Evidence of the new diagnostic criteria of diabetes mellitus in Japan
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Background
Many countries have examined the use of HbA1c for the diagnosis of diabetes mellitus (DM). The same trial has also been made in Japan. However, in considering oral glucose tolerance test (OGTT) as the golden standard for the diagnosis of DM, the correlation between OGTT 2-hour plasma glucose level (2-h PG) and fasting plasma glucose level (FPG) is extremely important and diabetic complications should also be taken into consideration.

Results
1) With regard to the correlation among HbA1c, FPG, and OGTT 2-h PG in 6,658 examinees < 60 years who underwent OGTT, HbA1c showed a remarkably high correlation to FPG and 2-h PG. From regression equation, for HbA1c of 6.1% FPG level was calculated to be 124.4 mg/dl and 2-h PG to be 199.3 mg/dl. The rate of retinopathy in 36,267 cases by HbA1c was 0.52% when HbA1c was <4.5%, but with the elevation of HbA1c the prevalence rate of retinopathy also increased. For HbA1c of 6.1~6.5% there was about a three-fold increase in the rate by HbA1c < 4.5%. In comparing using deciles the retinopathy rate by HbA1c, FPG and 2-h PG in OGTT, the rate elevated from FPG of 126 mg/dl, from 2-h PG of 198 mg/dl and from HbA1c of 6%. The rate of retinopathy was compared by quintile in 5,040 cases with DM under treatment more than 6 years. For HbA1c <5.5% the retinopathy rate was 1.0%, but when HbA1c value reached 6.0~6.5%, the rate showed a significant elevation to 2.2%.

2) As for the incidence of retinopathy, for HbA1c of 6.1~6.5% it was 42 fold higher than that for HbA1c < 5.6%. The cumulative incidence of retinopathy was observed for a period of 15 years. In examining the cumulative incidence rate in 1,139 cases whose HbA1c <6.5% was maintained for 15 years and that in 163 cases whose HbA1c value was 6.5~10% 10 years thereafter, their incidence rate was 6.9% and 18.4%, respectively, and 15 years thereafter their incidence rate was 9.1% and 29.2%, respectively.

Conclusion
A high correlation was observed between HbA1c and FPG. When HbA1c value was <6.5%, the prevalence and incidence of retinopathy were extremely low. The use of HbA1c is recommended for screening of DM, but for diagnosis of DM blood glucose value should be included.

Current diagnostic criteria of diabetes: Perspective from a physician in the clinical/research laboratory
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For decades glucose in blood, plasma or serum has constituted the diagnosis of diabetes mellitus. Whether fasting or post–glucose ingestion, the concentration of glucose has related very well to the development of retinopathy or nephropathy, as has hemoglobin A1c (HbA1c). For these 2 complications of diabetes, it has been relatively easy to establish cut-points between the “normal” state and diabetes. A recent “Expert Committee” of international scholars under the auspices of the American Diabetes Association has recommended the diagnosis of diabetes preferably be established with HbA1c. The guidelines strongly emphasized HbA1c, and predictably they have generated controversy. From a technical perspective in the clinical or research laboratory, HbA1c is the preferential analyte to measure. Modern methods permit measurements of HbA1c with a consistency at least equal to those for glucose. Furthermore, HbA1c has no constraints on when a blood sample can be collected; i.e., there is no need for fasting or post–glucose ingestion conditions. Additionally the reduction of glucose concentration in samples containing circulating cells leads to the requirement for prompt separation of cells from plasma or serum prior to analysis of glucose. All these “preanalytic” issues potentially and seriously compromise blood, plasma or serum glucose measurements for accurate assessment of a person’s glycemic status. Finally there are laboratories in many, if not most, countries which can measure Hb1c with great skill. The pending WHO Consultation will not recommend the use of HbA1c, rather saying it may be used in the diagnosis of diabetes mellitus, but does not replace glucose. Thus, hopefully, worldwide, all laboratories with technical and quality control capabilities to measure HbA1c will offer this assay to their patients.
Japan Diabetes Society (JDS) is definitely going to include the HbA1c as one of the parameter of the diagnostic criteria for diabetes mellitus. For the measurements of HbA1c, the immunological and the HPLC methods have been widely used in Japan. Recently, International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) proposed the peptide mapping method (Enzymatic method) as a reference method for the measurement of HbA1c. After then, a modified IFCC method (modified enzymatic method) was also developed in Japan and has been practically used.

For the use of HbA1c as a diagnostic criteria, the precision of the measurement should be satisfactory, especially around the value of HbA1c 6.1% (A1C 6.5%). The HbA1c values obtained from these three methods (HPLC, immunological and modified enzymatic methods) should also be standardized. From such points of view, we will present our results of precision of these methods and the difference of the HbA1c values among these three methods, especially the difference between HPLC and immunological methods.

Nationwide surveillance study on hemoglobin A1C by JDS in 2009

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Standardization of hemoglobin A1C (HbA1c) measurement in Japan, the Glycohemoglobin Standardization Committee of JDS have been conducted with the establishment of reference material as JDS Lot 1 and the stable HbA1c as for the measurand until 1998.

At the same time, verification of the commutability of measurements based on JDS Lot 1 and JDS Lot 2 which turned into JDS Lot 1 had been performed in the nationwide surveillance study since the reference material was set. As a result, the commutability of inter-methods for measurement, marvelous improvement was found in CV 2.7 - 4.0% as of 2003 from CV 7.1 - 12.8% before the standardization.

On the basis of the reference material proceeding to JDS Lot 4 (JCCRM411-2) in 2009, the nationwide surveillance study in 2009 was performed this time subsequently. In particular, considering the four international societies compile a consensus statement about the international standardization of the HbA1c measurement in June, 2007 and it was constituted with focus to the IFCC value and NGSP value which widely used around the North America.

The study was performed in the end of March after February and the routine laboratories were divided distribution and the measurement with three cycles. Four kinds of specimen were used in 905 routine laboratories as frozen blood which constituted 3 concentration levels and whole blood as one level were measured. Also, in the fourth cycle, 21 manufacturers were measured with ten kinds of the specimen every 3 concentration levels with frozen blood and one whole blood. These specimens were distributed with randomly. The target values of these specimens were set with designated comparison method by JSAC and JDS, and the results of a measurement were evaluated with a bias. Also, the NGSP values by NETCORE of NGSP and the IFCC values by the IFCC method as reference values were evaluated.

In addition, we will present the detail of the results of this surveillance study on the symposium.
It is now well established that HbA1c level reflects mean hyperglycemic state for the past 1 to 2 months and the maintenance of HbA1c less than 6.5% is at least required to prevent the initiation and progression of diabetic microangiopathy. In Japan, standardization of HbA1c measurement was energetically worked since 1993, when the 1st official standardization committee was organized in JDS (Japan Diabetes Society). Until now, the standard material of HbA1c, referred to as National Glycohemoglobin Standardization Program (NGSP) has been firstly established in DCCT (Diabetes Control and Complications Trial) and this method is now used in many countries except Japan and Northern Europe. There is a significant difference between HbA1c values of NGSP and JDS, which is now problematic to compare the international clinical trial data of the effects of hyperglycemia on diabetic vascular complications with Japanese data. On June 6, 2007, ADA, EASD, IDF and IFCC officially announced that HbA1c test results should be standardized globally, including referencing and the result reporting. In the new IFCC reference system, the standard reference for HbA1c measurement is implemented using purified primary standard materials. The HbA1c results are recommended to be globally reported using IFCC units (mmol/molHb) and IFCC-derived NGSP (%) using the IFCC-NGSP master equation. As a result of intensive review of the measurement of HbA1c in Japan, we agreed to accept the recommendation of the consensus statements on international standardization of HbA1c measurement. However, the committee has also agreed that results should be reported as JDS (%) in clinical practice to avoid confusion due to different notation and also difference in NGSP (%) and JDS (%).

Recently, international expert committee has recommended a new guideline to diagnose diabetes mellitus using HbA1c. Thus, our JDS committee decided to re-evaluate international difference of HbA1c measurement and the measured values. Based on many evidences on measurement of HbA1c using our standard material of HbA1c and results of measurement of HbA1c (DCCT) samples to compare JDS and NGSP, we found that NGSP (%) is equivalent to JDS (%) +0.4 (%). Thus, the present consensus statement of international standardization of HbA1c from JDS committee on diabetes mellitus laboratory testing standardization will be summarized in this presentation.
A search for new anti-diabetic and anti-obesity drugs

In Japan, as new therapeutic agents for type 2 diabetes, dipeptidyl peptidase-4 inhibitors and glucagon-like peptide–1 receptor agonists have been recently introduced in addition to sulfonylureas, biguanides, thiazolidinediones, α-glucosidase inhibitors, and rapid–acting insulin secretagogues. Several large–scale clinical trials such as ACCORD, ADVANCE, or J-DOIT3 revealed that the level of HbA1c that could be achieved in the case of most patients with type 2 diabetes mellitus was 6.0% when these drugs were adequately combined. However, in daily clinical practice, because good glycemic control is difficult to achieve, many patients develop diabetic complications. Therefore, development of new anti-diabetic drugs is expected. Anti-obesity medication is another treatment strategy for diabetic patients. Weight reduction is the best therapy for most obese diabetic patients. However, weight reduction is difficult owing to changes in the life style of the patients. The efficacy and safety of the currently available antiobesity drugs are unsatisfactory; in addition, treatment with these drugs is inferior to treatment with gastric bypass surgery. As a result of the development in the understanding of the adipose tissues or appetite mechanism over the last 15 years, many anti-obesity drugs will be available in the near future.

In this symposium, we invite 5 speakers who will discuss anti-diabetic or anti-obesity drugs, most of which are under clinical trials and will be available in the near future. Mechanism, results of clinical trials, merits and demerits, impact of treatment with these drugs on diabetes, and combination with other drugs will be presented.

S2-2

Sodium–glucose transporter–2 inhibition as an antidiabetic therapy

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Sodium–glucose transporters (SGLT) are a family of transmembrane proteins specialised in the co-transport of sodium and glucose across different cell types. The isoform, SGLT–2, is preferentially expressed in the brush–border membrane of proximal renal tubular cells in the S1 segment. SGLT–2 is a low–affinity, high–capacity transporter, which transfers glucose and sodium from the lumen into the cytoplasm of tubular cells. At the basolateral membrane, a glucose transporter of a different family, GLUT–2, effects transfer of intracellular glucose to the interstitium by a facilitated transport process.

For a glomerular filtration rate of 180L/day and an average plasma glucose of 5.6mmol/L, the filtered glucose load is 180g/day, of which only little is eventually excreted. As the transport maximum for glucose (TmG) is exceeded, progressively greater fractions of the filtered load spill over into the urine. In patients with type 2 diabetes (T2D), TmG is increased by 20–40% and in their renal tubular cells expression, protein concentration and α–methylglucose transport capacity of SGLT–2 are all increased markedly. Thus, renal glucose reabsorption is increased in diabetes. In contrast, familial renal glycosuria is a rare disorder caused by mutations in the SLC5 gene. Affected patients, who excrete between 1–170g/day of glucose in the urine, do not develop renal disease even in the long term and are not reported to be prone to diabetes and obesity.

This experiment of nature has suggested that lowering hyperglycaemia through enhanced renal glucose excretion, i.e., independently of insulin, may correct the pathophysiological abnormalities of diabetes. In 90% pancreatectomised rats, phlorizin reverses peripheral and hepatic insulin resistance and restores β-cell function. As phlorizin is not absorbed by the gut, is non–specific and of undetermined toxicity, orally active small molecules with a high specificity for SGLT–2 (dapagliflozin, sergliflozin, remogliflozin, canagliflozin, et al.) have been in development for several years: the forerunners are now being tested clinically.
Discovery and mechanism of action of small molecule glucokinase activators

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Glucokinase (GK) is a hexokinase isoform (hexokinase IV) and it functions as a key initial and rate-limiting step for glucose metabolism. The enzyme is expressed predominantly in pancreatic β-cells and liver. It is also known to be expressed in other endocrine and neural cells including pancreatic α-cells, L and K-type entero-endocrine cells, and in cells of the arcuate nucleus in the hypothalamus. GK is thought to act as a ‘glucose sensor’ in several of these tissues. Genetic deficiency of GK (heterozygous loss-of-function) in man is a well-established cause of a rare autosomal dominant type 2 diabetes variant, MODY2. In contrast, several ‘gain-of-function’ mutations have been identified recently. The gain-of-function variants are associated with persistent hyperinsulinemic hypoglycemia of infancy (PIHI). These data provides genetic validation and insight into the role of GK in the regulation of glucose homeostasis in man.

The drug discovery research efforts at Merck Research Laboratories have resulted in the identification of small molecule compounds that allosterically activate GK. Co-crystal structure of GK and small molecule GK activator (GKA) has been solved, which provided mechanistic basis for activation of GK by glucose and GKa. Pharmacological assessment of GKAs revealed that GKAs enhance glucose-stimulated insulin secretion in pancreatic β-cells and glucose metabolism in liver, both in vitro and in vivo in animals. These data support a dual mechanism for lowering blood glucose concentrations. Notably, GKAs demonstrate both acute and chronic glucose lowering efficacy in number of animal models for type 2 diabetes. From these observations, an allosteric activation of glucokinase by GKA is one of the potential targets for type 2 diabetes mellitus.

PPARx: New developments

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The peroxisome proliferator-activated receptor (PPAR) subfamilies of ligand-activated nuclear hormone receptors have been models for scientific discovery and targets for potential new therapeutics for three decades. Several PPARα agonists are used clinically for lipid lowering and three PPARγ compounds have been approved for their glucose lowering properties. All of the PPAR agonist therapies have side effects, sometimes leading to serious adverse outcomes. Interestingly, a wide range of toxicities and unforeseen adverse clinical outcomes has contributed to the failure of a number of compounds in late stages of development despite promising results of pre-clinical and early stage clinical testing.

The pleiotropic effects of PPARγ activation have been attributed to the numerous potential binding sites and conformations within the receptor ligand binding pocket and the large number of response genes. Ultrastructure delineation of the PPARγ ligand binding pocket has permitted the development of new classes of agonists referred to as either “partial agonists” or “selective PPAR modulators (SPPARMs)”. An ideal SPPARM maintains glucose (or lipid) lowering properties but has a markedly reduced impact on fluid retention and other adverse effects compared to the current non-specific PPARγ agonists. At least one such SPPARM is progressing through clinical trials, and will be reviewed.

While highly specific ligands are being pursued, PPAR response genes continue to be studied for their anti-neoplastic, anti-inflammatory and anti-atherosclerosis effects. Thus far, the response of tumors to PPARγ agonists in animal models has been mixed, but there does appear to be promise when the currently available drugs are given in combination with some chemotherapeutic agents. If the anti-inflammatory effects could be isolated from the pleiotropic effects, there may be a role in some difficult to treat disease states. The translation of bench research to bedside therapeutics has never been more challenging than with the PPAR family of molecules.
GPR40 as a potential therapeutic target for type 2 diabetes
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GPR40 is highly and dominantly expressed in pancreatic beta cells and is activated by medium- to long-chained FFAs to potentiate glucose stimulated insulin secretion. While GPR40 is an attractive target for type 2 diabetes, there is an ongoing debate whether agonists or antagonists represent the best therapeutic approach.

To clarify the function of GPR40 in pancreatic beta cell, we generated GPR40 Tg mice under the control of the insulin II promoter. GPR40 overexpression in mice did not affect plasma glucose and insulin level in fed conditions, but was associated with lower fasting plasma glucose. Morphological abnormality was not observed in islets of GPR40-Tg mice. GPR40-Tg mice had improved glucose tolerance with augmented insulin secretion. Moreover, GPR40-Tg mice maintained improved glucose tolerance with increased insulin secretion in high fat diet for long term, without body weight change or beta cell dysfunction. Finally, GPR40 transgenic mice with a KK background, also had improved insulin secretion and glucose tolerance without body weight change. These data supported the concept that activation of GPR40 may improve type 2 diabetes by enhancing glucose dependent insulin secretion.

The role of GPR40 was further confirmed using a small molecule agonist of GPR40. TAK-875 is a potent, selective and orally available agonist of GPR40. In rat insulinoma INS-1 832/15 cells, TAK-875 dose dependently stimulated insulin secretion under high glucose concentration. In an oral glucose tolerance test in type 2 diabetic rats, TAK-875 (0.3-3 mg/kg) showed a clear improvement of glucose tolerance and potently augmented insulin secretion. Chronic treatment of TAK-875 (1-10 mg/kg/d) in the diabetic rats dose dependently decreased glycylated hemoglobin without body weight gain or beta-cell dysfunction.

In conclusion, our results support the concept that GPR40 agonist therapy may be an effective treatment of type 2 diabetes.

A novel oral hypoglycemic agent for the treatment of type 2 diabetes with obesity: bromocriptine
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Development of medications for the treatment of type 2 diabetes mellitus with obesity targeting to factors that precipitate weight gain has been thought to be promising. Metformin and thiazolidinediones reduce insulin resistance, but may not have good impact with regard to body weight reduction. Oral agents that promote weight loss include phenformine, sibutramine, orlistat, diethylpropion, mazindol, and fenfluramine. Fenfluramine is no longer available, and some agents are difficult to use because of their problems in their safety and effectiveness. Orlistat, an inhibitor of lipase, prevents the absorption of fats, has a significant demand despite of its side effect. In Japan, a phase 3 clinical trial of cetilistat, that has better tolerability, was completed.

In the USA the FAD approved bromocriptine mesylate in May, 2009 for the treatment of type 2 diabetes mellitus. This is the first diabetes drug approved under the FDA's new guidelines requiring clinical trials to demonstrate no increased cardiovascular risk. Bromocriptine is a dopamine D2 receptor agonist with the structure of ergot alkaloids.

In the Cycloset Safety Trial, including 3,070 patients lasted 52 weeks revealed the 40% reduction in cardiovascular event rate, significant reductions in HbA1c. The between-group difference in HbA1c was for patients with baseline HbA1c of at least 7.5% (-0.5%) and for those with baseline HbA1c of at least 8.0% (-0.7%). Changes in circadian activities that regulate metabolism and insulin sensitivity are the primary target of this medication when bromocriptine was developed. Pijl et al compared 15 subjects with bromocriptine vs 7 controls in its earlier study (Diabetes Care23: 1154-1161, 2000). While improvement in insulin sensitivity and HbA1c (-1.2% vs control) was observed, weight loss an reduction in blood pressure that were observed in long term studies, were not detected in this short-term study.

While other oral medications that are under development include orexine receptor agonists, beta 3 adrenergic receptor agonists, and so on, bromocriptine has a potency to have a solid position in the long-term treatment of type 2 diabetes mellitus with obesity.
The trimolecular complexes underlying species risk of type 1A diabetes

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We hypothesize that the "root" etiology of autoimmune diabetes are germline encoded sequences of trimolecular complexes enhancing T cell receptor recognition of peptides of insulin presented by high risk HLA alleles. In man, studies such as the DASY study, following children from birth, demonstrate early activation of islet autoimmunity primarily determined by known and as yet unknown susceptibility genes within the major histocompatibility complex, with a large series (>40) of non-MHC genes having a minimal influence on risk but implicating multiple pathogenic pathways. Once multiple islet autoantibodies appear in young children almost all will progress to diabetes with some children requiring as much as a decade to develop hyperglycemia. The time lag in progression to diabetes is likely due to asynchronous segmental destruction of islet beta cells in lobules of the pancreas as indicated by studies of new onset patients and the nPOD repository. In the NOD mouse model the three components of trimolecular complexes can be studied in detail, with extensive evidence that insulin peptide B:9-23 is a primary target dependent upon presentation by I-Ax. Mutating a single amino acid of B:9-23 prevents all diabetes. The B:9-23 peptide is recognized by germline encoded sequences of Vα (TRAV5D-4) with little conservation of the CDR3 region, Jalpha, and the beta-chain of the TCR. Retrigenic mice having only multiple different TRAV5D-4 sequences develop insulin autoantibodies, their T cells recognize the peptide B:9-23 and a subset develop diabetes. Within islets of single NOD mice there are thousands of T cell receptors with TRAV5D-4 able to encode insulin autoimmunity as retronics. Specific trimolecular sequences may provide a critical target for preventing autoimmune diabetes.
ゲノムワイド関連解析（GWAS）で明らかになった
SNPを含めた1型糖尿病感受性遺伝子の日本人における統合的解析

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【目的】1型糖尿病感受性遺伝子は、候補遺伝子解析および最近のGWASにより、真ん中では2009年10月現在で43遺伝子が同定されている。一方、日本人においては6遺伝子との関連が報告されている。遺伝子との関連が示唆されている。本報告では、多施設共同研究による日本人で判明した1型糖尿病遺伝子の統合的解析の結果を述べる。

【方法】HLA–DR1/B1, HLA–DQB1, INS（rs6897, CTLA 4rs3087243）, IL2RA（rs706778）, ERBB3（rs2292239）, CLEC16A（rs2903692）, IL7R（rs6897932）, IFIH1（rs1997062）を、1型糖尿病769例、コントロール93例について解析し、1型糖尿病および各サブタイプとの関連性。【発症】年齢を補正したロジスティック回帰分析により検討した。サブタイプとしては、[急性]、[緩徐]、「[急性的]、[慢性]」、「[甲狀腺自己免疫合併」（パセドウ病、橋本病、自己抗体陽性）の4病型とした。

【結果】(1) HLAに関しては、従来の報告の通り、全1型糖尿病、および急性、緩徐、甲狀腺自己免疫合併のサブタイプにおいて、DRB1*0405–DQB1*0401, DRB1*0901–DQB1*0303, DRB1*0802–DQB1*0302のハプロタイプが感受性、DRB1*1501–DQB1*0602, DRB1*1502–DQB1*0601が抵抗性として強く関連していた。劇症では、DRB1*0405–DQB1*0401のみが感受性として有意であった。

(2) 非HLAに関しては、全1型糖尿病では、INS（オッズ比[OR]3.64, p=3.2x10^-7), IL2RA（OR1.17, p=0.029）, ERBB3（OR1.48, p=6.5x10^-7）, CLEC16A（OR1.23, p=0.035）, IL7R（OR1.26, p=0.018）が有意に関連していた。サブタイプ別の解析では、急性においてはINS（OR6.28, p=0.0001）, ERBB3（OR1.50, p=5.5x10^-7）, 緩徐ではIL7R（OR1.35, p=0.038）, IFIH1（OR1.37, p=0.027）が有意であり、甲狀腺自己免疫合併ではHLA-4（OR1.48, p=0.008）, IL2RA（OR1.44, p=0.004）, ERBB3（OR1.70, p=0.0002）が、それぞれ有意に関連していたが、劇症ではすべて有意でなかった。

(3) 非HLAの7SNPのリスクアーサ数による解析では、リスクアーサが増えるにつれてORは増加し、12個以上の場合は全部以下の場合に比べてORは3.80であり、全体でリスクアーサ1個あたりの平均ORは1.22であった（p=3.7x10^-7）。

【結論】日本人1型糖尿病においても、真ん中で同定された非HLA感受性遺伝子の関連と1型糖尿病サブタイプにおける関連の違いも認められた。しかし、非HLA感受性遺伝子の関連は全体でもHLA–DR1/B1, HLA–DQB1と比較して弱く、今後、日本人において進行中のGWASの成果が期待される。また、GWASで検出できない比較的まれな変異の解析も必要である。この点に関して、次世代シーケンスによる解析を現在行っている。その結果も発表したい。

テキストの一部が破損しているため、正確な文脈を保つには一部の入力を必要とする。
シンポジウム3 : 1型糖尿病の新しい展開
Symposium3: New Aspects of Type 1 Diabetes Mellitus

S3-5

1型糖尿病の機序変：症例1型糖尿病と緩徐進行1型糖尿病の対照から
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【目的・方法】症例1型糖尿病は激急に発症する著明な高血糖とカテアシドーシスを特徴とすることが、その病因は明らかでない。我々はenterovirus感染をそれらに引き続くchemokine/cytokine応答について、症例1型糖尿病3例と緩徐進行型1型糖尿病（SPIDDM）の肺を対比しつつ、組織学的に検討した。

【結果】症例1型糖尿病では3例全例にenterovirus capsid protein（VPI）が陽性であり、それは結核の外分泌腺細胞を示す、SPIDDM、非糖尿病、慢性腎炎の肺ではVPIは認められなかった。症例1型糖尿病では3例ともislet量とB細胞量が著明に減少し、1例ではα細胞量も減少していた。2例もほぼ100%に肺島炎が見られ、浸潤細胞のほとんどはCD8陽性T細胞とmacrophageであった。SPIDDM例での肺島炎はごく軽微なものであった。以上1型糖尿病肺では、CXCL10に平均93%の肺島に強く発現しており、すべての肺島内内分泌細胞（β細胞、α細胞、その他の細胞）に発現がみられた。また、肺島内・周囲にはCXCL10の受容体（CXCR3）を発現したT細胞が浸潤しており、さらに興味あることに、β細胞はCXCL10を誘導するIFN-γをCXCL10とも共に発現していた。一方、このようなchemokine/cytokineの発現はSPIDDM例ではみられなかった。

CD11c陽性dendritic cellが症例1型糖尿病の肺島内・周囲に見られたが、SPIDDM例、非糖尿病例、慢性腎炎例の肺では見られなかった。MHClass II分子が症例1型糖尿病肺島B細胞に見られ、肺島内・周囲の血管内皮細胞にも過剰発現していた。さらに、MHC class II分子も肺島に強く発現していた。

【結論】enterovirusが肺に感染すると、肺島細胞がIFN-γ、CXCL10を発現し、CXCL10がCXCR3を介してautoreactive T cellやmacrophageを活性化・遊走する。これら細胞は肺島内でIFN-γなどのcytokineを産生してβ細胞を障害するだけでなく、残存β細胞におけるCXCL10の発現を誘導し、さらに炎症反応の維持増幅が行われる。このcircuitが症例1型糖尿病における激急なβ細胞障害を引き起こしているが、SPIDDMにはこの機序は働いていないと考えられる。

S3-6

GAD抗体陽性NIDDMにおける進展予知マーカーの検討
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【目的】GAD抗体陽性NIDDMは、しばしばインスリン依存状態へ陥ることが知られており、特に高GAD抗体価で高率である。しかし、その予知率は60〜70%とされており、効率的かつ効果的な進展予防のために、他の因子と組み合わせたより正確な進展予知が必要である。今回我々は、新たな進展予知マーカーの同定を目的として“自己抗体の量と質”の面から検討した。

【対象と方法】食事・経口血糖降下薬治療中のGAD抗体陽性NIDDM 47例（M/F=22:25、平均糖尿病診断年齢48.1±12.5歳、平均罹病期間4.9±5.8年）において、IAA、IA-2抗体、ZnT8抗体、GAD65抗体エピトープのインスリン治療開始予知における有用性を検討した。GAD抗体に加えIAA、IA-2抗体、ZnT8抗体のいずれか陽性の場合、multiple islet Abs陽性と表現した。

【結果】追跡期間中（1〜9年）、17例（36%）において血糖コントロールのためインスリン治療が開始された。患者登録時血糖値におけるIAA、IA-2抗体、ZnT8抗体の陽性率は、それぞれ12例（26%）、7例（15%）、9例（19%）であり、17例（36%）がmultiple islet Abs陽性、30例（64%）がGAD抗体単独陽性であった。また、30例（64%）がGAD65の抗体、エピトープ、アミノ酸245〜306とC末端エピトープ（アミノ酸443〜585）を認識し、それらのうち2例（23%）がN末端エピトープ（アミノ酸1〜245）を認識していた。インスリン治療開始後の関係をKaplan-Meier法で解析したところ、IAA陽性例が陰性例に比べ早期にインスリン治療が開始されていたが、統計学的に有意差は認められなかった。log-rank値でIA-2抗体とZnT8抗体との間違は示されなかった。さらに、高GAD抗体価（P=0.003）、GAD65中央エピトープを認識するGAD抗体の存在（P=0.002）、multiple islet Abs陽性（P=0.002）がインスリン治療開始と強く関連していた。しかし、GAD抗体価、GAD65中央エピトープ保有例のどちらにおいても、GAD抗体陽性例では、multiple islet Abs陽性例に比べインスリン治療開始時期は有意に低く（<30%）、ロジスティック解析では、multiple islet Abs陽性が唯一の独立した危険因子であった（OR, 13.8, P=0.001）。

【結論】GAD抗体陽性NIDDMにおける進展予知においては、GAD抗体価やGAD抗体エピトープよりもIAA、IA-2抗体、ZnT8抗体の測定が重要であることが明らかになった。このようなハイリスク患者では、内因性インスリン分泌機能の経過を見ながら、インスリン療法開始のタイミングを見越しなければならないことが重要と考えられる。
シンポジウム3: 1型糖尿病の新しい展開

Symposium3: New Aspects of Type 1 Diabetes Mellitus

座長 花房 俊昭 小林 哲郎

1型糖尿病に対する臨床で島移植の最前線
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Baylor University Medical Center, Dallas, USA
金沢大学大学院医学系研究科臓器機能制御学

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松本 優一1

脾島移植は、1型糖尿病患者をインスリン注射から開放できる可能性がある。しかし、脾島分離成功率が低い事、インスリン離脱のため複数の脾島を必要とする事、長期インスリン離脱の維持が難しい事が問題である。当施設では、日本で心停止ドナーのための脾島分離方法を脳死ドナーに適応化することを、脾島分離成功率をほぼ100%にすることに成功した。最近では、サイモグロプランを免疫抑制の導入に用い、IL-1βおよびTNF-αをブロックすることで1回の移植でインスリン離脱を達成できた。

今回は、長期インスリン離脱を目指し、慢性期のグラフ特異性の原因を検討した。当施設で2007年以降に実施した5例の脾島移植患者は全例インスリン離脱を達成したが、2例は慢性期のグラフ特異性によりインスリン治療を再開している。全例で同様の維持免疫抑制療法を実施しているため、慢性期特異の主因は同種移植の症例でも慢性期の再燃と考え、移植後の脾島抗原特異的Ｔ細胞の反応について検討を行った。

脾島移植後1年以上が経過した1型糖尿病患者4例を対象とし、インスリン離脱中に我々が開発したEpitope Maximum（EpiMax）を実施した。方法は患者の末梢血単核球をIL-2の存在下にGAD65抗原のペプチドブートラック（GAD65−pp; #1−#14, 585アミノ酸配列を網羅）で刺激し、サイトカイン分泌をCytokine−multiplex systemで、CD4/CD8陽性T細胞内のサイトカイン産生をフローサイトメトリーにて解析した。移植後脾島機能は、空腹時Cペプチドおよび生着脾島数を反映するSUITO indexにて評価した。

GAD65特異的T細胞の反応について、2例はIFN-γ、TNF-α、IL-13の分泌が亢進しており、GAD65−pp−#4にてピークを示していた（陽性群）。細胞内サイトカイン解析では、陽性群の2例においてGAD65−pp−#4特異的なCD4およびCD8陽性T細胞の両方にてIFN-γの産生が見られた。移植終了1年後の空腹時Cペプチドは、陽性群で215±35, 陰性群で0.70±0.14 (ng/ml), SUITO indexは陰性群で65.7±15.4, 陽性群で15.4±4.0であり、陰性群の2例のみがインスリン離脱を維持していた。

EpiMaxを用いる事で、インスリン離脱中に慢性期のグラフ特異性に脾島抗原特異的T細胞の反応が関与している事が確認できた。これにより早期に移植後の自己免疫再燃を発見でき、慢性期のグラフ特異性克服できる可能性があると考えられた。
シンポジウム4：膵内分泌機能の再生～移植から再生医療まで～

Symposium4: Revitalization of Pancreatic Endocrine Function: From Medical Transplantation to Regenerative Medicine

座長 荒木 栄一 稲垣 暢也

S4-1

ES/IPS細胞から膵細胞系譜への分化誘導
熊本大学発生医学研究所

私たちは、現在はマウス・ヒトES細胞およびIPS細胞を用いて膵臓の分化誘導研究に取り組んでいる。ES/IPS細胞を用いて、正常な発生分化過程を試験管内で再現させながら、分化誘導の分子機構について解析できる系統を構築し、再生医療への応用を目指している。

ES/IPS細胞から膵臓前駆細胞を効率よく誘導する
M15細胞を用いた支持細胞系の方法では、特異的に液性成熟因子を添加することで、3細葉由来の細胞を特異的に誘導することが可能となった。とりわけ膵臓への分化誘導の分子機序について様々な検討の結果、基底膜成分であるラミンからのシグナルが重要な役割を担っていることが分かった。さらに人工基底膜（sBM）を用いることで、ES細胞から内胚葉、膵臓前駆細胞、インスリン産生細胞への分化誘導を再構築した。様々な解析の結果、sBMおよびES細胞由来の基底膜分子による細胞外被小環境（ニッチ）の形成が重要であることが示唆された。ES細胞からの分化誘導の分子機構について考察しながら、我々の最近の結果について紹介したいと思います。

S4-2

分化転換によるインスリン分泌細胞の分化誘導
神戸大学大学院医学研究科細胞分子医学1、神戸大学大学院医学研究科糖尿病、代謝・内分泌科学2、戦略的創造研究推進事業（CREST）3

南 幸太郎1、清野 進1,3

インスリン欠乏状態の糖尿病に対する治療としてインスリンが用いられている。しかしながら、実際の膵β細胞が血糖変動に対応してインスリンの分泌量を細かく調節できるのに対して、インスリンでは血糖値を生理的範囲にコントロールすることは困難であるため、糖尿病によって引き起こされる様々な合併症を完全に阻止することは困難である。そこで期待されるのが、いわゆる幹細胞からインスリン分泌細胞を誘導して移植治療する再生医療である。膵臓幹（ES）細胞から実際にインスリン分泌細胞を誘導できることが報告されているが、その使用は臨床応用の面でいくつかの障害を抱えている。かつて話題のIPS細胞についても倫理的問題を除けば、インスリン分泌細胞の作製においてES細胞と同様の問題が残る。一方、膵臓管細胞やoβ細胞、骨髄細胞などの成体に存在する非β細胞からインスリン分泌細胞を誘導できるとの報告もある。成体の膵β細胞はβ細胞自体の複製によって維持されるとの報告がなされていたが、in vitroで異なるメカニズムでもβ細胞を誘導できる可能性があると考えるべきであろう。我々は、成体マウス膵外分野組織に存在する膵臓前細胞がin vitroでインスリン分泌細胞へ分化転換できることを直接的に証明した。膵臓前細胞は膵臓で最も動量に存在する細胞種であり、膵臓移植の際にはその副産物として大量に入手することが可能である。膵臓の細胞はすべて共通の幹細胞から分化することから、膵臓前細胞から移植可能なインスリン分泌細胞（膵β細胞）を作製することは現実性が高いと思われる。実際に、in vivoでは遺伝子導入によって膵臓前細胞がβ細胞と区別のつかない細胞に変化することが報告された。しかしながらこれまでのところ、膵臓前細胞からin vitroで誘導したインスリン分泌細胞は、グルコースなどその他の刺激によってインスリンを分泌するものの、その分泌量は実際の膵β細胞に比較すると低いという問題がある。実際の膵β細胞が3次元の構造である腎臓を形成していることを考えると、細胞間相互作用の形成が膵β細胞の機能的な最終分化に必要なものかもしれない。さらに、膵臓前細胞からインスリン分泌細胞への分化転換のメカニズムを明らかにするとともに、ヒトの組織を用いた検討を進めることができ糖尿病再生医療の実現のために重要であると考えている。

—S-15—
シンポジウム4：膵内分泌機能の再生 ～移植から再生医療まで～
Symposium4: Revitalization of Pancreatic Endocrine Function: From Medical Transplantation to Regenerative Medicine
座長 荒木 栄一 稲垣 暢也

S4-3

膵管上皮細胞からβ細胞への分化メカニズム
九州大学大学院医学研究院幹細胞ユニット糖尿病遺伝子分野
稲田 明理

生後のβ細胞の供給源については、多くの学者によって様々な仮説が提唱されてきた。我々は膵管上皮細胞に注目し、膵管上皮細胞が幹細胞と考えられるので、ライス島が生後常に膵管上皮細胞に篭接して存在することと急激にβ細胞数が増加する成長期や成体の組織再生時において、インスリン陽性細胞が膵管上皮細胞に多くみられるからである。また、膵管上皮細胞からインスリンやクサゴンがbuddingしている現象（Neogenesis）は、肥満のヒトやIFN-γ過剰発現トランスジェニックマウス、TGF-α過剰発現トランスジェニックマウスにおいても報告されている。この他に、ヒトの膵管上皮細胞を長期培養するとインスリン陽性細胞が得られることが報告されているからの報告は、β細胞を供給する幹細胞が膵管上皮に存在する可能性が高いことを示唆している。しかし、別の細胞から分化した可能性や、他の細胞が混じっていたという疑問が残り、膵管上皮細胞からβ細胞に分化した過程は理解に至らなかった。そこで我々は、膵管上皮細胞そのものを標識して追跡し、生後成長期や成長にともないβ細胞の分化が有意に高い時期には、膵管上皮細胞に存在するβ細胞の前駆細胞からβ細胞が生成され供給されることを直接的に証明した。

そこで、今回の研究ではその続きとして、膵管上皮細胞からβ細胞へ分化するメカニズムを明らかにすることを目的とする。

膵管上皮細胞からβ細胞へ分化するためには必要不可欠の遺伝子の発現である「転写因子Pdx-1」に注目した。

Pdx-1は膵臓の発生やendocrine cellの分化に必要な転写因子であり、生後は主にβ細胞に存在するが、肥満のヒトやマウスにおいては生後成長期や成長再発時には膵管上皮細胞にPdx-1蛋白質の発現が見られることが報告されている。そこで、「膵管上皮細胞中のPdx-1の発現がβ細胞への分化に必要ではないか」と仮説を立てて、膵管上皮細胞特異的Pdx-1欠損マウスを作製し、膵管上皮細胞に発現するPdx-1の役割を検討した。

その結果、膵管上皮細胞特異的Pdx-1欠損マウスでは生後の成長期後β細胞数が不足し、血糖値が上昇していた。組織再生実験においては、欠損マウスでは膵管上皮中のインスリン陽性細胞や新生したβ細胞が非常に少なかった。

以上の結果から、膵管上皮細胞中のPdx-1の発現がβ細胞への分化に必須であり、この供給が生体のホモオステシスに貢献していることが明らかとなった。

β細胞の源となる細胞から再生・増殖が可能になるれば、糖尿病患者に長期にわたりβ細胞を供給することができる。
シンポジウム4：腸内分泌機能の再生～移植から再生医療まで～

Symposium 4: Revitalization of Pancreatic Endocrine Function: From Medical Transplantation to Regenerative Medicine

座長
荒木 栄一
稲垣 暢也

S4-5

わが国の腸臓移植の現状と課題
国立病院機構九州東病院臨床研究センター

腸臓移植は、内因性インスリン分泌の枯渇した1型糖尿病患者の根治療法として既に臨床応用されており、わが国においても脳死、心停止、生体ドナーから
の腸臓移植が行われている。今回、わが国の腸臓移植の現状と課題に触れ報告する。【症例数】1997年の腸
臓移植施行以来に脳死腸臓移植が4例、心停止腸臓移植が1例施行されている。腸臓移植術後、2010年1
月末までに64例の脳死腸臓移植、2例の心停止腸臓移植が実施された。生体腸臓移植2004年に本邦1例目
が国立病院機構九州東病院で施行され、2009年12月ま
でに20例行われている。【方法】脳死・心停止腸臓移植はドナーにより全例・十二指腸潰瘍を指摘し、軽いインスリ
ン依存型2型糖尿病である。生体腸臓移植で、ドナ
ーの膵体尾部を摘出し、膵臓および肺臓で保存する
場合がある。生体腸臓移植にて、膵臓・肺臓・生
体ともに50%以上が膵・腎同時移植である。免疫抑制
剤は、カールクニヒルリンヒトリマフィン、MMF、ステ
ロイド、抗CD25抗体の4剤併用療法とする。生体で
ABO血液型不適合の場合には、腎移植と同時にプ
ートコールで行う。【成績】64例の脳死腸臓移植では手
術死亡例はなかったが、移植後急性期に血栓症で5例
が移植腸臓摘出術となった。他に、もともとインスリン離
脱した。移植腎は3例が透析再導入。1例が二次移植を
受けた。36例まで長期成績をみると、患者年生
存率100%、腎臓5年生存率80.7%、腎臓5年生存率（腎
臓同時移植の場合は）90.6%と欧米を凌駕する成績であ
る。生体腸臓移植も良好な成績を得ており、当院の
15例では手術死亡例はなく、14例がインスリン離
脱し（93.3%）、全例透析離脱した（100%）。初期の3例に
脳液漏を認めた。また感染症としてサイトメガロウイ
ルス、PC肺炎がみられた。現在3例が少量のインスリ
ンを使用しているが、低血糖発作は認めていない。
ABO不適合同生体腸臓移植は当院で5例施行したが、現
在まで抗CD20関連性拒絶反応はなく、適合例と同等の
成績を示している。【考察・結論】腸臓移植は末期腎
不全を伴った1型糖尿病患者に対し腎臓同時移植の形
で施行されることが多く、低血糖発作の消失、インス
リン離脱、透析離脱により患者QOLの改善のみならず、
予後の改善も期待され、極めて有効な治療法である。
課題は、他の臓器移植と同様に感染症の安全性と、ド
ナー不足である。臓器移植術の成熟を進め今後のド
ナー増加が期待される。また重要な適応検討を行っ
た上で施行する、生体腸臓移植も、安全性、有効性が
臨床的に確認され、ABO不適合例でも十分に可能で、
脳死腸臓移植待機困難な重症患者に対してのオプショ
ンとして意義を有する。

S4-6

腸島移植の現状と課題
京都大学医学部附属病院臓器移植医学部

腸臓移植は、重症インスリン依存状態糖尿病患者に
対して、血糖調節手段としてインスリンを介する移植
臓器の採用が期待される。2005年に報告されたエドモントンコレド移植の報告以来、2006年
に報告された京都大学医学部附属病院での新たな腸
臓移植術例が発表されて以来、腸臓移植の安全性と有
効性が示唆されている。京都大学医学部附属病院で
の腸臓移植術例は、2006年に報告されており、腸
臓移植の安全性が示唆されている。京都大学医学
部附属病院での腸臓移植術例は、2006年に報告されており、腸
臓移植の安全性が示唆されている。京都大学医学
部附属病院での腸臓移植術例は、2006年に報告されており、腸
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部附属病院での腸臓移植術例は、2006年に報告されており、腸
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部附属病院での腸臓移植術例は、2006年に報告されており、腸
Integrating metabolic control by the NAD$^+$ sensor SIRT1
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A century after the identification of a co-enzymatic activity for NAD$^+$, NAD$^+$ metabolism has come in the spotlight again due to the potential therapeutic relevance of a set of enzymes whose activity is tightly regulated by the balance between the oxidized and reduced forms of this metabolite. In fact, the actions of NAD$^+$ have been extended from being an oxidoreductase cofactor for single enzymatic activities to acting as a substrate for a wide range of proteins. These include NAD$^+$-dependent sirtuin protein deacetylase, poly (ADP-ribose) polymersases, and transcription factors that affect a large array of cellular functions. Through these effects NAD$^+$ provides a direct link between the cellular redox status and the control of signaling and transcriptional events. Of particular interest within the metabolic/endocrine arena are the recent results, which indicate that the regulation of these NAD$^+$-dependent pathways may have a major contribution to oxidative metabolism and lifespan extension. I will provide an integrated view on how the control of NAD$^+$ production and cycling alters transcriptional pathways via NAD$^+$’s commanding role on a cofactor network that involves SIRT1 and PGC-1α. As such the modulation of NAD$^+$-producing and -consuming pathways have a major physiological impact and hold promise for the prevention and treatment of metabolic disease.

In vivo imaging reveals adipose tissue remodeling in obesity, and thrombosis: Parenchymal and stromal cross talks
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西村 之, 長崎 実雄1，真鍋 一郎2，
江藤 浩之3, 門脇 孝1，永井 良三3

Metabolic syndrome is a major risk factor of cardiovascular events, and obese visceral adipose tissue remodeling based on chronic inflammation play a central role. To assess dynamic interplay between multiple cell-types in obese adipose, a visualization technique in vivo was developed. Using this technique, we identified inflammatory cell clusters containing angiogenesis and adipogenesis in obese adipose tissue (Nishimura et al, 2007 Diabetes). We also found increased leukocyte–platelet–endothelial cell interactions in obese adipose tissue microcirculation, which were indicative of local chronic inflammation (Nishimura et al, 2008 J Clin Invest). Local platelet activation and up-regulation of adhesion molecules contributed to this inflammatory processes.

Recently, we also found that large numbers of CD8+$^+$ effector T cells infiltrated into obese adipose tissue (Nishimura et al, 2009 Nat Med). The infiltration of CD8+$^+$ T cells preceded the accumulation of macrophages. Immunological and genetic depletion of CD8+$^+$ T cells reduced inflammatory (M1) macrophage infiltration and adipose tissue inflammation, and ameliorated systemic insulin resistance. Co-culture and other in vitro experiments revealed major interactions between CD8+$^+$ T cells, macrophages, and hypertrophied adipocytes. Infiltration of CD8+$^+$ T cells is essential for inflammatory macrophage recruitment into obese adipose, and the initiation and development of adipose inflammation.

This imaging technique also visualizes single platelet kinetics in vivo, which enable us to analyze cell kinetics thrombus formation in single platelet level by laser-induced–injury. Using this technique, we revealed Lnk/Sh2b3 regulates integrin alpha-IIb-beta–3 outside–in signaling in platelets leading to stabilization of developing thrombus in vivo (Nishimura et al, 2009 J Clin Invest). In addition, we elucidated the functional role of human–iPS–derived–platelet in vivo (under review).

Our results clearly demonstrated the power of our imaging technique to analyze complex cellular interplays in vivo, especially parenchymal and stromal cell cross talks, and to evaluate new therapeutic interventions against them.
Regulation of body weight and insulin sensitivity by hypothalamic activation of interleukin-10 signaling

Interleukin-10 (IL-10) is a well known anti-inflammatory cytokine. Several human studies have reported that the expression level of IL-10 is correlated to insulin sensitivity, but the underlying mechanisms are not fully understood. In this study, we first examined the effect of adenovirus-mediated systemic overexpression of IL-10 (adeno-IL-10) on body weight, and insulin sensitivity in mice. Aeno-IL-10 lowered glucose profiles in insulin tolerance test (ITT) and glucose tolerance test (GTT). Body weight was lower without any changes of food intake. The expression of genes involved in mitochondrialOXPHOS and β-oxidation in skeletal muscle was increased, which might be related to the reduced body weight of the mice. Because treatment with recombinant IL-10 did not increase those genes in cultured L6 myotube, the existence of indirect mechanisms was suggested in adeno-IL-10-injected mice. Because STAT3 phosphorylation in hypothalamus was increased in the mice, we next examined the role of hypothalamic IL-10 signaling in the regulation of body weight and insulin sensitivity. We performed intracerebroventricular (ICV) injection of neutralizing anti-IL-10 antibody in the adeno-IL-10-injected mice. Body weight was increased and the increased expression of those genes in skeletal muscle was diminished by the ICV injection of the antibody. Food intake was not altered and glucose profile in ITT and GTT was elevated. These results suggest that the effects of adeno-IL-10 on body weight and insulin sensitivity is mediated, at least in part, by the activation of IL-10 signaling in hypothalamus. To directly know the effects of activation of IL-10 signaling in hypothalamus, we next injected recombinant IL-10 intracerebroventricularly. Body weight was decreased and the expression of the mitochondria–related genes in skeletal muscle was increased. Food intake was not altered and glucose profile in ITT and GTT was lowered in these mice. In conclusion, the activation of IL-10 signaling in hypothalamus decreases body weight with increased expression of β-oxidation and OXPHOS-related genes in skeletal muscle and improved whole body insulin sensitivity.

Oxidative stress and glutathione metabolism in adipose tissue

Oxidative stress plays an important role in obesity-related metabolic diseases. Fat accumulation correlated with systemic oxidative stress in humans and mice. Production of ROS increased selectively in adipose tissue of obese mice, accompanied by augmented expression of NADPH oxidase. In cultured adipocytes, oxidative stress caused dysregulated production of adipocytokines, including adiponectin, plasminogen activator inhibitor–1, IL-6, and monocyte chemotactic protein–1. Glutathione peroxidase (GPX) is an antioxidant enzyme downregulated in adipose tissue of obese mice. Obese mice showed not only a decrease in cellular activity of GPXs (GPX1, −4, and −7) but also an increase in expression of gamma-glutamylcysteine synthetase, a rate-limiting enzyme for de novo GSH synthesis, resulting in increased GSH contents in adipose tissue. These alterations in glutathione metabolism were also observed during differentiation of 3T3-L1 cells and their exposure to insulin, FFAs, or H2O2. Inhibition of GPX activity, addition of GSH resulted in impaired insulin signaling in 3T3-L1 adipocytes. These results suggest that oxidative stress, decreased GPX activity and increased GSH content are involved in the pathogenesis of metabolic syndrome.
S5-5

**Influence of visceral adiposity on insulin secretory aberrations: A lipotoxic process**

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島袋 充生，益崎 裕章

Excess abdominal fat is predictive of insulin resistance and the presence of related metabolic abnormalities currently referred to as the metabolic syndrome (MetS). Despite the fact that abdominal obesity is a highly prevalent feature of MetS, the mechanisms by which abdominal obesity is causally related to the MetS are not fully elucidated. Amount of visceral adipose fat, not that of subcutaneous abdominal fat, is a quite dominant correlate of metabolic abnormalities observed in overweight/obese patients. When categorized by whole-body distribution of adiposity, insulin sensitivity is well explained by ectopic fat deposition in insulin-sensitive non-adipose tissue. Obese subjects constantly deliver more lipids and dysregulated adipocytokine than normal lean subjects; visceral fat obesity can produce more pro-atherogenic adipocytokine, including free fatty acids (FFA), than subcutaneous fat obesity. The contribution of enhanced delivery of FFA to pancreatic B cells and other insulin-sensitive organs have been considered as pathophysiological mechanism(s) of metabolic derangements. Experimentally, this phenomenon, named as lipotoxicity, has been shown to be one possible mechanism of obesity-related consequences. Here, we studied influence of visceral adiposity and FFA on insulin secretory aberrations and insulin sensitivity in a large clinical population, and we will discuss the possibility of visceral adiposity in lipotoxic process.

S5-6

**Pathophysiological roles of adiponectin and AdipoRs in metabolic syndrome**

東京大学大学院医学系研究科糖尿病・代謝内科

山内 敏正，門脇 孝

Type 2 diabetes has increased dramatically in Japan, which appears to be associated with high-fat diet and underexercise.

We identified adiponectin as therapeutic target adipokine for insulin resistance, metabolic syndrome and type 2 diabetes by using the combination of genome-wide scanning and DNA chips, and showed that adiponectin exerts its insulin sensitizing action via several key molecules such as AMPK and PPAR. Genetic studies on SNP of adiponectin gene as well as functional analyses including transgenic or knock-out mice suggested that reduced adiponectin levels play a causal role in the development of metabolic syndrome and type 2 diabetes.

We discovered adiponectin receptors (AdipoR1 and R2) by expression cloning and found that AdipoRs are also decreased in obesity. We generated AdipoR1 and R2 double knockout mice and showed that AdipoR1 and R2 serve as the major receptors for adiponectin in vivo and play important roles in the regulation of glucose and lipid metabolism, inflammation and oxidative stress. From these data, we have been proposing “adiponectin hypothesis” that reduced plasma adiponectin levels as well as reduced adiponectin receptors play important roles in insulin resistance, metabolic syndrome and type 2 diabetes linked to obesity.

Finally, we found that osmotin, present in fruits and vegetables, activated AMPK via AdipoRs in myocytes. Moreover, the human adiponectin mutations analyses led to identification of high molecular weight (HMW) adiponectin as most active form, and then we developed an ELISA system and showed that measurement of HMW is useful for the prediction of insulin resistance and metabolic syndrome.
糖尿病予防のための戦略研究J-DOIT1,2,3
国立国際医療研究センター4, 国際協力医学会研究振興財団5
野田 光彦1,2, 加藤 昌之1, 泉 和生1

糖尿病は心血管疾患のリスクを高め、神経障害、腎臓、心臓、足部などの合併症を併発する。2007年
の国民健康栄養調査によると、わが国20歳以上の
健診で見出された糖尿病
のハイリスク群2,904人の登録被験者を対象として、
糖尿病について一般的な情報提供を受ける自立群（対
照群）と、それに加えて個別に非対立型の生活習慣介
入を1年間受ける支援群（介入群）の2群にクラスター
ランダム化して糖尿病の発症を比較する。17の地域・職
域（43クラスター）の健康診断実施団体が参加し、
3年間の追跡で、非対立型の生活習慣介入による糖尿
病発症率低下効果を検証している。

J-DOIT2は2型糖尿病患者の治療中断を減らすため
の研究である。本格的な研究の実施に先立って、1,585
人の被験者により2007年12月までバイオニック型が実
施され、その結果を踏まえて大規模研究の計画が策定
された。大規模研究では、かかりつけ医に通院する2
型糖尿病患者2,750人を15の医師会から登録する計画
とし、1医師会を2クラスターに分割してクラスター単
位での介入を行うこととしている。実際には11の医師
会の参加を得て進行している。

2型糖尿病の血管合併症に対する予防法を検討する
のがJ-DOIT2である。生活習慣、血圧、血圧、血圧
を強力に管理することにより、通常治療より大血管合
併症の発症、進展抑制に優れたことを検証する。血
圧、血圧、血圧を目標値を、従来治療群では日本糖尿
病学会で定める目標値として、強化治療群ではこれらを
より高く（厳しく）設定している。強化治療群では血
圧、血圧、血圧が目標に達するまでプロトコールに従っ
たステップアップ治療を行うこととし、81の医療機
関、登録被験者2,542人（従来治療群1,271人、強化療
法群1,271人）により2013年3月までの追跡期間で研究
が進行中である。

以上、本発表では現在実施している「糖尿病予防の
ための戦略研究」について概説する。
シンポジウム6：国内大規模臨床試験
Symposium 6: Diabetes-related Large-scale Clinical Trials in Japan
座長 山田 信博 斎藤 重幸

S6-3

2型糖尿病発症阻止をめざし、IGTに対する vogliboseによる介入試験
順天堂大学医学部内科学
河盛 隆造

日本人IGT例を対象に、ボグリボースかプラセボを投與し、2型糖尿病への進展を比較検討するRCTを行った（Kawamori R et al, Lancet 2009; 373: 1607-1614）。多施設無作為割り付け、二次盲検試験とした。
1780名の対象者はWHOによるIGTの定義を満たした。
（1）重症性高血圧症
（2）脂質異常症
（3）BMI≥25。
（4）2型糖尿病の家族歴、のいずれかを有する例とし、これらに加え、（5）開始時のOGTT2h血糖値（170～199mg/dL）を割付因子とし、これらの個数が「2個以下」「3個以上」に分類し、対象患者をボグリボース（V）群（0.6mg/日、毎食前投与）897名、プラセボ（P）群883名に無作為に割り付け、登録時のOGTT全例（平均BMI 26弱）の平均血清インスリン値は、前94.9分25分50.8時間値96.0μU/mlであり、最近発表された米国のACTNow研究におけるIGT（平均BMI 34）のインスリン値と同等であったことは注目に値する。両群とも標準的な食事・運動療法を継続して实施した。
観察期間は2型糖尿病への進展か、OGTT結果が正常型へ移行した時点で終了とし、IGTのままで144週が經過した時点で終了とした。
評価項目は主要評価項目をIGTから2型糖尿病への移行、次に評価項目をIGTから正常型への移行とした。
年2回のOGTTと3ヶ月毎の空腹時血糖値測定で評価した。被験者背景にはV群、P群ともに有意差を認めなかった。
結果：2型DMへの移行率：エンドポイントまでの平均投與期間は339日。2型糖尿病累積移行率は18週の時点でP群9.4%、V群3.6%であった。スタディ終了までの2型糖尿病累積移行者はP群106/881、V群50/897名と、ボグリボース群で2型DM発症リスクが40.5%減少した（ハザード比0.595）。リスクファクター数3個以上の層ではV群で有意に2型DM発症リスクが軽減（P群に比し39.3%減）した。
OGTT正常型への移行率：全観察期間における正常型への移行者数はP群454/881、V群509/897名で、正常型移行率の比率1.539と、V群で有意に高かった。
大血管症発症率：ボグリボース群がプラセボ群を下回った（ボグリボース群12/897例 [1%]、プラセボ群18/881例 [2%]）。
2型糖尿病への進展因子、α-グロコースイズ阻害有効例の特質を抽出した結果を発表する。

S6-4

糖尿病患者の高血糖治療と糖尿病発症予防の高血糖治療
大阪大学大学院老年・腎臓内科
楽木 宏宏

糖尿病診療に関連する高血糖治療のエビデンスが本邦でも蓄積されてきた。
I. 糖尿病合併高血圧患者での降圧治療
降圧治療開始基準についての明確なエビデンスはなく、医師教育と介入試験での到達高血圧とイベント発症の関係から設定された目標血圧レベル（130/80mmHg未満）より高い血圧値の患者が降圧治療対象とされていている。
II. 某種の糖尿病合併高血圧患者16,806例を対象にエビデンスを提供するARB（アダルシン）長期投与による心血管合併症を検討した研究である。
HbA1cを6.5%未満、血圧を130/80mmHg未満にコントロールした群で有意な心血管合併症の発症抑制が認められた。
III. 糖尿病合併高血圧患者有意にイベント発症が多かった。さらに低い血圧レベルを目標とすべきか、これらの試験結果から不明である。

一方、75歳以上の高齢者を対象にしたエビデンスはほとんどなく、the lower the betterの観点に立って糖尿病合併症で130/80mmHg未満を目指すべきかどうかは個々の病態に基づいて判断する必要がある。

2. 第1選択薬と併用療法
糖尿病合併高血圧患者約2000例についてARB（カシタサルタ）群とCa拮抗薬（アズミンゾン）群を比較した。
CASE-Jでは、糖尿病合併高血圧198例について、130mmHg未満群でイベント発症に有意差を認めなかった。
併用療法にてうまくいかなかったエビデンスはまだない。

3. 糖尿病性腎臓の発症・進展抑制
MEDMINDでは、ACE阻害薬（エナプラリル）群とCa拮抗薬（レニフェジン）群でアルブミン尿に関する腎臓進展に差を認めなかった。一方、SMARTでは、ARB（パルサルタ）群がCa拮抗薬（アンジオテンシン）群よりアルブミン尿改善に優れていた。
INNOVATIONでは、ARB（アズミンゾン）群で降圧効果に依存せず用量依存性の用量アルブミン尿遮断を示した。試験において結果に差があるのは、対象病態、症例数、観察期間などの違いによる可能性も考えられる。

II. 糖尿病末梢症のメタボリックシンドロームを合併した高血糖患者での降圧治療
治療開始血圧基準について明確な根拠となるエビデンスはなく、インスリン抵抗性改善効果を後述する糖尿病新規発症抑制効果の観点からレニン-アンジオテンシン系合併薬が推奨されるが、心血管病発症抑制に関するエビデンスはない。
III. 高血糖患者における糖尿病新規発症抑制効果
CASE-JではCa拮抗薬群との比較で、HIJ-CREATE, KYOTO-HEART studyではプラセボ群との比較で、ARB群での糖尿病新規発症抑制効果が示されている。
シンポジウム6：国内大規模臨床試験
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S6-5

我が国における脂質関連大規模臨床試験
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横手幸太郎12

血清/ブラドウ糖が糖尿病の病態における中心的な役者であることに異論をもつ者はいない。一方、高脂肪食がインスリン抵抗性を招き、肥満や高脂血症が大血管障害（動脈硬化）を助長するなど、2型糖尿病において、脂質が患者の予後を左右することも、かつジョスリン博士が20世紀初頭すでに指摘していた事実である。

脂質異常の合併は、糖尿病患者における心血管リスクをさらに上昇させ、また、主にスタチンを用いた脂質低下治療がそのリスクを軽減することが、HPSやCARDSなど海外の大規模試験により証明されてきた。しかし、そもそも欧米人に比べて冠動脈リスクが低いとされる日本人において、脂質への介入が糖尿病の合併症予防に有効か否かは明らかでなかった。

糖尿病患者のみを対象とする脂質介入試験が我が国で実施されていないが、高脂血症全般を対象とした研究において、2型糖尿病またはそれに準ずるサブグループの解析結果が、近年、相次いで報告されている。高LDLコレステロール（LDLC）血症患者にスタチン投与した観察研究J-LITでは、41,801名の被験者のうち6,554名が2型糖尿病患者であり、非糖尿病者に比べて高い冠動脈リスクを示した（Oikawa et al. Atherosclerosis, 2007）。冠動脈疾患の既往がない高LDLC患者7,832名を食事療法単独または食事療法＋低用量ブラバスタチンに割り付け、平均5.3年観察したMEGA研究では、空腹時高血糖を示した2,210名のサブグループにおいて、スタチンの服用により有意な（P=0.03）心血管リスクの低下を認めた（Tajima et al. Atherosclerosis, 2008）。さらに、18,645名の高LDLC患者に低用量スタチンまたはスタチン＋イコサベンタエン酸エチル（EPA）投与し4.6年の追跡を行ったJELISでも、2型糖尿病または空腹時高血糖を認めた4,656名において、EPAは22%（p=0.048）の冠動脈リスク低下を示している（Oikawa et al. Atherosclerosis, 2009）。一方、慢性の腎機能障害が新たな心血管リスクとして注目されているが、我々の検討結果を含め、スタチンやEPAには高脂血症患者の腎機能を保護する作用があることも示唆される。

確実なLDLC低下作用を有する薬剤が数多く登場し、2型糖尿病の実地診療の中でも、脂質は血糖に比べて管理目標を達成しやすいパラメーターとなりつつある。それが実際の患者予後の改善にどれだけ効果かを改めて考察し、課題を含めた展望を試みたい。
Diabetic macroangiopathy: From endothelial dysfunction to impaired regeneration

Angelo Avogaro
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Type 2 diabetes mellitus is characterized by a 2 to 4-fold increased risk of cardiovascular disease (CVD). This is generally attributed to the adverse effects of hyperglycemia and oxidative stress on vascular biology. It has been also shown that patients with prediabetic conditions, such as IFG and IGT, are themselves at increased risk of CVD. This suggests that abnormalities in carbohydrate metabolism form a continuum that progressively worsens cardiovascular health; the first step of the adverse sequence of events that leads to the atherosclerotic process is the endothelial dysfunction (ED). Ongoing hyperglycemia exposes the endothelial cells to apoptotic process, leading to intimal denudation. Endothelial progenitor cells (EPCs) are immature cells capable of differentiating into mature endothelial cells. Tissue ischemia, through the release of growth factors and cytokines, mobilizes EPCs which, once in the peripheral circulation, specifically home on the ischemic sites to stimulate compensatory angiogenesis. Moreover, EPCs constitute a circulating pool of cells able to form a cellular patch at sites of endothelial injury, thus contributing directly to the homeostasis and repair of the endothelial layer. Both type 1 and type 2 diabetic patients have less circulating EPCs than matched healthy subjects. Moreover, diabetic EPCs display functional impairment, such as reduced proliferation, adhesion, migration and incorporation into tubular structures. The mechanisms underlying EPC reduction in diabetes include weak bone marrow mobilization, decreased proliferation and shortened survival in peripheral blood.

Hyperinsulinemia and atherosclerosis

Type 2 diabetes mellitus is an important risk factor for the development of atherosclerosis. In contrast to microvascular complications of diabetes, macrovascular complications are related to many factors including hyperglycemia, hypertension, and dyslipidemia so on. Epidemiological studies suggested that hyperinsulinemia or insulin resistance is another significant risk factor for cardiovascular events.

We have investigated the significance of hyperinsulinemia and the mechanisms how it develops atherosclerosis. We found that physiological concentration of insulin stimulates PI3-kinase in vascular smooth muscle cells. The continuous stimulation of the PI3-kinase results in the activation of transcription factor C/EBP-beta which increases in the gene expression of inflammatory cytokines such as MCP-1. On the other hand, insulin stimulates the activity of endothelial NO synthase (eNOS) in vascular endothelial cells. Interestingly, NO attenuates the mRNA expression of MCP-1 in vascular smooth muscle cells through the inhibition of the activity of C/EBP-beta by inducing the expression of C/EBP homologous protein (CHOP). Thus, insulin does not induce the MCP-1 expression in insulin-sensitive vascular tissue. However, in the insulin resistance state showing endogenous hyperinsulinemia, the hyperinsulinemia chronically stimulates the PI3-kinase in vascular wall but not eNOS activity leading to the elevated expression of MCP-1 in the aorta from insulin resistant rats. These results indicate the significance of insulin resistance and endothelial function in diabetes for the prevention of macrovascular complications.
Hyperglycemia/AGEs and atherosclerosis
大阪市立大学大学院医学研究科代謝内分泌病態内科学
小山 英則

Large randomized studies in type 1 and type 2 have established that early intensive glycemic control reduces the risk of diabetic microvascular complications, with less impact on macrovascular complications. However, follow-up data of these trials reveal a long-term influence of early metabolic control on longer cardiovascular outcomes, even though the influence on glycemic control has been immediately disappeared after the trials. This phenomenon has recently been defined as “hyperglycemic metabolic memory”. Potential mechanisms for propagating this “memory” are the non-enzymatic glycation of cellular and tissue proteins which are conceptualized as advanced glycation end-products (AGEs). Accumulation of AGEs leads to crucial biomedical pathway generating intracellular oxidative stress and inflammatory mediators through receptor for AGEs (RAGE), which could result in vicious circle through further amplification of the pathway involved in AGE generation. Several lines of evidence suggest that AGEs/RAGE axis can profoundly be involved in cardiovascular diseases. Recent advances in AGEs and RAGE measurements in clinical settings led us to be capable of understanding more about the role of AGEs/RAGE axis as a risk for cardiovascular diseases in diabetic patients. Indeed, tissue AGE levels, but not necessarily serum AGEs, are strongly associated with arteriosclerosis and are shown to be a predictor for cardiovascular events. In this symposium, I would like to summarize the recent findings of AGEs/RAGE axis as a crucial mediator of cardiovascular complications in diabetes, and to discuss their potential usefulness as therapeutic targets to overcome the effect of metabolic memory, and also as biomarkers for the cardiovascular diseases.

NCEH1 is a novel enzyme regulating foam cell formation and atherosclerosis
自治医科大学内科学講座内分泌代謝学部門
大須賀淳一, 石橋 俊

Macrophage (Mφ) -derived foam cells, which contain excess cholesterol esters (CEs), play integral roles in the pathogenesis of atherosclerosis. The breakdown of the CEs is controlled by the neutral cholesteryl ester hydrolase (nCEH). Several observations suggested that hormone-sensitive lipase (HSL) might account for the nCEH activity. However, the presence of additional nCEH (s) is suggested since peritoneal Mφ of HSL knockout (HSLKO) mice still show nCEH activity. We identified a novel nCEH (NCEH1, Neutral Cholesterol Ester Hydrolase 1) and generated NCEH1 knockout (NCEH1KO) mice to gain insights into the physiological roles.

NCEH1 accounted for 50% of the nCEH activity. Upon exposure to acetylated LDL (acLDL), the CE accumulation was increased by 50%, and cholesterol efflux was decreased by 35% in NCEH1KO Mφ. When NCEH1KO were crossedbred with apoEKO, the offspring demonstrated an increase of atherosclerotic lesion without affecting serum lipids (2.5 fold by en face analysis, and 1.8 fold by cross-sectional analysis).

Furthermore, we generated mice lacking both NCEH1 and HSL (DKO). The nCEH activity in the DKO was reduced to 10% of that in wild-type (HSLKO, 50% : NCEH1KO, 50% of wild-type). Deficiency of NCEH1 and HSL had an additive effect in the CE accumulation upon exposure to acLDL (HSLKO, 130% : NCEH1KO, 150% : DKO, 170% of wild-type) and cholesterol efflux (HSLKO, 90% : NCEH1KO, 80% : DKO, 55% of wild-type). ACAT activity in the DKO was reduced by 45%. Lack of either NCEH1 nor HSL affected lipoprotein uptake and degradation.

In conclusion, NCEH1 is involved in foam cell formation and atherosclerotic lesion development. NCEH1 and HSL are responsible for most of nCEH activity in Mφ.
The preventive effects of anti-diabetic drugs on atherosclerosis

Type 2 diabetes is a major risk factor for atherosclerotic diseases. Recently, several studies demonstrated that many factors such as hyperglycemia, insulin resistance and inflammation promoted atherosclerosis in diabetes.

Postprandial hyperglycemia is also considered as an independent risk factor for atherosclerosis (Otsuba et al. Diabetologia 2005). We previously demonstrated that repetitive postprandial hyperglycemia per se induced monocyte adhesion to endothelial cells compared with persistent hyperglycemia in the Goto-Kakizaki rat (Azuma et al. ATVB 2006). In addition, we reported that α-glucosidase inhibitors or nateglinide could efficiently reduce daily blood glucose fluctuation and decrease monocyte adhesion (Azuma at al. BBRC 2006, Tanaka et al BBRC 2006) and arteriosclerotic lesions (Mita et al. BBRC 2007). Furthermore, we demonstrated that strict glycemic control, especially targeting for postprandial hyperglycemia, with nateglinide attenuated the progression of intima-media thickness in patients with type 2 diabetes (Mita et al. ATVB 2007).

We also found that mice models of systemic and monocyte/macrophage specific insulin resistance showed the enhanced monocyte/macrophage accumulations in the vascular walls accompanied by the inflammatory reaction in macrophage independent of lipid profiles (Mita et al. in submission). This data suggests that insulin resistance and macrophage inflammation are important therapeutic targets to prevent the progression of atherosclerosis.

GLP-1 receptor agonists such as exendin-4 are currently used for the treatment of type 2 diabetes. Recently, several studies showed that exogenous administration of GLP-1 or GLP-1 receptor agonist had direct beneficial effects on the cardiovascular system independent of their glucose lowering effects. Very recently, we demonstrated that exendin-4 suppressed the inflammatory response in macrophages and attenuated atherosclerotic lesions without major effects on metabolic parameters in apoE −/− mice (Arakawa, Mita et al. Diabetes 2010).

In summary, our data suggest multiple targets and new strategy to prevent atherosclerosis in the treatment of type 2 diabetes.
広い視野をもった地域包括的糖尿病医療連携
篠田 純治

臨床現場においては糖尿病の放置あるいは通院中断例が数多く見られ、このような状況には、主婦や自営業者など健診を定期的に受けるない場合や、健診を受けなくても認知が著しくない場合もある。すなわち、1次予防から2次予防まで遅れててしまうことが現実的には多いと思われる。糖尿病地域連携について各地で検討されているが、事例をみる限りでは2次予防・3次予防の病診連携の内容が多く、健康維持オススメ数は非常に多いに比較的意欲ある患者が対象の中心になっていると思われる。病診・健康検診をしないたち、加齢によって放置しても人々、さらに、合併症を進行しないうちに地域全体として対処することが必要ではないかと考えて活動してきた。

豊田地域（愛知県豊田市）では、2005年から豊田市糖尿病対策地域連絡会議が設立され、1次から2次予防まで地域一体型の連携を目指して、行政（保健所・病院・診療所（医師会）・産業医・健康保険組合・歯科医師会が参加している。この構成メンバーはほとんどが住民の健診から病診まで関連する。この会議で診断後の発症予防の作、健康データの分析、アンケート調査などを行った。また、栄養士のいない診療所で栄養指導ができる体制について検討を重ねて、糖尿病だけでなく脂質異常症なども含めた生活習慣病対策栄養サポート体制を構築している。さらに地域向けにオープン化されている各施設の糖尿病教室を市のホームページにまとめ掲載した。これに加え、健康確認の後、糖尿病発症に関しては他の施設と連携しての各機関の役割分担と連携基準を作成しつつあり、地域で切れ目のない連携を目指している。

さらに別の側面からの地域の組織として調剤薬局を含む多施設のメディカルスタッフが全体的に貢献する豊田コミュニティ型糖尿病対策会が活動している。研鑽しながら地域内が胃腸で共有できる情報連携ツールを作成しつつあり、どの施設で薬剤を処方する初診患者向けの共同指導ツールを作成している。豊田市糖尿病対策地域連絡会議の役割を担っているようにしている。

当院は地域の中で糖尿病の初期診断や見直しの役割を果たし、2008年度の糖尿病の紹介、逆紹介数はそれぞれ308・581であった。また産業医と連携して長期短期体験入院を勧めており、このシステムを開始してから年々上昇傾向にある職域全体のHbA1c平均値が低下傾向に転じていることが確認されている。多忙で社会人に対するやるべき作業が重なる職域での対策も重要な地域対策であると考えている。

地域全体の糖尿病対策・地域連携に向けて、施設や組織の枠にとらわれない視点から人材も情報も共有して、単純な健康診断以上の多面的・包括的な政策が必要であると考える。

医師会主導による地域糖尿病医療連携
松浦クリニック附属糖尿病研究所 ① 東京都渋谷区医師会
松浦 卓彦 ① 中村 一彦 ② 山崎 隆夫 ③

はじめに：最近の知見によれば、糖尿病合併症の予防のためには早期からの強力な治療介入が不可欠と明確化しているが、今日の我々の国を支える多くの糖尿病対策が取り組むべき領域は、発症予防や合併症予防を含む予防・治療の観点においても、それらの合併症の頻度を高めている。何故なら、糖尿病の合併症は慢性疾患であり、合併症の合併症が生じるため、合併症を予防することが最も重要である。

目的：今回私たちは一つの試みとして、東京都渋谷区の医療環境を生かして地域社会に対し、地域で発見・治療するものを目指す。医師会主導の地域連携の重要性を示すため、地域住民が健康診断を行うことが目指す。

1. 予防医学として、地域住民が健康診断を行うことが目指す。
2. 非専門医・専門医連携の糖尿病治療の標準化マニュアルによる連携ツールを作成し、連携することを目的とする。さらに、地域住民の健康診断の重要性を示すために、地域住民が健康診断を行うことが目指す。
3. 予防医学として、地域住民が健康診断を行うことが目指す。

すなわち、地域住民が健康診断を行うことが目指す。

S-27
シンポジウム8: 新しい糖尿病治療戦略を見据えた地域医療の在り方を目指して
Symposium 8: Community Healthcare Focusing on Novel Therapeutic Strategies for Diabetes
座長 小林 正 貴田岡正史

S8-3

『大阪市西部ブロック糖尿病地域連携パス』の現状と
今後—糖尿病管理のこれから

関西電力病院糖尿病・栄養・内分泌内科1, 関西電力
病院栄養管理室2, 大阪市立住吉区医師会3

黒瀬 健1, 井部 大介2, 深渡 好胤2, 蓮澤 方洋2, 表 宏徳, 小野 直美2,
松下 正幸3, 清野 裕

2007年の厚生労働省の調査において、本邦の糖尿病
患者は890万人。予備軍は1320万人、その合計は約2210
万人と2002年の同調査に比べ約340万人（18％）の急
激な増加を認める。が、糖尿病は発症や失明、腎不全、
心筋梗塞などの合併症出現まで自覚症状が乏しいで、
治療を拒む患者が多く、糖尿病の受診者は43％。継続
治療者は45％にも満たない。日本医師会・日本血液
学会・日本糖尿病学会を中心に糖尿病対策推進会議な
ど国民への糖尿病の知識普及を目的に様々な活動が展
開されているが、「糖尿病専門医」だけではなく、「かか
りつけ医」や「地域行政」が一丸となり、糖尿病の教
育と診療を一向層強化していく必要がある。
このような経緯から『糖尿病地域連携パス（以下、
地域連携パス）』が注目される。特に、糖尿病が5大
疾患と位置付けたことから、緊急に『地域連携パス』を
普及・充実する必要に迫られる。「地域連携パス」は、
「病院」「かかりつけ医」「地域行政」等で

サポーターとしての役割を果たし、共に糖尿病の

控ええ

S8-4

循環型糖尿病医療連携症例のアウトカム

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大学院分子内分泌内科2

辻野 元祥1, 西田 賢司2, 櫻田 麻耶2,
佐藤 文紀1, 大橋 稔1, 山崎 徹2

中村 友香1, 平田結喜雄2

【背景と目的】北東部南部保健医療圏糖尿病医療連携
事業では、2004年から2008年度の4カ年計画で、圏内
の糖尿病医療連携推進のための取り組みを行ってきた。
初年度には、圏内の糖尿病医療実態調査を行い、
診療所の78.5％、病院の98.2％から、診断時
間の情報不足が円滑な糖尿病医療連携の妨げとなっているとの問
答を得た。それに基づき、圏内の市町で、糖尿病医療
連携マップを作成し、また、糖尿病医療連携パスを選
定するなどの事業を展開してきた。こうした保健医療
圏における広域の糖尿病医療連携体制を整備する一
方、当院では10年前より近隣の診療所との間で循環型
糖尿病医療連携を展開している。このシステムは月1
回診療所を受診する一方、6-12カ月毎に当院糖尿病
外来を受診、評価を行う様みで、糖尿病医療連
携パスを用いた循環型糖尿病医療連携の重要性は

サポーターとしての役割を果たし、共に糖尿病の

控ええ

【対象方法】2008年9月から2009年8月までの
間、当院を定期受診した循環型糖尿病医療連携として
フォローされている10例（男性60例、女性49例、平
均年齢65±12.3歳）を対象に、糖尿病医療連携パスを
適応した場合のアウトカムについて考察を行った。

サポーターとしての役割を果たし、共に糖尿病の

控ええ

【結果】患者背景は、1型糖尿病2例、2型糖尿病107例、
治療内容は、食事・運動療法のみ9例、経口血糖降
下薬27例、インスリン治療23例であった。連携先医療機
関は82施設中58例（93.5％）が糖尿病非専門医であっ
た。逆算した平均年数は27±1.7年（最短0.5年、
最長11年）であった。また、病院への定期来院時の
HbA1c 6.9±1.0％であった。バルーン発生率は10例
(9.2％)で主にHbA1c, 高値（8.0％）が8例、認知症
進行が2例、急性感染症（尿液感染症）1例、急性心筋
梗塞発症1例だった。

【結論】循環型糖尿病医療連携でフォローされている
症例ではバルーン発生率は9.2％と少なく、経過は
良好であった。十分な評価のためには、より多数例か
つ、より長期間を対象とした前向き研究による評価が
必要と考えられた。

—S-28—
シンポジウム8：新しい糖尿病治療戦略を見据えた地域医療の在り方を目指して
Symposium8: Community Healthcare Focusing on Novel Therapeutic Strategies for Diabetes

座長 小林 正 貴田原正史

千葉県における全県共用地域医療連携バスの取り組み
千葉県共用地域医療連携バスワークインググループ糖尿病部会

江本 直也、栗林 伸一、篠宮 正樹、
小谷野 重、関谷貞三郎、平井 愛山、
青柳 和美、藤田 伸輔、松田 一郎、
野村 隆司、長谷川正克、戸次 一寿、石川 広己

平成18年6月の第五次医療法改正を受けて、千葉県では平成20年4月、千葉県保健医療計画において2次医療に4病（がん、脳卒中、急性心筋梗塞、糖尿病）の循環型地域医療連携システムを策定することが明示された。このシステムを円滑に運用するためのツールとしての医療連携バスに注目し、調査を行ったところ、一部の医療機関ではすでに運用されているものの、医療機関間の多様な診療場面においての異なるバスの乱立した状況の懸念が多数意見を占め、そこで千葉県医師会、関係医療機関との医療連携団体が協働し、疾病ごとの全県共用の地域連携バス(例示モデル)を策定することとなった。例示モデルの作成によれば、疾病ごとのワークインググループを県医師会により設立され平成24年9月より検討を開始した。医療連携バスにおいては、県内外の様々な糖尿病地域連携バスのモデルを集めて検討を行った。提案によって示された方向性は、二次医療圏における医療資源の分布にバネがあり、その結果として基幹病院、診療所、糖尿病専門医の役割分担も異なったものになっていることから柔軟性をもっていたものでなければならないうちに、非専門の診療所への技術移転をサポートする一貫したシステムの中のツールの一つであるというコンセプトが生かされること。現行の糖尿病手帳は使用に有利に活用しその導入が必要とする方医療機関の負担が増えることを極力抑えなければならないことであるため、さらに千葉県の医療供給システムとして患者（県民）のコンセプトを形成するための努力を続けることも必要である。これらの方向性をもって、具体的な分析モデルの検討に入ると、患者用のオーバービューや、心筋梗塞や脳卒中による病患数使用するものを利用できる形態の治療方法への工夫が重視された。医療者用の記載方法はできるだけ現行の糖尿病手帳の形式を踏襲するものとしてスタートすることである。医療機関の混乱を避け、実際に使用しながら意見を取り入れて改善していくこともと。作成論文の過程で、各医療圏での糖尿病治療供給システムそのものの多くの問題も議論された。それらを連携バスのみで解決するのは困難であり、様々な問題を残しながらも例示モデルを完成させ、平成21年1月に県内の医療関係者に対して開示した研究会（シンポジウム）において公表し、地域保健医療協議会等や各地域での説明会、県のホームページ等を通じ、意見を募集し、3月に開催した千葉県医療審議会で承認を得て、4月から普及活動を開始している。全県共用型バスは全国でも初めての実験的なものであり、その経過について報告する。

長野県における医師、コメディカル、患者レベルでの糖尿病ネットワークとその運営
佐久市立国保冷地病院医療部1、信州大学医学部医学教育センター2、厚生連佐久総合病院内科3、長野県市民病院内科4、長野赤十字病院内科5独立行政法人国立病院機構小諸高原病院内科6、独立行政法人国立病院機構長野病院内科7

仲 元司1、相澤 彰2、大橋 明正1、
西井 裕1、大房 裕和、山内 忠史5、
佐々木恵理子5、田中 征雄5

長野県は県歌「信濃の国」にも通じる高い山に囲まれた四つの平(盆地)に分かれており、南北約220km、東西約128kmにおよぶ広さや地の広さの特徴は、この中にまじめに困難な地理的条件の中、糖尿病に携わる医師、コメディカル、患者(糖尿病患者)がこれまでの転換が模索されてきた。

最近のように医療機関のネットワークがリンクして県単位の大きな動きとなりつつあるものでそれを紹介したい。

医師：①都開医師会を中心とした2次医療圏の地域連携として佐久平糖尿病ネットワーク（SNDnet）を取り上げること。ここでは2次医療圏内の複数の病院と医師の診療所などで共通に使用できる地域連携バスを構築し、その適用地域をさらに広げつつある。②全県レベルでは信州大学を中心に全国にまじめにない日本の糖尿病学会認定専門医の研究会、長野県糖尿病専門医会が発足した。

コメディカル：①東信地域の糖尿病に携わるコメディカルの会として10年目を迎えた長野県糖尿病スタッフ研究会では多施設共同アカデミーなど医療機関間の連携を含む活動を続けてきたが、2007年同地域内の病院・診療所で共通に使用できる地域連携バスを構築し、その適用地域をさらに広げつつある。②2008年には信州スタッフ研究会や地域薬剤師会の要望が強く東北信地域糖尿病治療指導士（L-CDE）育成会が発足、認定事業を開始した。③全県レベルでは2008年から県内のCDE-Jを統合した長野県糖尿病治療指導士会が活動中である。

協会：①各病院間での交流としては、例えば2003年から東信地区の6分会が持ち回りで開催している東信交流マレットゴルフ大会がある。②全県レベルでは2006年の関東甲信越デミナー開催を機に、翌年から毎年信州糖尿病セミナーを県内5地域の持ち回りで開催している。

このように各地域での小さなまとまりが徐々に大きくなる中、全県レベルでの統合も進み、下から向けた動きと上からの動きが相まって現れている中、これらに加え医師、コメディカル、患者レベルでのネットワークとがリンクしてイベントへの協力などが盛んに行われるようになった。長野県糖尿病対策推進会議ではこれらを結びつけるリボンの役割を果たしているが、糖尿病患者の住民と正常な生活者への啓発活動、また児童の食育から高齢者介護者のケアまで視野に入れた拡大糖尿病対策推進会議へと進化しつづける。
シンポジウム8：新しい糖尿病治療戦略を見据えた地域医療の在り方を目指して
Symposium8: Community Healthcare Focusing on Novel Therapeutic Strategies for Diabetes

座長 小林 正 貴田岡正史

S8－7

糖尿病療養の担い手を地域で育て、療養指導の地域における標準化を図る
東京医科大学八王子医療センター医療情報室
植木 彬夫

【目的】糖尿病治療を行う薬物療法は血糖降下薬選択のマニュアルやガイドラインが、インスリン療法ではアルゴリズムに従ったインスリン剤の選択などの標準化が進んできている。一方、薬物療法に勝るとも劣らない治療法である食事療法や運動療法については病態管理などの理論は進んできているが、患者指導の指導方法や教育方法は必ずしも一定では無く、その担当者のスキルにより多大な差がある。また施設によっては専門のスタッフが存在しないことも多く施設間の差も認められる。したがってこれらの糖尿病治療戦略を考えるときに、食事療法や運動療法の担い手であるコメディカルのスキルアップは糖尿病療養の質の向上と等同に重要と考えることに。特に運動療法や食事療法は生活環境や地域に密着した指導が必要である。以上の観点から我々の地域では、地域における指導方法の標準化の構築とこれに基づいた食事療法や運動療法の担い手の育成とスキルアップを図って来た。今回は簡単なリソースを少ないう運動療法の担い手を地域として育成し同時に運動療法の標準化の試みを2006年よりおこなっているので報告する。【方法】1：「運動療法の担当者の育成」は地域で医療資質を持た、あるいは実際の運動指導に卓越した健康運動指導士を中心にして行う。2：運動療法の標準化のためには患者指導の骨格となる運動プログラムを作成する。3：運動プログラムは患者の認知（理解の）要因と方法（理解的）要因の抽出法と、現状の身体活動能力の評価法を「エクササイズ」と「メソッド」の概念を取り入れた構築した。この情報とに踏まえ、運動の3要素（運動量・筋抵抗運動・筋調整運動）を、患者が実行可能な数値モデル（強度・量・時間・頻度・場所・機会）を明確にしたプログラムとした。4：「担当者の育成」患者に対するプログラム指導は実施が伴わなければならない。そのためには担当者自らが有酸素運動、筋抵抗運動、筋調整運動がどのようであるか、どの筋肉で、どの筋肉を用いるかを知るために、歩行法、リズム運動、障害者向けの筋トレ、ストレッチなどを2日間にわたり習得するセミナーを開催した。【結果とまとめ】毎年地域のコメディカルに対して、テーラーメイドの運動療法プログラム作成が出来るようにになるセミナーを行い、現在までに130余名が地域の施設で療養指導に当たっている。地域特性を生かした運動療法を、一定のレベルに維持するため、運動療法の標準化を図り、担当者の育成を行うことで糖尿病治療の「運動療法」を地域で支えていくことが出来ている。

S8－8

かかりつけ医の受診中間抑制の研究（J-DOIT2）での糖尿病治療支援
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山崎 賢也

厚生労働省の糖尿病の戦略研究の課題2「かかりつけ医による2型糖尿病診療を支援するシステムの有効性に関する研究」（J-DOIT2）では、かかりつけ医を対象とした研究であり、糖尿病患者各介による受診中間抑制をめざした研究である。地区医師会に所属するかかりつけ医を対象に、医師会単位でかかりつけ医と糖尿病専門医、眼科専門医、腎臓専門医の連携システムを構築し、「糖尿病治療支援」を実施し、診療支援がかかりつけ医に通院する2型糖尿病患者の受診中間抑制を改善するかを検証する。同時に、かかりつけ医の糖尿病治療の変化や、糖尿病診療目標の実施率・達成率、HbA1c、血压・脂質などの患者間アカウントを調査。この研究を通じて、このような医療システム、患者へのアプローチが受診中間率を抑制できるのかを明らかにすることを目的とする。今後、このようなかかりつけ医を対象とした「大規模研究」が無いため、「大規模研究」に先行して「バイロット研究」が4医師会を対象として行われた、「バイロット研究」では、患者の診療支援として「診療支援サービスセンター」による診療支援サービスを実施した。「診療支援サービスセンターよによる診療支援サービス」は、かかりつけ医の指示に従って電話による患者に対する食事療法及び運動療法に関する支援サービス（療養指導）と受診促進支援サービス（受診促進）を中心に行った。その結果、この療養支援を実施し、食事や運動に関する行動変容ステージの改善が見られ、診療支援の受診中間は5.8％と通常診療者の7.1％と比べ低下傾向が見られた。また、かかりつけ医には「診療支援目標ITシステムによる診療支援」を行い、あらかじめ設定した「糖尿病診療目標」での選択割合の改善が見られるかを検討した。これら「バイロット研究」の結果を踏まえ、「大規模研究」の実施が開始した。「大規模研究」では11医師会が参加し、患者登録の後、本登録をおこなって、10月より1年間の予定で研究は行う。 「大規模研究」での診療支援は、「バイロット研究」と同様に7医師会ではバイロット研究で行った電話による「診療支援サービスセンターによる診療支援サービス」を行い、糖尿病療養指導士や管理栄養士などの診療支援サービスが可能な4医師会では、対面による診療支援サービスを行うこととした。J-DOIT2の研究を通して培われた診療支援のシステムのあり方、新たな糖尿病地域医療システムとして役立つことが期待される。
S9-1

Functional characterization of a novel T2DM risk factor, Cdkal-1

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Recent advances in genome wide association study (GWAS) have revealed a number of genes, which have strong correlation with type 2 diabetes mellitus (T2DM). Among these suspicious genes, Cdk5 regulator subunit association protein 1-like 1 (Cdkal-1) is one of the most reproducible genes, which are correlated with impaired glucose stimulated insulin release in T2DM patients from various racial groups. Despite the accumulating clinical evidence, the molecular characterization of Cdkal-1 is completely unknown. In this symposium, I focus on the molecular function of Cdkal-1 in vitro and in pancreatic beta-cells.

Cdkal-1 contains transfer RNA (tRNA) modification domains, which are conserved from bacteria to mammals. In bacteria, Cdkal-1 successfully modified tRNA at position 37 by catalyzing the biosynthesis of N6-threonylcarbamoyladenosine (t6A) to 2-methylthio-N6-threonylcarbamoyladenosine (ms2t6A). In Min6 cells, we also identified ms2t6A modification of tRNA, suggesting that tRNA modification domains of Cdkal-1 are functional. Because tRNA is a key component in the complex of protein synthesis, we investigated if Cdkal1 had a role in insulin synthesis. We manipulated Cdkal-1 protein level in MIN6 cells by transfection of Cdkal-1 cDNA and the specific siRNA. While overexpression of Cdkal1 had no effect with total insulin content in MIN6 cells, knockdown of Cdkal-1 significantly decreased the total insulin content. Transfection of a mutant of Cdkal1, in which functional domains were deleted, showed that the precise localization as well as its tRNA binding ability of Cdkal-1 was important for insulin synthesis. Finally, we generated pancreatic beta-cell specific knockout mice of Cdkal1. The mice show impaired blood glucose control during glucose tolerance test. Moreover, the plasma insulin level after glucose application was lower than that of wild-type mice. These results suggest that tRNA modification by Cdkal-1 may participate in insulin synthesis in pancreatic beta-cell, and modulation of insulin synthesis by Cdkal1 could be one mechanism underlying T2DM.

S9-2

Cell metabolism-dependent regulation of Kv channels in pancreatic β-cells

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Voltage-gated potassium channels (Kv channels) play a crucial role in the formation of action potentials in response to glucose stimulation in pancreatic β-cells. An increase in extracellular glucose concentration to 5.6 mM or more leads to elevation of cytoplasmic ATP or ATP/ADP ratio within a few minutes, depolarizes β-cell membrane as a consequence of closure of the Kv11.5 channel and induces bursting spike-like short action potentials at membrane potentials positive to −50～−40 mV. These action potentials are produced by orchestrated openings of voltage-dependent Ca2+ channels (VDCCs) and Kv channels. We examined whether the Kv channel is modulated by cellular metabolism in terms of mechanistic relations with phosphorylation of the channels. In rat pancreatic β-cells, inhibition of glucose metabolism by 2.8 mM or less glucose or by using metabolic inhibitors decreased the Kv2.1-channel activity at positive membrane potentials, whereas conversely the channel currents increased at potentials negative to −10 mV, suggesting voltage-dependent modulation of Kv channels by glucose metabolism. Similar regulations of the recombinant Kv2.1 channels expressed in HEK293 cells by 0 mM MgATP but not by 10 mM MgATP were observed. Both steady-state activation and inactivation kinetics of the channel were shifted toward negative potential by cytosolic dialysis of alkaline phosphatase in β-cells. Glucose–metabolism dependent modifications of the Kv-channel current–voltage relations were observed before and during glucose-stimulated electrical excitation. It is concluded that the cellular metabolism including MgATP production and/or dephosphorylation/dephosphorylation of the channels may physiologically underlie the regulation of the Kv2.1 channels during glucose–induced insulin secretion.
**S9-3**

K<sub>ATP</sub> channel and neonatal diabetes  
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ATP–sensitive potassium (K<sub>ATP</sub>) channels are a key link between glucose metabolism and insulin secretion. Changes in intracellular adenosine nucleotides by glucose metabolism close K<sub>ATP</sub> channels, thereby inducing cell membrane depolarization and causing voltage dependent Ca<sup>2+</sup> entry and insulin exocytosis.

Gain-of-function mutations in the pancreatic β-cell K<sub>ATP</sub> channel subunits Kir6.2 and SUR1 can cause neonatal diabetes. Neonatal diabetes is defined as diabetes that manifests within the first 6 months of life, and it can be either permanent or transient. By studying mutant K<sub>ATP</sub> channels expressed in *Xenopus* oocytes, we have shown that neonatal diabetes results from reduced inhibition by MgATP. We also found that in most cases, sulphonylureas (selective blockers of K<sub>ATP</sub> channels) remain effective at closing mutated channels. This has enabled many patients to switch from insulin injection to oral sulphonylurea therapy.

To understand the functional effects of these mutations in vivo, we used Cre-lox technology to generate a mouse expressing the Kir6.2 mutation V59M that causes neonatal diabetes in humans specifically in the β-cell.

Induction of Kir6.2–V59M in adult mice led to diabetes within 2 days. Insulin secretion from perfused isolated islets was totally inhibited in response to glucose but not to sulphonylureas. The induced diabetes could be reversed by implantation of a slow-release glibenclamide pellet on day 2 after hyperglycaemia was detected. Islets isolated from glibenclamide–treated mice failed to secrete insulin in response to glucose alone. However, in the presence of 2mM glibenclamide in the perfusate, basal insulin secretion was elevated and, importantly, glucose–stimulated insulin secretion was restored. Islets isolated from placebo–treated mice 2 weeks or more after pellet implantation failed to show glucose–stimulated insulin secretion despite containing glibenclamide. Insulin content decreased with time in placebo–treated mice, presumably due to the hyperglycaemia, but was preserved in glibenclamide –treated mice.

These results may provide insights into sulphonylurea therapy of patients with neonatal diabetes.

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**S9-4**

Epac2 is a direct target of both cAMP and sulfonylurea in insulin secretion  

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Insulin secretion is regulated by various intracellular signals including Ca<sup>2+</sup>, ATP, cAMP, and phospholipid–derived molecules. Although glucose–stimulated insulin secretion (GSIS) is the principal mechanism of insulin secretion, its potentiation by cAMP is also critical. cAMP is now known to potentiate insulin secretion in both a protein kinase A (PKA)–dependent and a PKA-independent manner, the latter involving Epac2 (also referred to as cAMP–GEFII), which possesses guanine nucleotide exchange factor (GEF) activity towards the Ras-like small GTPase Rap1. We previously reported that Epac2/Rap1 signaling is required for potentiation of the first phase of GSIS by cAMP.

To elucidate the Epac2–mediated signaling mechanisms, we developed a fluorescence resonance energy transfer (FRET)–based Epac2 sensor. FRET and binding experiments revealed that sulfonylureas, widely used anti-diabetic drugs, interact directly with Epac2. Sulfonylureas activated Rap1 specifically through Epac2. Sulfonylurea–stimulated insulin secretion is reduced both in vitro and in vivo in mice lacking Epac2. Sulfonylureas are well known to stimulate insulin secretion by closing pancreatic β-cell ATP–sensitive K<sup>+</sup> (K<sub>ATP</sub>) channels through binding to the sulfonylurea receptor SUR1, a regulatory subunit of the channel. Our study demonstrates that Epac2 also is a direct target of sulfonylureas and that it is required for sulfonylureas to exert their full effects in insulin secretion. Because Epac2 is also required for the action of incretins such as glucagon–like peptide 1 (GLP–1) crucial for potentiating insulin secretion, it may be a promising target for the development of anti-diabetic drugs.

References
Roles of Rab27a and its multiple effectors in insulin exocytosis

We have studied the function of the small GTPase Rab27a and its effector granophilin, which are preferentially expressed in pancreatic beta cells. Our analyses indicate that, although Rab27a and granophilin form a complex in pancreatic beta cells, these two proteins have distinct roles in insulin granule exocytosis. Granophilin is essential for the stable attachment (docking) of insulin granules to the plasma membrane and negatively regulates the final fusion reaction. By contrast, Rab27a positively regulates glucose-induced insulin exocytosis by stimulating the recruitment of insulin granules close to the plasma membrane. Differences in the insulin secretory phenotypes between pancreatic beta cells of granophilin-null and of Rab27a-mutated mice suggest involvement of other Rab27a effector proteins. In fact, it was reported by several laboratories that Rab27a effector protein candidates, such as exophilin 8/MyRIP, Noc2, and coronin 3, could function in the intracellular trafficking of insulin granules. We would like to present our data and to discuss possible roles of Rab27a effector proteins that are significantly expressed in pancreatic beta cells.

Autophagy regulates pancreatic beta cell death in response to Pdx1 deficiency and nutrient deprivation

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There are three types of cell death: apoptosis, necrosis and autophagy. The possibility that activation of the macroautophagy (autophagy) pathway may increase beta cell death is addressed in this study. Increased autophagy was present in pancreatic islets from Pdx1−/− mice with reduced insulin secretion and beta cell mass. Pdx1 expression was reduced in MIN6 cells by delivering shRNAs using a lentiviral vector. The MIN6 cells died after 7 days of Pdx1 deficiency and autophagy was evident prior to the onset of cell death. Inhibition of autophagy prolonged cell survival and delayed cell death. Nutrient-deprivation increased autophagy in MIN6 cells, mouse and human islets after starvation. Autophagy inhibition partly prevented amino acid starvation-induced MIN6 cell death. The in vivo effects of reduced autophagy were studied by crossing Pdx1−/− mice to Beclin1−/− mice. After 1 wk on a high fat diet, 4 wk old Pdx1+/−/Beclin1−/− mice showed normal glucose tolerance, preserved beta cell function and increased beta cell mass compared to Pdx1−/− mice. This protective effect of reduced autophagy had worn off after 7 wks on a high fat diet. Increased autophagy contributes to pancreatic beta cell death in Pdx1−/− deficiency and following nutrient-deprivation. The role of autophagy should be considered in studies of pancreatic beta cell death and diabetes and as a target for novel therapeutic intervention.
Functional brown adipose tissue in healthy humans
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Obesity has been described, rather drastically, as "the plague of our time" and to some extent rightly so since it is endemic in many regions of the world and a forerunner of several serious and sometimes fatal diseases such as ischemic heart disease, stroke, kidney failure and neoplasia. Although we know its origin – it results when energy intake exceeds energy expenditure – today, the only proven therapy is bariatric surgery (1, 2). A major abdominal procedure that, for reasons that are largely unknown (it cannot be explained solely by a reduction in ventricular volume), significantly reduces energy intake, but due to cost and limited availability, it will most likely be reserved for only a small fraction of all those who stand to gain from effective anti-obesity treatment. Clearly, alternative ways to treat obesity are needed. Another way to combat excessive accumulation of white adipose tissue (WAT) would be to increase energy expenditure. Rodents, hibernators, and human infants all have a specialized tissue – brown adipose tissue (BAT) – with the unique capacity to regulate energy expenditure by a process called adaptive thermogenesis (3). This process depends on the expression of uncoupling protein 1 (UCP1), which is a unique marker for BAT. UCP1 is an inner mitochondrial membrane protein that short-circuits the mitochondrial proton gradient, so that oxygen consumption is no longer coupled to ATP synthesis. As a consequence, heat is generated. Mice lacking ucp-1 are severely compromised in their ability to maintain normal body temperature when acutely exposed to cold and they are also prone to become obese (4, 5). We have shown that, in mice, BAT protects against diet-induced obesity, insulin resistance, and type-2 diabetes (6, 7). This is based on prevention of excessive accumulation of triglyceride in non-adipose tissues like muscle and liver. Ectopic triglyceride storage at these locations is associated with initiation of insulin resistance and, ultimately, development of type-2 diabetes (8).

BAT has been considered without physiological relevance in adult humans. Recently, this view was radically changed by identification of significant amounts of metabolically active BAT in healthy adults (hBAT). This was recently published by three groups (9–11), making BAT-mediated dissipation of excess energy in humans a real possibility. This new knowledge is part of an explosion of information regarding BAT function that has accumulated during the last few years and catapulted brown fat from a position of relative obscurity – as an animal-only tissue – to the center-stage of human physiology (12). Together, these advances will lead to a reassessment of brown adipose tissue in humans and its role in pathophysiology. Furthermore, these new data also afford new opportunities with regard to novel therapeutic possibilities for treatment of obesity and type-2 diabetes. Ways to increase the amount of and/or activity of hBAT appear interesting since they make use of a unique feature of BAT – to safely dissipating large amounts of energy.

S10-2

Adipose tissue inflammation and the metabolic syndrome
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In contrast to “acute inflammation” which resolves by an active termination program, “chronic inflammation” is characterized by persistent interaction between parenchymal and stromal cells in response to tissue stress or malfunction, thereby leading to functional maladaptation and tissue remodeling. The adipose tissue is capable of communicating with multiple organs or tissues by virtue of a large number of adipocytokines, and thus influences a variety of physiologic and pathophysiologic processes. Recent evidence has suggested that obesity is a state of a chronic low-grade inflammation; obesity–induced adipose tissue inflammation results in the dysregulation of adipocytokine production, thereby contributing to the development of the metabolic syndrome. On the other hand, obese adipose tissue is characterized by adipocyte hypertrophy, followed by increases in angiogenesis, macrophage infiltration, and pro-inflammatory adipocytokine production, which may be referred to as “adipose tissue remodeling”. Among stromal cells involved, macrophages may play a critical role in obesity–related adipose tissue inflammation. Using an in vitro co-culture system composed of 3T3-L1 adipocytes and macrophages, we have provided evidence that a paracrine loop involving saturated fatty acids and tumor necrosis factor α (TNFα), which are derived from adipocytes and macrophages, respectively, establishes a vicious cycle that aggravates inflammatory changes in obese adipose tissue. During the paracrine interaction between adipocytes and macrophages within obese adipose tissue, saturated fatty acids, which are released in large quantities from hypertrophied adipocytes via the macrophage–induced lipolysis, serve as an endogenous ligand for Toll–like receptor 4 (TLR4) complex, a major pathogen sensor, to activate macrophages for the regulation of metabolic homeostasis. Understanding the role of macrophages in adipose tissue remodeling would lead to the identification of novel therapeutic strategies to prevent or treat obesity–induced adipose tissue inflammation.

S10-3

Adipose senescence is critically involved in the regulation of insulin resistance
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Cellular senescence was originally defined as the finite replication of human somatic cells in culture. Various stimuli, such as telomere dysfunction and oxidative stress, can activate p53–dependent DNA damage signals and therefore induce cellular senescence. There is also evidence that senescent cells promote changes related to aging or age–related diseases. Aging is known to increase the prevalence of metabolic disorders like diabetes. Therefore, we hypothesized that cellular aging might influence insulin resistance and accelerate the development of diabetes. By using various genetic models, we show that p53 expression in adipose tissue is critically involved in insulin resistance, which underlies age–related cardiovascular and metabolic disorders. Telomerase–deficient mice developed insulin resistance when fed a high–calorie diet. The adipose tissue of these mice showed senescence–like changes, such as increases in activity of senescence–associated β–galactosidase and expression levels of p53. Resection of senescent adipose tissue improved insulin resistance in telomerase–deficient mice, whereas implantation of senescent adipose tissue into wild–type mice led to impairment of insulin sensitivity and glucose tolerance in the recipients. Up–regulation of p53 induced expression of pro–inflammatory cytokines and accumulation of macrophages in adipose tissue. We also found that excessive calorie intake led to the accumulation of oxidative stress in the adipose tissue of type 2 diabetic mice and promoted senescence–like changes including increased expression of p53. Inhibition of p53 activity significantly ameliorated these senescence–like changes of adipose tissue, decreased the expression of pro–inflammatory cytokines, and improved insulin resistance in type 2 diabetic mice as well as in telomerase–deficient mice. Conversely, up–regulation of p53 in adipose tissue caused an inflammatory response that led to insulin resistance. Our results demonstrate a previously unappreciated role of adipose tissue p53 in the regulation of insulin resistance and suggest that cellular aging signals in adipose tissue could be a novel target for the treatment of diabetes.
S10−4

Adipocyte/macrophage fatty acid-binding proteins and insulin resistance

It is proposed that infiltration of macrophages following T lymphocytes into adipose tissue initiates the alterations in expression and secretion of adipokines thereby inducing systemic insulin resistance in obesity. In collaboration with Department of Plastic and Reconstructive Surgery in Tokyo University Hospital, we have collected subcutaneous adipose tissue samples from non-diabetic patients who receive plastic surgery with normal body weight or mild obesity. Using these samples, which allow us to exclude the possible secondary effects by hyperglycemia and morbid obesity, we have extensively investigated gene expressions by cDNA microarray analysis, and identified several genes which have signal peptide sequence and whose expression was changed by obesity. Of these, we have picked up one potential adipokine and made adenovirus expression vector for this potential adipokine and the antisense oligonucleotide. We have assessed the effects of overexpression and suppression of this gene in wild-type mice and dab/dab mice. Together with in vitro experiments using RAW 264 cells and 3T3L1 adipocytes, the data suggest that this molecule may play a role in the development of obesity-induced insulin resistance. We are now confirming the role of this molecule in humans using visceral adipose tissue samples that we have started collecting.

S10−5

Adipocyte/macrophage fatty acid-binding proteins and insulin resistance

Inflammation and stress signaling is a critical mechanism underlying chronic metabolic disease, and both metabolic and immune cells are involved in regulation of metabolic homeostasis. Adipocyte-macrophage fatty acid–binding proteins (FABPs), aP2 (FABP4) and mal1 (FABP5) integrate inflammatory and metabolic responses in these cells and play a significant role in several aspects of metabolic syndrome including type 2 diabetes and atherosclerosis. We have recently demonstrated that an orally active small molecule inhibitor of aP2 is an effective therapeutic agent against severe atherosclerosis and type 2 diabetes in mouse models. In macrophage and adipocyte cell lines with or without aP2, we also showed the target specificity of this chemical intervention and its mechanisms of action on metabolic and inflammatory pathways. FABPs also offer a unique opportunity to address the role of macrophages and adipocytes in controlling different aspects of metabolic disease. To address the extent of contribution of macrophages in adipose tissue per se on systemic insulin sensitivity and metabolic homeostasis, we generated macrophage– or adipocyte–FABP-deficient mice through bone marrow transplantation to dissect the biology of macrophage and adipocyte FABPs on systemic insulin action and glucose metabolism. The experiments illustrated that neither macrophages nor adipocytes individually could account for the total impact of FABPs on systemic metabolism and that the interactions between these two cell types are critical for the inflammatory basis of metabolic deterioration. Quality change of adipose tissue by FABP-deficiency in macrophages led to a decrease in inflammatory responses independently of amount of macrophages. These findings indicate a central role for lipid chaperones in controlling the metabolic and inflammatory responses in the context of insulin resistance.
Cellular mechanism for GLP-1 specific insulino-otopic action in pancreatic β-cells

Incretins such as gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are secreted upon meal ingestion and potentiate insulin secretion in glucose dependent manner. Both GLP-1 and GIP activate cAMP signalling in pancreatic β-cells, however, their insulino tropic effect differ greatly in patients with type 2 diabetes mellitus, suggesting that each evokes its specific cellular signalling in the pancreatic β-cells. We previously found that K_{ATP} channel-deficient mice (Kir6.2/-/- mice) fail to elicit glucose-stimulated insulin secretion (GSIS). However, the perfusion experiments in Kir6.2/-/- mice revealed that apparent GSIS was evoked by the administration of GLP-1, but only marginally by GIP, indicating that GLP-1 and GIP require the K_{ATP} channel differently in the pancreatic β-cells. The perfusion experiments further revealed that the combination of cAMP (1 mM 8-Br-cAMP) plus glucose also evoked apparent insulin secretion in Kir6.2/-/- mice. Considering that both GLP-1 and GIP increase intracellular cAMP concentrations to a similar level, the difference in the insulino tropic effect of GLP-1 and GIP may be due to the temporal or special difference in cAMP signalling in the pancreatic β-cells.

Recently, we identified an adrenal gland-specific isoform of Epac2 (a cAMP-activated GEF protein against Rap1), which we designate Epac2B, while renaming the previously identified Epac2 Epac2A. Immunocytochemical analysis revealed that Epac2A was localized near the plasma membrane, while Epac2B was found primarily in the cytoplasm. Involvement of Epac2A and Epac2B on cAMP-induced insulin secretion was investigated in Epac2B-overexpressing MIN6 cells. Interestingly, hormone secretion was triggered by sub-stimulatory ranges (5.6 mM) of glucose concentrations plus cAMP in Epac2A overexpressing MIN6 cells but not in Epac2B, suggesting that subcellular localization of Epac2A near the plasma membrane is critical for triggering the cAMP-dependent GSIS. Taken together, GLP-1 and GIP may exert its specific effect through specially different cAMP signalling in the pancreatic β-cells.

Molecular mechanism by which incretin mimetics preserve pancreatic β-cell mass in diabetic mice

It is widely accepted that the β-cell function progressively deteriorates in patients with type 2 diabetes (T2DM). Development of treatment strategies has focused on ways to improve β-cell function and to prevent β-cell death. Glucagon-like peptide-1 (GLP-1) attracts considerable attention because it may alter the natural history of T2DM by preservation of functional β-cell mass. To investigate the molecular mechanism by which GLP-1 preserves pancreatic β-cell mass, we performed subcutaneous injection of a long-acting GLP-1 analogue, liraglutide (LIRA) or vehicle for 2 weeks (long-term) or 2 days (short-term) to diabetic db/db mice and normoglycemic m/m mice. After extracting pancreatic sections, we studied histochemical analysis and gene expression in the core of islets by using laser capture microdissection and real-time RT-PCR. The immunohistochemical analysis suggested that a long-term treatment with LIRA increased β-cell mass in db/db mice due to the enhancement of cellular proliferation and the inhibiting cellular apoptosis. The gene expression analysis after short- and long-term treatment of db/db mice with LIRA revealed that LIRA directly stimulates cellular differentiation and proliferation. In m/m mice, we also found that a long-term LIRA treatment increased β-cell mass and up-regulated expression of genes related with cellular differentiation and proliferation. Moreover, a long-term treatment of db/db mice with LIRA decreased the expression of gene related with endoplasmic reticulum (ER) stress and increased the expression of genes related with anti-oxidative stress. However, we observed that, unlike the direct effect of LIRA on gene expression involved in cellular differentiation and proliferation, a short-term LIRA treatment did not affect the gene expression related with ER stress and anti-oxidative stress in db/db mice. Taken together, we hypothesize that LIRA inhibits cellular apoptosis through suppressing oxidative/ER stress via amelioration of glucolipotoxicity as a chronic effect. In conclusion, the human GLP-1 analog liraglutide preserves β-cells by regulation of cell kinetics and suppression of oxidative/ER stress in diabetic mice.
Protective effect of GLP-1 on pancreatic β-cells and our attempt to evaluate β-cell mass
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GLP-1 and GIP are incretin hormones. They not only stimulate insulin secretion but also have various beneficial actions in the treatment of type 2 diabetes (T2DM). New anti-diabetic agents targeting the incretin system have been developed.

On the other hand, it has been demonstrated that existing anti-diabetic agents including insulin could not suppress the progression of T2DM, and the reduction of β-cell mass has been pointed out to be one of the possible reasons. Recent studies in which pancreas samples obtained by autopsy were analyzed showed significant reduction of β-cell mass in T2DM. In this context, it has attracted considerable attention whether incretin-related agents can modulate β-cell mass in human T2DM.

In the first part of my talk, I will present the cytoprotective effect of GLP-1 using islet transplantation model that is suitable for evaluating β-cell mass. We transplanted islets isolated from the transgenic mice, islet of which express GFP, and subsequently observed the β-cell mass. Administration of the GLP-1 receptor agonist exendin-4 significantly decreased the reduction of β-cell mass after islet transplantation. Thus, it should be important to investigate whether incretin-related agents have a beneficial effect on β-cell mass in human. Furthermore, the reduction of β-cell mass is one of the major causes of poor results in maintaining insulin independence for long period after clinical islet transplantation. Therefore, it also is interesting to investigate the cytoprotective effect of incretin-related agents on β-cells in clinical islet transplantation.

Need for non-invasive quantification of β-cell mass is increasing to clarify the pathophysiology of diabetes and to evaluate the cytoprotective and proliferative effects of various drugs including incretin-related agents on β-cells. However, such method has not been developed to date. We previously showed the possibility of the probe targeting GLP-1 receptor by using its antagonist, exendin (9-39), labeled with 125I. In the second part, I will introduce our study in which we are trying to evaluate β-cell mass using positron emission tomography imaging targeting GLP-1 receptor.

Elucidating the physiological actions of GLP-1:
Lessons from GLP-1 receptor knockout mice
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Classic actions of the incretin hormone glucagon-like peptide–1 (GLP–1) include the enhancement of glucose–stimulated insulin secretion from pancreatic β-cells. Pharmacological actions of GLP–1 also include inhibition of gastric emptying, engagement of neural circuits in the portal region and CNS, reduction of glucagon secretion, and expansion of β-cell mass. GLP–1R activation also lowers plasma lipid levels and may regulate regulatory T cell function in the immune system. The extent to which these actions also reflect the endogenous physiological actions of low circulating levels of GLP–1 remain unclear. We review herein new insights from GLP–1 receptor knockout mice that define the physiological importance of GLP–1 in different tissues. Unexpectedly, GLP–1 receptor signaling is essential for allocation of immune subsets and for the control of lymphocyte proliferation. Endogenous GLP–1R signaling also regulates intestinal lipid synthesis in the postprandial state. In contrast to the GIPR, basal GLP–1R signaling maintains prosurvival gene expression in the basal state in murine islets. Despite the importance of GLP–1R–regulated neural circuits for control of glucose disposal, transgenic restoration of hGLP–1R expression in pancreatic β-cells and ductal cells of Glp1r−/− mice results in normalization of glucose tolerance in the absence of any effect on gastric emptying or CNS glucose homeostasis. Hence global deletion or tissue–selective restoration of GLP–1R signaling provides useful insights into the diverse physiological actions of GLP–1 in vivo.
History of GLP-1: From discovery to clinical application in humans. Human pathophysiology

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GLP-1, a brain and gut product of the proglucagon, was searched for because of data indicating that glucose–dependent insulinotropic polypeptide (GIP) could not be the only incretin hormone. Neither of the 2 glucagon–like sequences (GLPs) identified in the predicted proglucagon precursor were insulinotropic, but the natural peptide (Holst et al FEB-Sletters 1987), a truncated, amidated peptide (GLP-1 7–36amide) strongly stimulated insulin secretion and inhibited glucagon secretion, and has powerful glucose lowering effects in patients with T2DM. It is an incretin hormone and the incretin effect is lost in T2DM. This is because both GLP-1 has lost insulinotropic potency, while GIP loses insulinotropic efficacy. In addition, meal–stimulated GLP-1 secretion is frequently impaired. Both defects appear to be secondary to T2DM: similar losses are seen early in secondary diabetes (chronic pancreatitis) and induction of glucose intolerance (gestational diabetes, glucocorticoid treatment) leads to a similar loss of incretin effect. Intensive metabolic control in T2DM improves incretin function and activity of GLP–1 and GIP. Conclusion: The loss of incretin effect contributes importantly to diabetic hyperglycemia, and restoration of the effect (with GLP–1 agonists) greatly improves metabolic control. Conversely, accelerated gastric emptying causes exaggerated GLP–1 secretion and explains postprandial reactive hypoglycemia in insulin sensitive individuals.

GLP–1 is now known to be a physiological negative regulator of appetite and food intake, and chronic administration leads to weight losses. BMI is inversely correlated to meal–induced GLP–1 secretion, and loss of gut–derived appetite regulation may represent a pathophysiological trait of obesity. Gastric–bypass operations in morbidly obese diabetic subjects are associated with rapid transfer of nutrients to the distal small intestine and hugely elevated (up to 10– fold) GLP–1 responses. Because of its effects on appetite and metabolic control, GLP–1 is likely to contribute importantly to the beneficial results of these operations.
Mechanism of albuminuria in diabetic nephropathy—urinary intact albumin and incomplete tubular compensation model

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Extent of albuminuria (or proteinuria) is one of the most important predictors of renal survival and mortality both in patients with diabetic nephropathy and in general population. However, there are a number of unsolved, fundamental issues concerning how urinary excretion of albumin is regulated.

Neutrophil gelatinase–associated lipocalin (NGAL) is a 25-kDa globular protein, whose renal expression and urinary excretion is highly elevated in the very early phase of acute kidney injury (Mori et al. Kidney Int 2007). We reported that urinary NGAL is potentially useful for the monitoring of disease activity and treatment efficacy of chronic kidney disease (Kuwabara et al. Kidney Int 2009). Urinary NGAL and albumin levels are elevated in streptozotocin–induced diabetic mice and high fat diet further increases the levels. Impaired reabsorption at the proximal tubules appears to play a role in these findings (Tojo et al. Hypertens Res 2003). Comper and Russo et al. have proposed that, in healthy humans, a gram level of albumin is excreted into the urine as albumin fragments (after degradation at proximal tubules) which cannot be detected by routine antibody-mediated methods (Diabetes 2000, J Am Soc Nephrol 2009). They claim that ratio of intact to fragmented albumin is increased in diabetic nephropathy, which does not fit well with the current understanding that glomerular hyperfiltration is the primary abnormality found in early diabetic nephropathy. Here we propose a model in which glomerular hyperfiltration of albumin, (insufficiently) increased overall endocytotic activity of proximal tubules, reduced efficiency of albumin reabsorption, and increased urinary excretion of intact albumin occurs. This concept may be applied to other low-molecular-weight urinary biomarker proteins, in general.

Monotham or Onozato et al. reported that angiotensin receptor blocker improves peritubular capillary blood flow and reduces oxidative stress in proximal tubules (J Am Soc Nephrol 2004, Kidney Int 2002), raising a possibility that reagents that increase albumin reabsorption may improve albuminuria in diabetic nephropathy.

Microinflammation in the pathogenesis of diabetic nephropathy

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Pathogenesis of diabetic nephropathy involves many factors in the downstream of hyperglycemia. We present our hypothesis that low-grade inflammation, “microinflammation” is one of the common pathways of development of diabetic vascular complications and a candidate of therapeutic target for diabetic nephropathy in this symposium.

We previously demonstrated that macrophages are accumulated in the kidney of patients with diabetic nephropathy. ICAM-1 is up-regulated in renal tissues of diabetic animals and diabetic patients (Diabetes 46, 1997). ICAM-1 deficient mice are resistant against renal injuries after induction of diabetes (Diabetes 52, 2003). DNA microarray revealed up-regulated gene expression of proinflammatory molecules including macrophage scavenger receptor-A (SR-A). SR-A knockout mice were also resistant against diabetic renal injuries (Diabetes 56, 2007). Statin, thiazolidinedione, macrolide and methotrexate prevents the renal injuries through anti-inflammatory actions in diabetic animals (Nephrol Dial Transplant 18, 2003, Diabetologia 48, 2005, J Am Soc Nephrol 16, 2005, Am J Physiol 292, 2007). Moreover we have found that GLP-1 receptor is expressed on glomerular endothelial cells and monocytes. GLP-1 receptor agonist, Exendin-4, prevented renal injuries in streptozotocin induced diabetic rats through anti-inflammatory effects. These findings suggest that modulation of microinflammation may be beneficial for the therapy of diabetic nephropathy.

We also demonstrated that serum and urinary concentrations of proinflammatory cytokines are elevated in type 2 diabetic patients. Serum level of IL-18 was correlated with baPWV, carotid IMT and albuminuria (Diabetes Care 28, 2005). We have recently found that gene expression of TNF-α is up-regulated in circulating monocytes of type 2 diabetic patients using DNA microarray study. TNF-α was up-regulated by high glucose condition and down-regulated by statin in cultured human monocytes.

Our findings suggest that microinflammation is one of the common pathogenesis of diabetic vascular complications and might be a therapeutic target for diabetic nephropathy.
Remission of diabetic nephropathy with intensive multifactorial therapy
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Diabetic nephropathy in type 2 diabetes is a leading cause of end-stage renal disease worldwide. Its early clinical sign is microalbuminuria and proteinuria, which are not only a predictor to end-stage renal disease but also an independent risk factor for cardiovascular disease. Thus, diabetic nephropathy is an important therapeutic target to improve the prognosis in diabetic patients. A few decades ago, we believed that diabetic nephropathy was a progressive and irreversible chronic complication. Thus, the main therapeutic objective for type 2 diabetic patients with nephropathy at the time was to prevent the progression to the advanced stage of nephropathy. However, recent clinical trials in type 2 diabetic patients with nephropathy showed that intensified intervention on multiple risk factors reduced the risk of both microvascular and macrovascular events, and the renin-angiotensin blockade drugs induced a decrease in albuminuria, such as SMART and INNOVATION studies. We also reported that a reduction in microalbuminuria of Japanese patients with type 2 diabetes mellitus was more frequent than progression to overt proteinuria in a prospective observational follow-up study and that multifactorial control approach was important to the reduction of microalbuminuria. Furthermore, we showed that the eight-year cumulative incidence rate of renal and cardiovascular events was significantly lower in patients with remission than in those without. The annual decline rate of estimated glomerular filtration rate in patients with remission was also significantly slower. Remission of nephropathy is therefore considered to be possible with intensive therapy and to be an important therapeutic objective in type 2 diabetic patients. However, in the diabetic patients with sever cardiovascular complications, the intensive therapy was reported to induce adversely cardiovascular events. These evidences suggest that the benefit of the intensive therapy is not uniform across all diabetic patients. Thus, we should consider the target levels for intervention on risk factors according to the condition of each diabetic patient.

Significance of blood pressure control and use of RAS inhibitors in treating diabetic nephropathy
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Diabetic nephropathy is currently the most common reason for patients to start dialysis treatment. Moreover, elucidation of the pathology and establishment of treatment methods are urgently needed. Under diabetic conditions, advanced glycation end products increase intracellular reactive oxygen species (ROS), increasing inflammation, proliferation and apoptosis, ultimately inducing nephropathy. ROS also increase the production of intrarenal angiotensinogen; and the production of angiotensin II (Ang II), and this Ang II further aggravates ROS production, forming the ROS-RAS-ROS vicious cycle. Administration of renin–angiotensin system (RAS) inhibitors, therefore, is believed to block this process and suppress intrarenal ROS. The increase in this intrarenal Ang II, moreover, increases constriction of the cortical superficial efferent arterioles, raises the intraglomerular pressure, and increases albuminuria. Treatment using RAS inhibitors, therefore, can help reduce the excretion of albumin from the cortical superficial glomeruli into the urine. Under diabetic conditions, furthermore, the afferent arterioles are in a contractile dysfunctional state, and are therefore susceptible to the influence of systemic blood pressure (BP). Juxtamedullary glomeruli, in particular, are able to be influenced due to their anatomical location. When BP rises, therefore, the pressure inside the juxtamedullary glomerulus similarly rises, and urinary albumin excretion from these glomeruli increases. Since this urinary albumin excretion from the juxtamedullary glomerulus can be suppressed by strict BP control, BP reduction and administration of RAS inhibitors are believed to be a treatment suited to the pathology of diabetic nephropathy. In diabetes, exacerbation of the sympathetic nervous system, reduction of NO production, aggravation of RAS, hyperinsulinemia, sodium reabsorption and vascular resistance are also induced. BP rises as a result. In addition to treatment using RAS inhibitors, the concomitant use of low-dose diuretics and calcium channel blockers is effective for treating the hypertension that develops in association with diabetes.
Renin-Angiotensin System inhibition in the primary prevention of diabetic nephropathy
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Renin-Angiotensin System (RAS) inhibition has proven to be valuable adjunctive therapy to conventional blood pressure (BP) control in slowing the rate of progression to end-stage renal disease in type 1 (T1DM) and type 2 (T2DM) patients with established diabetic nephropathy (DN) and elevated serum creatinine, and this is claimed to be independent of BP control. However, there is much controversy regarding the efficacy of RAS blockade (RASB) at earlier stages of DN. In order to demonstrate primary prevention with RASB it would be necessary to demonstrate benefits on (1) the rate of development of important DN lesions in normoalbuminuric patients; or (2) prevention of microalbuminuria which is durable, i.e., persistent after discontinuation of the RASB; and (3) that these benefits are demonstrable in comparisons with patients under equal BP control with other agents.

The Renin-Angiotensin System Study (RASS) could not demonstrate benefits of RASB on slowing the early lesions of DN in normoalbuminuric, normotensive T1DM patients and other studies have not met the criteria listed above for either T1DM or T2DM. Thus RASB is not proven to provide DN prevention in normotensive normoalbuminuric T1DM patients. Nor has RASB been shown to provide durable benefits which were independent of its BP lowering effects in hypertensive normoalbuminuric T2DM patients.
New trends of insulin pumps in the USA
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Studies have shown that the overall risk of micro- and macro-vascular complications associated with both type 1 and 2 diabetes. Implementation of intensive diabetes management (using insulin pumps or MDI along with increased SMBG) is expensive though there is a significant reduction in risk of long term complications and cost. Although the benefits of optimal glucose control seem clear, the risk of severe hypoglycemia can be a barrier to achieving this goal.

For insulin requiring patients with type 1 or 2 diabetes basal–bolus Rx is the best choice as it imitates the normal physiology.

The current basal insulins (insulin glargine and detemir) do not replicate the normal physiology and the rapid–acting insulins (aspart, lispro and glulisine) must be taken much earlier to match the food absorption. Thus use of MDI results in significant post–prandial hyperglycemia and delayed hypoglycemia.

The ideal basal–bolus treatment strategy can be best implemented with insulin pump Rx. Several studies using insulin pumps have shown reduction in insulin dose, hypoglycemia and significantly better A1C values as compared to MDI. It is important to consider increased cost and risk of diabetic ketoacidosis if patients do not monitor SMBG regularly. Currently about 16–20% of type 1 diabetes patients are on insulin treatment in the USA. In type 2 diabetes only about 37,000 patients are using pump treatment in part due to poor reimbursement and lack of resources to initiate pumps in such patients.

The wider availability and use of CGM recently in USA has further highlighted the need for pump Rx in insulin–requiring patients. The future (5–10 years) might see semi–close/close–loop by integrating CGM with pumps with the first step being auto–shut off of the pump when the blood glucose fall below 70 mg/dl. For this to happen sooner the CGM technology must improve, new algorithms must be created, new ultra–rapid acting insulins and/or smart insulins (reacting to changes in blood glucose levels) must become available so that insulin pump treatment can be proactive rather than being reactive.

Current status of insulin treatment in France
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The treatment recommended by the American Diabetes Association for type 1 diabetes consists in basal and prandial insulin analogs, administered either with multiple daily injections (MDI) or in insulin pump. Two French national surveys, conducted among diabetologists, respectively in 1998, and in 2002, have shown that basal bolus insulin regimen was effective respectively in 38%, and 47% of patients who were followed by a specialist, and 10% were treated with pump. The ENTRED survey conducted in 2007 in a representative national sample, demonstrated that only 51% of the 134,000 T1DM were followed by a diabetologist. Their estimated mean HbA1c was 7.9%. About 15,000 patients were treated with pump. Two main concerns are still pending:
1- The spread of Flexible insulin therapy
2- The subset of patients poorly controlled chronically, even though they benefit from intensive insulin regimen with a specialized followed–up: in an exhaustive regional pump registry, HbA1c was >9% for 11% of patients. For these "high–risk" patients, a multidisciplinary intensive follow–up is proposed but submitted to scarce funding. An active electronic diary up–loaded in a smartphone, associated with a telemedicine follow–up could be part of the solution as this system gave a 0.9% HbA1c improvement over controls in the Telediab1 multicenter French study. The generalization and the reimbursement of such a system by the national French health insurance is still being discussed.
There are 2,200,000 subjects with T2 diabetes in France, whose mean estimated HbA1c is 7.1%: 17% of these patients are treated with insulin, either combined with OAD (10%) or alone (7%) HbA1c is >8% for 35% of the patients. The main reasons of this failure are:
1- Insufficient titration of basal insulin by GPs. An active electronic diary, with an automatic adjustment of basal insulin coupled with a telemedicine follow–up, could partly solve the problem. A coaching focused on dietary and physical exercise has also been added to the system. The Telediab 2 multicenter trial, which objective is to assess this system is still on–going.
2- Lack of guidelines for patients with a well–titrated basal insulin but with HbA1c still above the targets. Different strategies are proposed: "basal + " or "basal bolus" seem more accurate than premix, and basal analog associated with short acting GLP–1 are still under evaluation.
S13－3

Current status of insulin therapy in Japan （from the study of JDDM）

Japan Diabetes Clinical Data Management Study Group (JDDM) has collected the clinical data from study group members of diabetes clinics and hospitals in Japan with the use of diabetes management software CoDiC and analysed them to produce clinically useful results for better treatment of diabetes. We report the yearly change of insulin therapy and glycemic control to show the recent insulin treatment in Japan. We collected the data of HbA1c and insulin therapy in May-July from 2004 to 2007 and examined the data from 1,700 patients with type 1 diabetes and 8,100 patients with type 2 diabetes among about 40,000 registered diabetic patients.

In type 1 diabetes, the mean HbA1c value was 7.6% in 2007. Fifty to 60% of these patients were treated by insulin 4 times injections a day and 27% by 5 times injections a day. Among the patients treated by 4 times injections a day, 54% of the patients were treated by Q (ultrarapid insulin analog) 3 times injections + G (glargine) once, the most frequent combination. In 5 injections a day group, Q 3 times + NPH twice injections was 49% in 2004, but Q 3 times + G twice gradually increased to 47% in 2007.

In type 2 diabetes, the mean HbA1c value was 7.5% in 2004, and decreased to 7.4% in 2007. OHA + insulin was administered in 44% and the percentage of patients using analog insulin was increased from 40% in 2004 to 65% in 2007. Insulin therapy with twice injection a day was 40% in 2007, the most common in this group. Three times injections a day was gradually increased, and 4 times injections a day was around 25%. In twice injections a day group, Qmix was 80%. Among 3 times injections a day group. Qmix 3 times gradually increased and became more frequent than Q 3 times injection a day in 2007.

At the time of starting insulin therapy in type 2 diabetes, the mean HbA1c value was 8.88% in 2007. Qmix (30) twice a day was 19%, the most common, and Q 3 times a day + OHA was 16%. Further study showed that the lower HbA1c at the insulin start the better glycemic control afterward, suggesting that early introduction of insulin therapy is recommended in the patients with poor glycemic control.

S13－4

Incretin-based therapy: Possibility as a surrogate therapy of basal-supported oral therapy in Japanese T2DM

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In type 2 diabetes (T2DM), capacity of insulin secretion by pancreatic beta cells (≒ beta cell mass) has been reported to be decreased to nearly 50% at the time of diagnosis. Recent advance in insulin injection therapy and blood glucose measuring or monitoring system has enabled us to treat both fast- and post-prandial hyperglycemia in inadequately controlled patients with T2DM treated with oral anti-diabetic drugs to protect the beta cell function and prevent the development of both micro- and macroangiopathy in the way such as early introduction of BOT (basal-supported oral therapy). Unfortunately, the timing of introduction of insulin injection in such patients appeared to be delayed in Japan compared to those in Caucasian population probably due to the strongly negative image of insulin.

Newly developed therapy for diabetes, so-called incretin-based therapy such as GLP-1 receptor agonists and DPP (dipeptidyl peptidase)-4 inhibitors may change the present status of diabetes treatment in Japan. GLP-1 has multi-potential effects on blood glucose control in patients with type 2 diabetes, showing not only glucose-dependent insulinitropic effect (incretin effect) and glucagonostatic effect, but also, suppression of food intake (appetite), gastric emptying, and glucose output in the liver, and stimulation of glucose uptake in skeletal muscle, adipocytes, and perhaps hepatocytes. Furthermore, GLP-1 has protective/proliferative effects on beta cells, and promotes the beta cell differentiation/neogenesis from pancreatic duct cell epithelium at least in the animal model.

According to our analysis on the islet pathology, “beta cell mass” does not always mean the decrease in the number of beta cells in Japanese non-obese T2DM, suggesting that in addition to well-known incretin effect of GLP-1-related therapy, the trophic effects on beta cells, if existed in human, may surrogate the early introduction of insulin therapy aiming to protect progressive loss or maintenance of beta cell function. The propriety of this possibility will be discussed from clinical and experimental data in view of the positioning of incretin-based therapy in Japanese T2DM.
Insulin treatment optimized using CGM

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When managing diabetes, the therapeutic goal is to maintain a blood glucose level as close to the normal range as possible, in order to both prevent the onset and progression of diabetic complications.

Currently, Self Monitoring of Blood Glucose (SMBG) is frequently used worldwide for glycemic management in diabetes. However, while this provides an indicator of blood glucose levels, these can change dynamically, particularly after meals. Therefore it is important to recognize that SMBG determines the blood glucose level at a specific time point, and it is extremely difficult to understand whether the blood glucose level at that time point is increasing, stable, or decreasing.

Continuous Glucose Monitoring (CGM), which solves the problem associated with SMBG by continuously measuring glucose levels, appeared in the late 1990s. Over the past several years in Japan, there have been applications for approval of CGM devices with an application finally approved in October 2009. The CGM device approved at that time was the CGMS System Gold (CGMS) by Medtronic. CGMS performs measurements every 10 seconds and records the mean value every 5 minutes. Therefore, 288 measurements are recorded daily, providing sufficient information to understand the diurnal variation in blood glucose.

Insulin preparations are basically classified into 5 categories, and there are wide variations in administration methods using a combination of these preparations. It is believed that using CGMS to monitor the glucose variability in patients treated with insulin will result in improved glucose control, and that this control will be better than that achieved using an insulin administration pattern optimized for each individual based on SMBG. In fact the insulin administration pattern determined with a CGMS device is often far different from the one previously used based on SMBG. Today, we report a case in which insulin treatment was optimized using CGMS.

It is expected that the future will see an era of so-called made-to-order medicine in which the optimal combination of insulin will be determined based on CGM patterns.
S14–1

Pluripotent stem cells for type 1 diabetes modeling and cell replacement therapy
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Type 1 diabetes (T1D) is the result of an autoimmune destruction of insulin producing, pancreatic beta cells. The events leading to the disease have usually occurred long before diagnosis and are based on complex interactions between genes and the environment. In addition to finding new cell sources for T1D cell replacement therapy we need to protect transplanted cell from an autoimmune response. We propose to use pluripotent stem cells as a novel source of beta cells and to model T1D. To this end, we have generated type 1 diabetes patient specific pluripotent stem cells. Combined with directed differentiation into key cell populations implicated in T1D we aim to recapitulate the disease in a patient specific manner. The long term goal is to recapitulate the disease in a patient specific manner and to identify novel treatment strategies.

S14–2

The roles of FoxO1 in pancreatic cell differentiation

芳原貴子

We previously reported that FoxO1 protects against β cell failure induced by hyperglycemia (glucose toxicity) (Kitamura, Y. et al, Cell Metab 2005). However, on the other hand, we also reported that FoxO1 inhibits β cell proliferation via suppression of Pdx1 transcription (Kitamura T. et al, J Clin Invest 2002). Therefore, FoxO1’s functions in β cells seem to be like a double-edged sword. To clarify the roles of FoxO1 in pancreatic β cells more precisely in vivo, we generated pancreas specific FoxO1 transgenic (Tg) and pancreas specific FoxO1 knockout (KO) mice. We show that Tg mice develop severe diabetes due to decreased β cells. Consistent with these observations, we also show that KO mice have improved glucose tolerance compared to control mice under high fat diet condition. We found that the numbers of small islets and insulin-positive duct cells were increased in KO mice, indicating increased β cell neogenesis. However, when we crossed KO mice with db/db mice, the mice exhibited more severe glucose intolerance compared to control db/db mice, indicating that FoxO1 deficiency enhanced β cell dysfunction in the state of hyperglycemia. These results support our hypothesis that FoxO1’s functions are like double-edged sword. On the other hand, besides β cell reduction, Tg mice have some other interesting phenotype. Tg mice display severe hypoplasia of pancreatic acinar cells and marked increase in duct-like structure, which leads to the formation of pancreatic cysts at old age. More interestingly, Tg mice exhibit islet hypervascularties, which is associated with increased VEGF level in β cells. By using luciferase promoter assay, we show that FoxO1 directly regulates VEGF transcription in β cells. Taken together, our results suggest that FoxO1 plays important roles in pancreas development as well as β cell growth and function. We propose that conditional manipulation of FoxO1, either genetically or pharmacologically, may contribute the development of new strategies for the treatment of diabetes.
The role of epigenetic dysregulation in beta-cell failure

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The abnormal intrauterine milieu of intrauterine growth retardation (IUGR) permanently alters gene expression and function of pancreatic β-cells leading to the development of diabetes in adulthood. Expression of the pancreatic homeobox transcription factor Pdx1 is permanently reduced in IUGR and epigenetic modifications are responsible for this decrease. Exendin-4 (Ex-4), a long-acting glucagon-like peptide 1 (GLP-1) analog, given on days 1–6 of life increases Pdx1 expression and prevents the development of diabetes in the IUGR rat. Here we show that Ex-4 increases USF-1 and PCAF association at the proximal promoter of Pdx1, thereby increasing histone acetyl transferase (HAT) activity leading to a permanent increase in histone H3 acetylation and H3K4 methylation. Normalization of these histone modifications precludes DNA methylation thereby preventing silencing of Pdx1 in islets of IUGR animals. These studies demonstrate a novel mechanism whereby a short treatment course of Ex-4 in the newborn period prevents diabetes in adulthood by restoring Pdx1 promoter chromatin structure thus preserving Pdx1 transcription.

The role of glucokinase in the regulation of beta cell proliferation

中村 昭伸, 寺内 康夫

While evidence has mounted that insufficient adaptation of beta cell mass is symptomatic of type 2 diabetes, we demonstrated that glucokinase and insulin receptor substrate-2 (IRS-2) are critically required for beta cell hyperplasia to occur in response to high-fat (HF) diet-induced insulin resistance. To elucidate the glucokinase–dependent pathway, we focused on small molecule glucokinase activator (GKA). GKA has been identified to stimulate both insulin secretion and glucose utilization in the liver and has antidiabetic efficacy in rodents. Here, we investigated glucose homeostasis and beta cell mass of wild-type and the haploinsufficiency of beta cell–specific glucokinase (Gck−/−) mice challenged with a HF diet with or without GKA. The results of our study indicate that GKA is able to improve glucose metabolism and has an effect on beta cell proliferation. Moreover, we investigated the role of IRS-2 in the regulation of beta cell proliferation in response to GKA. Our results suggest that glucokinase activation by GKA increased IRS-2 expression through the cAMP–responsive element–binding protein (CREB) phosphorylation, thereby leading to beta cell proliferation via cell cycle signaling molecule such as cyclin D2. Thus, GKA should also have substantial merit in respect to the stimulation of beta cell proliferation.

By contrast, when we evaluated beta cell mass in Gck−/− and IRS-2−/− knockout mice during pregnancy, we noted that beta cell mass was significantly increased by pregnancy in both genotypes of mice. These data clearly demonstrate the existence of glucokinase– and IRS-2–independent signal transduction pathway in beta cell proliferation during pregnancy. To investigate the molecular mechanism, systemic gene expression profiling study by DNA microarray was carried out. Interestingly, a novel peptide gene expression was increased in pregnant mice, but not in HF diet–induced insulin resistant mice. The results of our study raise the possibility that this novel peptide may be involved in pancreatic beta cell proliferation in pregnancy.

We believe that these results could lead to novel therapeutic strategies that will increase beta cell mass.
Role of autophagy in beta cells

One of the earliest signs of beta cell failure in the natural history of type 2 diabetes is a specific loss of glucose induced insulin secretion. Beyond this functional defect, pathological studies demonstrated that the patients with type 2 diabetes mellitus have decreased islet size and decreased numbers of insulin-producing cells. One of these studies also pointed out that the subjects with impaired glucose tolerance already shows significant decrease of the islet mass, as the patients with type 2 diabetes mellitus. These results suggest that the decrease in beta-cell mass as well as dysfunction of each islet are main features of beta cell failure in type 2 diabetes.

Pancreatic beta cells are specifically differentiated cells to secrete insulin with proper response to various secretagogues. Although large-scale protein synthesis and degradation are essential for this function, the mechanism underlying the dynamic protein turnover in beta-cells remains largely unknown. While in terms of protein degradation, two principle systems, the ubiquitin-proteasome system and autophagy-lysosome system function to maintain cellular homeostasis, recent studies have shown the importance of autophagy in regulating beta cell function.

Studies using Beta−Atg7KO mice demonstrates the importance of autophagy in the preservation of the architecture and function of pancreatic beta-cells. Intriguingly, the typical features of type 2 diabetes were phenocopied by Beta−Atg7KO mice: these mice exhibited impaired glucose induced insulin secretion under normal diet, while they developed overt diabetes accompanied by the decrease of beta-cell mass when they fed high-fat diet. These observations suggest that loss of beta-cell autophagy could be predisposing factor for type 2 diabetes. On the other hand, it is possible that the acceleration of beta-cell autophagy may enhance beta cell death in special condition. Taken together, imbalance of autophagy may be involved in the basic mechanism of beta cell failure. To elucidate molecular mechanisms controlling beta-cell autophagy, and its proper manipulation may help to establish a novel therapeutic strategy for diabetes.
The physiological and pathophysiological roles of hepatic IRS1 and IRS2 in the glucose and lipid metabolism

Insulin receptor substrate (Irs) mediates metabolic actions of insulin. Although both insulin receptor substrate (Irs) 1 and Irs2 are abundantly expressed in the liver, their respective roles remain controversial. Here, we show that hepatic Irs1 and Irs2 function in a distinct manner in the regulation of glucose homeostasis. The PI3K activity associated with Irs2 began to increase during fasting, reached its peak immediately after refeeding, and decreased rapidly thereafter. By contrast, the PI3K activity associated with Irs1 began to increase a few hours after refeeding and reached its peak thereafter. The data indicate that Irs2 mainly functions during fasting and immediately after refeeding, and Irs1 functions primarily after refeeding. In fact, liver specific Irs1–knockout mice failed to exhibit insulin resistance during fasting, but showed insulin resistance after refeeding, associated with decreased expressions of glucokinase and SREBP1c; conversely, liver–specific Irs2–knockout mice exhibited insulin resistance during fasting but not after refeeding, associated with increased expression of PEPCK and G6Pase. Moreover, liver–specific Irs1/Irs2 double–knockout mice showed insulin resistance in both during fasting and after refeeding, and developed type 2 diabetes. There seems to be a functional relay between Irs1 and Irs2 in hepatic insulin signaling during fasting and feeding. Understanding the molecular basis of hepatic insulin signaling in the regulation of glucose metabolism may be expected to provide a basis for a better understanding of the pathogenesis and treatment of type 2 diabetes mellitus.

Regulation of hepatic lipid metabolism by transcription factor Stra13

Regulation of energy metabolism in the liver is largely dependent of control of the expression of genes related to carbohydrate and lipid metabolism, and insulin play an essential role in the regulation of such gene expression. We have found that hepatic expression of Stra13, a member of bHLH family transcription factors, was stimulated by insulin both in vitro and in vivo. In cultured hepatocytes, overexpression of Stra13 stimulates the expression and the promoter activity of SREBP1c, a master regulator of lipogenesis in the liver. The reduction of Stra13 with the use of short hairpin RNA (shRNA) inhibits insulin–induced expression of SREBP1c. These results indicate that Stra13 contributes the regulation of the SREBP1c expression by insulin. Overexpression of Stra13 in mice liver augmented the increase in hepatic expression of SREBP1c and the reduction Stra 13 in with the use of an adenovirus vector encoding shRNA of Stra13 decreased the expression of SREBP1c. In obese KKAy mice, the hepatic abundance of Stra13 as well as of SREBP1c is greater than those of control mice. The reduction of Stra13 in the liver with the use of an adenovirus vector encoding shRNA of Stra13 decreases the expression of SREBP1c along with those of the downstream genes of SREBP1c including stearoyl–CoA desaturase1 or fatty acid synthase. The reduction of hepatic Stra13 in KKAy mice markedly ameliorates hypertriglyceridemia and hyperinsulinemia. These results suggest that Stra13 controls hepatic lipid metabolism thorough the regulation of SREBP1c and that the Stra13 pathway serves as a potential therapeutic target of dyslipidemia.
S15–3

The transcriptional coactivator Cited2 regulates hepatic gluconeogenesis by controlling PGC–1α activity

Hepatic gluconeogenesis is critical in the adaptation to fasting conditions and contributes to fasting hyperglycemia under hepatic insulin resistance in diabetes. Many transcription factors and coregulators cooperatively induce and maintain the gluconeogenic program during fasting. It has been reported that the cAMP-responsive element–binding protein binding protein (CBP) /p300–binding transcriptional coactivator Cited2 binds hepatocyte nuclear factor 4 α (HNF4α) and plays a crucial role during liver development. Despite the physiological importance of HNF4α and CBP in controlling gluconeogenesis, the mechanisms by which Cited2 regulates gluconeogenesis remain unknown.

We investigated the role of Cited2 in hepatic gluconeogenesis by using gain– and loss–of–function approaches in vitro and in vivo. In hepatocytes, the overexpression of Cited2 resulted in a 2–fold increase in cAMP–induced expression of gluconeogenic genes such as G6Pase and Pepck, leading to a 2–fold increase in glucose levels in the media. Cited2 deletion in hepatocytes attenuated cAMP–dependent induction of gluconeogenic genes. Hepatic expression of Cited2 in vivo increased gluconeogenic gene expression and blood glucose levels under both fasting and fed conditions. cDNA microarray analysis revealed that Cited2 overexpression in vitro enhanced cAMP–dependent induction of genes regulated by peroxisome proliferator–activated receptor γ coactivator–1α (PGC–1α), such as Ppara, Cpt1a, Cyp17a1, as well as G6Pase and Pepck. Therefore, we next examined the effect of Cited2 on gluconeogenic gene expression induced by PGC–1α in vitro. Overexpression of Cited2 enhanced PGC–1α–induced gluconeogenic gene expression, but Cited2 deletion attenuated the expression. These data strongly suggest that Cited2 upregulates PGC–1α function, and the molecular mechanism underlying the upregulation is currently being investigated.

S15–4

The role of endoplasmic reticulum stress and lipogenesis in the development of hepatic steatosis

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Hepatic steatosis is associated with the metabolic syndrome, included type 2 diabetes and obesity. Lipogenesis, which is the metabolic pathway metabolizing glucose into fatty acids, has been shown to be one of the major actors implicated in hepatic steatosis development. Lipogenesis is highly dependent on nutritional conditions for its activation. It is induced by high–carbohydrate feeding and inhibited by fasting. Lipogenic gene expression is controlled by SREBP–1c, a transcription factor which requires proteolytic cleavage to be activated. Activation of SREBP–1c by insulin during carbohydrate feeding involves 2 mechanisms: activation of SREBP–1c transcription and increase in proteolytic cleavage of the SREBP–1c precursor form embedded in the membranes of the ER. Although highly dependent on insulin for its activation, lipogenesis is paradoxically very active in the liver of obese rodents such as ob/ob mice, which are characterized by severe hepatic insulin resistance. We hypothesized that SREBP–1c could be activated by another mechanism than insulin and we investigated the role of the endoplasmic reticulum stress in SREBP–1c and lipogenesis activation. We showed that an activation of the ER stress pathway stimulates SREBP–1c cleavage and induces its lipogenic target genes in vitro in rat hepatocytes. In ob/ob mice, as shown previously by others, the hepatic ER stress pathway is activated. Selective inhibition of hepatic ER stress using an adeno viral overexpression of the chaperone Bip/GRP 78, leads to a marked decrease of ER stress markers and consequently to a decrease in SREBP–1c processing and expression and to an inhibition of lipogenesis. In turn, this reduces hepatic steatosis and serum triglycerides and restores insulin sensitivity.

We concluded that the ER stress pathway has a major role in the development of hepatic steatosis in insulin resistant states by activating SREBP–1c and thus hepatic de novo lipogenesis. A pharmacological inhibition of the ER stress pathway could be a way to reduce hepatic steatosis and to improve glucose homeostasis.
New aspect of hepatic insulin resistance: fatty acid composition

Insulin resistance is often associated with obesity and precipitates metabolic syndrome and type 2 diabetes. To date, most known approaches which improve insulin resistance are preceded by amelioration of obesity and hepatosteatosis. However, abnormal tissue lipids involves two aspects: quantity and quality. For instance, SREBP-1c is a bHLH type transcription factor that controls lipid synthesis and is induced during over-nutrition to facilitate the conversion of glucose to fatty acids and triglycerides for storage of the excess energy. Activation of nuclear SREBP-1c in the liver causes hepatosteatosis, hypertriglyceridemia, and hepatic insulin resistance through direct suppression of insulin signaling pathways. SREBP-1c also seems to be linked to various pathological processes such as pancreatic beta cell function, diabetic nephropathy, parasympathetic response of heart, and hypertrophy of adipocytes in obesity.

While this scenario involves quantitative aspect of abnormal tissue lipids linking to obesity-related diseases, quality of tissue lipids could be another factor. Here, we show that insulin resistance can be improved by modifying hepatic fatty acid composition, despite persistent obesity and hepatosteatosis. Mice deficient for Elov1-6, the elongase that catalyzes conversion of palmitate to stearate, become obese and develop hepatosteatosis when fed a high-fat diet or when mated to leptin-deficient ob/ob mice. However, they exhibited marked protection from hyperinsulinemia, hyperglycemia, and hyperleptinemia. Hepatic fatty acid composition is a novel determinant for insulin sensitivity independent of cellular energy balance. Inhibition of this elongase could be a new therapeutic approach for insulin resistance, diabetes, and cardiovascular risks circumventing obesity issues.

Glycolipid metabolism regulation by fibroblast growth factors

The energy sources of animal body shift from glucose to fatty acid and ketone body during energy deprivation status such as fasting. We show that fasting induces FGF21 mRNA levels thorough PPARα, which plays a crucial role in regulating energy stores during fasting. FGF21 is induced directly by PPARα in liver in response to fasting and PPARα agonists including the fibrate dyslipidemia drugs. FGF21 in turn stimulates lipolysis in white adipose tissue and ketogenesis in liver. FGF21 also regulates glucose metabolism as well as lipid metabolism. On the other hand, we present that FGF 15 is an FXR target gene in small intestine and regulates bile acid synthesis in liver as an endocrine factor. These studies demonstrate that FGF21 and FGF15 are the endocrine signals regulating diverse metabolism in the body.
S16-1

Incretin response in Japanese type 2 diabetes and healthy controls: Towards standardization of assays for GLP-1 and GIP

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Beneficial effects of incretins, GLP-1 and GIP on glucose homeostasis primarily through its regulation of beta cell function and viability have been establishing incretins as highly attractive therapeutic targets for diabetes. There are, thus, strong demands for reliable assays to measure levels of intact and total (i.e. intact plus DPP-4–metabolized) incretins in human subjects in order to study their secretion and processing, to evaluate effects of DPP-4 inhibitors, and to further develop better incretin-related therapies.

However, immunoassays for GLP-1 and GIP, especially those to measure their intact forms, require specific antibodies and have not been widely available. In addition, large variability in intact GLP-1 levels determined by different commercially-available immunoassays leads to confusion and hampers our precise understanding of the incretin biology. Recent studies including ours have revealed an importance of an additional extraction step (i.e. ethanol or solid–phase extractions) prior to immunoassays for intact GLP-1. The extraction removes interferences with unknown identity, which give rise to large variations in intact GLP-1 levels, from plasma to obtain results less variable among different human subjects. Furthermore, the extraction improves correlation across the different immunoassays for intact GLP-1.

In this presentation, we first provide results demonstrating the importance of the ethanol or solid–phase extraction to measure intact GLP-1. We also report characterization of various immunoassays for intact and total GLP-1 and GIP, which is valuable for standardizing incretin immunoassays. With refined assays, we then demonstrate that intact GLP-1 levels are considerably low in both Japanese type 2 diabetes (T2DM) patients and healthy controls, and that meal–induced enhancement of GLP-1 response is negligible in both groups. Our results could account for effectiveness of incretin-related therapies in Japanese compared to other ethnicities. We also provide our recent data on incretin response in Japanese T2DM patients with various anti–diabetic drugs including DPP-4 inhibitor sitagliptin and alpha–glucosidase inhibitor acarbose.

S16-2

Defects in the incretin system in Caucasians with type 2 diabetes

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The incretin effect, that is, the postprandial augmentation of insulin secretion by gastrointestinal hormones, mediates approximately 50–70% of the overall insulin responses after a mixed meal or glucose ingestion in healthy subjects. In patients with type 2 diabetes, the incretin effect is markedly reduced, and this has been attributed to defects in the secretion and insulinotropic action of the two main incretin hormones, namely gastric inhibitory polypeptide (GIP) and glucagon–like peptide 1 (GLP–1). It has been speculated that a reduced incretin effect might precede the onset of hyperglycaemia in patients with type 2 diabetes. However, the secretion and action of GIP and GLP–1 is relatively unaltered in normal glucose–tolerant individuals at high risk for type 2 diabetes (e.g. first–degree relatives) and a diminished incretin effect is also detectable in other types of diabetes, thereby arguing against such reasoning. This presentation will describe the defects in the incretin system in patients with type 2 diabetes, summarise their relevance in the development of hyperglycaemia and discuss the potential individual roles of GIP and GLP–1 in the pathogenesis of type 2 diabetes.
Factors related with fasting and postprandial plasma incretin levels in Japanese type 2 diabetic patients

We investigated factors that would be related with plasma incretin levels in Japanese type 2 diabetic patients (T2Ds). We recruited 45 Japanese T2Ds (means of age, HbA1c, and BMI were 83.7 yrs, 7.0% and 25.4, respectively) with only diet therapy or diet + oral anti-diabetic agents without alpha-glucosidase inhibitors that affect plasma incretin levels. Plasma active glucagon-like peptide-1 (GLP-1) and total gastric inhibitory polypeptide (GIP) were measured by ELISA kits (Linco Research) 0, 30, 60 and 120 min after a mixed meal. Relationships between plasma incretin levels (fasting and incremental area under the curve [AUC]) of GLP-1 and GIP and several clinical characteristics, such as gender, age, duration of diabetes, fasting plasma glucose (FPG), HbA1c, 1,5-anhydroglucitol (1,5-AG), uses of metformin, pioglitazone or statin, BMI, serum lipids profile, HOMA-B, HOMA-R and serum total adiponectin, were evaluated. Mean plasma active GLP-1 and total GIP levels were 3.3, 7.0, 6.4 and 5.6 (pmol/L) at 0, 30, 60 and 120 min after the meal, respectively, indicating significant augmentation of both plasma incretin levels. In a simple linear regression analysis, I-AUC-GLP-1 correlated with 1,5-AG with tendency toward weak positive correlation (r=0.286, p=0.056). Fasting GIP and I-AUC-GIP positively correlated with increase in PG (0-120 min) (r=0.326, p<0.05) and I-AUC-PG (r=0.311, p<0.05), respectively. I-AUC-GIP in patients with statin use was higher than in those without (p<0.01). In a multiple linear regression analysis with forward stepwise method, significant independent variables for higher levels of fasting GLP-1, I-AUC-GLP-1, fasting GIP and I-AUC-GIP were statin use, 1,5-AG, increase in PG (0-120 min) and statin use, respectively (corrected by gender, age, diabetes duration and BMI). In conclusion, glycemic control, increase in PG after the meal and statin use were correlated with plasma incretin levels in our present study. Previous studies of statin with increase in cholesterol absorption marker and DPP-4 inhibition ability might explain our present results of the relationships between statin use and plasma incretin levels.

GLP-1 Action on glucagon and insulin secretion in patients with type 2 diabetes

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When glucose is taken in orally, insulin secretion is stimulated significantly more than after isoglycemic intravenously glucose infusion. This effect is called the incretin effect and is conveyed by the two incretin hormones: glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). In healthy subjects the incretin effect has been shown to account for up to 70% of the insulin response following oral ingestion of glucose, but in patients with type 2 diabetes, the incretin effect is markedly impaired. The mechanisms of the impaired incretin effect have been found to involve reduced secretion of GLP-1 and a severely impaired effect of GIP.

GLP-1 has beneficial anti-diabetic effects on both pancreatic beta- and alpha cells. Focus has previously been on the improved beta cell function, while the inhibition of alpha cell secretion has received less attention. Recent findings suggest that the inhibition of glucagon secretion is a very important part of the anti-diabetic, glucose-lowering effect of GLP-1. In most clinical studies of type 2 diabetes treatment with GLP-1-based therapy, where glucagon levels have been reported, there have been significant reductions both in the fasting and after e. g. meal stimulation, whereas in general, insulin levels have been less affected. However, this should be considered in the light of the often pronounced reductions in PG levels observed concomitantly. Therefore, regarding insulin secretion, the effect of GLP-1 seems to consist of enhancement of glucose-induced secretion, resulting in a rather unchanged absolute secretion rates, whereas glucagon secretion, which would have been expected by increase with falling PG levels, actually decreases. In many patients with type 2 diabetes both fasting and postprandial glucagon levels are abnormally elevated, possibly due to a decreased ability of glucose to inhibit glucagon secretion appropriately. Thus effects of GLP-1 may be viewed as restoration of the ability of the alpha cells to react to glucose which has recently been demonstrated in hyperglycemic clamp studies. Together, these results supports that both glucose-induced insulin secretion and the inhibition of glucagon secretion by GLP-1 constitutes very important parts of the hypoglycemic effects of GLP-1 in the clinical setting.
Development and clinical trial of intranasal GLP-1

While glucagon like peptide-1 (GLP-1) and GLP-1 receptor agonist are expected to be multi-potential anti-diabetic agents, subcutaneous or intravenous injection is necessary. Because intranasal administration is noninvasive and easy method for the drug delivery, we developed a new device and medication for intranasal administration of human native GLP-1. To investigate the safety, tolerability, and effectiveness of intranasal GLP-1, we conducted a physician initiated, randomized double-blind trial to type 2 diabetic patients.

The medication and the device were developed by Asubio Pharma (Tokyo, Japan) and SPG Technology (Miyazaki, Japan), respectively. GLP-1 was fixed around the calcium carbonate core, 60 μm in diameter, and the minute powder was filled in a capsule containing 1.2 mg GLP-1. GLP-1 or placebo was administered intranasally just before each meal. Meal tolerance test was carried out on day 1, then the patients continued the administration of nasal GLP-1 for 14 days.

Twenty-six patients were enrolled. They were 60.5 ± 12 years old (M=13), and their body mass index and HbA1c were 26.4 ± 0.9 and 7.2 ± 0.1%, respectively. Plasma active GLP-1 levels rapidly increased from 7.0 ± 0.9 to 82.5 ± 17.0 pg/ml 5 min after the administration, then gradually decreased to the similar levels of placebo group. Serum insulin concentration in GLP-1 group was significantly higher than placebo group at 15 min, and was similar to placebo group after 30 min. Area under the curve of glucose at 0 to 180 min after taking the meal tended to be low in GLP-1 group, but it did not reach statistically significance. Plasma glucagon levels increased after taking the meal in both groups, but the increment was significantly low at 30 and 180 min in GLP-1 group. On day 14, serum glycoalbumin level significantly decreased and 1.5-AG level significantly increased in GLP-1 group, but there were no changes in placebo group. Body weight, appetite score and hunger score did not change in both groups. Though nausea was seen in three patients in GLP-1 group, all patients completed 14 days protocol. Hypoglycemia was not observed through this study.
Sleep disorders in diabetes mellitus: Clinical significance
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There is both laboratory and epidemiologic evidence to indicate that inadequate sleep duration and poor sleep quality may increase the risk of diabetes. In laboratory studies of young, healthy volunteers, sleep restriction or reduced sleep quality resulted in marked decreases in insulin sensitivity without adequate beta-cell compensation. Several prospective epidemiologic studies have observed that individuals reporting difficulty initiating or maintaining sleep have a greater risk of developing diabetes and that short sleep durations (≤5 or ≤6 hours) are also associated with an increased risk of incident diabetes relative to 7–8 hours, even after controlling for many covariates. In patients who have type 2 diabetes, self-reported sleep duration and quality are significant predictors of glycemic control, as assessed by hemoglobin A1c (HbA1c). Obstructive sleep apnea (OSA) has been identified as a highly prevalent co-morbidity of type 2 diabetes (T2DM), affecting roughly 75% of patients. OSA is a well-documented risk factor for type 2 diabetes, independent of adiposity. In our recent study of 60 diabetic patients who underwent a laboratory polysomnography, increasing OSA severity was associated with poorer glucose control, after controlling for age, sex, race, BMI, number of diabetes medications, level of exercise, years of diabetes and total sleep time. Compared to patients without OSA, the adjusted mean HbA1c was increased by 1.49% (p=0.0028) by mild OSA, 1.93% (p=0.0033) by moderate OSA, and 3.69% (p<0.0001) by severe OSA. These effect sizes are comparable to those of widely used hypoglycemic drugs. Treating OSA in T2DM may have clinically significant beneficial effects on glucose control and reduce the number of drugs needed and/or their dose regimen.

Comprehensive management of diabetes and dementia

Cognitive dysfunction of elderly subjects with type 2 diabetes has received considerable attention in connection with dementia because diabetes increases the risk of vascular dementia, as well as Alzheimer’s disease (AD). Because cognitive impairment often disturbs successful treatment of diabetes and because chronic hyperglycemia worsens the cognitive dysfunction vice versa, comprehensive management of diabetes and cognitive decline is crucial for diabetic elderly. In routine care, however, mild cognitive dysfunction remains undetected and untreated in a considerable proportion of patients, resulting in several difficulties when treating diabetic elderly.

In this symposium, we would present some representative cases of diabetic elderly with AD, and discuss the points for successful management of diabetes and dementia in the elderly, including glycemic control, medication and daily care. Second, we would present our screening maneuver for early dementia among diabetic elderly. We have developed a model for predicting mild to moderate AD using a self-reported questionnaire and by evaluating vascular risk factors for dementia. When considering a total scheme for detecting AD in diabetic elderly subjects, high-risk individuals can be selected using this warning index for AD. Finally, we would present the long-term effects of pioglitazone (thiazolidine) on the cognitive function of diabetic elderly with AD. Thiazolidine can be expected to restore the progression of AD pathology by improving insulin resistance, which is crucial for development of diabetes and AD.

Conclusively, it should be mentioned that cognitive decline in the diabetic patients can be protected and occasionally reversed, at least in part, by successful management of diabetes and dementia. Substantial attention is thus needed for the cognitive dysfunction in patients with type 2 diabetes.
Bone fragility in diabetic patients: Close relationship between osteoporosis and lifestyle-related diseases

There has been accumulating evidence about increased risk of hip fracture in type 2 as well as type 1 diabetic patients. Our studies revealed that Japanese type 2 diabetic patients have increased risk of vertebral fractures despite higher bone mineral density (BMD), indicating increased bone fragility independent of BMD. In recent years, attention has focused on bone quality, a term that encompasses the bone strength–determining factors other than BMD. As factors associated with bone quality, microarchitecture, turnover, microdamage, mineralization as well as bone matrix like collagen cross-links are listed. A suggested mechanism of increased bone fragility in diabetic patients is the accumulation of advanced glycation end-products (AGEs) in collagen cross-links. Our studies indicate that AGEs–receptor for AGEs (RAGE) system is related to deterioration of bone quality and that serum levels of pentosidine, one of AGEs and endogenous secretory RAGE (esRAGE) as well as serum IGF-1 are useful for assessing risk of vertebral fractures in diabetic patients. Attention has also focused on bone–vascular linkage, namely on the fact that osteoporosis is closely linked to arteriosclerosis. Hyperhomocysteinemia is cited as one of proposed connecting factors and indicated to induce not only vessel but also bone fragility via accumulation of AGEs.

The possibility of two-way relationships between bone and energy metabolism is attracting attention. Adipokines regulate bone metabolism, while osteocalcin produced by osteoblasts may be involved in glucose and lipid metabolism. Indeed, our study suggests that serum osteocalcin level is associated with energy metabolism including glucose metabolism. These findings indicate close relationship between osteoporosis and lifestyle–related diseases in terms of organ cross-talk. From the viewpoint of medical treatment, possible influences of agents for lifestyle–related diseases on fracture risk also become apparent.

Molecular pathology underlying insulin resistance in type 2 diabetic liver

The liver plays a central role in energy homeostasis and contributes to the pathophysiology of diabetics by sensing nutrient stimuli and producing bioactive substances (Diabetologia 2004 & 2007). Here, we focus on nonalcoholic fatty liver disease (NAFLD) as a systemic disease causing insulin resistance.

1. Pathology of fatty liver disease and insulin resistance

Hepatic steatosis is an independent predictor of insulin resistance in Japanese patients with NAFLD (J Gastroenterol 2007). By establishing the rodent models of nonalcoholic steatohepatitis (NASH) (Gastroenterology 2007, Hepatology 2007), we proved that insulin resistance promotes the pathology of NASH, and screened potential agents that ameliorate pathology of NASH (Eur J Pharmacol 2008, Hepatology 2008).

2. Toxic lipids that cause hepatic insulin resistance

High fat feeding itself does not cause steatohepatitis, but accelerates the pathology of cholesterol–induced steatohepatitis (Hepatology 2007). In visceral obesity, excessive fatty acids flux into the liver via the portal vein. Increased oxidative stress in the liver precedes the onset of high–fat diet–induced insulin resistance (Metabolism 2008). In vitro fatty liver system showed that mitochondria–derived reactive oxygen species induced by palmitate might be major contributors to JNK activation and insulin resistance (J Biol Chem 2009).

3. Lessons from comprehensive gene expression analyses of type 2 diabetic liver

By using serial analysis of gene expression (SAGE) and DNA chip analyses of human liver tissues (Curr Pharm Biotechnol 2008), we have found that genes involved in mitochondrial oxidative phosphorylation are coordinately up–regulated in the liver of patients with type 2 diabetes (Diabetologia 2007), especially in association with obesity (Obesity 2008).

Among a variety of secreted proteins produced from the liver, we have identified the selenoprotein P (WO 2008/013234) the expression levels of which are associated with insulin resistance and hyperglycemia in patients with type 2 diabetes (Curr Pharm Biotechnol 2008). Possible roles of the selenoprotein P, a redox–associated hepatokine, in the development of insulin resistance will be discussed.
Primary care for diabetic erectile dysfunction

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The prevalence of erectile dysfunction (ED) in men with diabetes is reported to range from 26 to 35%. Diabetic ED, considered to be a form of organic ED, is progressive, and treatment of diabetic ED is extremely effective if the condition is diagnosed and treated early. Recently, the importance of the patient’s quality of life (QOL) has garnered much attention, and QOL can no longer be ignored even by internal medicine physicians. We discuss herein primary care for diabetic ED.

History taking regarding diabetic ED should start by determining whether the patient is experiencing sexual problems. Next, it is extremely important to distinguish ED due to organic dysfunction from psychosomatic issues. It is highly likely that patients with long-term control problems and complications such as cardiovascular problems, hypertension, renal dysfunction, neurological disorders, and retinopathy will eventually develop organic ED. However, diabetes and LOH syndrome may occur simultaneously; therefore, total serum testosterone (or free testosterone) should be measured.

The treatment of choice for diabetic ED is administration of phosphodiesterase (PDE)-5 inhibitors, which suppress cGMP metabolism in smooth muscle cells, causing intracellular cGMP accumulation and facilitating the development of penile erection. However, PDE-5 inhibitors are ineffective in the absence of release and/or synthesis of NO in the corpus cavernosum of the penis due to sexual excitation. PDE-5 inhibitors are also ineffective in psychosomatic ED, in which effective sexual arousal cannot be attained, and in organic ED, in which NO cannot be synthesized. If PDE-5 inhibitors are effective, they should be continued; patients in whom they are ineffective, provided that they are willing, should be referred to an ED treatment specialist. As coadministration of some drugs, such as nitrates, with PDE-5 inhibitors is contraindicated, all of the patient's medications must be confirmed at the time of prescription of a PDE-5 inhibitor. In addition, because the incidence of painless myocardial infarction is high in patients with diabetes, it is necessary to exercise adequate caution when treating those with a history of cardiovascular disorders.

For patients in whom PDE-5 inhibitors are ineffective, possible therapeutic options include use of a penile vacuum constriction device (VCD) and self-injection of prostaglandin E1 into the corpus cavernosum of the penis. When conservative therapy is impossible, penile prosthesis implantation may be indicated.

Reality of periodontal disease as the sixth complication of diabetes mellitus

Periodontal diseases represent chronic inflammatory responses to a bacterial biofilm infection. Evidence suggests that periodontal tissues destruction is mainly due to the host's inflammatory response to the bacterial challenge. In addition, other risk factors such as diabetes mellitus have been shown to modify the host response to the bacterial challenge. Recently, periodontal diseases have been described as the 'sixth complication of diabetes'. Some meta-analysis concluded that the majority of studies about the relationship between diabetes and periodontitis demonstrate a more severe periodontal condition in diabetic adults than in adults without diabetes.

To validate a relationship of these two diseases, biologically plausible mechanisms must be evident to explain the pathobiology of the interactions. In individuals with sustained hyperglycemia, proteins become irreversibly glycated to form advanced glycation end products (AGEs). Higher levels of AGEs accumulation in periodontal tissues are found in diabetic patients than in non-diabetic individuals. The interaction between AGES and the receptor RAGE in periodontal tissues is thought to explain, in part, the marked elevation in gingival crevicular fluid levels of IL-1β, TNF-α, and PGE2 seen in diabetic patients. These proinflammatory cytokines contribute to the pathogenesis of periodontal diseases and probably play a major role in patients with diabetes.

We have performed an animal model study of the experimental periodontitis using cDNA microarray to investigate global gene expression in inflamed gingival tissue of diabetic ZDF rats. A significant progression of alveolar bone resorption was observed in ZDF rats compared to lean rats as the result of micro-CT analysis. The gene expression of LPS binding protein was up-regulated in ZDF rat, while those of IL-10 and IL-24 were down-regulated. These results suggest that modulating role of diabeties in bone destruction of periodontitis may involve increased level of LBP and reduced level of Th2 cytokines.
S18-1

The roles of glycogen synthase kinase-3β in the regulation of β-cell mass in insulin resistant diabetes models

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The capacity of pancreatic β-cells to adapt to insulin resistance is critical for glucose homeostasis and is a factor in the development of type 2 diabetes. The insulin receptor signaling in β-cells plays a crucial part in regulating β-cell mass and function. Impairment of this pathway results in elevation of negatively regulated substrates such as Gsk-3β. When activated this enzyme has anti-proliferative and pro-apoptotic properties, whereas it was initially identified as a regulator of glycogen synthesis. Recently, we have shown that genetic inhibition of Gsk-3β rescued diabetes in mice models of Insulin resistance. This talk will focus on the contribution of Gsk-3β to the regulation of β-cell mass.

Mice lacking one allele of the insulin receptor exhibit Insulin resistance and a doubling of β-cell mass. Gsk-3β haploinsufficiency in these mice results in reduced β-cell mass as a consequence of augmented whole body glucose disposal. In the second model, mice missing two alleles of the insulin receptor substrate 2 (Irs2−/−) are Insulin resistant and develop diabetes as a consequence of profound β-cell loss. Crossing the mice having haploinsufficiency for Gsk-3β rescued the diabetes of Irs2−/− mice, in part by preserving β-cell mass associated with restored proliferation and attenuated apoptosis. More importantly, β-cell specific Gsk-3β deficiency was sufficient to improve hyperglycemia in Irs2−/− mice, indicating a pivotal role of β-cell adaptation to insulin resistance. The beneficial effects of Gsk-3β haploinsufficiency on β-cell mass observed in Irs2−/− mice were explained, at least in part, by restoration of Pdx1 expression and decreased p27kip1 levels. By contrast, overexpressing constitutively active form of Gsk-3β in β-cells induced impaired glucose tolerance and decreased β-cell mass and proliferation that was associated with decreased cyclin D1 and Pdx1 levels. Taken together, these results suggest an important role of Gsk-3β in the regulation of β-cell replication and apoptosis. Understanding the mechanisms by which Gsk-3β modulates β-cell mass and function helps developing new strategy to the treatment of type 2 diabetes.

S18-2

Molecular mechanism for pancreatic beta-cell glucose toxicity

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Under diabetic conditions, chronic hyperglycemia gradually leads to deterioration of pancreatic beta-cell function. This process is known as "beta-cell glucose toxicity" and clinically often observed in type 2 diabetic patients.

It has been shown that under diabetic conditions oxidative stress is provoked and thereby involved in the beta-cell glucose toxicity. When beta-cells are exposed to oxidative stress, insulin biosynthesis and secretion are suppressed, accompanied by inactivation of pancreatic transcription factor PDX-1. Furthermore, activation of the JNK pathway is involved in the beta-cell dysfunction by oxidative stress. Therefore, oxidative stress and subsequent activation of the JNK pathway are likely involved in beta-cell glucose toxicity.

On the other hand, it has been drawing attention that GLP-1 plays a crucial role in beta-cells and that stimulation of insulin secretion by GLP-1 is reduced in type 2 diabetic patients. It seems that this impairment is, at least in part, due to a defect at the receptor level induced by hyperglycemia. Indeed, GLP-1 receptor mRNA and protein levels in beta-cells are significantly decreased under diabetic conditions. In addition, perfused islets isolated from hyperglycemic rats showed reduced insulin response to GLP-1. Therefore, we assume that decrease of GLP-1 receptor level by hyperglycemia explains the impaired incretin effects and is also involved in beta-cell glucose toxicity found in type 2 diabetes.
S18－3

Transcriptional and translational control in adaptive stress responses in pancreatic β cells
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Recent studies have shown decreased pancreatic β cell mass to be a common feature of subjects with type 2 diabetes mellitus. Stress-mediated apoptosis is considered as one of the causes of β cell loss. To cope with stress conditions, β cells reprogram gene expressions by translational and transcriptional mechanisms.

The regulation of translation is often used under stress conditions because it allows immediate changes in protein levels. Thus, the first step against these stress stimuli is to suppress protein synthesis through activation of eIF2α kinases. The global attenuation of protein biosynthesis then paradoxically increases expressions of several proteins including the transcription factor ATF4. We have reported that ATF4-mediated induction of the translational suppressor 4E-BP1 is important for pancreatic β cell survival under ER stress. In contrast to transient nature of translational suppression due to eIF2α inhibition, 4E-BP1 seems to exert chronic translational suppression in β cells.

Transcriptional regulation is another means to cope with stress conditions. During the course of study in ATF4-mediated induction of the Eif4ebp1 gene, encoding 4E-BP1, we have noticed that transcriptional induction of 4E-BP1 is much stronger in MIN6 cells as compared to that in other cell lines. Detailed analysis of transcriptional regulation revealed that the cell-type specific transcriptional induction is due to different accessibility of ATF4 to intron 1, where two C/EBP:ATF composite sites exist.

ATF6α is another important but not yet fully examined player in transcriptional control of the ER stress response. We have analyzed roles of ATF6α in glucose homeostasis using ATF6α KO mice. ATF6α KO mice maintained normal β cell function, indicating that under normal conditions, β cells have capacity to deal with the large amount of insulin without ATF6α. When Ins2WT/C96Y mice were crossed with the KO mice, blood glucose levels were worsened with reduced pancreatic insulin content in both male and female mice. These data indicate that ATF6α protects pancreatic β cells from ER stress-induced cell damage.

S18－4

Ablation of C/EBPβ alleviates ER stress and pancreatic β cell failure through the GRP78 chaperone in mice
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Pancreatic β cell failure is thought to underlie the progression from glucose intolerance to overt diabetes, and endoplasmic reticulum (ER) stress is implicated in such β cell dysfunction. We demonstrated that the transcription factor CCAAT/enhancer-binding protein β (C/EBPβ) accumulated in the pancreatic islets of mouse models of diabetes even during the prediabetic stage. We also showed that C/EBPβ expression was induced by ER stress in pancreatic β cells. Transgenic overexpression of C/EBPβ specifically in pancreatic β cells of mice reduced pancreatic β cell mass and lowered plasma insulin levels in a manner dependent on its expression level, resulting in the development of diabetes. Conversely, genetic ablation of C/EBPβ in the pancreatic β cells of mouse models of diabetes, including Akita (Ins2<sup>Wt/C96Y</sup>) and Lepr<sup>−/−</sup> mice, resulted in an increase in pancreatic β cell mass and ameliorated hyperglycemia. C/EBPβ ablation in pancreatic β cells promotes the induction of the molecular chaperone GRP78 (BiP) expression and thereby confers tolerance to ER stress. Conversely, the accumulation of C/EBPβ likely increase the vulnerability to pancreatic β cells to ER stress. Activating transcription factor 6α (ATF6α) has been identified as the main inducer of GRP78. We further demonstrated that excessive accumulation of C/EBPβ blocks ATF6α-mediated GRP78 transcription in pancreatic β cells and thereby prevents the induction of GRP78 expression. Our results thus indicate that the accumulation of C/EBPβ in pancreatic β cells contributes to pancreatic β cell failure in mice by enhancing susceptibility to ER stress.
Role of ER stress in pancreatic beta-cell death  
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Increasing evidence indicates that cellular stress caused by the dysregulation of endoplasmic reticulum (ER) homeostasis, termed ER stress, is involved in beta-cell dysfunction and death during the progression of type 1 and type 2 diabetes, and Wolfram syndrome, a genetic form of diabetes. To counteract ER stress, beta cells activate cellular signaling pathways termed the unfolded protein response (UPR).

When the UPR fails to restore ER homeostasis and attenuate ER stress, UPR activation induces apoptosis. This unresolvable ER stress can be caused by genetic mutations as well as environmental factors. One example is observed in Wolfram syndrome, which is an autosomal recessive disorder characterized by insulin-dependent diabetes mellitus caused by non-autoimmune loss of beta cells and neurological dysfunctions. Here we show that WFS1 negatively regulates a key transcription factor involved in ER stress signaling, activating transcription factor 6α (ATF6α), through the ubiquitin–proteasome pathway. WFS1 suppresses expression of ATF6α target genes and represses ATF6α-mediated activation of the ER stress response (ERSE) promoter. WFS1 stabilizes the E3 ubiquitin ligase HRD1, brings ATF6α to the proteasome, and enhances its ubiquitination and proteasome–mediated degradation, leading to suppression of ER stress signaling. Consistent with these data, beta cells from WFS1-deficient mice and lymphocytes from patients with Wolfram syndrome exhibit dysregulated ER stress signaling through upregulation of ATF6α and down-regulation of HRD1. These results reveal a role for WFS1 in the negative regulation of ER stress signaling and in the pathogenesis of diseases involving chronic, unresolvable ER stress, such as pancreatic beta cell death in diabetes.

Growth factor signaling and the regulation of mitochondrial function in pancreatic beta cells  
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The pancreatic islets play a critical role in the maintenance of glucose homeostasis by regulating hormone secretion and synthesis from the endocrine pancreas.

While it has been recognized that a paucity of beta cell mass is central in the pathogenesis of both type 1 and type 2 diabetes, the regulatory mechanisms, signaling pathways and key proteins that regulate beta cell mass are not fully explored. Furthermore, several insulin target tissues manifest alterations in mitochondrial biology in type 2 diabetes. In this presentation experiments aimed at understanding the link between growth factor (insulin and insulin–like–growth–factor–1) signaling and mitochondrial function in human islets and mouse models of type 2 diabetes will be discussed. Given the significant role of mitochondria in metabolism and apoptosis, these data have direct implications for the maintenance of beta cell mass and secretory function.
Glucose transport in the 21st century
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Since the mid 1950s it has been known that glucose uptake in muscle and fat is stimulated by insulin, but the mechanism of this regulation remained elusive until the discovery of the translocation phenomenon by two independent laboratories in 1980. Nearly a decade later, several laboratories identified Glut4 as a distinct glucose transporter isoform responsible for insulin-sensitive transport in these tissues. Much has been learned in the past 30 years concerning the subcellular trafficking routes followed by Glut4, but the precise steps that are affected by insulin to increase the plasma membrane content of the transporter remain ill-defined. Several amino acid motifs that appear to influence the trafficking itinerary of Glut4 have been identified, but how they interact with regulatory proteins to control the movement of the transporter through different subcellular compartments remains unknown. A novel subcellular trafficking motif within the C-terminus of Glut4 will be described that appears to be essential for the movement of Glut4 to the plasma membrane under basal or insulin-stimulated conditions. Progress has been made in our understanding of the insulin-signaling pathway that leads to the redistribution of Glut4 to the plasma membrane, but our knowledge in this area remains incomplete. It is clear, however, that low molecular weight G proteins and their associated regulatory proteins play an essential role in the regulated trafficking of Glut4. Recently, we discovered that an Akt regulated GTPase complex, AS250/KIAA1219, functions in the intracellular retention of Glut4 in the basal state and that this complex appears to act at a different intracellular site than does AS160. Unlike AS160, AS250/KIAA1219 is only present in large intracellular Glut4 vesicles that also contain Syntaxin-6, suggesting that the complex may regulate a small G-protein in a trans-Golgi-like membrane compartment.

GLUT4-vesicle fusion: Role of SNARE regulator DOC2b and calcium
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Insulin stimulates glucose uptake in skeletal muscle and adipose tissues primarily by stimulating the translocation of vesicles containing a facilitative glucose transporter, GLUT4, from intracellular compartments to the plasma membrane. The formation of stable SNARE [soluble N-ethylmaleimide-sensitive fusion protein (NSF) attachment protein receptor] complexes between VAMP-2 and syntaxin4 initiates GLUT4-vesicle docking and fusion processes. In addition to the core SNARE proteins, a number of related factors (collectively called SNARE regulators) are required for the regulation of GLUT4 vesicle fusion. In the last 10 years, three SNARE related proteins (i.e. munc18c, tomosyn, and synip) were reported to be negative regulators to inhibit glucose transport. Despite numerous investigations, the positive SNARE regulators have not been adequately clarified.

Recently, we identified a new SNARE regulator DOC2b, a SNARE related protein containing double C2 domains but lacking a transmembrane region, is translocated to the plasma membrane upon insulin stimulation and directly associates with syntaxin4 in an intracellular Ca^{2+} dependent manner. Furthermore, this process is essential for triggering GLUT4-vesicle fusion. Expression of DOC2b in cultured adipocytes enhanced, while expression of the Ca^{2+} interacting domain mutant DCO2b or knockdown of DOC 2b inhibited insulin stimulated glucose uptake.

These findings indicate that DOC2b is a positive SNARE-regulator for GLUT4 vesicle fusion and mediates insulin stimulated glucose transport in adipocytes.
S19-3

Exploring GLUT4 translocation from a different angle: Regulation of insulin signal by the ECM rigidity

The molecular mechanism of insulin–stimulated GLUT4 translocation has been extensively studied. One of the cell types that had been frequently used for those studies are 3T3-L1 adipocytes mainly due to easiness to handle and robust response to insulin stimulation. We previously reported that these cells sense the rigidity of the extracellular matrix (ECM) and their sensitivity toward insulin stimulation is optimized when cells are seeded on a 250 Pa (pascal) ECM, which mimics the rigidity of white adipose tissues (WAT). Previous studies by others have shown upregulation of collagen in WAT in insulin resistance and knockdown of collagen improved insulin sensitivity in model rodents despite the uninhibited expansion of adipocytes. Hence we hypothesized that adipocyte expansion due to excess lipid intake induces mechanical stress between adipocytes and the ECM, which initiates signals that lead to abnormal adipocyte cellular functions observed in insulin resistance. To test this, we seeded 3T3-L1 adipocytes on polyacrylamide gels with various rigidities. In our gel system, a mixture of collagen type 1 and fibronectin is covalently linked to the surface of gels through crosslinkers. However, once gels are made, crosslinkers remaining unbound to ECM ligands are inactivated so that no additional ECM ligand in the medium binds to the gel and participates in cell adhesion. Palmitate treatment increased intracellular triglyceride content regardless of the rigidity of the gel, indicating an ability of cells to transport lipid and cause hypertrophy on all gel types. We looked at the effect of ECM rigidity on palmitate–induced insulin resistance in 3T3-L1 adipocytes, such as attenuated insulin–stimulated GLUT4 translocation. We also looked at the effect of ECM rigidity on palmitate–induced change in the profile of adipokines production/secretion, which affects insulin–stimulated GLUT4 translocation in the neighboring cells. We now propose a new factor, a mechanical property of the ECM, in regulating insulin action.

S19-4

Identification of functional sites of insulin-mediated GLUT4 trafficking using quantitative single molecule imaging

Despite major advances in understanding the molecular basis of insulin receptor signals responsible for GLUT4 translocation, the most fundamental unanswered question remains, i.e. what are the actual step(s) at which insulin signals directly converge and impact the process of dynamic GLUT4 trafficking events? This lack of progress is due at least in part to technical limitations of past studies relying on GLUT4–fusing fluorescent proteins, e.g. enhanced green fluorescent protein (EGFP) which is limited by dim fluorescence and its sensitivity to photo-bleaching which makes it unsuitable for investigating GLUT4 behavior at high spatial and temporal resolution.

Recently, we have developed a novel method that allows direct analysis of intracellular GLUT4 dynamics at the single molecule level using Quantum dot technology, quantitatively establishing the behavioral nature of GLUT4 in great detail (on a nanometer-scale). Our data demonstrate the predominant mechanism for intracellular GLUT4 sequestration in the basal state to be “static retention”, rather than “dynamic retention”, in 3T3-L1 adipocytes. We could directly observe insulin–induced behavioral alterations in GLUT4 molecules and thereby successfully defined three distinct insulin–stimulated GLUT4 trafficking processes: (1) release from the putative GLUT4 anchoring system in storage compartment (s), (2) the speed at which transport GLUT4–containing vesicles move and (3) the tethering/docking steps at the plasma membrane.

In addition, these quantitative analyses allowed us to to directly examine aberrant alterations in intracellular GLUT4 trafficking behavior in an insulin–resistant state, which obviously operates in addition to the insulin receptor signaling defects. Indeed, under insulin resistance in 3T3-L1 adipocytes experimentally induced by endothelin treatment, obvious derangements in all theses insulin–responsive GLUT4 behavioral regulation were detected by using the Qdot–based single molecular analysis. Thus, our novel method provides fundamental and novel insights necessary for further elucidating the molecular basis of GLUT4 regulation and its derangements under insulin–resistant conditions.
S19–5

The past, present and future of the 'Translocation Hypothesis'

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In the 30 years since Cushman and Kono published the translocation hypothesis to explain insulin regulated glucose transport in the fat cell we have come a long way. The transporter involved has been identified as GLUT4. GLUT4 is unique in that in insulin’s absence it resides in specialised intracellular storage vesicles from where it moves to the plasma membrane (PM) via a dose-dependent quantal release mechanism. In addition to releasing these storage vesicles, insulin also speeds their delivery to the PM and under some conditions slows GLUT4 endocytosis, collectively resulting in a substantial increase in PM GLUT4. The signal transduction components used by insulin to orchestrate this, have been identified with a major role for Akt and its substrate the RabGAP AS160. AS160 putatively regulates the GTP loading of a putative Rab GTPase possibly at the PM that facilitates the docking/fusion of GLUT4 vesicles with the PM. While this step is likely the major regulated step our knowledge of the molecular components is still incomplete. The actin cytoskeleton, together with myosin motors, is involved at or close to the PM prior to SNARE complex assembly. It is unlikely that SNAREs represent the major regulated step since their involvement is promiscuous as indicated by the paucity of effects in single knock out studies. High-resolution microscopy techniques, such as total internal reflection fluorescence microscopy combined with the use of novel reporters and computational analytical methods, are yielding new insights. Major questions are – what is the major insulin regulated step? How does AS160 regulate GLUT4 translocation? Which part of this process is defective in insulin resistance and what is the contribution of defects in GLUT4 translocation in muscle and fat to the metabolic syndrome?