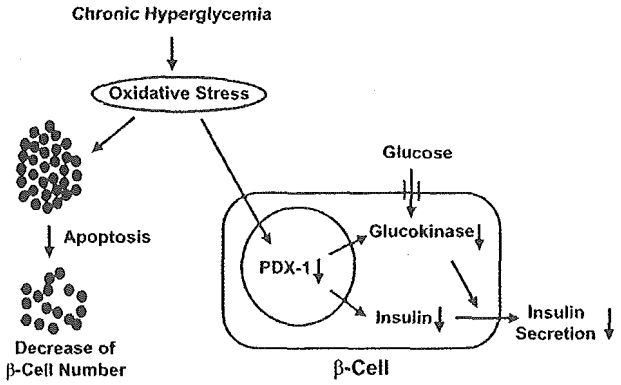

FIGURE 1. Involvement of oxidative stress in β-cell dysfunction found in type 2 diabetes.

SUPPRESSION OF INSULIN GENE EXPRESSION AND REDUCTION OF PDX-1 ACTIVITY MEDIATED BY OXIDATIVE STRESS

To understand the molecular mechanisms of β-cell glucose toxicity, we evaluated the potential effects of glycation and the consequent increase of ROS on β-cell function.[9] Among various reducing sugars that have a potential to induce glycation, D-ribose is a very strong reducing sugar and thus is often used in vitro studies as an inducer of glycation. Previously, we showed that the induction of glycation with D-ribose suppresses the insulin gene transcription in β cells, indicating that glycation and the ROS-dependent suppression of the insulin gene promoter cause the impairment of insulin biosynthesis in β cells. Based on the results of the reporter gene analyses, the insulin gene promoter was highly sensitive to glycation; when HIT-T15 cells were exposed to D-ribose, insulin gene promoter activity was markedly suppressed, whereas no such changes occurred with the β-actin gene promoter. It was suggested that ROS production via the glycation reaction suppressed the insulin gene promoter because suppression was neutralized with aminoguanidine, an inhibitor of the glycation reaction, or *N*-acetyl-L-cysteine, an antioxidant. The glycation-dependent suppression of the insulin gene promoter also caused the reduction of its transcripts, which was also neutralized with aminoguanidine or NAC. In addition, the insulin content in the cells was decreased after exposure to D-ribose.

As a possible cause of the reduction in the insulin gene promoter activity by oxidative stress, we found that the DNA-binding activity of pancreatic and duodenal homeobox factor-1 (PDX-1) (also known as IDX-1/STF-1/IPF1, an important transcription factor for insulin gene transcription and pancreas development[21–25]) is sensitive to the glycation reaction and the resulting oxidative stress. When HIT-T15 cells were exposed to D-ribose, marked reduction of PDX-1 binding to the insulin gene was observed.[9] The presence of aminoguanidine or NAC in the medium prevented this decrease, indicating that D-ribose suppressed the PDX-1 DNA-binding activity in a glycation and ROS-dependent manner. However, we were aware that the decrease in the PDX-1 binding activity alone may not lead to the suppression of the insulin gene transcription. In our previous study,[26] the suppression of the PDX-1 expression in β cell–derived MIN6 cells using an antisense oligodeoxynucleotide (ODN) did not lead to a decrease of any β cell–specific genes, such as insulin and glucokinase. Therefore, the PDX-1 amount per se may not function as a rate determinant of the insulin gene transcription in β cells, at least under normal conditions. Instead, some posttranslational modifications may play a role in the suppression of its activity under diabetic conditions.

PROTECTION OF β CELLS AGAINST GLUCOSE TOXICITY BY ANTIOXIDANTS

Diabetic conditions produce oxidative stress and also may cause in β-cell dysfunction. Therefore, treatment with antioxidants may be beneficial for diabetes treatment. There are several reports describing a potential usefulness of antioxidants in treatment of type 2 diabetes.[12,13,16] We used diabetic C57BL/KsJ-db/db mice as a model for type 2 diabetes. In these mice, antioxidant treatment was started at 6 weeks of age and its effects were evaluated at 10 and 16 weeks of age. Results of intraperitoneal glucose tolerance tests indicated that the treatment with antioxidants preserved glucose-stimulated insulin secretion with moderately decreased blood glucose levels. No effect on insulin secretion was observed when the same antioxidants treatment was given to nondiabetic control mice. Histologic analyses of the pancreata revealed that the β-cell mass was significantly larger in the mice treated with the antioxidants. These results may indicate that the antioxidant treatment prevented apoptotic death of β cells without changing the rate of β-cell proliferation. The antioxidant treatment also preserved the amounts of insulin content and insulin mRNA, and histologically insulin degranulation was less evident. Furthermore, the PDX-1 expression was more clearly visible in the nuclei of the islet cells treated with antioxidants. Taken together, these data indicate that

the antioxidant treatment exerts beneficial effects on diabetes with better preserved β-cell function. Our data in mice suggest a potential usefulness of antioxidants for treatment of diabetes and also further support the implication of oxidative stress in β-cell dysfunction in diabetes.

INVOLVEMENT OF JNK IN OXIDATIVE STRESS–MEDIATED SUPPRESSION OF INSULIN GENE EXPRESSION

Several signal transduction pathways, including c-Jun N-terminal kinase (JNK) (also known as stress-activated protein kinase [SAPK]), p38 mitogen-activated protein kinase (p38 MAPK), and protein kinase C (PKC), are activated by oxidative stress in several cell types including β cells. We recently reported that activation of JNK is involved in the reduction of the insulin gene expression by oxidative stress and that the suppression of the JNK pathway can protect β cells from oxidative stress.[27] Subjecting rat islets to oxidative stress, we found that the activation of JNK, p38 MAPK, and PKC preceded the decrease of insulin gene expression. Adenovirus-mediated overexpression of dominant-negative type (DN) JNK, but not the p38 MAPK inhibitor SB203580 nor the PKC inhibitor GF109203X, protected insulin gene expression and secretion from oxidative stress. Moreover, the wild-type (WT) JNK overexpression suppressed both insulin gene expression and secretion. These results were correlated with changes in the binding of PDX-1 to the insulin promoter and the PDX-1 DNA binding activity preserved by the adenoviral overexpression of DN-JNK in the face of oxidative stress, whereas the WT-JNK overexpression decreased PDX-1 DNA binding activity. We then examined whether suppression of the JNK pathway can protect β cells from the toxic effects of hyperglycemia. Rat islets were infected with the DN-JNK–expressing adenovirus or control adenovirus and transplanted under renal capsules of streptozotocin-induced diabetic nude mice. In mice receiving DN-JNK–overexpressing islets, insulin gene expression in the islet grafts was better preserved and hyperglycemia was ameliorated as compared with control mice.

In addition, we recently reported that PDX-1 is translocated from the nuclei to the cytoplasm in response to oxidative stress.[28] When β cell–derived HIT-T15 cells were exposed to oxidative stress, both intrinsically expressed PDX-1 and exogenously introduced green fluorescent protein (GFP)–tagged PDX-1 moved from the nuclei to the cytoplasm. The addition of DN-JNK inhibited the oxidative stress–induced PDX-1 translocation, suggesting an essential role of JNK in mediating this phenomenon. Whereas the nuclear localization signal (NLS) in PDX-1 was not affected by the oxidative stress, leptomycin B, a specific inhibitor of the classic, leucine-rich nuclear export signal (NES), inhibited nucleocytoplasmic translocation of PDX-1 induced by an oxidative stress. Indeed, we located an NES at position 82-94 of the mouse PDX-1 protein. Thus, these results revealed a novel mechanism that negatively regulates the PDX-1 function. The identification of the NES, which overrides the function of the NLS in an oxidative stress–responsive, JNK-dependent manner, supports the complicated regulation of PDX-1 function in vivo and may lead to further understanding of the β-cell pathophysiology in diabetes.

It is known that β-cell destruction by various cytokines[29,30] can be prevented by the inhibition of the JNK pathway,[31,32] implying that JNK plays a role in autoimmune β-cell destruction found in the early stage of type 1 diabetes. Also, it was reported that levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a marker for oxidative stress, are increased in islets of type 2 diabetic animal models[11] and that JNK activation by oxidative stress in islets actually reduces the PDX-1 DNA binding activity and insulin gene transcription.[22] Thus, we assume that JNK is involved in deterioration of β-cell function in both type 2 diabetes and the early stage of type 1 diabetes. Taken together, these results provide new insights into the mechanisms through which the oxidative stress suppresses insulin gene transcription in β cells. The finding that this adverse outcome can be prevented by DN-JNK overexpression suggests that the JNK pathway in β cells could become a new therapeutic target for diabetes (Fig. 2).

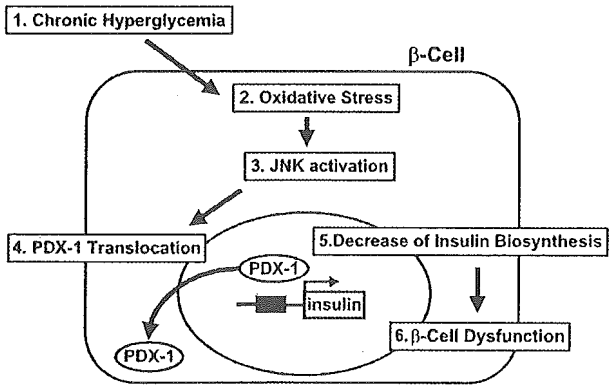

FIGURE 2. Possible mechanism for impaired β-cell function in chronic hyperglycemia.

CONCLUSION

Oxidative stress–mediated activation of the JNK pathway triggers nucleocytoplasmic translocation of PDX-1 and suppresses insulin gene expression, which likely leads to progression of β-cell dysfunction found in diabetes.

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Beneficial effects of nateglinide on insulin resistance in type 2 diabetes

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Abstract

Nateglinide, a rapid insulin secretagogue, is known to facilitate the early phase of insulin secretion and has been used for the treatment of type 2 diabetic patients with postprandial hyperglycemia. The aim of this study is to evaluate the effect of nateglinide on insulin resistance as well as insulin secretory defects in type 2 diabetic patients. Insulin secretion ability was evaluated by the hyperglycemic clamp test, and insulin sensitivity was evaluated by the euglycemic hyperinsulinemic clamp test, using an artificial pancreas. The hyperglycemic clamp test showed that a 7-day treatment with nateglinide significantly increased insulin secretion in response to high glucose. Interestingly, although nateglinide is known to facilitate insulin secretion, daily urinary C-peptide excretion was decreased after nateglinide treatment. Moreover, in the euglycemic hyperinsulinemic clamp test, glucose infusion rate was significantly increased by nateglinide treatment, indicating that nateglinide functions to decrease insulin resistance. Nateglinide ameliorates insulin resistance as well as insulin secretory defects in type 2 diabetic patients.

Keywords: Nateglinide; Insulin resistance; Insulin secretion; Hyperglycemic clamp; Euglycemic hyperinsulinemic clamp

1. Introduction

Insulin secretory defects and insulin resistance due to hereditary and/or environmental factors are observed in type 2 diabetic patients. To date, insulin secretagogues, insulin sensitizers and insulin injections have been used for the treatment of type 2 diabetic patients. It was reported that postprandial hyperglycemia is an independent risk factor of cardiovascular disease (DECODE study) [1], and that the improvement of postprandial hyperglycemia by an α-glucosidase inhibitor decreased the risk of death (STOP-NIDDM)

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[2]. An important cause of postprandial hyperglycemia is impairment of the early phase of insulin secretion [3]. Nateglinide, a rapid insulin secretagogue, facilitates the early phase of insulin secretion [4] and has been used for the treatment of type 2 diabetic patients with postprandial hyperglycemia. However, the effect of nateglinide on insulin resistance remained unknown. In this study, we examined the effect of nateglinide on insulin resistance as well as insulin secretory defects in type 2 diabetic patients.

2. Methods

We recruited seven Japanese inpatients in Osaka University Hospital with type 2 diabetes for this study after obtaining written informed consent. The study was approved by the Ethics Committee for Human's Studies at Osaka University Hospital. These patients were treated with a standard diet (27–29 kcal/kg), exercise (approximately 10,000 steps per day) and low dose insulin (less than seven units per day) for 3 weeks to reduce the influence of glucose toxicity on glucose metabolism and insulin secretion. After achievement of good glycemic control before meal (below 130 mg/dl), their 2-h postprandial blood glucose levels were still above 180 mg/dl. To determine the degree of glucose tolerance and insulin secretion ability of each patient, a 75 g oral glucose loading test was performed after an overnight fast. The patients were then given 90 mg of nateglinide before each meal (270 mg/day) for a week. We examined each patient's daily profile of blood glucose levels and daily urinary C-peptide excretion before and after the treatment with nateglinide. Insulin secretion ability was evaluated by a hyperglycemic clamp test, and insulin sensitivity was evaluated by a euglycemic hyperinsulinemic clamp test, using an artificial pancreas (Model STG-22, Nikkiso, Tokyo, Japan) [5]. Briefly, three cannulae were positioned intravenously, one in an antecubital vein for blood glucose monitoring, another inserted distally in the same arm for extraction of venous blood samples and the third inserted in an antecubital vein in the contralateral arm for glucose and insulin infusion. Experiments consisted of a 90-min hyperglycemic clamp period and a 120-min euglycemic hyperinsulinemic clamp period. During the hyperglycemic clamp period, an exogenous glucose infusion was given to achieve steady-state blood glucose levels (200 mg/dl) within 5 min, and serum insulin levels were measured at 0, 5, 10, 20, 30, 60 and 90 min after commencement of the clamp test. After the 7-day treatment with nateglinide, 90 mg of nateglinide was given 10 min prior to the hyperglycemic clamp test. During the consecutive euglycemic hyperinsulinemic clamp period, patients were given a primed-constant infusion of regular insulin (1.12 mU/(kg min)) (Eli Lilly, Indianapolis, IN, USA) and an exogenous glucose infusion to achieve the desired steady-state serum insulin level (100 μU/ml) and to maintain blood glucose levels within the euglycemic range (100 mg/dl), according to the method of DeFronzo et al. [6]. When the rate of exogenous glucose infusion reached a steady-state level, we evaluated insulin sensitivity as the average glucose infusion rate during the last 30 min (*M*-value). Age-matched six healthy individuals (age 52 ± 2.4 years, body mass index 23.4 ± 1.8, fasting plasma glucose 99 ± 5 mg/dl) were subjected to the hyperglycemic clamp and euglycemic hyperinsulinemic clamp as control. Each healthy control subject represented normal glucose tolerance by 75 g oral glucose loading test and no overt abnormalities of liver function test and lipid profile.

Blood glucose levels were determined with an autoanalyzer using a glucose oxidase method, and immunoreactive insulin levels were measured by an enzyme immunoassay. To determine the effect of nateglinide on inflammation markers, which are related to insulin sensitivity in type 2 diabetic subjects, serum levels of tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) were measured using ELISA kits (BioSource International, CA, USA and Alpha Diagnostic, TX, USA), and high sensitive C-reactive protein (hs CRP) concentrations were determined using a latex-enhanced immunophelometer (Dade Behring, Newark, DEI). Data are represented as mean ± S.D.

3. Statistical analysis

Statistical comparisons between before and after treatment with nateglinide were assessed using Student's paired two-tailed *t*-test. Multiple comparisons among before and after nateglinide treatment,

and healthy control subjects were tested using the one-way analysis of variance (ANOVA). These comparisons were performed on a personal computer with Statview SE (Brainpower, Calabasas, CA, USA). A p-value of less than 0.05 was considered statistically significant.

4. Results and discussion

Clinical characteristics and metabolic parameters of the type 2 diabetic patients used in this study are as follows: age 56.0 ± 3.5 years, duration 4.7 ± 2.1 years, body mass index (BMI) 23.0 ± 1.8, hemoglobin A_{1C} (HbA_{1C}) 9.0 ± 2.2%, fasting plasma glucose 117 ± 15 mg/dl. All subjects were classified into diabetes category based on the criteria proposed by the American Diabetes Association. The insulinogenic index, an indicator of the early phase of insulin secretion, which is determined by a 75 g oral glucose loading test, was 0.08 ± 0.02, showing that the early phase of insulin secretion was markedly decreased in these patients. Before treatment with nateglinide, fasting blood glucose levels were 118 ± 7 mg/dl, and 2-h postprandial glucose levels were 216 ± 21, 229 ± 19 and 180 ± 11 mg/dl after breakfast, lunch and dinner, respectively, in these patients. As expected, the 7-day treatment with nateglinide significantly reduced postprandial blood glucose levels (Fig. 1A). To examine the effect of nateglinide on insulin secretion, we performed the hyperglycemic clamp test on these patients. As shown in Fig. 2A, impaired insulin secretion during the early phase of insulin secretion was observed in type 2 diabetic patients compared with age-matched non-diabetic subjects ($p < 0.05$). In addition, the hyperglycemic clamp test showed that treatment with nateglinide significantly increases insulin secretion in response to high glucose ($p < 0.05$) (Fig. 2A).

Interestingly, although nateglinide is known to function as an insulin secretagogue, daily urinary C-peptide excretion was significantly decreased after nateglinide treatment (from 53.0 ± 9.1 to 26.7 ± 4.7 μg/day, $p < 0.05$) (Fig. 1B). A possible mechanism of reduced daily insulin requirement by nateglinide is an improvement in the early phase of insulin secretion [7] and/or amelioration of insulin resistance. To further evaluate the effect of nateglinide on insulin resistance,

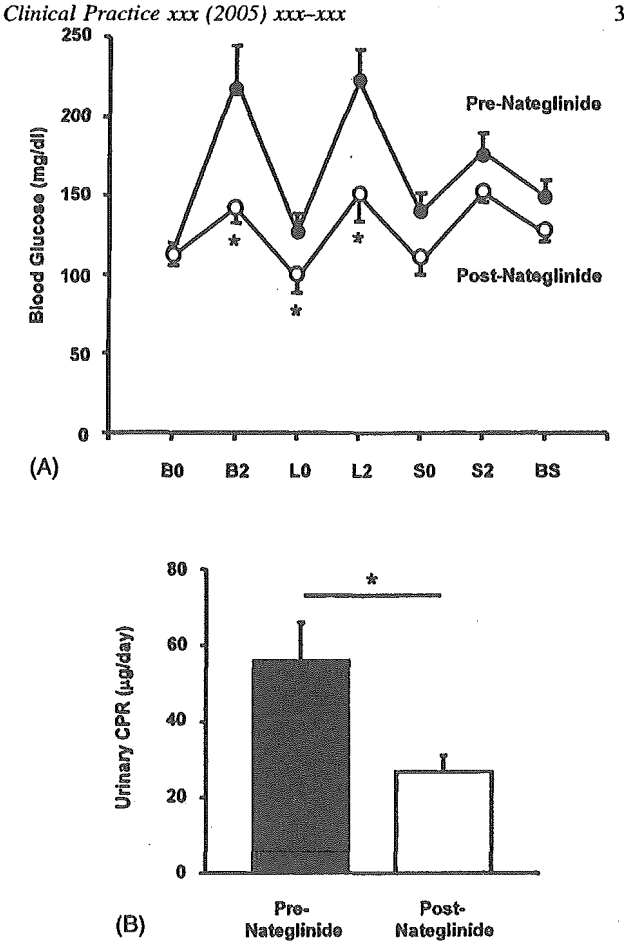

Fig. 1. The effect of nateglinide on daily profile of blood glucose and daily urinary C-peptide excretion. (A) Daily profile of blood glucose before (●) and after (○) treatment with nateglinide in type 2 diabetic patients. (B) Daily urinary C-peptide (CPR) excretion before (■) and after (□) treatment with nateglinide in type 2 diabetic patients. Data are expressed as mean ± S.D. $^{*}p < 0.05$, after (post-nateglinide) vs. before treatment with nateglinide (pre-nateglinide).

we performed the euglycemic hyperinsulinemic clamp test on type 2 diabetic patients before and after nateglinide treatment. As shown in Fig. 2B, M-value, an indicator of insulin sensitivity, in the type 2 diabetic patients was significantly lower compared with age-matched non-diabetic subjects ($p < 0.05$). In addition, interestingly, the M-value was significantly increased by nateglinide treatment (from 6.3 ± 0.9 to 7.6 ± 0.6 mg/(kg min), $p < 0.05$) under similar serum insulin levels (109 ± 14 μU/ml versus 127 ± 22 μU/ml, ns), indicating that nateglinide functions to decrease insulin resistance. Taken together, these results indicate that nateglinide ameliorates insulin resistance as well as

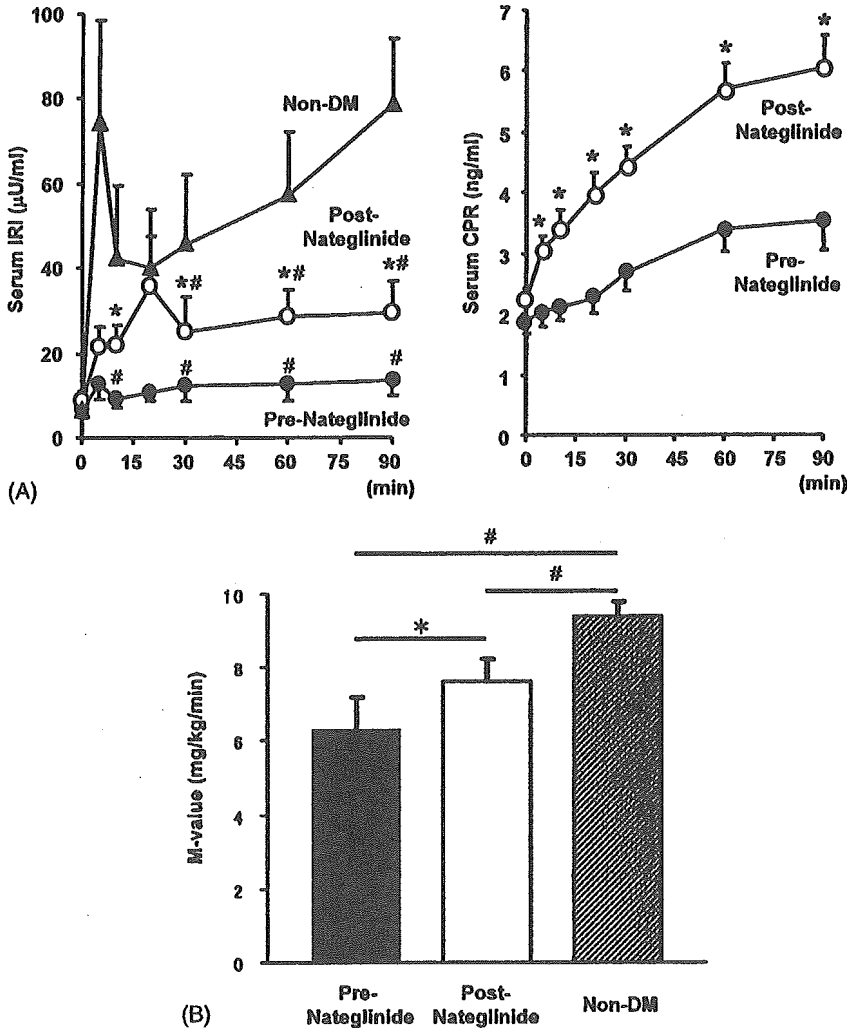

Fig. 2. The effect of nateglinide on insulin secretion and insulin sensitivity. (A) Concentrations of serum insulin (IRI) and C-peptide (CPR) during the hyperglycaemic clamp before (pre-nateglinide, ●) and after (post-nateglinide, ○) treatment with nateglinide in patients with type 2 diabetes, and age-matched healthy controls (non-DM, ▲). (B) *M*-value during the euglycemic hyperinsulinemic clamp test in patients before (pre-nateglinide) and after (post-nateglinide) treatment with nateglinide, and age-matched healthy controls (non-DM, ▲). Data are expressed as mean ± S.D. $^{*}p < 0.05$ vs. pre-nateglinide. $^{\#}p < 0.05$ vs. healthy control.

insulin secretory defects found in patients with type 2 diabetes.

Although insulin resistance was improved by nateglinide treatment as shown in this study, the actual mechanism by which nateglinide affects insulin sensitivity has not yet been demonstrated. Since, we have not determined insulin sensitivity after discontinuation of nateglinide, it was possible that improved glycemic control during hospitalization gradually decreased insulin resistance and insulin requirement before and during nateglinide treatment. To minimize the influence of glucose toxicity on insulin resistance and insulin requirement, the patients were treated with standard diet, regular exercise and small dose of insulin for 3 weeks before nateglinide treatment. Therefore, improved glycemic control, especially postprandial hyperglycemia and/or amelioration of insulin secretory pattern by nateglinide may contribute to improve insulin resistance. Several insulin-providing therapies by sulfonylurea, such as glimepiride [8] and glicrazide [9], but not glibenclamide, were reported to improve insulin action, so-called extrapancreatic effect. This effect was thought to be a drug-specific effect independent of insulin secretory ability,

but extra-pancreatic effect of nateglinide has not been clarified. On the other hand, attenuation of postprandial hyperglycemia by α-glucosidase inhibitor ameliorated insulin resistance [10]. Taken together, the treatment with nateglinide could improve insulin resistance mainly due to improved glycemic profile. Further studies will need to clarify extra-pancreatic effect of nateglinide.

It is known that increased oxidative stress and various inflammatory cytokines are associated with postprandial hyperglycemia in patients with type 2 diabetes and are possibly involved in the pathogenesis of type 2 diabetes [11]. To examine the possible involvement of such factors in the improvement of insulin resistance by nateglinide, there were no significant differences in serum levels of TNF-α, IL-6, and hs CRP before and after nateglinide treatment (TNF-α, 4.86 ± 2.16 pg/ml versus 4.76 ± 1.57 pg/ml; IL-6, 1.83 ± 0.92 pg/ml versus 1.97 ± 0.75 pg/ml; hs CRP, 371 ± 206 ng/ml versus 300 ± 127 ng/ml). Nateglinide has been reported to have improved endothelial dysfunction after an oral glucose load in type 2 diabetic patients [12]. Thus, although not examined in this study, we speculate that amelioration of insulin resistance is in part due to the improvement of endothelial function by the improvement of postprandial hyperglycemia. Further studies examining the detailed molecular mechanisms by which nateglinide ameliorates insulin resistance using endothelial cells will be necessary in the future.

In conclusion, nateglinide improves postprandial hyperglycemia via amelioration of insulin resistance as well as insulin secretory defects in type 2 diabetic patients.

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Hepatic insulin resistance induced by chronic hindlimb ischemia

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Abstract

Peripheral vascular disease (PVD) has been reported to cause deterioration in insulin sensitivity. The precise mechanism of insulin resistance induced by PVD has not been clarified. To elucidate the mechanism causing impaired insulin action and glucose metabolism under peripheral ischemic conditions, we determined glucose turnover and glucose tolerance in hindlimb-ischemic (FAL) rats.

The right femoral artery was ligated in hindlimb-ischemic (FAL) rats, while the artery was only exposed in the Sham operated (Sham) rats used as a control. Two weeks after the ligation, glucose tolerance was impaired and plasma insulin levels were significantly increased in FAL rats compared with Sham rats after intraperitoneal glucose loading (2 g kg^{-1}). Under euglycemic hyperinsulinemic clamp conditions, the glucose infusion rate was significantly lower in FAL rats compared with Sham rats, but there was no significant difference in the glucose disappearance rate between the two groups. Hyperinsulinemia suppressed endogenous glucose production by 50% in Sham rats, while the suppression was 20% in FAL rats, indicating hepatic insulin resistance in FAL rats. mRNA analysis of isolated liver after the clamp experiment revealed that glucokinase mRNA, but not PEPCK and glucose-6-phosphatase mRNA, was significantly lower in FAL rats compared with Sham rats.

In conclusion, chronic hindlimb ischemia impaired glucose tolerance associated with insulin resistance in the liver rather than the peripheral tissues.

Keywords: Hepatic insulin resistance; Hindlimb ischemia; Endogenous glucose production; Glucokinase

1. Introduction

Peripheral vascular disease, defined as lower extremity arteriosclerosis, is a common complication in type 2 diabetic patients. Diabetes mellitus increases

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the risk of peripheral vascular disease and peripheral vascular disease increases the risk of death and the need for lower extremity amputation in diabetic patients.

Non-diabetic patients with arteriosclerosis obliterans (ASO) exhibit insulin resistance with compensatory hyperinsulinemia [1] and diabetic patients with ASO are accompanied with severe insulin resistance [2]. A number of studies demonstrated that insulin resistance is linked to the development of arteriosclerosis in diabetic and non-diabetic subjects. Therefore, the insulin resistance observed in these patients is considered to be the cause of peripheral arterial disease. On the other hand, limitation of movement, degradation of skeletal muscle volume and impaired muscular blood flow in the ischemic foot provoke further insulin resistance in these patients. Therefore, insulin resistance in patients with peripheral arterial disease may be both a cause and a result of the development of arteriosclerosis.

To elucidate the pathological feature and mechanism of acquired insulin resistance and impaired glucose metabolism caused by peripheral vascular disease, we performed the intraperitoneal glucose tolerance test (IPGTT) and the euglycemic hyperinsulinemic clamp test on a rodent model of chronic hindlimb ischemia produced by surgical ligation of the right femoral artery.

2. Materials and methods

2.1. Animals and surgical procedures

This study was approved by the Institute of Experimental Animal Science of Osaka University Medical School. Experiments were conducted with 31 male Sprague–Dawley rats weighing 294 ± 14 g at 10 weeks of age. All rats were fed standard chow (Oriental Yeast, Tokyo, Japan) and housed in an environmentally controlled room with a 12-h light:12-h dark cycle. The rats were randomized into two groups, 15 femoral artery ligated (FAL) rats and 16 Sham-operated (Sham) rats used as a control. In FAL rats, we exposed the right femoral artery and ligated it at 5 mm distal from the inguinal ligament. In Sham rats, only exposure of the right femoral artery was performed. Four days before the euglycemic hyperinsulinemic clamp experiment, a silicon catheter (Phicon Tube®, Fuji-Systems, Tokyo, Japan) was inserted into the right jugular vein for infusion and a polyethylene catheter (PE-50®, Clay Adams, Sparks, MD, USA) was inserted into the left carotid artery for blood sampling under general anesthesia with sodium pentobarbital.

2.2. Glucose tolerance test

Two weeks after ligation of the femoral artery, intraperitoneal glucose tolerance tests were performed to evaluate glucose tolerance. After an overnight fast, rats were injected intraperitoneally with 2.0 g kg^{-1} body weight of glucose. Blood samples for determining glucose levels were taken from the tail vein at various time points (0–120 min).

2.3. Euglycemic hyperinsulinemic clamp tests

Euglycemic hyperinsulinemic clamp studies were performed on rats under conscious and unstressed conditions after an overnight fast. The rats were used in the experiments only if they had a good appetite and less than 10% body weight loss after the surgery. Each experiment consisted of a 90-min tracer equilibration period (from −120 to −30 min), a 30-min basal sampling period (from −30 to 0 min, basal period) and a 120-min euglycemic hyperinsulinemic clamp period (from 0 to 120 min, clamp period).

A priming infusion (1.5 mg kg^{-1} min^{-1} for 10 min) followed by continuous infusion (0.15 mg kg^{-1} min^{-1}) of [6,6-$^2H_2$]glucose (Cambridge Isotope Laboratories, Andover, MA, USA) was started through the jugular vein at −120 min. To prevent a decrease in the enrichment of [6,6-$^2H_2$]glucose during the euglycemic hyperinsulinemic clamp period, the infusion rate of [6,6-$^2H_2$]glucose was increased to 0.5 mg kg^{-1} min^{-1} at 0 min and continued until 120 min. At 0 min, a priming infusion (60 μU kg^{-1} min^{-1} for over 5 min) followed by constant infusion (6 μU kg^{-1} min^{-1}) of regular human insulin (Eli Lilly, Indianapolis, IN, USA) was started and continued during the clamp period. During this period, the arterial blood glucose level was monitored every 5 min and the rate of glucose (20% dextrose) infusion into the jugular vein was adjusted to maintain arterial blood glucose levels at the fasting levels as described by Kraegen et al. [3]. At the end of

the experiment, some rats (FAL rats $n = 5$, Sham rats $n = 6$) were anesthetized with an intravenous injection of pentobarbital, their abdomens quickly opened and the liver was freeze-clamped and stored at −80 °C until the assay.

Two hundred microliters of blood samples was taken at −120, −30, −15, 0, 90, 105 and 120 min to determine the isotopic enrichment of [6,6-$^2H_2$]glucose. To measure plasma insulin, 300 μl blood samples were taken at −120, 0 and 120 min. To measure plasma C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α) and Interleukin-6 (IL-6), 600 μl of blood samples was taken at 120 min.

2.4. Analytical procedures

Plasma glucose levels were measured by the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman, Fullerton, CA, USA). Blood glucose levels were measured regularly with a portable glucose meter (Sanwa-kagaku, Aichi, Japan). Plasma rat insulin concentrations were measured using an ELISA kit (Levis-insulin-rat, Shibayagi, Gunma, Japan). Plasma human insulin concentrations were measured at 120 min using a double antibody solid-phase technique radio immunoassay kit. Serum concentrations of TNF-α, IL-6 and CRP were measured using an ELISA kit (BioSource International, CA, USA and Alpha Diagnostic, TX, USA).

The enrichment of plasma [6,6-$^2H_2$]glucose was determined as described previously [4,5]. In brief, after deproteinization by 99.5% ethanol, the plasma sample was derivatized by *N*-methyl-bis-trifluoroacetamide (Pierce, Rockford, IL, USA) and trifluoroacetylated glucose was measured using a gas chromatography–mass spectrometer (Model TSQ-700, Finningan-MAT, San Jose, CA, USA) with a silicon SE-30 capillary column (Gasukuro Kogyo, Tokyo, Japan).

2.5. Calculation of glucose fluxe

The rates of glucose appearance (Ra) and disappearance (Rd) were calculated according to Steele's non-steady-state equations [6]. Endogenous glucose production (EGP) was calculated as the difference between the tracer-derived Ra and exogenous infusion rates of glucose (GIR) and tracer. The insulin-mediated suppression ratio of EGP (%ΔEGP) was calculated by the following equation:

$$\%\Delta EGP = \frac{EGP_{basal} - EGP_{clamp}}{EGP_{basal}} \times 100(\%)$$

where EGP_{basal} and EGP_{clamp} are the EGP in the basal and the clamp period, respectively. The results are represented as the average of metabolic parameters from −30 to 0 min in the basal period and from 90 to 120 min in the clamp period.

2.6. Measurement of hindlimb blood flow by the use of colored microspheres

In some rats (FAL rats $n = 5$, Sham rats $n = 5$), a colored microsphere method was used to determine regional blood perfusion of the limb muscles 2 weeks after ligation of the femoral artery. After the clamp study, Dye-Trak microspheres (15 μm in diameter, Triton Technology, TX, USA) were injected into the jugular artery. Animals were scarified and muscle samples were obtained from the medial thigh of both limbs. Samples from both limbs were digested with potassium hydroxide and microspheres were reclaimed with a vacuum filter. The dye from the microspheres was extracted with dimethyl formamide. These dye samples were then analyzed with a spectrophotometer. On the basis of the optical density (OD) measurements, the percent limb flow (flow in the ischemic limb expressed as a percentage of that in the contralateral normal limb) was calculated using the following equation: [7]

$$\frac{\text{OD of the ischemic limb}}{\text{OD of the normal limb}} \times \frac{\text{tissue weight of the normal limb}}{\text{tissue weight of the ischemic limb}} \times 100(\%).$$

2.7. Northern blot analyses

Total RNA was extracted from rat liver. Northern blotting was performed following standard procedures using 20 μg of total RNA, with the buffer conditions as described previously [8]. Reverse transcription-PCR was performed using primers [sense 5′-TGGTCTG-GACTTCTCTGCCAAG-3′ and antisense 5′-ACCGT-CTTGCTTTCGATCCTGG-3′] for PEPCK (PCR pro-

duct 258 bp), [sense 5′-TGTCTTGGTGTCTGTGATCGCTG-3′ and antisense 5′-AAGTGAGCCGCAAGGTAGATCC-3′] for glucose 6-phospholase (G6Pase) (PCR product 441 bp) and [sense 5′-TGCAGAAGGAGATGGACCGT-3′ and antisense 5′-CAGGGAAGGAGAAGGTGAAG-3′] for glucokinase (GK) (PCR product 351 bp) [8]. The probed membranes were exposed to an imaging plate, BAS-MS 2040 (Fujifilm, Tokyo, Japan). The hybridization intensity was quantified using a BAS2500 system (Fujifilm, Tokyo, Japan). To correct the loading differences of RNA, each membrane was re-probed with the mouse β-actin cDNA probe [9].

2.8. Statistical analyses

Data are represented as means ± S.D. Statistical comparisons between the FAL and Sham rats were assessed using the Student's two-tailed unpaired *t*-test. Comparisons of repeated measurements within each experimental group were assessed using ANOVA or Student's two-tailed paired *t*-test where appropriate. A statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Limb blood flow

Sham operations did not affect blood flow of the hindlimb. At 2 weeks after ligation of the femoral artery, blood flow to the ischemic limbs in FAL rats was reduced to 52 ± 12% of that of the normal limb (Fig. 1).

3.2. Fasting glucose and insulin levels and glucose tolerance

At 2 weeks after ligation, body weight and fasting blood glucose levels in FAL rats were similar to those in Sham rats. However, fasting insulin levels in FAL rats were significantly higher than those in Sham rats (19.8 ± 1.6 μU ml^{-1} versus 13.0 ± 1.2 μU ml^{-1}, $p < 0.05$) (Fig. 1) indicating the presence of insulin resistance in FAL rats. Two weeks after ligation of the femoral artery, we performed the intraperitoneal glucose tolerance test and found that glucose tolerance was impaired and plasma insulin levels were increased

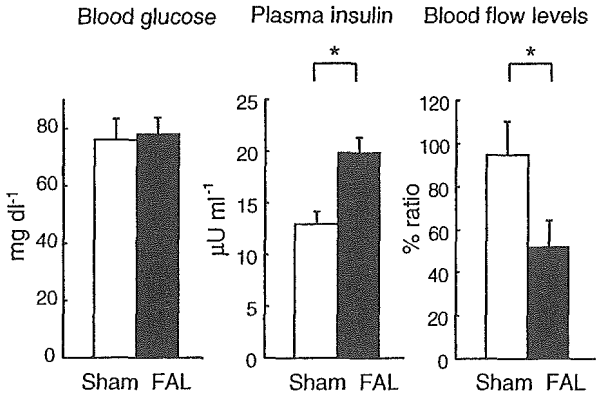

Fig. 1. Blood glucose, plasma insulin and blood flow levels in FAL rats (closed columns) and Sham rats (open columns). Blood flow levels of ischemic or Sham-operated limbs are expressed as percent flow of the contralateral normal limb. Data are expressed as means ± S.D. $^{*}p < 0.05$ vs. Sham rats.

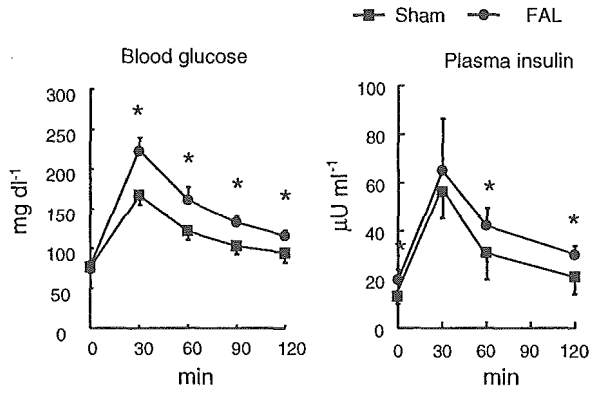

Fig. 2. Glucose tolerance in FAL rats (closed circles) and Sham rats (closed squares) after intraperitoneal glucose loading. Data are expressed as means ± S.D. $^{*}p < 0.05$ vs. Sham rats.

significantly in FAL rats (Fig. 2) compared with Sham rats after intraperitoneal glucose loading at 60 and 120 min.

3.3. Glucose clamp test

To further investigate the insulin resistance in FAL rats, we performed the hyperinsulinemic euglycemic clamp test.

Results of the basal period: At 0 min, basal plasma glucose levels in FAL rats were similar to Sham rats (FAL versus Sham, 95.0 ± 12.0 mg dl^{-1} versus 95.3 ± 10.1 mg dl^{-1}) (Fig. 3). However, insulin levels in FAL rats were significantly higher than those in

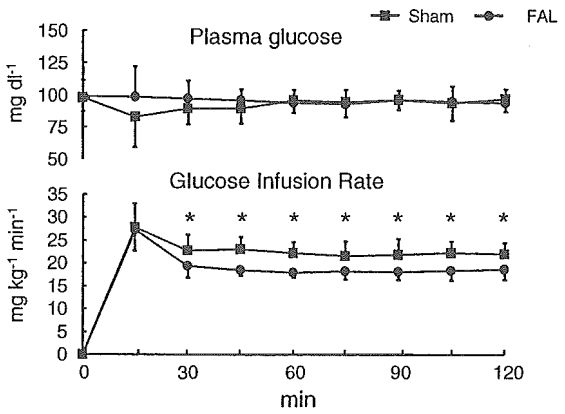

Fig. 3. Glucose infusion rate and plasma glucose level in FAL rats (closed circles) and Sham rats (closed squares) during the euglycemic hyperinsulinemic clamp test. Data are expressed as means ± S.D. $^{*}p < 0.05$ vs. Sham rats.

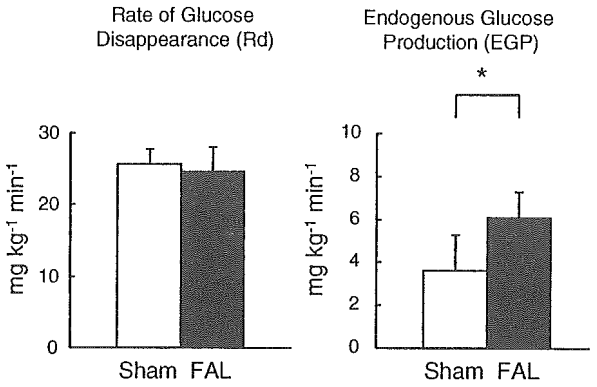

Fig. 5. Rates of glucose disappearance (Rd) and endogenous glucose production (EGP) during the clamp period in FAL rats (closed columns) and Sham rats (open columns). Data are expressed as means ± S.D. $^{*}p < 0.05$ vs. Sham rats.

Sham rats (15.0 ± 2.3 μU ml^{-1} versus 8.3 ± 1.3 μU ml^{-1}, $p < 0.05$). During the basal period from −30 to 0 min, plasma enrichment of [6,6-$^{2}H_2$]glucose was comparable between the two groups (1.86 ± 0.26% versus 1.84 ± 0.25%). Under basal conditions, there was no significant difference in the rate of glucose disappearance (Rd) and endogenous glucose production between the two groups (Fig. 4).

Results of the clamp period: During the clamp period, stable and comparable arterial blood glucose levels were achieved in both groups (Fig. 3). Arterial plasma human insulin levels at 120 min were comparable between the two groups (124.1 ± 1.8 μU ml^{-1} versus 121.7 ± 1.9 μU ml^{-1}). Under clamp condition, the glucose infusion rate was significantly lower in FAL rats than in Sham rats from 30 to 120 min ($p < 0.05$) (Fig. 3). The average glucose infusion rate from 90 to 120 min was lower in FAL rats than Sham rats (18.3 ± 2.6 mg kg^{-1} min^{-1} versus 21.8 ± 2.8 mg kg^{-1} min^{-1}, $p < 0.05$) (Fig. 3). During the clamp period, plasma enrichment of [6,6-$^{2}H_2$]glucose was comparable between the two groups (1.97 ± 0.17% versus 1.92 ± 0.23%). Under hyperinsulinemic conditions, the rate of glucose disappearance increased by three-fold compared to the basal period, but there was no significant difference between FAL rats and Sham rats (24.5 ± 2.2 mg kg^{-1} min^{-1} versus 25.6 ± 3.7 mg kg^{-1} min^{-1}). As shown in Fig. 5, endogenous glucose production in FAL rats as significantly greater than that in Sham rats (6.1 ± 1.7 mg kg^{-1} min^{-1} versus

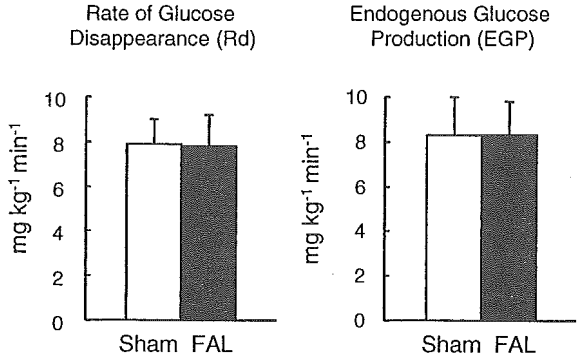

Fig. 4. Rates of glucose disappearance (Rd) and endogenous glucose production (EGP) during the basal period in FAL rats (closed columns) and Sham rats (open columns). Data are expressed as means ± S.D.

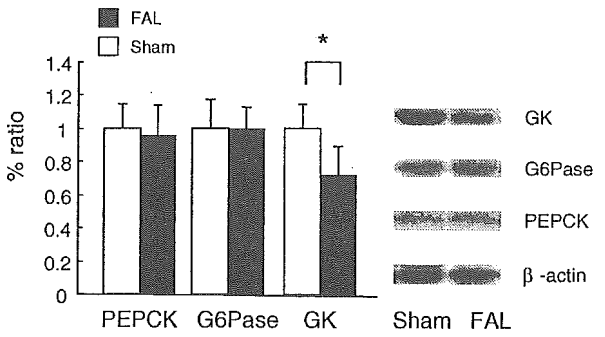

Fig. 6. Hepatic mRNA expression of PEPCK, glucose-6-phosphatase (G6Pase) and glucokinase (GK) in FAL rats (closed columns) and Sham rats (open columns). Data are expressed as means ± S.D. $^{*}p < 0.05$ vs. Sham rats.

3.6 ± 1.2 mg kg^{-1} min^{-1}, $p < 0.05$) and the insulin-mediated suppression ratio of endogenous glucose production in FAL rats was significantly lower than in Sham rats ($23.2 \pm 8.1\%$ versus $53.3 \pm 16.8\%$, $p < 0.05$).

3.4. Northern blot analyses

We evaluated hepatic mRNA expression of key enzymes of glucose metabolism in the operated rats. In FAL rats, mRNA expression of PEPCK and G6Pase as similar to that of Sham rats, while mRNA expression of glucokinase was significantly lower than that in Sham rats (Fig. 6).

3.5. TNF-α, IL-6 and CRP levels

To determine whether peripheral ischemia could induce systemic inflammation that could be the cause of hepatic insulin resistance, we measured plasma TNF-α, IL-6 and CRP levels in the blood of operated rats. There was no significant difference in these values between FAL and Sham rats (data not shown).

4. Discussion

To determine the impact of chronic hindlimb ischemia on glycemic control and insulin action, we investigated the glycemic status and insulin sensitivity in rats with unilateral hindlimb ischemia 2 weeks after femoral artery ligation. We first demonstrated that rats with impaired peripheral blood flow exhibit fasting hyperinsulinemia and glucose intolerance associated with insulin resistance in the liver, rather than in the peripheral tissue itself.

It has been reported that acute vasoconstriction and hindlimb ischemia impairs insulin action in the muscle [10]. On the other hand, in the present study, chronic hindlimb ischemia did not impair insulin-stimulated glucose utilization in peripheral tissues under physiological high insulin levels. Since basal insulin levels in FAL rats were higher than those in Sham rats, unilateral hindlimb ischemia might reduce peripheral glucose utilization at relative low insulin levels. It has been reported that the blood flow ratio of ischemic leg to non-ischemic leg immediately decreased to 10% and gradually recovered to 50 and 80%, 2 and 4 weeks after ligation [11]. In our study, the blood flow of unilateral ligated legs determined by the microsphere method was identical with those findings at 2 weeks post-ligation. Therefore, the influence of impaired blood flow on peripheral glucose utilization disappeared by increasing the collateral flow and maximizing insulin-stimulated intracellular glucose utilization.

To clarify the possible mechanism of impaired insulin action on endogenous glucose production by the liver, we evaluated hepatic mRNA expression of key enzymes of glucose metabolism. Endogenous glucose production consists of positive fluxes of glycogenolysis and gluconeogenesis and a negative flux of glucokinase [12,13]. The mRNA expressions of key enzymes of gluconeogenesis, such as PEPCK and G6Pase in liver isolated after the euglycemic hyperinsulinemic clamp study were identical between FAL and Sham rats. Hepatic glycogen was almost completely depleted after the overnight fast in our previous study [5]. Therefore, a decrease in glucokinase levels may increase glucose output by reducing glucose influx into the liver independent of gluconeogenesis and glycogenolysis. Indeed, it was demonstrated that liver-specific disruption of glucokinase gene increased fasting plasma glucose, which could reflect hepatic overproduction of glucose, without altering insulin levels [14]. Since fasting insulin levels are higher in FAL rats, such an increase in insulin levels may compensate for the decreased in glucokinase mRNA and hepatic glucose overproduction.

It was reported that acute ischemia of the hindlimb induced the expressions of various genes, mainly inflammation-related genes in the ischemic muscle, but that these increases in gene expression and histological abnormalities returned to their original levels 3 days after ligation [15]. We also could not find any increase in circulating inflammatory markers such as CRP, IL-6 and TNF-α 2 weeks after ligation. Therefore, it is unlikely that acute inflammation in the ischemic leg induces glucose intolerance and hepatic insulin resistance. It was demonstrated that the expression levels of several members of the insulin-like growth factor (IGF) axis were increased until 2 weeks after ligation in the ligated hindlimb [15,16]. Since IGF mimics insulin action in the liver as well as in the muscle, it is also unlikely that a local increase in IGF levels in the muscle impairs insulin action in the

liver. In addition, fasting hyperinsulinemia, impaired glucose tolerance and hepatic insulin resistance in FAL rats disappeared by 4 weeks after femoral artery ligation (data not shown), when the decreased femoral artery perfusion of the ligated leg had almost returned to that of the non-ligated leg. Therefore, impaired blood flow of the unilateral ischemic hindlimb mainly increased hepatic glucose production under basal and high insulin levels up to 2 weeks after the femoral artery ligation. In this study, it remains unclear as to how an ischemic leg affects hepatic insulin sensitivity, but there are several possible mechanisms, such as hormonal and neural factors, for hepatic insulin resistance induced by chronic hindlimb ischemia. These factors are well-known to play crucial roles on homeostasis of whole body glucose metabolism. The further examinations are necessary to determine the influence of these factors to hepatic insulin resistance in FAL rats.

In conclusion, chronic hindlimb ischemia induces glucose intolerance mainly associated with hepatic insulin resistance. We should take caution and be aware that the insulin resistant state is not only the cause of peripheral vascular disease, but also a result of it. Thus, the evaluation and treatment of insulin resistance in patients with peripheral vascular disease is an important issue.

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Possible novel index determined by the glucose clamp test for selection of a suitable therapy for each type 2 diabetic patient

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Abstract

The hallmark of type 2 diabetes is insulin resistance and insufficient insulin secretion, and appropriate therapy should be selected for each patient. In this study, to establish some index to select suitable therapy for each patient, we evaluated insulin sensitivity and insulin secretion with euglycemic hyperinsulinemic clamp and hyperglycemic clamp tests, respectively, and found that specific GIR index (GIR × IRI (90)) could be a useful marker to select suitable therapy for each type 2 diabetic patient (GIR: glucose infusion rate in euglycemic hyperinsulinemic clamp test; IRI (90): plasma insulin level 90 min after starting the hyperglycemic clamp test).

Keywords: Insulin resistance; Insulin secretion; Glucose clamp test

Type 2 diabetes is mainly caused by insulin resistance and/or insufficient insulin secretion, and suitable therapy should be selected for each type 2 diabetic patient, based on the pathological conditions of the individual. Therefore, it is very important to precisely estimate the pathological conditions of each individual patient, especially regarding the extent of insulin resistance and insufficiency of insulin secretion. Several methods to evaluate either insulin resistance or secretion have been developed and enforced; insulin sensitivity has been estimated by HOMA-IR, log(HOMA-IR), QUIKI (1/log(HOMA-IR)), the minimum model, steady-state plasma glucose (SSPG) and the euglycemic hyperinsulinemic clamp test, and insulin secretion has been estimated by the oral glucose tolerance test (OGTT), the glucagon loading test, and daily urinary secretion of C-peptide (u-CPR) [1–5]. These tests are very useful to separately evaluate insulin sensitivity or secretion, but do not necessarily provide enough information to

Abbreviations: OGTT, glucose tolerance test; u-CPR, urinary secretion of C-peptide; GIR, glucose infusion rate; SU, sulfonylurea

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determine suitable treatment (diet and/or exercise, oral hypoglycemic agents, or insulin therapy) for each patient with type 2 diabetes. The ultimate goal of therapy for type 2 diabetic patients is to normalize blood glucose levels and to prevent various diabetic complications. To achieve good glycemic control, type 2 diabetic patients usually start diet and/or exercise, and, if necessary, treatment with oral hypoglycemic agents or insulin is added. However, when glycemic control is not good enough, it is often difficult to determine whether the cause is due to inadequate diet or medication. Therefore, it would be very beneficial for both patients and physicians if an index for estimating the necessity of suitable medication according to the extent of insulin secretory ability and insulin sensitivity of each patient would be established.

In this study, to establish an index to determine suitable treatment for type 2 diabetic individuals, insulin secretion and sensitivity were determined simultaneously by a dual glucose clamp test using an artificial endocrine pancreas (STG-22; Nikiso Co., Shizuoka, Japan) [4]; we evaluated insulin secretory capacity by the hyperglycemic clamp test and also estimated insulin sensitivity by the consecutive euglycemic hyperinsulinemic clamp test. The study was approved by the Ethical Committee for Human Studies at Osaka University Graduate School of Medicine. We enrolled forty type 2 diabetic patients who were hospitalized in Osaka University Hospital (mean age: 51.9 years-old; duration: 11.1 years; HbA1c: 9.6%) and treated with diet (27–29 kcal per standard body weight per day), exercise (approximately 10,000 steps per day) and insulin. After a full explanation of this study with document, written informed consent was obtained from each subject. After 3-week intensive therapy with multiple insulin injection to remove glucose toxicity, insulin secretion and sensitivity were determined by the dual glucose clamp test. Experiments consisted of a 90-min hyperglycemic clamp period and a 120-min euglycemic hyperinsulinemic clamp period. During the hyperglycemic clamp period, an exogenous glucose infusion was given to achieve steady-state blood glucose levels (11 mmol/l) within 5 min, and serum insulin levels were measured at 0, 5 and 90 min after commencement of the clamp test (IRI (0), IRI (5), and IRI (90)), which reflect basal insulin secretion and early and late phases of additional insulin secretion, respectively. During the consecutive euglycemic hyperinsulinemic clamp period, patients were given a primed-constant infusion of regular insulin (1.12 mU/(kg min)) and an exogenous glucose infusion to achieve the desired steady-state serum insulin level (100 μU/ml) and to maintain blood glucose levels within the euglycemic range (5 mmol/l). When the rate of exogenous glucose infusion reached a steady-state level, we evaluated insulin sensitivity as the average glucose infusion rate (GIR). After the glucose clamp test, treatment of all patients was switched from insulin to sulfonylurea (SU), namely 2.5–5 mg glibenclamide per day. When blood glucose levels were within well-controlled levels (less than 7 mmol/l before a meal and 10 mmol/l, 2 h after a meal), treatment was not changed ("SU" group, $n = 12$). When 1-week treatment with SU failed to maintain blood glucose levels within the above levels (greater than 7 mmol/l before a meal or greater than 10 mmol/l, 2 h after a meal), the patients were treated with multiple insulin injections ("Insulin" group, $n = 13$). When hypoglycemia was observed upon SU treatment, SU treatment was discontinued ("Diet" group, $n = 10$); and when blood glucose levels were not within the above levels (more than 7 mmol/l before a meal or 10 mmol/l, 2 h after a meal), the patients were treated with 15–30 mg per day of pioglitazone, an insulin sensitizer ("Sensitizer" group, $n = 5$). In addition, we performed conventional examinations such as OGTT, HOMA-IR, the glucagon loading test and u-CPR.

Clinical characteristics of patients in each group were shown in Table 1. The patients treated with insulin sensitizer were significantly younger than the patients in other groups. BMI in "Insulin" group was significantly lower than those in "Sensitizer" and

Table 1
Patients characteristics in each group

	Insulin	SU	Sensitizer	Diet
Age	56.0 ± 2.6*	54.6 ± 4.2*	35.8 ± 4.6	50.0 ± 2.8*
Gender (M/F)	10/3	8/4	5/0	9/1
BMI	21.6 ± 0.3*,†	23.3 ± 1.0	26.3 ± 1.7	25.3 ± 1.8
HbA_{1C} (%)	9.9 ± 0.6	8.5 ± 0.4	9.2 ± 0.9	9.2 ± 0.6

Data are means ± S.E.M.
* $P < 0.05$ vs. sensitizer.
† $P < 0.05$ vs. diet.

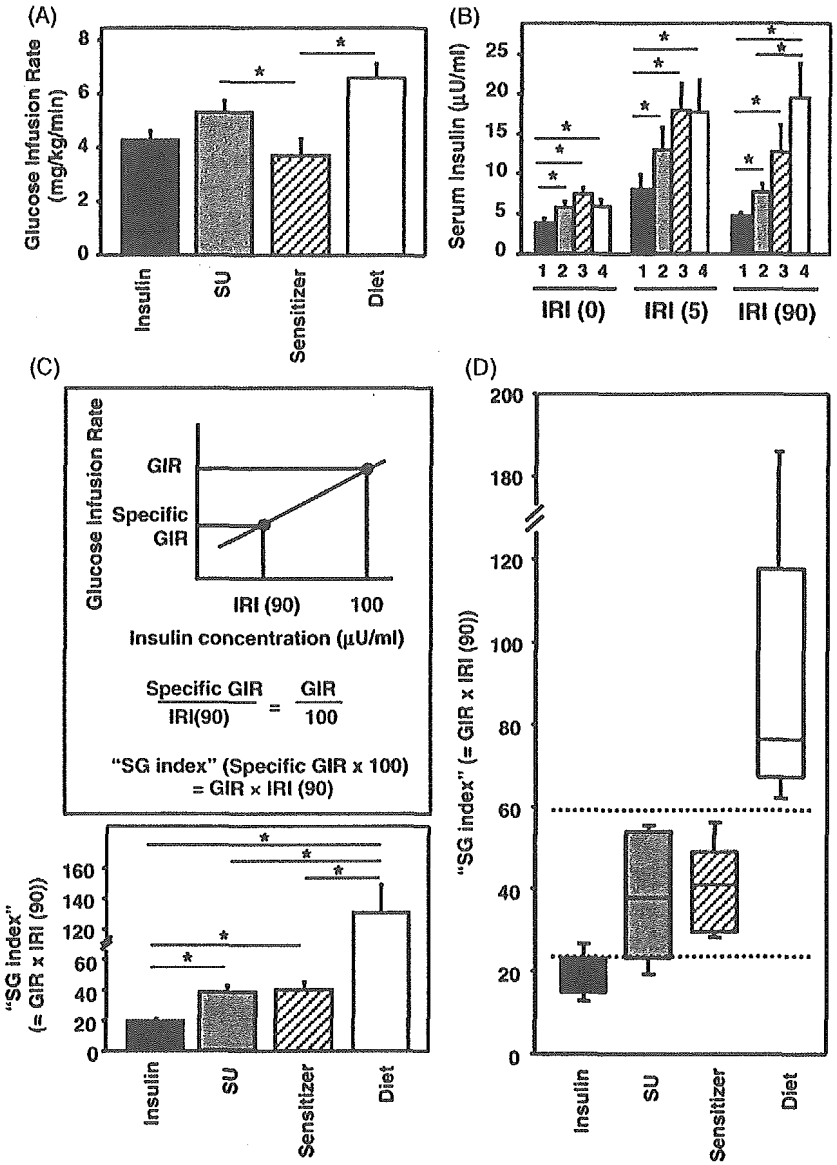

Fig. 1. Glucose infusion rate, insulin secretion, and "SG index" (=GIR × IRI (90)) estimated by the euglycemic hyperinsulinemic clamp and hyperglycemic clamp tests in type 2 diabetic patients. (A) Glucose infusion rate estimated by the euglycemic hyperinsulinemic clamp test. (B) Insulin secretion estimated by the hyperglycemic clamp test. (C) "SG index" (=GIR × IRI (90)) estimated by the euglycemic hyperinsulinemic clamp and hyperglycemic clamp tests. (D) Box and whiskers plot of the "SG index". Data are means ± S.E.M., $^{*}P < 0.05$.

"Diet" groups. These findings suggested that the treatment with insulin sensitizer is suitable for young obese patients with type 2 diabetes, and treatment with insulin is proper to lean type 2 diabetic patients. Regarding insulin sensitivity, the GIR in the "Sensitizer" group was significantly lower than that in the "SU" and "Diet" group (Fig. 1A). Regarding insulin secretion, IRI (0) and IRI (5) in the "Insulin" group were significantly lower than those in any of the other groups (Fig. 1B). In addition, there was a significant difference between the "SU" and "Diet" group in IRI (90) (Fig. 1B). These data suggest the importance of insulin resistance and secretion in diabetic treatment, but do not provide enough information to determine suitable treatment for each patient. Indeed, the 95% confidence interval for GIR, IRI (0), IRI (5), or IRI (90) overlapped and was difficult to clearly distinguish among these groups (data not shown). To determine

suitable treatment for diabetic individuals, we should consider the pathological conditions of them as the combination of pancreatic β-cell dysfunction and insulin resistance. Therefore, we attempt to establish a new index, representing the extent of insulin secretion capacity and insulin resistance simultaneously, from the results of glucose clamp test. GIR mainly shows the glucose disposal rate in peripheral tissues with serum insulin concentrations under 100 μU/ml. IRI (90) represents the late phase of insulin secretion during moderate hyperglycemia (11 mmol/l) observed in postprandial condition. Since physiological conditions, GIR is in proportion to serum insulin concentration [6], we suppose that the "SG index" (specific GIR × 100) (=GIR × IRI (90)) reflects the ability of glucose disposal within the physiological range of serum insulin in each patient (Fig. 1C, upper panel). As shown in Fig. 1C, lower panel, there was a significant difference in the "SG index" among the "Insulin" group, "SU or Sensitizer" group (single oral agent group), and "Diet" group. Furthermore, as shown in Fig. 1D, the 95% confidence intervals for the "SG index" were 15.6–22.3 ("Insulin" group), 28.7–48.2 ("SU" group), 25.9–54.5 ("Sensitizer" group) and 64.3–171.1 ("Diet" group), and we found a significant difference among the 3 groups ("Insulin" group, "SU or Sensitize" group (single oral agent group), and "Diet" group). Thus, we were able to clearly distinguish these 3 groups using this index; <25: insulin treatment; 25–60: oral hypoglycemic agent; >60: diet and exercise. We also estimated insulin sensitivity and secretion with conventional methods, such as HOMA-IR, QUIKI, and OGTT, but any combination of these conventional indexes was not as good as the "SG index" for determination of suitable treatment for type 2 diabetic individuals.

The "SG index" (=GIR × IRI (90)) determined by the dual glucose clamp test appeared to be the most useful index for distinction among diet, oral hypoglycemic agents, and insulin, and was much better than GIR, and IRI (0), IRI (5), IRI (90) alone from the glucose clamp test and any other results from conventional methods. We assume that there are several reasons for this; one reason is that the euglycemic hyperinsulinemic clamp test is the most accurate method for evaluation of insulin sensitivity, and another reason is that with the hyperglycemic glucose clamp test we can estimate insulin secretory capacity at the identical glucose concentration, which is impossible with OGTT. In addition, IRI (90), reflecting the late phase of insulin secretion, was much better than IRI (0) or IRI (5), reflecting basal or early phase of insulin secretion, for determination of a suitable treatment for type 2 diabetic individuals. We assume that this is at least in part because deterioration of the second phase of insulin secretion contributes to progression of type 2 diabetes.

In conclusion, the "SG index" (=GIR × IRI (90)) measured by the glucose clamp test appears to be a very useful marker for selection of a suitable treatment for each type 2 diabetic patient, and thus we would like to propose the "SG index" as a new index for determination of a suitable treatment for each type 2 diabetic patient.

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