatic steatosis, which might involve its anti-inflammatory effect in adipose tissue and direct inhibition in liver.

An elevated plasma homocysteine (Hcy) level is denoted hyperhomocysteinemia (HHcy), which is an important and independent risk factor for several disorders, including atherosclerosis, diabetes and fatty liver. Liver dysfunction can lead to increasing levels of Hcy and in turn aggravates liver damage though mechanisms such as endoplasmic reticulum (ER) stress, oxidative stress and inflammation. We previously reported that Hcy induced sEH expression in vascular endothelial cells and inhibition of sEH could attenuate Hcy-induced cardiovascular diseases. We further established HHcy animal model by feeding C57/6J mice with high methionine diets (2% wt/wt) for 8 weeks, while using sEH inhibitor to intervene. The results showed that plasma concentrations of Hcy were significantly elevated in HHcy mice and obvious steatosis. Administration of sEH inhibitor decreased the contents of triglyceride and lipid accumulation in the mice by increasing the expression of PPARα and its target genes involving in fatty acid oxidation and attenuated hepatic steatosis. Thus, sEH may be a therapeutic target for diet-induced hepatic steatosis in inhibiting expression of sEH and systemic inflammation.

# OR-21 Central Activating Transcription Factor (ATF4) Regulates Hepatic Insulin Resistance in Mice Via S6K1 Signaling and the Vagus Nerve

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My lab has been interested in molecular mechanisms underlying control of energy homeostasis and insulin sensitivity. Recent studies have revealed that central nervous system (CNS), particularly the hypothalamus, is critical for regulating insulin sensitivity in peripheral tissues. In this seminar, I will present data showing a novel central pathway regulating hepatic insulin sensitivity that is mediated by hypothalamic Activating Transcription Factor (ATF) 4/mammalian target of rapamycin (mTOR)/S6K1 signaling and the vagus nerve. Important results include: 1) overexpression of ATF4 in the hypothalamus resulting from intraventricular (icv) injection of adenovirus expressing ATF4 induces hepatic insulin resistance in mice and that inhibition of hypothalamic ATF4 by icv adenovirus expressing a dominant-negative ATF4 variant has the opposite effect; 2) hypothalamic ATF4-induced insulin resistance is significantly blocked by selective hepatic vagotomy or by inhibiting activity of mTOR downstream target S 6K1; 3) inhibition of hypothalamic ATF4 reverses hepatic insulin resistance induced by acute brain endoplasmic reticulum (ER) stress. Taken together, our study demonstrate an important role for hypothalamic ATF4 in regulating hepatic insulin sensitivity. These results may lead to the identification of novel therapeutic targets for treating insulin resistance.

# OR-22 The Suppression of Erythropoietin Production by Long-Chain Saturated Fatty Acids: Lipid Nephrotoxicity and ER Stress

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Dyslipidemia is an important risk factor of cardiovascular disease (CVD) as well as chronic kidney disease (CKD) in general population. It indeed contributes to morbidity and mortality of these patients in the world. Lipid and their metabolites derange various kinds of cellular functions through the induction of pathogenic stress responses, such as endoplasmic reticulum (ER) stress. However, the effect of dyslipidemia on anemia is still unclear.

Hear, we investigated the link between dyslipidemia, ER stress, and erythropoietin (EPO) production. ER stress inducer (tunicamycin or thapsigargin) suppressed transcription of EPO, a representative HIF target gene, in HepG2. This suppression was inversely correlated with an activation state of ER stress signal, unfolded protein response (UPR): expression of UPR chaperon, GRP78, was increased when the EPO expression was suppressed by ER stress inducers. This suppression was restored by an ER stress inhibitor, salubrinal, in association with normalization of the UPR state. We then examined the effect of long-chain saturated fatty acid, palmitate, on EPO transcription. Palmitate also suppressed EPO mRNA expression in a dose dependent manner. The decreased EPO expression by palmitate was also associated with an increase in ER stress. Importantly, the alteration of EPO production was observed in HepG2 overexpressing UPR activating transcription factor (ATF) 4. Overexpression of mutated ATF4 that lacks the transcriptional activity did not alter EPO transcriptional regulation. Transcriptional activity of the EPO 3'- enhancer, which is mainly regulated by hypoxia inducible factor (HIF), was abolished by both ER stress and ATF4 overexpression, while nuclear HIF accumulation or expression of other HIF target genes was not suppressed. Chromatin immunoprecipitation analysis identified a novel ATF4 binding site (TGACCTCT) within the EPO 3'-enhancer region, suggesting a distinct role for ATF4 in UPR-dependent suppression of the enhancer. Induction of ER stress in rat liver and kidney by tunicamycin, decreased the hepatic and renal mRNA and plasma level of EPO.

Collectively, ER stress selectively impairs the transcriptional activity of EPO but not of other HIF target genes. This effect is mediated by suppression of EPO 3'-enhancer activity via ATF4 without any direct effect on HIF, indicating that UPR contributes to oxygen-sensing regulation of EPO. Palmitate might influence EPO transcriptional regulation as an ER stress inducer via alteration of EPO 3'-enhancer activity. It indicates that elevated level of long-chain saturated fatty acids might affect EPO production. Inappropriate EPO production causes anemia in patients with CKD, and epidemiological studies suggest that patients with diabetes show lower production of EPO associated with early development of anemia compared with patients due to non-diabetic kidney disease. We speculate that this suboptimal production of EPO in patients with diabetes may due to dyslipidemia often observed in these patients. Our results indeed highlight the lipotoxic mechanism in kidney disease, namely "lipid nephrotoxicity".

# OR-23 Pro-atherogenic Effects of Retinol-binding Protein 4 on Vascular Smooth Muscle Cells

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A high level of retinol-binding protein 4 (RBP4) in circulation is thought to be associated with obesity, insulin resistance and type 2 diabetes, which are independent risk factors for coronary artery diseases. However, direct effects of RBP4 on vasculature remain largely unclear. We evaluated the potential roles of RBP4 in vascular smooth muscle cells (SMCs) and its implications in atherosclerosis. Quantitative reverse-transcriptase PCR revealed that recombinant human RBP4 significantly induced the expression of pro-inflammatory genes cyclooxygenase-2 (COX2) and inducible nitric oxide synthase (iNOS) in cultured rat aortic SMCs in a time- and dose-dependent manner. Meanwhile, RBP4 increased nuclear translocation of nuclear factor-κB p65 and the activity of NF-κB-driven luciferase reporter. Infection with Ad-IκBα inhibited RBP4-induced expression of COX2 and iNOS. In apoE-deficient mice, high-fat diet increased the plasma level of RBP4. In addition, oxidized low-density lipoprotein (ox-LDL) also induced the expression of RBP4 in vascular SMCs. In cultured SMCs, RBP-4 promoted the migration of SMCs via an Akt-mediated mechanism. Moreover, immunohistochemical study demonstrated the presence of RBP4 protein in the atherosclerotic lesions of apoE-deficient mice and in the human disease, in which RBP4 was associated predominantly with SMCs. In conclusion, our results demonstrated that RBP4 may exert pro-inflammatory and pro-migratory effects in vascular SMCs via the NF-κB and Akt signaling pathways, suggesting that RBP4 may participate in the pathophysiologic process of atherosclerosis.

## OS-05 Characteristics of Adiponectin as abundant defense protein

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Adipose tissue expresses a variety of genes for bioactive secretory proteins conceptualized as 'Adipocytokines'. We identified an adipose-specific protein named 'Adiponectin' from human fat cDNAs. Hypoadiponectinemia underlies the pathogenesis of multiple diseases related to obesity and visceral fat accumulation, including insulin resistance, diabetes, dyslipidemia, hypertension, atherosclerosis, cardiac failure, hepatic steatosis, chronic kidney disease, COPD, osteoporosis, and some types of cancers.

Recent analysis is revealing that adiponectin works as a defense protein toward tissue damage. Adiponectin associates with many factors related to tissue damage and inflammation. Among them, we have revealed functional interference of adiponectin and C1q, and their direct binding in vitro and in blood. Adiponectin/C1q complex in blood associates the risk for metabolic syndrome and atherosclerotic diseases.

It has been a mystery why adiponectin needs to exist very high in blood. This issue will be touched from recent works.

# OR-24 FIBROBLAST GROWTH FACTOR 19 IN RELATION TO OBESITY AND LIPID METABOLISM IN MAN

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**Objective**—Fibroblast growth factor-19 (FGF19) is derived from the ileum and plays an important role in inhibiting bile acid synthesis and regulating lipid metabolism in animal models. Mouse models overexpressing FGF19 have been shown to have reduced weight and adiposity. Glucose-lowering effect was also demonstrated in obese diabetic mouse models. However, the clinical relevance of these findings is still unknown in humans. The objective of this study is to examine the relationship of serum FGF19 levels with serum lipid profiles and other metabolic parameters in a large population-based community cohort.

**Methods and Results**—1925 subjects, who attended the Hong Kong Cardiovascular Risk Factors Prevalence Study and had complete anthropometric and biochemical data, were included for analysis. Serum FGF19 concentrations were measured using enzyme-linked immunosorbent assays. Serum FGF19 correlated positively with low density lipoprotein-cholesterol (LDL-C, $p<0.001$), triglyceride (TG, $p<0.05$), gamma glutamyl transpeptidase ($p<0.05$) and age ($p<0.05$), and negatively with body mass index (BMI, $p=0.015$) and waist circumference ($p=0.017$), but not with indices of insulin resistance or glycemic status. On stepwise multiple linear regression analysis, only BMI ($p<0.001$) and LDL-C ($p<0.003$) were independently associated with FGF19. Subjects with BMI$\geq$25 kg/m$^2$ had significantly lower ($p=0.005$) serum FGF19 levels [median 163.5 pg/ml (IQR 109.8-252.8); n=705] than those with BMI<25 kg/m$^2$ [median 172.0 pg/ml (IQR 120.4-268.3); n=1200]. On the other hand, subjects with elevated LDL-C$\geq$3.4 mmol/L had significantly higher ($p=0.038$) serum FGF19 levels [median 173.5 pg/ml (IQR 121.3-271.9); n=836] than those with lower LDL-C< 3.4 mmol/L [median 163.1 pg/ml (IQR 112.5-259.1); n=1051].

**Conclusion**—The study provides the first clinical evidence in a general population that serum FGF19 levels are independently associated with LDL-C levels and BMI, but not with indices of insulin resistance or glycemic status. This suggests a predominant role for serum FGF19 in cholesterol metabolism in humans under physiological conditions. There is also an independent inverse relationship between BMI and serum FGF19, with overweight/obese subjects having lower FGF19 levels than lean subjects.

# OR-25 CALORIE RESTRICTION VERSUS PERIODIC FASTING TO REDUCE RISK MARKERS FOR DIABETES AND CARDIOVASCULAR DISEASE IN HUMANS

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Calorie restriction (CR) improves insulin sensitivity, and reduces the incidence of type 2 diabetes and cardiovascular disease (CVD), and increases maximal lifespan in animal models. Evidence in mice suggests that intermittent or periodic fasting (PF) will also reduce aging related diseases and increase lifespan, even without a reduction in overall calorie intake. This seminal discovery indicates that periodic cellular energy deprivation, rather than a daily reduction in energy intake and weight loss, can act as the stimulus required to improve health. Whether this observation is true for human physiology, particularly in comparison to CR, and the mechanism/s responsible are unknown.

We have shown that alternate day (24-hour) fasts are feasible in free-living humans for 3-weeks. In this study, participants lost weight, indicating that they could not consume enough food on feasting days to maintain body weight. We also observed increased HDL-cholesterol and reductions in serum triglyceride and insulin response to a liquid meal. Importantly, we noted a number of differences between PF and a CR study that was running concurrently in the laboratory. In particular, resting metabolic rate (RMR) was not changed in response to periodic fasting with 3% weight loss. This result is of great interest, considering the rapid reduction in RMR that typically occurs following CR. We also observed PF provided a greater stimulus to increase whole body fat oxidation (43% versus 8%) and we observed that cells treated with PF serum were better able to survive following heat shock.

We are currently conducting a randomised controlled trial to directly compare 8 weeks of 100%PF and 70%PF versus daily 70%CR on energy metabolism and insulin sensitivity in obese but otherwise healthy women. Our preliminary data from this study suggest not only that periodic fasting is a feasible alternative to CR, but that 70%PF results in identical weight loss and improvements in insulin sensitivity by euglycemic hyperinsulinemic clamp and reductions in fasting glucose as compared to 70%CR. Further numbers are required to confirm these findings.

# OR-26 ADIPONECTIN GENETIC VARIANTS IN THE PREDICTION OF CORONARY ARTERY DISEASE IN CHINESE: A 16-YEAR PROSPECTIVE STUDY

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**Background:** Adiponectin is an insulin-sensitizing adipokine predominantly secreted from adipocytes. Circulating adiponectin is shown to be reduced in obesity and various insulin resistant states, which predispose to endothelial dysfunction, atherosclerosis and subsequent cardiovascular events. Adiponectin has been suggested to play a protective role in the development of coronary artery disease (CAD). Low circulating adiponectin levels have been shown to be associated with greater CAD risk in cross-sectional studies. However, recent studies suggested that high adiponectin levels are associated with an increased risk of cardiovascular mortality or CAD in elder populations or cohorts with prevalent CAD, suggesting that high levels in established disease state may act as a compensatory mechanism to limit further vascular injury.

**Objective:** Study of the genetic variants of the adiponectin gene (*ADIPOQ*) may provide further insights into the specific role of adiponectin in the development of CAD. Our major objective was to examine the prospective relation between the genetic variants of *ADIPOQ* and incident CAD in a 16-year prospective population-based cohort of Southern Chinese.

**Methods and Results:** A 16-year prospective study was conducted in 2196 CAD-free subjects from the Hong Kong Cardiovascular Risk Factors Prevalence Study (CRISPS). 184 subjects developed CAD (cumulative incidence rate=5.4/1000 person-years) during 33,862 person-years of follow-up. Nine *ADIPOQ* genetic variants with potential functional relevance or shown to be associated with adiponectin levels and/or CAD were investigated. Among these genetic variants,+276G>T (rs1501299) was found to be independently associated with incident CAD in male but not in female subjects, even after adjustments for different sets of traditional cardiovascular risk factors ($P_{adjusted}$=5.5x$10^{-3}$ to 0.023; Hazard ratio [HR]=1.39 to 1.54). Moreover, in 1676 subjects with available plasma samples for analysis, the TT genotype of +276G>T was significantly associated with reduced plasma adiponectin level (P=0.027; β[95%CI]=-0.05[-0.10, -0.01]).

**Conclusions:** In this study, we have demonstrated that the +276G>T variant was an independent predictor of CAD development in a population-based cohort and was associated with lower adiponectin level. Our findings have provided evidence for the role of an *ADIPOQ* variant, +276G>T, in the development of CAD, likely through a reduction in adiponectin expression. Therefore, this study would support a protective role of adiponectin in the development of CAD in the general population.

## OR-27 Targeting gp130 to prevent inflammation and promote insulin action

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Obesity results in a state of chronic low grade inflammation, characterized by the elevation of pro-inflammatory cytokines in both the circulation and in metabolically active tissues. Work in our laboratory has focussed on the role of the cytokine interleukin-6 (IL)-6 and other IL-6-like cytokines that signal through the gp130 receptor complex. We have focussed on the role of blocking IL-6 trans-signaling to prevent inflammation on the one hand, and activating membrane bound signaling to promote insulin sensitivity on the other. Since the cloning of the IL-6, a pattern has emerged associating IL-6 to a number of diseases associated with inflammation including rheumatoid arthritis (RA), Crohn's disease and several cancers. Accordingly, tocilizumab, an IL-6 receptor-inhibiting monoclonal antibody is now useful for the treatment of RA. However, this may not be the most optimal strategy to block inflammation associated with IL-6 and may result in unwanted side effects that, paradoxically, could actually promote metabolic disease. This presentation with discuss the complex biology of IL-6/gp130 receptor signaling in the nexus between inflammation and metabolism in obesity and nutrient overload.

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# OR-28 Exploring the Foregut Hypothesis Using Continuous Subcutaneous Glucose Monitoring, Metabolomics and Proteomics

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**Background:** Bypass of foregut secreted factors promoting insulin resistance is hypothesized to be one of the mechanisms by which diabetes resolution follow roux-en-y gastric bypass (GBP) surgery.

**Aims:** To determine whether GBP had a greater impact among patients with type 2 diabetes (T2D) in the first few days after surgery on (a) lowering glycemia and glycemic variability (b) lowering insulin resistance (c) lowering proteins or metabolites linked with insulin resistance, compared with sleeve gastrectomy (SG) with intact foregut.

**Methods:** Six day subcutaneous continuous glucose monitoring (CGM) recordings were obtained from T2 D patients beginning 3 days before GBP (n=11), SG (n=10) or fasting in caloric matched control group without surgery (n=10). GLP-1, insulin, and glucose was measured during 75g oral glucose tolerance testing at the start and end of each CGM. Plasma from 15 of these subjects were analysed 3 days before and after GBP or SG surgery. Samples were depleted of abundant plasma proteins, trypsin digested and labeled with iTRAQ™ isobaric tags prior to liquid chromatography-tandem mass spectrometry analysis. Gas chromatography-mass spectrometry was used for metabolomic analysis.

**Results:** Post-operative hyperglycaemia occurred after both GBP and SG in the first 6 hours, with a more rapid decline in glycaemia after GBP ($p<0.001$). Beyond 24 hours post-operatively, continuous overlapping net glycaemia action (CONGA), reduced from baseline after GBP (median [interquartile range] 1.6 [1.2-2.4] to 1.0 [0.7-1.3] and after SG 1.4 [0.9-1.8] to 0.7 [0.7-1.0] $p<0.05$), similar to controls (2.2 [1.7-2.5] to 1.3 [0.8-2.8] $p<0.05$). Higher logGLP-1 increment post oral glucose, occurred after GBP (mean±SE, 0.80±0.12 vs 0.37±0.09, $p<0.05$), but not after SG or after matched caloric intake. Log EIR or HOMA-IR did not change 3 days after any of the interventions except in the subgroup with baseline hyperglycemia where HOMA-IR reduced following GBP.

In the subgroup of patients (n=15) who had proteomic and metabolomic analysis, mean reduction in HOMA-IR was greater following GBP than SG although not statistically significant (3.55 vs 0.47, $p=0.39$). Proteomic analysis yielded 7 proteins which decreased after GBP only, including Fetuin A and Retinol binding protein 4 (RBP4), both known to be associated with insulin resistance. Decrease in Fetuin A (25.7± 3.8%, $p=0.02$) and RBP4 (50.5±6.9%, $p=0.02$) after GBP were confirmed using ELISA and immunoassay respectively. Metabolomic analysis identified significant decrease of citrate, proline, histidine and decanoic acid specifically after GBP.

**Conclusions:** GBP and SG has a similar acute impact on reducing glycaemia to caloric restriction, however in those with baseline hyperglycaemia, GBP may have a superior impact. Greater decrease of fetuin A, RBP-4, and several metabolites occur early after GBP compared to SG, independent of weight loss, and may contribute to enhanced T2D remission observed following foregut bypass (GBP) procedures.

# OR-29 UNDERSTANDING METABOLIC REMODELING OF SKELETAL MUSCLE IN TYPE 2 DIABETES

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In type 2 diabetes, skeletal muscle displays transcriptional and metabolic remodeling that is thought to contribute to impaired oxidative metabolism, lipid accumulation and insulin resistance. The impact that skeletal muscle metabolic remodeling has on the overall disease state in type 2 diabetes remains unresolved. In addition, the molecular mechanisms contributing to this response are also unknown. Here we present evidence to suggest that accumulation of the class IIa histone deactylases (HDACs) in skeletal muscle contributes to the transcriptional and metabolic remodeling seen in type 2 diabetes. Increasing class IIa HDAC expression phenocopies muscle in the diabetic state, while genetic ablation of class IIa HDAC activity prevents muscle remodeling in diabetic mice. Comparative genomic approaches reveal that the increase in class II HDACs and metabolic remodeling seen in diabetes occurs to prevent skeletal muscle apoptosis by metabolic insults. However, regulation of metabolism and protection from apoptosis by the class IIa HDACs occurs via distinct enzymatic functions. We are currently designing novel small molecules that exploit this divergence in class IIa HDAC function to enhance metabolism, without altering muscle cell sensitivity to apoptosis, as a potential therapeutic strategy to normalize muscle metabolism in type 2 diabetes.

## P-01 Analysis of GLP-1 signaling and class IA phosphatidylinositol 3-kinase in pancreatic β cells

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Type 2 diabetes is induced by insulin resistance and pancreatic β cell failure. GLP-1 is important for regulation of insulin secretion in pancreatic β cells but the signaling of GLP-1 is not well known. We previously reported that inhibition of class IA phosphatidylinositol 3-kinase (PI3K), using a mouse model lacking the pik3r1 gene specifically in β cells and the pik3r2 gene systemically (βDKO mouse), shows glucose intolerance and reduced early insulin secretion. We investigated the relation of GLP-1 and PI3K in pancreatic β cells. The administration of GLP-1 analog in βDKO mice didn't recover the early insulin secretion. The microarray analysis showed the down-regulation of lipid and mitochondria related genes and GLP1 receptor gene. This result indicated that PI3K in pancreatic β cells is important for lipid and mitochondria homeostasis and regulate expression of GLP-1 receptor.

# P-02 PLASMA SOLUBLE LEPTIN RECEPTOR LEVELS ARE ASSOCIATED WITH β-CELL FUNCTION IN PATIENTS WITH TYPE 2 DIABETES

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A soluble form of the leptin receptor (sOb-R) is the main binding protein for leptin in circulation and modulates its bioactivity. Plasma sOb-R levels are positively correlated with leptin sensitivity to body weight and energy expenditure, and inversely with the risk of developing type 2 diabetes (T2D). Hyperinsulinemia in mice lacking leptin signaling in β-cells indicates that leptin acts on β-cell to inhibit insulin secretion. In this study, we investigated the clinical association of plasma sOb-R levels with β-cell function in T2D patients. Two hundred and eighty nine T2D patients without renal dysfunction or insulin therapy were included in this study (mean age, 61 years; duration of diabetes, 7.8 years; BMI, 25.4 kg/m$^2$). Fasting plasma leptin and sOb-R concentrations were measured by ELISA. The median (interquartile range) of plasma leptin or sOb-R level was 3.5 (1.8-6.6) or 23.7 (19.7-28.5) ng/ml. Plasma sOb-R was negatively correlated to HOMA-R ($r=-0.232$, $p<0.0001$), HOMA-β ($r=-0.507$, $p<0.0001$), and C-peptide index (CPI) ($r=-0.514$, $p<0.0001$), whereas plasma leptin was positively to each index ($r=0.615$, $p<0.0001$, $r=0.556$, $p<0.0001$, and $r=0.425$, $p<0.0001$, respectively) in simple regression analysis. Multiple regression analyses including age, sex, duration of diabetes, BMI, blood pressure, serum creatinine, glycated hemoglobin, lipid profile, leptin, and sOb-R as independent variables revealed that sOb-R independently and negatively contributed to HOMA-β ($β=-0.206$, $p<0.001$) and CPI ($β=-0.341$, $p<0.0001$), but not to HOMA-R ($β=0.004$, $p=0.940$), whereas leptin did positively to HOMA-R ($β=0.667$, $p<0.0001$), HOMA-β ($β=0.531$, $p<0.0001$), and CPI ($β=0.489$, $p<0.0001$). These data indicate that plasma sOb-R is associated with decreased β-cell function, whereas plasma leptin is associated with insulin resistance and hyperinsulinemia, independently of obesity and other metabolic parameters, in T2D patients. In conclusion, plasma sOb-R levels are associated with β-cell function in T2D.

# P-03 EFFECT OF ALLOGLIPTIN AND METFORMIN COMBINATION THERAPY: A PILOT STUDY

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**Background**

As insulin resistance and impaired insulin secretion are 2 major features of type 2 diabetes, combination therapy with dipeptidyl peptidase-4 inhibitors, which stimulate insulin secretion, and biguanides, which enhance insulin sensitivity, is a desirable treatment for diabetes. We selected alogliptin and metformin as representative drugs of these classes and evaluated the efficacy of their combination.

**Methods**

Continuous glucose monitoring (CGM) was performed for 2 patients with type 2 diabetes and 1 control subject throughout the study. Starting with no medication, all subjects received 3 patterns of medication; alogliptin alone, alogliptin and metformin co-administration, and metformin alone. Blood was sampled on the representative days with no medication, alogliptin alone, alogliptin and metformin co-administration, and metformin alone, before and 1 hour after breakfast and lunch, respectively. Serum insulin, plasma glucagon, glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic peptide (GIP) were measured.

**Results**

CGM results showed that the combination of alogliptin and metformin attenuated the escalation and fluctuation of glucose levels. The patterns of insulin and glucagon secretion with alogliptin alone, alogliptin and metformin co-administration, and metformin alone varied among subjects. When alogliptin and metformin were co-administered, GLP-1 levels 1 hour after lunch were higher than those at any other time point in all subjects. Postprandial GIP levels varied according to medication and subject.

**Conclusions**

CGM revealed that a combination of alogliptin and metformin effectively reduces postprandial glucose fluctuation and stabilizes blood glucose levels. The study subjects exhibited completely different response patterns of insulin, glucagon, GLP-1, and GIP with medications alone or in combination, suggesting that individual hormone-dependent glycemic responses to these drugs are complicated and multifactorial.

# P-04 Adipocyte Dysfunction and Metabolic Disease

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Adipocyte dysfunction is thought to play an important role in the development of metabolic diseases including type 2 diabetes. However, the mechanism connecting adipocyte dysfunction and these diseases is unclear. Adipocytes possess various functions such as lipid and glucose handling as well as adipokine secretion, and most of these functions are regulated by insulin signaling in these cells. PDK1 (3-Phosphoinositide-dependent protein kinase 1) plays a critical role in mediating metabolic actions triggered by insulin downstream of PI 3-kinase. In this study, we generated adipocyte-specific PDK1 knockout mice (Adipo-PDK1 KO mice) and studied the influence of adipocyte dysfunction on the development of type 2 diabetes and other metabolic diseases.

We generated Adipo-PDK1 KO mice by crossing PDK1-floxed mice with Adiponectin-Cre mice. In these mice, insulin signaling downstream of PI 3-kinase is blunted specifically in adipocytes. On a standard diet, fat mass of Adipo-PDK1 KO mice was 40% smaller than that of control mice, and the variability in size of adipocytes was much greater. Adipo-PDK1 KO mice manifested a severe impairment of lipolysis and lipogenesis during fasting and re-feeding, respectively. Serum adiponectin and leptin levels as well as the expression of these genes in adipose tissue were markedly decreased in these mice. These results collectively suggest that Adipo-PDK1 KO mice exhibit functional impairment of adipocytes.

Adipo-PDK1 KO mice on a standard diet exhibited a significant increase in blood glucose level, a marked increase in serum insulin concentration, and insulin resistance evaluated by insulin tolerance test. To elucidate the mechanism for this metabolic alteration, we performed immunostaining for macrophages in adipose tissue, and found that macrophage infiltration was apparent in Adipo-PDK1 KO while such infiltration was barely detectable in control mice. Since JNK pathway plays a central role in the induction of chronic inflammation, we tested the activation of this pathway in adipose tissue. Adipo-PDK1 KO mice showed a significant increase in JNK activity compared to control mice. These data indicate that insulin signaling mediated by the PDK1 pathway might control chronic inflammation in adipose tissue through the regulation of stress signaling such as JNK pathway. Besides the alteration in glucose metabolism, Adipo-PDK1 KO mice developed hepatic steatosis even on a standard diet. Histological examination of liver in Adipo-PDK1 KO mice at age of 37 weeks old on this diet revealed hepatocyte ballooning, immune cell infiltration, and fibrosis, which were common pathological features observed in nonalcoholic steatohepatitis (NASH). The expression of genes related to lipid synthesis such as SREBP1c and PPARγ, a pro-inflammatory cytokine TNFα, and a fibrosis marker COL1A1 was significantly increased in liver of these KO mice.

In conclusion, interruption of insulin signaling by targeting PDK1 in adipocytes led to functional impairment of these cells. This adipocyte dysfunction causes not only glucose intolerance and dyslipidemia but NASH in mice. Adipo-PDK1 KO mice are thus a unique model that exhibits metabolic diseases including NASH even on a standard diet, and may contribute to understanding the pathogenesis and to the development of new therapy for these diseases.

# P-05 FEEDING BEHAVIOR IN MICE WITH HEREDITARY PREDISPOSITION TO HIGH FAT-DIET INDUCED GLUCOSE INTOLERANCE

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Background and aims

Recently, we established two lines of mice with different susceptibilities (prone and resistant) to high fat diet (HFD)-induced glucose intolerance by selective breeding (designated SDG-P and SDG-R, respectively). Under HFD feeding, SDG-P mice had significantly higher food intake and greater body weight gain. After 5-week HFD, SDG-P mice showed evidently higher blood glucose levels as compared with SDG-R mice. Here, we performed a pair-feeding study for elucidating the role of feeding behavior on the differences in susceptibility to glucose intolerance between SDG-P and SDG-R mice.

Materials and methods

Male mice of SDG-P and SDG-R were given HFD (32% energy as fat) ad libitum for 5 weeks (from 5 to 10 weeks of age, AL-SDG-P and AL-SDG-R, respectively). In addition, a group of SDG-P mice were subjected to pair-feeding (PF-SDG-P; daily food intake was matched to that of AL-SDG-R). Food intake (under a normal chow), whole-body metabolism (by indirect calorimetry), and locomotive activity were evaluated before the HFD feeding. During the HFD feeding period, individual food intake was monitored daily and body weight was measured weekly. Glucose tolerance and insulin sensitivity were evaluated by oral glucose tolerance test and insulin tolerance test, respectively.

Results

Before the HFD feeding, SDG-P mice had slightly higher food intake and modest glucose intolerance as compared to SDG-R mice. No differences were observed in oxygen consumption, respiratory ratio, and locomotor activity. Over the 5-week HFD feeding, AL-SDG-P mice consumed more food than AL-SDG-R mice. The hyperphagic behavior in AL-SDG-P mice became more evident in accordance with body weight gain. On the other hand, PF-SDG-P mice had similar body weight to AL-SDG-R mice over the HFD feeding. Whereas AL-SDG-P mice developed overt glucose intolerance after the HFD challenge, PF-SDG-P mice kept modest glucose intolerance as shown before the HFD feeding. AL-SDG-P mice also had increased epididymal fat pad mass, higher plasma leptin concentration, and marked insulin resistance as compared to AL-SDG-R and PF-SDG-R.

Conclusion

Spontaneous hyperphagia in SDG-P mice directly contributed to the HFD-induced weight gain and insulin resistance. Calorie restriction by pair-feeding ameliorated these metabolic impairments. These results indicate that hereditary feeding behavior can determine the susceptibility to HFD-induced glucose intolerance.

# P-06 LIPIN-1 IN 3T3-L1 ADIPOCYTES: ITS REGULATION AND ROLE IN OBESITY

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Lipin-1 is a multifunctional metabolic regulator involved in the synthesis of triacylglycerol and glycerolipid as an enzyme, and in the regulation of transcription as a co-factor. Lipin-1 is highly expressed in adipocytes. Lipin-1 deficient mice exhibited lipodystrophy and insulin resistance. On the other hand, mice with adipocyte-specific lipin-1 overexpression exhibited improvement of insulin sensitivity under high-fat feeding. In human, adipose lipin-1 mRNA expression is decreased in obesity compared with lean subjects, and its expression levels positively correlate with systemic insulin sensitivity. Thus, adipose lipin-1 is implicated in the pathogenesis of obesity. However, the mechanism of lipin-1 regulation and the significance of depleted lipin-1 expression in obesity have not been fully understood.

Since TNF-alpha is deeply involved in the pathogenesis of obesity and insulin resistance, we first investigated the role of TNF-alpha on lipin-1 expression in 3T3-L1 adipocytes. TNF-alpha suppressed lipin-1 mRNA expression in time- and dose- dependent manners in mature adipocytes. This suppressive effect was attenuated by the treatment with a Jak2 inhibitor, AG490. These results suggest that TNF-alpha could be involved in obesity-induced lipin-1 suppression in adipocytes via Jak2 signaling.

Recently, endoplasmic reticulum (ER) stress is implicated in obese adipose tissue. We next investigated the role of ER stress in lipin-1 expression in 3T3-L1 adipocytes. Treatment to the adipocytes with ER stress inducers (tunicamycin and thapsigargin) suppressed lipin-1 mRNA and protein expression. Thapsigargin was not altered lipin-1 mRNA decay rates under the treatment with actinomycin D, suggesting that ER stress suppresses lipin-1 expression at the transcriptional level. We also showed that constitutive lipin-1 expression could be maintained by peroxisome proliferator-activated receptor-gamma in 3T3-L1 adipocytes. Activation of peroxisome proliferator-activated receptor-gamma recovered the ER stress-induced lipin-1 suppression. These results suggest that ER stress might be involved in the pathogenesis of obesity through lipin-1 depletion.

We finally investigated whether and how reduced lipin-1 expression affects adipocyte function. We

knocked down lipin-1 mRNA expression by introducing small interfering RNA, and then assessed mRNA levels of various genes related to adipocyte metabolism. The results showed that mRNA and protein expression levels of monocyte chemoattractant protein-1 (MCP-1) were significantly increased. Conditioned medium from the cells were able to promote monocyte chemotaxis in a Transwell system. The increase in MCP-1 expression was prevented by the treatment with quinazoline or salicylate, inhibitors of nuclear factor-kappa B activation. These results suggest that reduced adipose lipin-1 expression in obesity may exacerbate adipose inflammation and insulin resistance via up-regulation of MCP-1.

Collectively, TNF-alpha and ER stress reduced lipin-1 expression in adipocytes, whereas knockdown of lipin-1 expression led to increase MCP-1 expression. MCP-1 attracts monocytes into adipose tissue and exacerbates adipose inflammation partly by producing TNF-alpha. These molecules may mutually affect with each other through the mediation of lipin-1, thereby contributing exacerbation of adipose inflammation. Amelioration of the lipin-1 depletion might improve overall metabolism. On the basis of our current findings, we would like to propose the concept that adipose lipin-1 has a crucial role in the “vicious cycle” of adipose inflammation in the pathogenesis of obesity.

# P-07 Role of DNA Methylation for Leptin Gene Promoter in 3T3-L1 Adipocytes

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[Background] Leptin plays a major role in the regulation of energy intake and expenditure. The peptide hormone is expressed in mouse white adipose tissue (WAT) but hardly expressed in 3T3-L1 adipocytes. With bisulfite sequencing, we found CpG islands in leptin promoter region were highly methylated in 3T3-L1 adipocytes as compared with in WAT. DNA methylation at CpG sites typically suppresses gene expression by inducing chromatin condensation and preventing transcription factor binding. Therefore, we investigated the role of DNA methylation on leptin expression in 3T3-L1 cells.

[Methods] To examine the effects of DNA demethylation on leptin expression, we exposed 3T3-L1 cells to 5 μM 5-azacytidine (5-aza-C), a DNA methyltransferase inhibitor, for 7 days. After 5-aza-C treatment, leptin mRNA level and methylation status of promoter region were analyzed. We also examined adipocyte specific genes such as adiponectin, PPAR gamma, C/EBP alpha and aP2 gene expression. The degree of adipocyte differentiation was determined by lipid accumulation and clonal expansion, several rounds of mitosis during adipogenesis. We next checked the effect of insulin, glucocorticoid and thiazolidinedione on leptin expression in 5-aza-C treated 3T3-L1 cells. Finally, we investigate whether down-regulation of Forkhead box protein O1 (FoxO1), one of the insulin targeted transcription molecules, affects leptin mRNA level in these cells.

[Results] 5-aza-C treatment in mature adipocytes did not affect methylation status of leptin promoter and its expression. Treatment of preadpocytes with 5-aza-C induced DNA demethylation, but did not change leptin expression. However, after adipocyte differentiation, preadipocytes with 5-aza-C treatment showed markedly increased leptin mRNA with lowered methylation status in CpG islands of leptin. Slight increase of some of the adipocyte specific gene expressions were found in 5-aza-C pretreated 3T3-L1 adipocytes (3T3-L1 AZ), but the increase of leptin gene mRNA was remarkable, suggesting that leptin gene is strongly affected by DNA methylation status in 3T3-L1 adipocytes. No differences were found between 5-aza-C treated and untreated 3T3-L1 adipocytes in lipid accumulation and clonal expansion. Stimulation of insulin, glucocorticoid and thiazolidinedione in 5-aza-C untreated 3T3-L1 adipocytes did not affect the gene expression. On contrary, 3T3-L1AZ adipocytes showed increased expression of leptin in response to insulin, but decreased expression to glucocorticoid and thiazolidinedione. Finally, FoxO1 knock down, but not C/EBP alpha overexpression, induced leptin mRNA in 5-aza-C treated preadipocytes.

[Discussion] These results suggest that leptin expression in 3T3-L1 adipocytes requires at least 2 steps; (1) DNA demethylation before adipocyte differentiation, (2) transcriptional activation during the differentiation of adipocytes. Established 3T3-L1AZ adipocytes can be useful tool for analyzing transcription and function of leptin in cultured condition. 3T3-L1AZ adipocytes are likely to provide further insights into biological functions of leptin.

# P-08 Autophagic flux is suppressed via ROS in differentiated and hypertrophic adipocytes

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Obesity represents an expansion of adipocytes, which store energy in the form of triacylglycerols (TG) in white adipose tissue, and it is closely associated with the low-grade chronic inflammatory response. Adipocytes not only store TG, but also secrete various biologically active molecules, called adipokines. Enlarged and hypertrophic adipocytes alter their adipokine expression and secretion, and these changes are linked to inflammatory responses, insulin resistance and obesity-associated complications. It has been reported that the alteration in the adipokine profile in obesity is partly mediated by the elevation in oxidative stress. Therefore, modulation of oxidative stress in adipocytes is a potentially attractive therapeutic strategy against obesity and its complications.

Autophagy is a major cytosolic catabolic process operating through the lysosomal machinery, playing an important role in cellular and/or organism homeostasis against diverse pathologies. In this process, autophagy is initiated by autophagosome formation, surrounding cytoplasmic components with a double-membrane. Then, the autophagosome fuses with a lysosome to form an autolysosome that subsequently degrades the intramembrane contents.

We recently reported that in differentiated and hypertrophic adipocytes autophagosomes accumulate probably due to the reduced autophagic flux, and suggested that autolysosome formation and/or lysosome functions may be impaired in hypertrophic adipocytes *in vitro* and *in vivo* (Mikami et al, BBRC, 427:758-63, 2012). To investigate the autophagic flux in adipocytes, first, we used 3T3-L1 adipocytes overexpressed RFP-GFP-LC3 transgene. When we compared 3T3-L1 adipocytes differentiated for 12 days (day 12 hypertrophic adipocytes) with those differentiated for 4 days (day 4 adipocytes), autophagic flux was decreased and reactive oxygen species (ROS) production was increased in day 12 hypertrophic adipocytes, suggesting that the reduction of autophagic flux was attributed to lysosomal function because the oxidative stress damages lysosomal membrane permeabilization. Next, to evaluate the lysosome function in differentiated and hypertrophic adipocytes, we assayed lysosomal pH and activity. As a result, lysosomal pH was higher and cathepsin B activity was lower in day 12 hypertrophic adipocytes than in day 4 adipocytes. Moreover, Western blotting analysis for cathepsin B suggested that its maturation was suppressed in day 12 hypertrophic adipocytes. In addition, we clarified a link between ROS production and the decreased autophagic flux by lysosomal impairment in differentiated and hypertrophic adipocytes. An electron microscopic observation found that day 12 hypertrophic adipocytes have more swelled mitochondria than day 4 adipocytes. A double staining with CM-H2DCFDA and MitoTracker Red, which is a marker of ROS production and mitochondria, respectively, showed that both fluorescences were significantly overlapped in Bafilomycin-treated day 12 hypertrophic adipocytes. Taken together, we suggested that autophagosome accumulates due to the lysosomal impairment in differentiated and hypertrophic adipocytes. Moreover, increased ROS production derived from mitochondria might affect the lysosomal integrity in differentiated and hypertrophic adipocytes.

# P-09 Effects of *trans*-Tiliroside Isolated from Rosehip on Glucose Tolerant and Lipid Metabolism through the Liver Functions

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**[Objective]** Presently, 30% of the Japanese population is considered to be obese. Prevalence of obesity is increasing more than 1.5 fold compared to 20 years ago. The number of cases with fatty liver is also escalating, as the prevalence rate of fatty liver was 10% in the 1980s and presently has reached 30%. It is recognized that fatty liver is a significant risk factor for serious liver disease, diabetes, metabolic syndromes, and cardiovascular diseases. In our previous studies, *trans*-tiliroside, one of the major constituents in fruits of Rosa *canina* (Rosaceae), was revealed to have potent anti-obese effect. Oral administration of *trans*-tiliroside not only prevented gain of body weight and accumulation of visceral adipose tissues significantly, but improved glucose tolerance, and reduced liver fat in mice. In this study, structure-activity relationships (SAR) of *trans*-tiliroside on glucose and lipid metabolism using human hepatocarcinoma cell line, HepG2, were examined.

**[Experiments and Results]** *Effects on glucose tolerant; trans*-Tiliroside, obtained as the principal constituent of fruits of *R. canina*, was administered to ddY mice (male, 11 w) fed a standard chow once a day for 14 days. After 20 h fasting, a glucose solution was intraperitoneally administered to the mice (1 g/kg). As the results, *trans*-tiliroside (0.1-10 mg/kg/d) potently inhibited the gain of body weight, especially visceral fat weight, and significantly reduced blood glucose levels of glucose loaded mice. Furthermore, a single oral administration of *trans*-tiliroside at a dose of 10 mg/kg increased the expression of PPAR-α mRNA of liver tissue in mice. As kaempferol 3-O-β-D-glucopyranoside, kaempferol, and p-coumaric acid showed no anti-obese effects, both kaempferol 3-O-β-D-glucopyranoside and p-coumaroyl moieties were found essential for the potent anti-obese activity of this flavonol. Furthermore, we examined the effects of constituents on glucose and lipid metabolism in vitro. Effects on glucose metabolism; HepG2 cells were cultured with a test sample for 6 days. The medium was exchanged with high glucose (HG)-contained DMEM and incubated for 20 h. Glucose concentration in the medium was determined by the mutarotase-GOD method. Effects on lipid metabolism; HepG2 cells were cultured in HG-contained DMEM to store triglyceride (TG) in the cells. Then, the cells were cultured in DMEM with a test compound for 20 h, and were homogenized by sonication. The TG concentration of the homogenate was determined by the GPO DAOS method. Among the 28 samples tested, trans-tiliroside was found to enhance glucose consumption in the HepG2 cells most effectively. However, kaempferol 3-O-β-D- glucopyranoside slightly stimulated the glucose consumption, and kaempferol itself showed no activity. These results suggested that the acyl group was essential for the potent activity. The SAR studies of these acylated flavonol glycosides on TG contents in the cells revealed different behavior. Both the acylated flavonol glycosides and flavonol itself significantly reduced TG contents. Whereas, their flavonol glycosides showed no activity.

# P-10 SREBP-1c IS REQUIRED FOR LIFE-LONG CALORIC RESTRICTION-INDUCED MITOCHONDRIAL BIOGENESIS IN WHITE ADIPOSE TISSUE OF MICE

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Caloric restriction (CR) is the most robust, reproducible and simple experimental manipulation that can extend lifespans, and delay the onset of several age-related patho-physiological changes. It has been suggested recently that the beneficial actins of CR are involved in growth hormone/IGF-1 signal, sirtuin, mitochondrial biogenesis and oxidative stress, but exact molecular mechanisms are still unclear. CR also activated lipid metabolism, up-regulated Sirt1 and sirt3 expressions, enhanced mitochondria biogenesis and reduced oxidative stress in white adipose tissue (WAT). Therefore, we thought that these metabolic alterations, so-called WAT remodeling, might be important for the beneficial actions of CR. Previously, we found that CR up-regulated sterol regulatory element binding protein-1c (Srebp-1c), a master transcriptional factor that regulates fatty acid biosynthesis, in a growth hormone/IGF-1 signal-independent manner in WAT (Chujo et al., AGE, in press). To clarify an impact of Srebp-1c in CR, in this study, we monitored several biomarkers in both Srebp-1c$^{+/+}$ (Wd) and $^{-/-}$ (KO) mice.

CR extended longevity in Wd, but did not in KO. In WAT, CR reduced adipocyte size in both Wd and KO equally. Predictably, Srebp-1c was required for the CR-associated up-regulation of protein expressions involved in fatty acid synthesis in WAT. Surprisingly, in addition, Srebp-1c was essential for the CR-enhanced mitochondria biogenesis in WAT probably via the up-regulation of peroxisome proliferator-activated receptor-γ coactivator α (Pgc-1α) expression, a transcriptional coactivator for energy metabolism. Srebp-1c was also required for the reduction of oxidative stress in WAT of CR mice. Most of these Srebp-1c-associated metabolic alterations were not found in the liver.

Our findings suggest that the CR-induced up-regulation of Srebp-1c enhanced mitochondria biogenesis and suppressed oxidative stress in WAT. Therefore, Srebp-1c and its regulating signals in WAT might play a pivotal role for the beneficial actions of CR.

# P-11 Serum Ketone Body Level Correlates with Daily Carbohydrate Intake in Low Carbohydrate Diet (LCD)

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[Background] In Japan, the standard method of diet therapy has been calorie restriction (CR) for long years. However, low carbohydrate diet (LCD) has recently become an effective therapy in patients with obesity, diabetes and metabolic syndrome.

[Patients and Methods] We have treated more than 2000 patients with obesity and diabetes so far, and high percentage of those had successfully reduced weight with LCD treatment. Through strict LCD meal, patients showed high serum concentration of ketone body associated with the amount of daily carbohydrate intake. Study-A: Ten subjects with diabetes (type 2 DM, T2DM) on LCD (30-40g/day, ten days after start of LCD) were chosen for the serum level of ketone body. Study-B: 61 year-old female with T2DM was on strict LCD for 8 months with complete data of daily carbohydrate intake. The Data include 1) total ketone body concentration (normal range 28-120 micro mol/L), 2) acetoacetic acid (AcAc, 14-68 micro mol/L), 3) 3-hydroxy butyric acid (3-OHBA, 0-74 micro mol/L).

[Result & Discussion] Result-A: The level of 1) was 513+/-267 (246-912) micro mol/L, and the percentages were 22.8+/-3.6% in 2), 77.2+/- 3.6% in 3). Result-B: The correlation of daily carbohydrate intake and 1) were: 19-26 g/day -- 2000-3000micro mol/L, 23-34g/day -- 1000-2000 micro mol/L, 32-44g/day -- 500-1000 micro mol/L, 37-50g/day -- 300-500 micro mol/L. Those results would become the fundamental data for the analysis of pathophysiology of ketone body metabolism with carbohydrate intake amount, and would contribute the development and evolution of LCD in the future.

# P-12 EFFECT OF LONGITUDINAL CHANGES IN VISCERAL FAT AREA ON INCIDENCE OF METABOLIC RISK FACTORS

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**Introduction**

Cardiovascular disease (CVD) is one of the leading causes of morbidity and mortality in the world. Previous reports have shown that obesity plays a significant role in increasing cardiovascular risk. Some indicators of obesity, such as the visceral fat area (VFA) are more strongly associated with the risk of CVD than other indicators of obesity, such as waist circumference, body mass index, or subcutaneous fat area (SFA). However, no study has measured the longitudinal change in VFA among the same subjects twice, at baseline and at follow-up, to examine its relation to the incidences of metabolic risk factors in a large cohort study. We studied the incidences of metabolic risk factors according to changes in VFA in a large Japanese population.

**Methods**

The subjects were 973 men who received a computed tomography (CT) examination in both 2004 and 2007, and not having two or more of metabolic risk factors (except for the waist circumference) at the baseline survey. VFA, SFA, and waist circumference were measured using CT.

The three-year change in the participants' VFA (designated as ΔVFA) was categorized into seven groups (Group 1, $\leq$-50 $cm^2$; Group 2,>-50 $cm^2$ and $\leq$-30 $cm^2$; Group 3,>-30 $cm^2$ and $\leq$-10 $cm^2$; Group 4,>-10 $cm^2$ and< 10 $cm^2$; Group 5, $\geq$10 $cm^2$ and<30 $cm^2$; Group 6, $\geq$30 $cm^2$ and<50 $cm^2$; Group 7, $\geq$50 $cm^2$). Odds ratios (95% confidence intervals; CI) of the three-year incidence of each metabolic risk factor and/or clustering of metabolic risk factors according to the seven groups of ΔVFA were estimated with the use of a multiple logistic regression analysis, where adjustments were made for the following potential confounders: age, VFA, and each parameter of the metabolic risk factors (high blood pressure, hyperglycemia, high triglycerides, or low HDL cholesterol) at baseline. P value for trend across the seven groups was calculated by applying consecutive integers to the categories in the logistic regression model.

**Results**

The odds ratios for the incidences of the clustering of metabolic risk factors according to the ΔVFA groups adjusted for were 0.45, 0.63, 0.74, 1.00 (ref.), 0.70, 1.04, and 3.91, respectively (trend p<0.001). The odds

ratios (95% CI) of the incidences of hyperglycemia, high triglycerides, and low HDL cholesterol for the group with the largest increase in ΔVFA (50 $cm^2$ or more) were 2.98 (1.33-6.68), 4.88 (2.51-9.47), and 3.36 (1.26-8.91), respectively. In subjects with a decrease in ΔVFA, the incidences were low for high triglycerides and low HDL cholesterol, but did not change for high blood pressure and hyperglycemia. Of the components of the metabolic syndrome, especially the incidences of high triglycerides and low HDL-cholesterol paralleled with ΔVFA (trend $P<0.05$).

**Discussion**

The associations between change in VFA and metabolic risk factors were most pronounced for the risk of a high triglycerides and low HDL cholesterol. The adoption of a lifestyle that does not increase the VFA is important for preventing metabolic syndrome.

# P-13 Effects of Physical Activity on Insulin Sensitivity and Pancreatic β-Cell Function Among Japanese Obese Adults: The Saku Control Obesity Program (SCOP)

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Background: We investigated the effects of physical activity on insulin sensitivity and pancreatic β-cell function in a randomized controlled trial among Japanese obese adults.

Methods: We randomized obese individuals into a lifestyle intervention group and a control group. Participants in the intervention group underwent exercise and diet intervention for 1 year. We evaluated physical activity-related energy expenditure (PAEE), insulin sensitivity (insulin sensitivity index: ISI), pancreatic β-cell function (oral disposition index: DI), and hemoglobin A1c (HbA1c) levels both at baseline and after 1 year. On the basis of the results from a 75-g oral glucose tolerance test, we calculated the ISI using the insulin and glucose levels measured at 0, 60, and 120 minutes. The DI was calculated by multiplying the insulinogenic index $(I_{30}-I_0)/(G_{30}-G_0)$ and the ISI. To examine the effects of physical activity on insulin sensitivity and pancreatic β-cell function, we first compared the changes (Δ) in ISI, DI, and HbA1c levels between the intervention group and control group. Then, using generalized linear models with adjustments for baseline age, sex, intervention group, visceral fat area, PAEE, and total energy intake, and Δtotal energy intake, we examined the associations between ΔPAEE and ΔISI, ΔDI, and ΔHbA1c levels.

Results: This study included 215 obese adults (intervention group, 102; men, 107) with a mean age of 54.0 years, mean body mass index of 30.5 kg/m$^2$, and mean HbA1c levels of 6.1%. The intervention resulted in an increase in PAEE (control group, 0.12±0.88 metabolic equivalents (METs)-h/day; intervention group, 0.74±1.22 METs-h/day; $P<0.001$) and improved ΔISI (+1.92; $P<0.001$) and ΔHbA1c (-0.2%; $P=0.004$), but no difference was observed for ΔDI. After adjusting for potential confounding factors including Δtotal energy intake, ΔPAEE (+1.38 METs-h/day) was found to be associated with an improvement in ΔISI (+1.92; $P<0.001$) and ΔHbA1c (-0.1%; $P=0.017$) but not with ΔDI ($P=0.36$).

Conclusions: Our findings suggested that physical activity may largely exert its effects on glucose metabolism by improving insulin sensitivity, but it may not substantially influence pancreatic β-cell function.

# P-14 PREVALENCE OF OBESITY FROM BIRTH TO INFANCY: A LONGITUDINAL STUDY AT AN ARBAN CITY, NARA, JAPAN

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Child obesity often beginning during infancy is thought to track to adulthood at a high rate. However, the longitudinal study of obesity in infancy is still limited. The aim of the present study is to investigate the prevalence of obesity from birth to infancy longitudinally paying attention to the relationship with the obesity of their parents.

1668 infants aged at 4-5 years old (boys; 849, girls; 819) attending to kindergartens in Nara prefecture were enrolled with their parents' consent. Present height and weight of both children and their parents were obtained by the questionnaire. Children's heights and weights from birth to infancy at every year were obtained by the records of mother-child health notebooks. By body mass index (BMI), child's and parental obesity was defined as $\geq 90^{th}$ percentile based on the standard of the Japanese Association for Human Auxology and $\geq 25$ ($m^2/kg$), respectively.

The prevalence of obesity at birth and 1.5 years was 9.9% and 9.4%, respectively, demonstrating no statistical differences between ages. In addition, there was not any difference between genders (Chi-squre test). Then, the prevalence was found to decrease statistically in both genders ($p<0.01$, Cochrane-Armitage test). At 4-5 years, the prevalence of obesity in boys and girls was 5.9% and 4.9%, respectively. In the follow-up analysis of the same children, approximately 10% and 25% of obese children at birth and 1.5 years, respectively, were found to be still obese at 4-5 years old. Statistically, the association of obesity at 1.5 years old with obesity at 4-5 years old was more striking ($p<0.05$, Fisher's exact test). When both or either parents were obese, their children had a slightly higher risk of obesity (OR: 1.59; 95% CI: 0.98-2.59; $p=0.06$, Chi-square test) compared with children of having not obese parents.

Obesity at 4-5 years old has a significantly stronger relationship with that at 1.5 years than a birth time. In addition, a weak association between parental obesity and a risk of obesity in infancy was found. It is important to manage body weight from an early stage of infancy to avoid health problems caused by obesity in the future.

## P-15 Association between BMI and High-Sensitivity C-Reactive Protein

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C-reactive protein (CRP) is produced mainly in the liver, and increases rapidly in the blood when inflammation occurs in the body. In the USA, hs-CRP of>3.0 mg/l is thought to be a high risk for heart disease. Meanwhile, in Japan, hs-CRP of>1.0 mg/l is considered to be an indicator for the risk of future heart disease. Hence, with subjects divided into those having hs-CRP of ≤1.0 mg/l and>1.0 mg/l, we conducted a cross-sectional survey to investigate factors related to hs-CRP>1.0 mg/l among male Japanese workers.

Tests of hs-CRP were performed concurrently with a workplace health checkup in all the 212 male workers. A self-completed questionnaire survey was also conducted. Basic attributes and lifestyle were investigated, including age, work pattern, whether living with family, job type, overtime working, mean sleeping hours, regular exercise, smoking habits, and alcohol consumption. Fasting blood including hs-CRP was collected during the morning of daytime work (08:30-17:00) from daytime workers, and from shift workers during the morning of afternoon shift among three shifts of morning shift (07:00-14:00), afternoon shift (14:00-22:00), and night shift (22:00-07:00). Hs-CRP was measured using latex agglutination turbidimetric immunoassay with an automatic analyzer (Hitachi 7700, Japan). People being treated for diabetes or other inflammatory conditions were excluded. People who could not undergo blood tests in a fasting state on the day of the health checkup were also excluded from analysis. As a result, the subjects for analysis were 183 men (age, 19-62 years; mean, 38.2±12.9 years). All participants consented to the study and signed a consent form. This study was approved by the ethics committee of the Nagoya University School of Medicine.

Among daytime workers, the median hs-CRP was 0.32 mg/l with the interquartile range (IQR) of 0.12 mg/l to 1.06 mg/l. Among shift workers, the median was 0.32 mg/l (IQR; 0.12 - 0.83 mg/l). The levels did not differ statistically between the groups. With subjects divided into those having hs-CRP of ≤1.0 mg/l and>1.0 mg/l, basic attributes and lifestyle were investigated by Mann-Whitney U test, $\chi^2$ test and $t$-test. The results showed that the subjects with hs-CRP>1.0 mg/l had significantly higher BMI ($p<0.01$), age ($p<0.05$) and fasting blood glucose ($p<0.05$), and significantly lower HDL-cholesterol ($p<0.05$), when compared to those with hs-CRP≤1.0 mg/l. The levels of hs-CRP increased with an increase in BMI ($p<0.001$); hs-CRP>1.0 mg/l was seen in 38% of subjects with BMI≥25 kg/m$^2$ and 75% with BMI≥30 kg/m$^2$.

In the present study the level of hs-CRP increased with BMI. Hs-CRP of>1.0 mg/l was found in about 40% of subjects with BMI≥25 kg/m$^2$, and 75% with BMI≥30 kg/m$^2$. The findings have suggested that male Japanese workers with BMI≥25 kg/m$^2$ are more likely to be at risk for heart disease. This agrees with the obesity guidelines of BMI≥25 kg/m$^2$ for Japanese people. The present findings have suggested that hs-CRP>1.0 mg/l can be an indicator for obesity-related risks in male Japanese.

# P-16 TRAJECTORY OF BODY MASS INDEX BEFORE THE DEVELOPMENT OF DIABETES IN JAPANESE MEN: TORANOMON HOSPITAL HEALTH MANAGEMENT CENTER STUDY (TOPICS)

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**OBJECTIVE:** To date longitudinal data on the trajectory of general adiposity with repeated clinical measurements before the actual onset of type 2 diabetes are sparse, especially in Asian individuals who are not predominantly overweight or obese. Evidence has shown rapidly increasing levels of glycemic concentrations could be observed before the diagnosis of diabetes. However, it remains unknown in which period of the natural history of diabetes an elevated BMI, increasing BMI or stable overweight/obesity could be observed among individuals who eventually develop type 2 diabetes. We aimed to investigate the trajectory of BMI and BMI histories before the development of type 2 diabetes in Japanese men.

**METHODS:** We retrospectively examined data on 2068 non-diabetic Japanese men who underwent health examinations at the Health Management Center, Toranomon Hospital. Mean BMI and incident cases of diabetes (diabetes indicated by fasting plasma glucose level≥7.0 mmol/L, self-reported clinician-diagnosed diabetes, or HbA1c≥6.5%) were assessed on an annual basis over 8- to 10 years after the baseline examination. We assessed the maximum BMI during the follow-up time and a history of excessive obesity was indicated by BMI≥27.5 kg/m$^2$ in this study. Duration of overweight/obesity was evaluated according to whether a participant experienced BMI≥25.0 kg/m$^2$ or BMI≥27.5 kg/m$^2$ for 3 or more consecutive annual examinations.

**RESULTS:** Mean (SD) BMI at the time of diagnosis was 24.4 (3.2) kg/m$^2$ among diabetic individuals (n= 241). An increasingly high BMI was associated with the 8- to 10-y pre-diagnosis period and diabetic individuals experienced a prolonged and stable elevated BMI of≥24 kg/m$^2$ (range 24.1-24.4) over the 8 y prior to the diagnosis of diabetes. The mean BMI among the non-diabetic individuals did not exceed 23.2 kg/m$^2$ throughout the period. When we assessed the maximum BMI and the history of excessive obesity (BMI ≥27.5 kg/m$^2$), the mean maximum BMI was higher among diabetic individuals than non-diabetic individuals ($\rho$<0.001). The prevalence of individuals with a history of excessive obesity was also significantly higher among diabetic individuals (16.2% vs. 10.4%; p=0.007). Results of our assessment of the duration of overweight/obesity showed that diabetic individuals were more likely to have a history of stable overweight/obesity.

**CONCLUSIONS:** Japanese men who eventually developed diabetes during the 10-year observation period were not characterized as obese but had stable high-normal BMIs during the 8 y prior to onset of diabetes. Previous evidence indicated that values for glycemic markers rapidly increased before the development of diabetes; however, the present study showed a slight gain in BMI in the earlier stage of the natural history of diabetes followed by a prolonged period of overweight. Our findings would contribute to efforts to prevent the further deterioration of the glycemic state through a better understanding of the trajectory of general adiposity before the onset of diabetes.

# P-17 Prediction of the Development of Hypertension by Body Mass Index History in Japan: Toranomon Hospital Health Management Center Study (Topics)

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**OBJECTIVE:** It has not been fully clarified whether overall adiposity in early adulthood such as at age 20 y or at the lifetime maximum would irreversibly provide a residual risk of hypertension after considering the risk associated with current adiposity. We aimed to investigate the roles of BMI histories including the BMI at age 20 y, at its maximum and at the time of baseline examination as predictors for future hypertension in Japanese men and women.

**METHODS:** Enrolled were 4485 men and 1780 women without hypertension (hypertension indicated by systolic blood pressure (SBP)≥140 mm Hg, diastolic blood pressure (DBP)≥90 mm Hg or a self-reported history of medical treatment for hypertension). The risk of the development of hypertension 4 y after the baseline examination was investigated using BMI at age 20 years (BMIage20y), the lifetime maximum BMI (BMImax), or BMI at the baseline examination (BMIbaseline). Logistic regression analysis was performed to calculate the odds ratio (OR) (95% CI) for the development of hypertension across quartile (Q) categories of BMIbaseline, BMIage20y and lifetime BMImax. We investigated the combined effect of the BMIbaseline and BMIage20y or BMIbaseline and BMImax by categorizing participants into 4 groups.

**RESULTS:** Elevated values (highest quartile group) for BMIbaseline or BMImax, rather than BMIage20y years, were strongly associated with the development of hypertension among men and women. Men with both elevated BMIbaseline and BMIage20y had the highest OR of 2.50 (95% CI 1.87, 3.35) compared to men without those two factors. Among women, a low BMIage20y but an elevated BMIbaseline the OR was high at 2.63 (95% CI 1.72, 4.03) for the development of hypertension compared to women without the two factors. In results of a combination of BMIbaseline and BMImax, we observed that participants with an elevated BMIbaseline and who also had a history of elevated BMImax had a significantly high risk of future hypertension (OR 2.26 (95% CI 1.69, 3.02) in men; OR 2.41 (95% CI 1.60, 3.65) in women) compared to those without the two factors (elevated BMIbaseline and BMImax). When we stratified participants by either<50 y or≥50 y of age at the baseline examination, increasingly higher values for BMIage20y were predictive of the development of hypertension particularly among younger males. BMIbaseline or BMImax was positively associated with the development of hypertension among men and women, even those aged≥50 y.

**CONCLUSIONS:** Elevated values of BMIbaseline or the lifetime BMImax, rather than of BMIage20y, were strongly associated with the development of hypertension in Japanese men and women. After considering the risk of hypertension associated with BMIbaseline, having a history of elevated BMIage20y or BMImax might further provide a residual risk of hypertension, particularly in male participants with an elevated BMI at the baseline examination. Although the risk of hypertension associated with the past weight history is considered to be non-modifiable, individuals with an elevated BMIbaseline, particularly that which is close to the lifetime BMImax, should be considered to be offered an appropriate weight management program.

# P-18 Comparison of Various Body Mass Index Histories to Identify Undiagnosed Diabetes in Japanese Men and Women: Toranomon Hospital Health Management Center Study

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**OBJECTIVE:** To compare current body mass index (BMI) and various aspects of BMI history as pre-screening tools for undiagnosed diabetes in Japanese individuals.

**RESEARCH DESIGN AND METHODS:** This cross-sectional study included 15143 men and 6674 women aged 30-75 y without a self-reported history of clinician-diagnosed diabetes. We estimated the probability of having undiagnosed diabetes (fasting plasma glucose≥7.0 mmol/L and/or HbA1c≥6.5%) for the following: current BMI, BMI at age 20 years ($BMI_{20y}$), maximum BMI ($BMI_{max}$), change between BMI at age 20 years and current BMI ($\Delta BMI_{20y\text{-}cur}$), change between BMI at age 20 years and maximum BMI ($\Delta BMI_{20y\text{-}max}$), and change between maximum and current BMI ($\Delta BMI_{max\text{-}cur}$).

**RESULTS:** Prevalence rate of individuals with undiagnosed diabetes was 3.3% among total participants (n=730/21817). $BMI_{max}$, $\Delta BMI_{20y\text{-}max}$ and current BMI (1-SD increments) were more strongly associated with undiagnosed diabetes than the other factors (multivariate odds ratio 1.56 [95% CI: 1.45-1.68] (in men)/1.61 [1.38-1.87] (in women) for $BMI_{max}$; 1.46 [1.36-1.57]/1.58 [1.37-1.82] for $\Delta BMI_{20y\text{-}max}$; 1.46 [1.35-1.57]/1.58 [1.35-1.85] for current BMI). Individuals with both the highest tertile of $BMI_{max}$ and greatest $\Delta BMI_{20y\text{-}max}$ had a markedly higher probability of having undiagnosed diabetes. A substantially low likelihood of undiagnosed diabetes was observed among individuals with the lowest and middle tertiles of current BMI (<24.5 kg/m$^2$ in men and<22.4 kg/m$^2$ in women).

**CONCLUSION:** Past maximum BMI and BMI changes from age 20 years were strongly associated with undiagnosed diabetes. Further adding data on BMI histories into current BMI data would contribute to improving the identification of undiagnosed diabetic patients.

# P-19 Evaluation of Food Preference in Childhood Using A Picture Choice Method: A Relationship with Body Habitus

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Background: Food preference, one of the major factors for one's lifestyle, is thought to be associated with development of obesity. However, there has not been an appropriate method to evaluate it in childhood. That seems because children are not able to understand questions for evaluation usually employed in adults. Hence we have invented a picture choice method designed to evaluate childhood food preference and then compared it with their body habitus.

Methods: In total, 486 children aged 6-12 years (231 boys, 255 girls) were enrolled in the study. To evaluate "interest in diet", the subjects were requested to choose any 10 from 36 pictures on a panel depicting 26 objects normally around them and 10 different foods. The number of food chosen was set for the "interest in diet" score (point). The test was performed within 30 minutes after meal. To evaluate "food preference", they were also requested to choose any 10 from 36 pictures on the other panel depicting 36 different foods epidemiologically reported to be their favorites, which were staple foods, main greens, second greens, fruits, cakes, taste drinks and fast foods. On the 10 foods chosen, "fast-food score (point)", "Japanese food score (point)", "energy density (kcal/100g of food)", "lipid content (g/100g of food)" and "fat energy ratio (%)" were calculated according to the average ingredient amount in the foods. This test was performed between meals to avoid postprandial effect. These "food preference" indices were then compared with "interest in diet" score or with their body habitus which was evaluated by a relative weight index calculated by one's height, weight measurements and a standard weight for one's sex, age and height.

Results: "Fast-food score", "Japanese food score","lipid content", "fat energy ratio" as well as "interest in diet" score were significantly higher in the boys than in the girl (1.4±0.9 vs 1.0±0.8, 1.4±1.1 vs 1.2±1.1, 10.3±3.2 vs 9.5±3.3, 41.5±6.4 vs 39.3±6.9, 3.4±2.3 vs 2.3±1.9, $p<0.05$). As for age differences, "interest in diet" score was significantly higher in the 5-6th grader than in the 1st-2nd grader of primary school children (1st-2nd grader: 2.5±1.9 vs. 5-6th grader: 3.1±2.3, $p<0.05$). A positive correlation was found between "interest in diet" score and "fast-food score", "energy density", "lipid content" or "fat energy ratio" ($r=0.16$, 0.23, 0.25, 0.22, respectively, $p<0.05$). As for body habitus, the numbers of the subjects whose "interest in diet" score or "lipid content" were in the upper half were significantly greater in the underweight (lighter than 85 percent of their standard weight) subjects than in the rest ($p<0.05$).

Conclusions: The findings in the study could be supportive for the usability of the picture choice method as a practical evaluation method for childhood food preference which must be an accurate predictor of that in adulthood.

## P-20 Factors influencing the body image of Japanese college students

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Background: Frequent and often unnecessary weight-loss practices in young adults have been a public health concern in Japan. However, a limited number of studies have comprehensively assessed influences of environment factors on the body image and weight management practices in college students.

Purpose: To clarify influencing factors to the body image of young Japanese college students.

Methods: A self-reported questionnaire that includes questions on lifestyle and body image was administered to 687 college students (354 females and 333 males, mean age±S.D: 19.5±1.3 yrs). The participants were divided into two groups: those perceived themselves as "slim" or "normal" (S/N: females; n=104, males; n=207) and those perceived themselves as "slightly overweight" or "overweight" (OW: females; n=250, males; n=126). Statistical comparisons between the groups were performed with Tukey's multiple comparison adjusted by grade, specialty, and assessment time. The difference of the distribution was examined by Cochran-Mantel-Haenszel adjusted by grade, specialty, and assessment time.

Results: 67% of the S/N group was males, and 67% of the OW group was females. Self-reported BMI for the S/N group and OW group were 20.2 $kg/m^2$ and 24.2 $kg/m^2$ in males, and 18.9 $kg/m^2$ and 20.8 $kg/m^2$ in females, respectively ($p<0.01$). The ideal BMI was 20.8 $kg/m^2$ in males and 18.2 $kg/m^2$ in females. The S/N group's EAT 26 score was higher than that of the OW group in females (8.2 vs 4.6, $p<0.01$). A greater proportion of the OW group reported frequent dieting practices (2% vs 10% in males, $p<0.01$, 8% vs 39% in females, $p<0.01$). A greater proportion of the OW group expressed their body image concern than the S/N group and their major reasons included to have a confidence (42% vs 71%, $p<0.01$, in female), fashion-related (69% vs 89%, $p<0.01$, in female, 38% vs 49%, $p<0.05$, in male), and neighboring people get thinner (9% vs 43%, $p<0.01$, in female, 2% vs 16%, $p<0.01$, in male). More OW individuals compared one's physique with the media presents ideal (5% vs 25%, $p<0.01$, in female), family/classmate (10% vs 42%, $p<0.01$ in female, 8% vs 21%, $p<0.01$, in male), and the others (9%, 35%, $p<0.01$, in females). Also the OW participants expressed more dissatisfaction with their abdomen (42% vs 87%, $p<0.01$, in female, 22% vs 61%, $p<0.01$, in male), legs (52% vs 84%, $p<0.01$ in female, 28% vs 44%, $p<0.01$, in male), arms (29% vs 71%, $p<0.01$, in female), and face (35% vs 50%, $p<0.05$, in male), etc. than the S/N counterparts. More than 60% college students reported TV influenced the body image regardless of genders and their body image.

Conclusion: Japanese college students are over-concerning their own physique regardless of the genders. The media might be influence the body image of young Japanese, especially in females those perceived themselves as overweight.

## P-21 Aging-like skin changes occurred through mineralocorticoid receptor signaling pathway in a mouse model for metabolic syndrome

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Purpose

Skin aging process may be accelerated by fatty tissue inflammation in patients with metabolic syndrome (MetS). Various molecular pathways such as oxidative stress are involved in this pathological change. Recently, mineralocorticoid receptor (MR) signaling was suggested to play a pivotal role in development of inflammatory tissue changes in MetS; MR overexpression was observed when skin tissue atrophy occurred. However this result might not fully elucidate the physio-pathological functions of MR in MetS. We have previously developed the aging-like skin model by single ultraviolet (UV) irradiation in the MetS mouse model, to study the effects of fat metabolism disorder on aging (UV-MetS mouse model). In this study, skin tissues in the UV-MetS mouse model were examined to investigate the role of MR in skin aging.

Methods

The skin samples were obtained from TSOD (MetS) and TSNO (control) mice after UV irradiation (24 hours), for histological and immunohistochemical evaluation (Hematoxylin & Eosion, 8OHdG, MR). The expression of mRNA (Hmox-1, Cox2, MR and Sgk1) was analyzed to assess oxidative stress and inflammatory changes. Antioxidant (Tempol) and MR antagonist (spironolactone) were applied to some mice. Long-term UV irradiation was conducted in TSNO mice to study if different mechanisms for development of aging-like skin damage exist between presence and absence of obesity.

Results/Discussion

Mineralocorticoid receptor (MR) signaling was involved in development of UV-induced skin changes if fat metabolism was impaired. Aging-like skin damages were induced after UV irradiation only in TSOD mouse (Hmox-1, Cox2 genes were up-regulated). The expression of MR and its effector gene were obvious in TSOD mice (not in TDNO mice). These markers were suppressed by MR antagonist (spironolactone) application and damaged skin was restored. Inflammatory changes, equivalent to the UV-MetS (TSOD) mouse model were obtained in TSNO mice after 2 week-irradiation protocol. Interestingly, no changes of MR and Sgk1 expression were observed in the TSNO mice.

Conclusion

MR signaling might be involved in skin aging in MetS.

## P-22 Diabetes Disrupts Homeostasis against Alzheimer's Disease

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**[Background]** Emerging evidence suggests that diabetes increases the risk of onset of Alzheimer disease (AD). However, the underlying mechanisms have not been fully elucidated. Retrospective studies using AD brains indicated that diabetes did not increase senile plaques. On the other hand, a Japanese cohort, the Hisayama study suggested that insulin resistance in midlife increased the risk of development of amyloid plaques. Therefore, our aim is to understand the molecular mechanisms by which diabetes increase the risk of AD by dividing them into two phases; before and after the development of senile plaques. **[Methods]** To test whether insulin resistance increases Abeta accumulation in the brain, we fed wild-type mice with high-fat diet and measured the levels of Abeta in brain and plasma. To investigate the effects of diabetes on AD, we further analyzed the phenotypes of APP+ob/ob mice, which showed the increased cerebral amyloid angiopathy and impaired insulin signaling (Takeda, Sato, et al. Proc Natl Acad Sci U S A, 107, 7036-41, 2010), focusing on tau phosphorylation. **[Results]** Six months high fat diet increased Abeta40 in B6C mice brain. Eighteen months old APP+ob/ob mice showed highly increased level of tau phosphorylation in the brain. On the other hand, high fat diet increased plasma Abeta levels in APP/PS1 mice, but not in wild type mice. **[Conclusion]** Tau phosphorylation is increased by diabetes in APP mice, suggesting that Abeta is prerequisite, but insufficient to cause tau phosphorylation in vivo. Abeta accumulation, insulin signaling and tau phosphorylation might play essential roles in the pathological interaction between AD and diabetes. Of note, a vicious cycle likely underlies the interaction between AD and diabetes. High fat diet-induced elevation of plasma Abeta level might be involved in a mutual pathological interaction between the diseases. These results suggest that diabetes disrupts homeostasis against AD.

# P-23 Chewing Betel Quid (Areca Nut) and Risk of Metabolic Disease, Cardiovascular Disease, and All-Cause Mortality: A Meta-Analysis

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Background:

Asia already has 60% of the world's diabetic population and diabetes is increasing more rapidly in Asia than anywhere else. Such metabolic diseases have a crucial influence on public health, since a modest increase in the risk of morbidity and mortality translates into a substantial social burden, so prevention of these diseases is extremely important.

Betel nut (Areca nut) is the fruit of the Areca catechu tree. Approximately 700 million individuals regularly chew betel nut (or betel quid) worldwide and it is thought to be the fourth most commonly used psychoactive substance in the world. Betel quid is a known risk factor for oral cancer and esophageal cancer.

It was recently proposed that there is an association between inflammatory oral conditions and systemic disorders. Numerous studies have shown that chewing Betel Quid is associated with the risk of various systemic diseases (including metabolic disease, cardiovascular disease, and all-cause mortality), as well as oral diseases, and have generally identified a positive association, although its magnitude has varied.

Clarifying the relationship between chewing Betel Quid and metabolic disease may be important for the development of preventive strategies. Accordingly, we performed a meta-analysis to confirm the influence of chewing Betel Quid on metabolic disease, cardiovascular disease, and all-cause mortality.

Methods:

We searched Medline, Cochrane Library, Web of Science, and Science Direct for pertinent articles (including the references) published between 1951 and 2013. The adjusted relative risk (RR) and 95% confidence interval were calculated using the random effect model. Sex was used as an independent category for comparison. To assess the validity of the studies thus identified, each report was appraised with reference to the STROBE statement (an established checklist of items that should be included in articles reporting observational research comprising several study designs and many topic areas). The Newcastle-Ottawa Scale for assessing the quality of nonrandomized studies in meta-analyses was also used to quantify the validity of each study.

Results:

Of 580 potentially relevant studies, 17 studies from Asia (5 cohort studies and 12 case-control studies) covering 388,134 subjects (range: 94 to 97,244) were selected. Seven studies (N=121,585) showed significant dose-response relationships between betel quid consumption and the risk of events. According to pooled analysis, the adjusted RR of betel quid chewers vs. non-chewers was 1.47 (P<0.001) for obesity (N=30,623), 1.51 (P=0.01) for metabolic syndrome (N=23,291), 1.47 (P<0.001) for diabetes (N=51,412), 1.45 (P=0.06) for

hypertension (N=89,051), 1.2 (P=0.02) for cardiovascular disease (N=201,488), and 1.21 (P=0.02) for all-cause mortality (N=179,582).

Conclusion:

Betel quid chewing is associated with an increased risk of metabolic disease, cardiovascular disease, and all-cause mortality. In addition to preventing oral cancer, stopping betel quid use could be a valuable public health measure for metabolic diseases that are showing a rapid increase in South-East Asia and the Western Pacific.