(insulin-dependency). As both basal and stimulated insulin secretion from pancreatic β-cells is completely abolished in the majority of patients with type 1 diabetes, the blood glucose level remains unstable despite all effort to use optimal exogenous insulin.

In diabetic patients, blood glucose levels are considerably high or low in comparison with optimal levels, and the amplitude of glycemic excursions are large. The former is assessed by the *M*-value [1] and the latter by the mean amplitude of glycemic excursions (MAGE) [2]. In diabetic patients with metabolic instability, the *M*-value and MAGE are significantly high [1–3]. Such patients are at increased risk of progression of diabetic complications [4], life-threatening hypoglycemia unawareness and even "dead-in-bed syndrome" [5].

Since the success of islet transplantation with the corticosteroid-free protocol from Edmonton [6], islet transplantation has become more common radical therapy for type 1 diabetes mellitus [7]. The Edmonton protocol, in addition to optimizing islet isolation and modifying the immunosuppressive regimen, includes multiple infusions of islets from different donors. This therapy generally aims at insulin independence as well as the avoidance of severe hypoglycemia. Under present conditions, when cadeveric donors are used, multiple injections are required to attain insulin independence except in one report [8]. However, in many countries, the donor pool is extremely small, making it impractical to aim at insulin independence. Accordingly, we have investigated the effect of a single transplantation of the islets on glycemic lability.

Using even a non-heart-beating donor, a single transplantation of the islets can achieve metabolic stability probably by effective supply of basal insulin secretion regardless of the achievement of insulin independence, suggesting the therapeutic value of a single transplantation of the islets for patients with insulin-dependency in cases of donor shortage.

2. Methods

2.1. Subjects

Five patients (three women, two men) (patients one to five) (median age, 44 years; range, 36–58) who had had type 1 diabetes mellitus for a median of 21 years (range, 14–29) and a 27-year-old woman (Patient 6) who had had pancreatic diabetes for 12 years underwent islet transplantation in Kyoto University Hospital between April 7 2004 and January 19 2005. Diagnostic basis for type 1 diabetes in five of the six recipients was completely depleted insulin secretion (negative C-peptide [<0.1 ng/ml] prestimulation and post glucagon stimulation) and clinical course. The donors of five patients (Patients one to five) with type 1 diabetes mellitus were non-heart-beating donors, and a donor of a patient (Patient 6) with pancreatic diabetes was a living donor who was a mother of the patient. Each patient received islet transplantation from one or two pancreases [9,10].

The study was approved by the ethics committee of Kyoto University Graduate School and Faculty of Medicine.

2.2. Assessment of glycemic control

The *M*-value [1] and the mean amplitude of glycemic excursions (MAGE) [2], the indexes of glycemic lability, of the six patients were measured before and 1 month after a first islet transplantation.

There is a predictable relationship between plasma glucose and HbA_{1c}. Thus, the estimated HbA_{1c} levels were calculated by the corresponding seven-point plasma glucose profiles (premeal, postmeal and bedtime) before and 1 month after the first transplantation: estimated HbA_{1c} = mean plasma glucose (mg/dl)/35.6 + 1.87 [11,12].

The *M*-value, MAGE and HbA_{1c} levels of 52 type 1 diabetic patients (31 women and 21 men) (median age, 43 years; range, 16–79) and 102 type 2 diabetic patients (43 women and 59 men) (median age, 65 years; range, 28–79) admitted to Kyoto University Hospital from January 2002 to June 2005 were also examined.

2.3. Exogenous insulin requirement

We defined the total amount of premeal regular insulin or rapid-acting insulin analogue as the supplemental dose of stimulated insulin and that of neutral protamine Hagedorn (NPH) insulin, long-acting insulin analogue, or basal dose of continuous subcutaneous insulin infusion (CSII) as the supplement of basal insulin. The supplemental dose of basal and stimulated insulin were compared before and 1 month after the first islet transplantation.

2.4. Statistical analyses

Results are expressed as medians and ranges. Wilcoxon signed-rank test was used to compare the *M*-value, MAGE, the estimated HbA_{1c} levels and the amount of exogenous insulin before and 1 month after islet transplantation.

Mann–Whitney's *U*-test was used to compare the *M*-value, MAGE, and the HbA_{1c} levels of type 1 and type 2 diabetic control patients. Significance was taken at a P value of <0.05.

3. Results

3.1. Glycemic lability is higher in type 1 diabetic patients

The median HbA_{1c} level of 102 type two diabetic patients who had been admitted to Kyoto University

Hospital was 8.6% (range, 5.3–14.5%). Their *M*-value and MAGE at discharge was 12.2 (range, 1.2–66.2) and 5.2 mM (range, 0.8–12.7 mM), respectively (Fig. 1A and B). The median HbA_{1c} level of 52 type 1 diabetic patients admitted to Kyoto University Hospital was 8.3% (range, 5.0–16.3%), not significantly different from that of type 2 diabetic patients. The *M*-value and MAGE of 52 type 1 diabetic patients at discharge was 22.0 (range, 1.3–127.6, $P < 0.05$) and 6.7 mM (range, 2.8–16.6 mM, $P < 0.05$), respectively, significantly higher than those of type 2 patients (Fig. 1A and B).

Glycemic lability was improved after the first islet transplantation. The *M*-value (Fig. 2A) and MAGE (Fig. 2B) before islet transplantation was 53.0 (range, 8.9–91.0) and 8.5 mM (range, 4.8–11.7 mM), respectively. These medians were higher than the third quartiles of the 52 type 1 diabetic control patients. The *M*-value (Fig. 2A) decreased significantly to 4.2 (range, 0.6–8.8, $P < 0.05$), and MAGE (Fig. 2B) also significantly decreased to 3.3 mM (range, 2.0–4.5 mM, $P < 0.05$) 1 month after islet transplantation. These values were lower than the first quartile of the 102 type 2 diabetic control patients.

The estimated HbA_{1c} level before islet transplantation was 7.9% (range, 5.7–10.9%). The estimated HbA_{1c} level 1 month after islet transplantation decreased significantly to 5.4% (range, 4.7–5.9%, $P < 0.05$). There was no worsening of the secondary complications of diabetes due to the decrease of HbA_{1c} levels in all six patients.

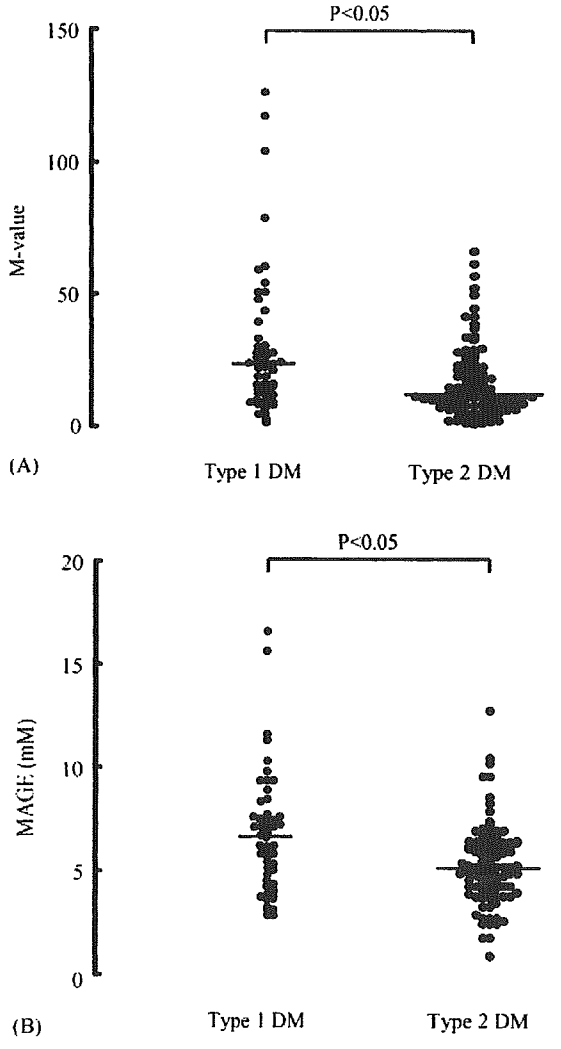

Fig. 1. (A) *M*-value of 52 type 1 diabetic patients and 102 type 2 diabetic patients at discharge who were admitted to Kyoto University Hospital. Horizontal lines represent medians. (B) MAGE of 52 type 1 diabetic patients and 102 type 2 diabetic patients at discharge who were admitted to Kyoto University Hospital. Horizontal lines represent medians.

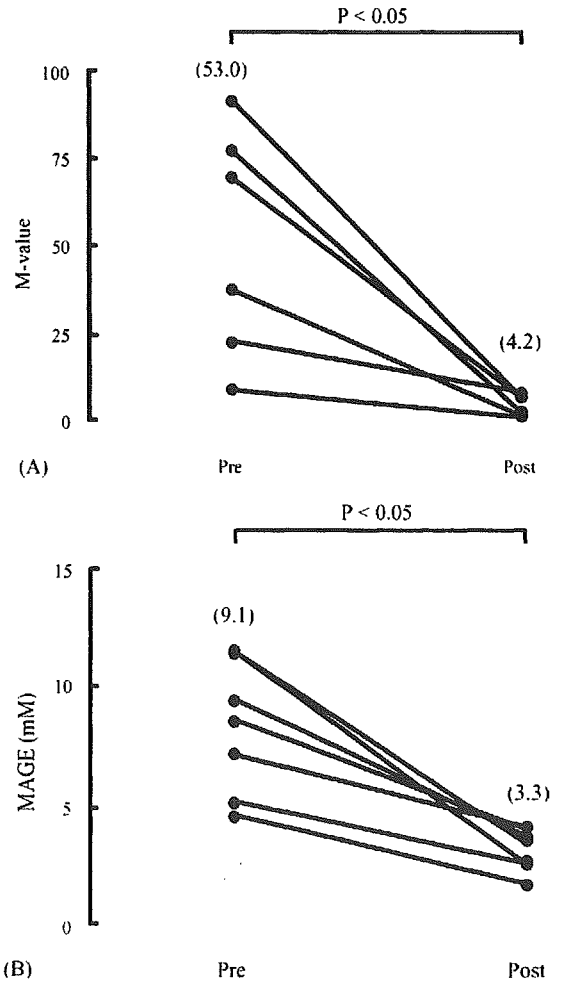

Fig. 2. (A) *M*-value before and 1 month after islet transplantation. Median values in parentheses. (B) MAGE before and 1 month after islet transplantation. Median values in parentheses.

3.2. Daily exogenous insulin use before and after islet transplantation

The total amount of exogenous insulin decreased gradually in all cases, stabilizing 1 month after transplantation. The supplemental dose of stimulated insulin (Fig. 3A) did not change significantly from 0.28 units/kg/day (range, 0.13–0.51 units/kg/day) to 0.21 units/kg/day (range, 0–0.41 units/kg/day); the supplement of basal insulin (Fig. 3B) decreased significantly by a median of 43% from 0.31 units/kg/day (range, 0.16–0.37 units/kg/day) to 0.18 units/kg/day (range, 0–0.22 units/kg/day, $P < 0.05$).

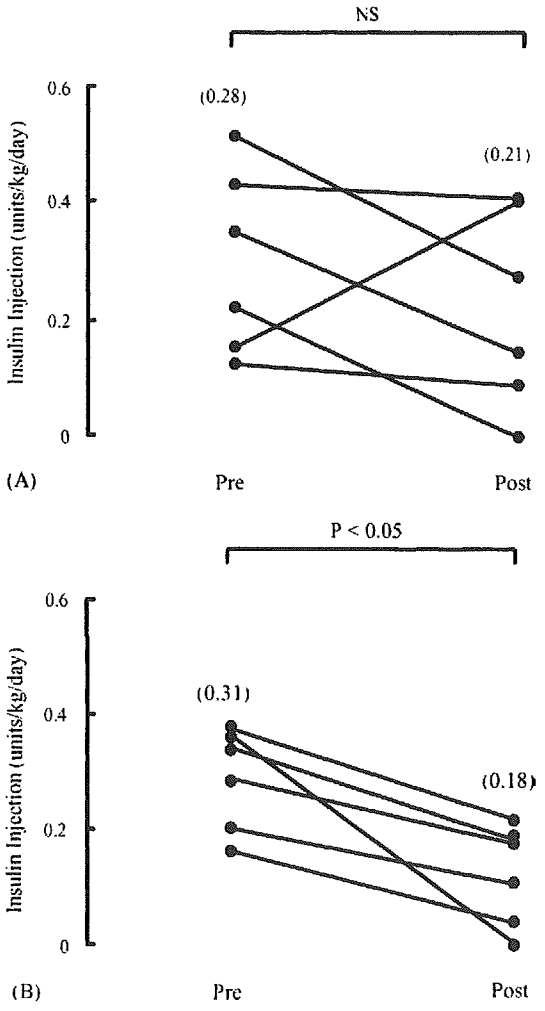

Fig. 3. (A) Daily supplement of stimulated insulin (units/kg/day) before and 1 month after islet transplantation. Median values in parentheses. (B) Daily supplement of basal insulin (units/kg/day) before and 1 month after islet transplantation. Median values in parentheses.

4. Discussion

Intensive insulin therapy can decrease HbA_{1c} and thereby delay the onset and slow progression of secondary complications of diabetes in both type 1 and type 2 diabetic patients [13,14]. However, our results showed that, in many patients with type 1 diabetes, blood glucose levels remained unstable despite optimal insulin therapy in hospital. The levels remained considerably high or low in comparison with the optimal levels, assessed by the *M*-value, and the amplitude of glycemic excursions remained large, assessed by MAGE, even though the HbA_{1c} levels of type 1 and type 2 diabetic patients on admission were nearly the same. In addition, before islet transplantation, all six patients exhibited hypoglycemia unawareness and life-threatening hypoglycemia despite all effort to optimize insulin therapy. Like this, in type 1 diabetic patients, it is quite difficult to achieve good glycemic control with exogenous insulin even if it is optimized.

Islet transplantation has become an alternative therapy for type 1 diabetes, the Edmonton protocol of multiple transplantations from different donors aiming at insulin independence as well as the avoidance of severe hypoglycemia. However, in some countries, the donor pool is extremely small, and many patients are on waiting lists. We evaluated the effects on glycemic lability in patients with insulin-dependency 1 month after a single transplantation of the islets, when the total amount of exogenous insulin became stable.

Glycemic lability in all six patients was improved significantly 1 month after the first islet transplantation. The *M*-value and MAGE became better than in most of the type 2 diabetic control patients. This indicates that a gain of endogenous insulin secretion resulted in non-insulin-dependent status. Furthermore, episodes of severe hypoglycemia have not occurred for at least 6 months after islet transplantation [10], even though the overall blood glucose level continues to be considerably lower than that before transplantation.

Only 5 years have passed since the Edmonton protocol was started, and the loss of insulin independence in many patients several years after transplantation has been reported [15]. Thus, the duration of insulin-independent status established by transplantation is uncertain. However, maintaining the blood glucose level close to the normal range for a certain period by transplantation can be beneficial: the epidemiology of diabetes interventions and complications (EDIC) study [16–20] indicates a reduced risk of onset and progression of diabetic complications for

several years, even after glycemic control becomes fair or poor due to graft failure.

As glycemic lability in type 1 diabetes is due to a complete loss of both basal and stimulated insulin secretion from β-cells, the supplemental dose of basal and stimulated insulin required before and after islet transplantation was compared. The supplement of basal insulin of all six patients decreased significantly 1 month after transplantation, indicating that a single transplantation of the islets is an effective therapy to supply basal insulin secretion.

The treat-to-target trial and related trials have shown that the uniform supplementation of basal insulin throughout the day significantly reduces risk of hypoglycemia and achieves good glycemic control in type 2 diabetic patients [21–23]. Our findings apparently support this. The supplemental dose of stimulated insulin was not changed significantly, partly due to the toxic effects on the islets of the immunosuppressive drugs. Indeed, we have shown that tacrolimus suppresses glucose-induced insulin release from pancreatic islets by reducing glucokinase activity [24]. In addition, sirolimus significantly impairs glucose-induced insulin secretion in islets [25,26]. Accordingly, the development of a new immunosuppressive regimen less toxic to β-cells may be required.

These results demonstrate that only a single transplantation of the islets using even a non-heart-beating donor can be sufficient to achieve metabolic stability, avoid life-threatening severe hypoglycemia, and improve the quality of life in patients with insulin-dependency regardless of the achievement of insulin independence. Thus, a single transplantation of the islets using one donor may enable a greater number of diabetic patients to benefit from islet transplantation therapy where donor shortage is a serious problem.

Acknowledgements

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Maternal Weight Gain Is Associated With Infant Insulin Concentrations During the 1st Year of Life

Since hyperinsulinemia tracks from childhood to adulthood and is associated with diabetes risk, identifying modifiable conditions during gestation that may impact insulin metabolism in offspring is important. We conducted a pilot study to investigate associations between maternal weight gain and infant insulin concentrations in an underserved population at high risk for diabetes. Mexican or Native American women with an infant <1 year of age provided written consent. Infant weight-for-age Z scores (WAZ) were calculated, and nonfasting plasma samples were analyzed for insulin by standard assay. Pearson's bivariate test was used to assess relationships between variables, and the unpaired *t* test was used to examine differences between means.

A total of 16 women ([means ± SE] 21.8 ± 1.7 years) and their infants (6.4 ± 0.9 months; 9 males and 7 females) completed the study, and medical records were available for 9 of these pairs. Based on combined self-reports and medical records, the mean prepregnancy weight was 71.5 ± 4.0 kg, and the mean pregnancy weight gain was 10.7 ± 2.4 kg. Infants were full term with birth weights ranging from 2,495 to 4,309 g (3,381 ± 121.0 g); WAZ scores averaged 0.47 ± 0.23. Blood insulin concentrations averaged 11.5 ± 1.6 mU/l. Gestational weight gain was significantly correlated to infant insulin concentrations (r = 0.662; P = 0.005); however, for nondiabetic women with verifiable pregnancy weight gain (n = 8), this association was strengthened (r = 0.763, P = 0.028; Fig. 1). Infant insulin concentrations (n = 16) were not associated with birth weight, infant age, WAZ scores, prepregnancy weight, or maternal age.

These data show that maternal weight gain predicted infant insulin concentrations, explaining nearly 60% of the variance in these values. Diabetes during pregnancy has been associated with cord blood insulin and with insulin concentrations in adolescence (1), and in nondiabetic pregnancies, maternal weight gain was related to cord blood insulin in macrosomic neonates (2). Currently, a weight gain of 6.8–11.5 kg is recommended for overweight women, and obese women are advised to gain a minimum of 6.8 kg. In obese, nondiabetic women, minimal gestational weight gain (<5 kg) normalized obstetric outcomes, including hypertension, cesarean section, induction of labor, and macrosomia, and did not adversely affect fetal outcomes (3). Utilizing an emerging obstetric outcome, infant insulin concentrations, our preliminary data support the contention that gestational weight gain should be carefully considered in overweight populations at high risk for diabetes. Differential analyses of our data show that minimal gestational weight gain in the nondiabetic women (≤5 vs. >5 kg) was associated with lower infant insulin concentrations (7.2 ± 0.6 vs. 13.4 ± 2.0 mU/l; P = 0.013). Together, the available data indicate that controlling weight gain during obese pregnancies may be advantageous and that more studies of this nature are warranted.

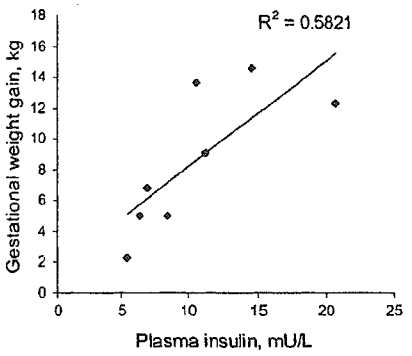

Figure 1—*Correlation of maternal weight gain and infant insulin concentrations (n = 8 mother/infant pairs; r = 0.763, P = 0.028).*

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Soluble Tumor Necrosis Factor Receptor 1 Is Strongly and Independently Associated With Serum Homocysteine in Nonobese Japanese Type 2 Diabetic Patients

The major clinical consequence of type 2 diabetes is mortality and morbidity from atherosclerotic vascular disease. With regards to the risk factors responsible for the evolution of atherosclerosis, Bierman (1) estimated that typical risk factors, including smoking, cholesterol, and blood pressure, can account for no more than 30% of excess cardiovascular risk factors in diabetic patients. Thus, other factors seem to play a key role in the progression of atherosclerosis in diabetes.

One potential factor is homocysteine. Homocysteine has been shown to contribute to the development of atherosclerosis in diabetic patients (2). Whereas the deficiencies of folate and vitamin B_{12} lead to hyperhomocysteinemia, these deficiencies alone do not completely account for atherosclerotic changes induced by homocysteine in diabetic patients.

Tumor necrosis factor (TNF) is a potent candidate involved in the pathogenesis of atherosclerosis. Rauchhaus et al. (3) demonstrated that elevated soluble TNF receptor 1 (sTNF-R1) has shown to be predictive of cardiovascular mortality in patients with chronic heart failure. We

found that sTNF-R1 is independently associated with albuminuria in type 2 diabetic patients (4). To the best of our knowledge, however, it is not clear whether serum homocysteine is associated with TNF receptor in type 2 diabetic patients. The aim of the present study was therefore to investigate the relationships between serum homocysteine and TNF receptor in patients with type 2 diabetes.

Fifty nonobese Japanese type 2 diabetic patients were studied. Their BMI, HbA_{1c}, and serum creatinine were 22.6 ± 0.3 kg/m2 (range 17.6–26.2), 7.8 ± 0.2% (5.5–12.3), and 0.70 ± 0.02 mg/dl (0.46–0.98), respectively. They had not been treated with insulin or any medications known to alter homocysteine level. In conjunction with homocysteine, systolic and diastolic blood pressure, HbA_{1c}, glucose, lipids, serum creatinine, TNF-α, sTNF-R1, and sTNF-R2 were measured after an overnight fast.

With univariate analysis, serum homocysteine was positively correlated with age ($r = 0.361$, $P = 0.012$), diabetes duration ($r = 0.292$, $P = 0.045$), serum creatinine ($r = 0.623$, $P < 0.001$), sTNF-R1 ($r = 0.415$, $P < 0.005$), and sTNF-R2 ($r = 0.371$, $P < 0.01$). Other variables including TNF-α, however, were not associated with homocysteine. Multiple regression analyses showed that serum homocysteine was independently associated with serum creatinine ($F = 20.1$) and sTNF-R1 ($F = 6.9$), which explained 49.3% of the variability of homocysteine. Thus, TNF system activity may be responsible for the evolution of atherosclerosis induced by homocysteine in nonobese Japanese type 2 diabetic patients.

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Phenformin-Induced Lactic Acidosis in an Older Diabetic Patient

A recurrent drama (phenformin and lactic acidosis)

Editor's note: The authors had the following statement in their letter to me, with which I agree, "Most physicians are aware of the risk of lactic acidosis in patients taking phenformin. However, this side effect is continuously observed because phenformin is still used in Italy, Brazil, and China. We believe that the publication of our observation in an important journal like Diabetes Care *may help to prompt governments of these countries to ban phenformin, just like in the rest of the world. This is the only way to prevent further cases of this avoidable, unacceptable and life-threatening complication."*

A 73-year-old man with diabetes presented with upper-abdominal pain and nausea. He also had a history of hypertension, a pace-maker implant, and peripheral arterial disease treated with amputation of his left leg. His therapy included ticlopidine, enalapril, omeprazole, and 2 mg glibenclamide/30 mg phenformin b.i.d. The patient was alert and cognitively intact. Blood pressure and heart rate were 120/70 mmHg and 70 bpm, respectively. Radiographs of the chest and abdomen and an abdominal ultrasound study were normal. Laboratory tests disclosed a severe lactic acidosis (pH 6.8, pCO_2 14.1 mmHg, pO_2 108 mmHg, HCO_3 4.9 mmol/l, lactate 21 mmol/l, and anion gap 31 mmol/l). After phenformin discontinuation, the patient's conditions rapidly improved. He was treated with intravenous insulin and glucose (1) and discharged 7 days later in good condition.

This report confirms that phenformin-induced lactic acidosis (PLA) is still a public health problem (1,2). To our knowledge, phenformin is still used in Italy, China, and Brazil. In a Medline search, we found 12 cases that occurred in Italy between 1981 and 1998 (2). In two patients phenformin was even brought back into use soon after, thereby questioning the belief that PLA is adequately recognized (2). More importantly, according to data by Intercontinental Marketing Services (www.imshealth.com), 838,000 preparations of phenformin and a sulfonylurea have been sold in Italy between January and October 2005. Because PLA occurs in 1 of 4,000 patients (3) with a mortality rate of ~50%, these data raise worrying health care considerations. In fact, diabetic patients often have comorbid conditions known to favor PLA.

Phenformin was removed from the U.S. market in 1977, but, surprisingly, cases of patients who have been prescribed the drug abroad are continuously reported (1). Phenformin can also be illegally obtained online or through mail orders to replace metformin, which is more costly. Furthermore, herbal medicines containing phenformin are also consumed in developed countries. In February 2000, the Food and Drug Administration recalled five Chinese herbal medications containing phenformin (4), while Health Canada is currently warning consumers not to take "Shortclean," a phenformin-based Chinese "natural" medicine (5).

Phenformin can always be replaced by metformin, which should not be associated with a higher risk of lactic acidosis compared with nonbiguanide therapies (6). Despite most clinicians being aware of PLA, the only way for preventing further cases is to forbid phenformin in countries where it is still used.

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Factors responsible for deteriorating glucose tolerance in newly diagnosed type 2 diabetes in Japanese men

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Abstract

Hyperglycemia frequently continues to worsen even after the diagnosis of overt diabetes. The aim of this study is to evaluate the factors contributing to increasing glucose intolerance after onset of type 2 diabetes in Japanese subjects. Five hundred fifty newly diagnosed type 2 diabetic patients were classified into 3 degrees of hyperglycemia based on plasma glucose levels estimated by 75-g oral glucose tolerance test: diabetes mellitus with isolated fasting hyperglycemia (DM/IFH), DM with isolated postchallenge hyperglycemia (DM/IPH), and DM with fasting and postchallenge hyperglycemia (DM/FPH). In addition, the DM/IFH and DM/IPH groups were subdivided to clarify the determinants of fasting and postchallenge hyperglycemia. Insulin secretion was evaluated by insulinogenic index, and insulin sensitivity was evaluated by composite index of insulin sensitivity (ISI composite). The insulinogenic index in DM/IFH was highest of the 3 groups ($P < .0001$). The insulinogenic index in DM/IPH was higher than in DM/FPH ($P < .0001$). The international sensitivity index composite in DM/IPH was highest of the 3 groups ($P < .05$). Although impaired early-phase insulin secretion plays the crucial role in deterioration from DM/IFH to DM/FPH in Japanese subjects, impaired early-phase insulin secretion and decreased insulin sensitivity both are factors in deterioration from DM/IPH to DM/FPH. In addition, comparison of subgroups of DM/IFH and DM/IPH shows that although decreased early-phase insulin secretion plays the more significant role in postchallenge hyperglycemia in Japanese subjects, insulin sensitivity is the more important factor in fasting hyperglycemia.

1. Introduction

Type 2 diabetes is a heterogeneous disorder characterized by progressive elevation of plasma glucose (PG) levels. Although the occurrence of diabetes in Japan is increasing as in other countries, the hyperglycemia of Japanese subjects is typically because of factors that differ somewhat from those of other ethnic groups [1-5], impaired insulin secretion, and sensitivity most notably being differently involved. In previous studies, we found that impaired early-phase insulin secretion plays the more important role in deterioration from normal glucose tolerance (NGT) via impaired glucose tolerance (IGT) to type 2 diabetes in Japanese subjects [6]. This agrees with reports on the importance of impaired early-phase insulin secretion in type 2 diabetes in Japanese subjects [7,8]. These findings differ from those in Pima Indians, Mexican Americans, and Caucasian populations, in which increasing insulin resistance is clearly the more important factor in developing glucose intolerance [9,10]. In the present study, we investigated the factors responsible for decreased glucose tolerance after onset of diabetes and evaluated the contribution of these factors in fasting and postchallenge hyperglycemia.

We classified 550 Japanese men with newly diagnosed diabetes mellitus (DM) into 3 subgroups of glucose intolerance based on 75-g oral glucose tolerance test

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(OGTT): DM with isolated fasting hyperglycemia (DM/IFH), DM with isolated postchallenge hyperglycemia (DM/IPH), and DM with fasting and postchallenge hyperglycemia (DM/FPH) (Fig. 1A). Insulin secretion and insulin sensitivity measurements were compared to evaluate the factors involved in the deterioration of glucose tolerance in newly diagnosed type 2 diabetic Japanese subjects. The insulinogenic index (30 minutes) was used as the parameter of early-phase insulin secretion [11,12]; the composite index of insulin sensitivity (ISI composite) was used as the parameter of insulin sensitivity [13]. Subcategories of DM/IFH and DM/IPH were compared to evaluate the contributions of these factors in fasting and postchallenge hyperglycemia.

2. Materials and methods

2.1. Subjects

We recruited for closer evaluation 550 Japanese men undergoing 75-g OGTT who had positive urine glucose test, greater than 5.6 mmol/L fasting PG (FPG), greater than 5.0% HbA_{1c}, or family history of diabetes at initial examination for regular medical check-up at Kyoto University Hospital, Ikeda Hospital, Kansai-Denryoku Hospital, Kansai Health Management Center, and Kyoto Preventive Medical Center between 1993 and 2004. OGTT was performed within 3 months of the initial examination. All subjects were Japanese males with no signs of hypertension, hepatic, renal, endocrine, or malignant diseases. No subject had engaged in heavy exercise, had taken gastrectomy, or had taken any medication known to affect glucose metabolism before the study. The study was designed in compliance with the ethics regulations set out by the Helsinki Declaration.

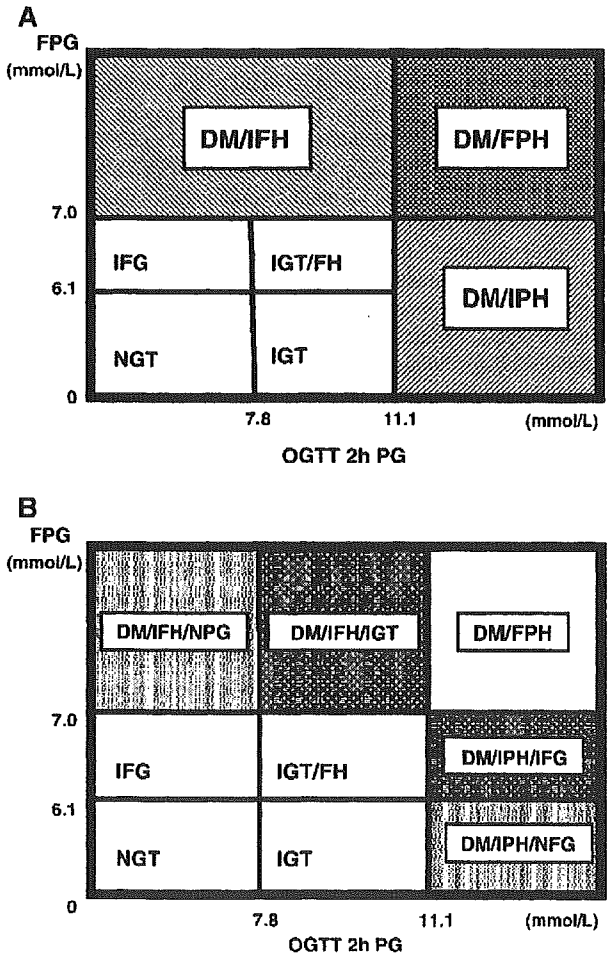

Fig. 1. A, Degrees of glucose intolerance: DM/IFH, DM/FPH, and DM/IPH. B, DM/IFH subdivided into 2 groups: DM/IFH/NPG and DM/IFH/IGT. DM/IPH subdivided into 2 groups: DM/IPH/NFG and DM/IPH/IFG.

Standard OGTT was administered according to the National Diabetes Data Group recommendations [14], which require the subjects to fast overnight for 10 to 16 hours. We obtained fasting, 0.5-, 1-, 1.5-, and 2-hour blood samples for measurement of PG, and fasting, 0.5-, 1-, and 2-hour samples for measurement of serum insulin after oral administration of 75-g glucose. Blood samples for measurements of HbA_{1c}, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride levels were collected after an overnight fast.

DM was defined by the 1998 World Health Organization diagnostic criteria [15]. Diabetic subjects were classified into 3 groups based on the results of OGTT: DM/IFH, FPG $\geq$7 mmol/L and 2-hour PG level <11.1 mmol/L (n = 66); DM/IPH, FPG <7 mmol/L and 2-hour PG level $\geq$11.1 mmol/L (n = 148); and DM/FPH, FPG $\geq$7 mmol/L and 2-hour PG level $\geq$11.1 mmol/L (n = 336) (Fig. 1A).

To evaluate the factors involved in fasting and postchallenge hyperglycemia, we subdivided DM/IFH and DM/IPH into DM/IFH with normal postchallenge glucose levels (DM/IFH/NPG), FPG $\geq$7 mmol/L and 2-hour PG level <7.8 mmol/L (n = 17); DM/IFH with IGT (DM/IFH/IGT), FPG $\geq$7 and 7.8 mmol/L $\leq$ 2-hour PG level <11.1 mmol/L (n = 49); DM/IPH with normal fasting glucose levels (DM/IPH/NFG), FPG <6.1 mmol/L and 2-hour PG level $\geq$11.1 mmol/L (n = 50); and DM/IPH with impaired fasting glucose (DM/IPH/IFG), 6. mmol/L $\leq$ FPG <7 mmol/L $\leq$ 2-hour PG level $\geq$11.1 mmol/L (n = 98). As shown in Fig. 1B, DM/IFH/NPG is characterized by increasingly impaired fasting glucose and 2-hour PG within normal limits, whereas DM/IPH/NFG is characterized by increasingly impaired 2-hour PG and fasting glucose within normal limits.

2.2. Measurements

PG level was measured by glucose oxidase method using Hitachi Automatic Clinical Analyzer 7170 (Hitachi, Tokyo, Japan). Serum insulin level was measured by 2-site radioimmunoassay (Insulin Riabead II, Dainabot, Tokyo, Japan), as reported previously [6]. Serum total cholesterol, HDL-C, and triglyceride levels were measured as reported previously [16].

Table 1
Clinical characteristics of DM/IFH, DM/IPH, and DM/FPH

	DM/IFH	DM/IPH	DM/FPH
n	66	148	336
Age (y)	52.3 ± 1.2*	55.7 ± 0.8**	52 ± 0.5
BMI (kg/m2)	24.4 ± 0.5	24.5 ± 0.2	24.8 ± 0.2
FPG (mmol/L)	7.5 ± 0.1*,**	6.4 ± 0.0	8.9 ± 0.1
2-h PG (mmol/L)	8.8 ± 0.2*,**	13.1 ± 0.1**	17.2 ± 0.2
Fasting insulin (pmol/L)	50 ± 4	46 ± 2	50 ± 1
HbA_{1c} (%)	6.2 ± 0.1**	6.1 ± 0.1**	7.5 ± 0.1
Triglycerides (mmol/L)	1.72 ± 0.16	2.35 ± 0.26	2.15 ± 0.15
Total cholesterol (mmol/L)	5.43 ± 0.13	5.51 ± 0.12	5.46 ± 0.06
HDL-C (mmol/L)	1.44 ± 0.07	1.36 ± 0.04	1.36 ± 0.02

Data are mean ± SE.
* $P < .05$ (vs DM/IPH).
** $P < .005$ (vs DM/FPH).

Insulinogenic index was used to measure the capacity of early-phase insulin secretion [11,12], and ISI composite was used to measure systemic insulin sensitivity [13], according to the following formulas:

Insulinogenic index

$$= [30\text{-minute serum insulin} - \text{fasting serum insulin (FI) (pmol/L)}] / [30\text{-minute plasma glucose} - \text{FPG (mmol/L)}] [11,12]$$

ISI composite

$$= 10000/[\text{FPG (mg/dL)} \times \text{FI}(\mu\text{U/mL})] \times [\text{mean OGTT PG (mg/dL)} \times \text{mean OGTT serum insulin } (\mu\text{U/mL})]^{0.5} [13]$$

2.3. Statistical analysis

All data are expressed mean ± SE. All statistical analyses were performed using STATVIEW 5 system (Abacus Concepts, Berkeley, CA). Age, body mass index (BMI), FPG, 2-hour PG, HbA_{1c}, triglycerides, total cholesterol, HDL-C, insulinogenic index, and ISI composite were compared among DM/IFH (DM/IFH/NPG, DM/IFH/IGT), DM/IPH (DM/IPH/NFG, DM/IPH/IFG), and DM/FPH groups by general analysis of variance. For comparison between 2 groups, unpaired Student t test was performed as post hoc analysis. $P < .05$ was considered statistically significant.

3. Results

3.1. Clinical characteristics

Table 1 shows the clinical and metabolic characteristics of the 550 Japanese men classified with DM/IFH, DM/IPH, and DM/FPH. The age and BMI (mean ± SE) were 53.0 ± 0.4 years and 24.7 ± 0.2, respectively. The mean age of the DM/IPH group was significantly higher than that of the other 2 groups ($P < .005$). There was no significant difference in BMI, triglycerides, total cholesterol, or HDL-C among the 3 groups. HbA_{1c} and the area under the curve of glucose (DM/IFH, 25056; DM/IPH, 26284; and DM/FPH, 33509) were significantly higher in the DM/FPH group than in the other groups ($P < .0001$, respectively).

3.2. Insulin secretion

The insulinogenic indices of the 3 groups are shown in Fig. 2A. The insulinogenic index in the DM/IFH group was significantly higher than in the other groups ($P < .0001$). There was a significant difference in the insulinogenic index between the DM/IPH and DM/FPH groups ($P < .0001$).

3.3. Insulin sensitivity

Fig. 2B shows the ISI composite of the 3 groups. ISI composite in the DM/IPH group was significantly higher than in the other groups ($P < .05$). There was no significant difference in ISI composite between DM/IFH and DM/FPH.

3.4. Comparison of DM/IFH/NPG and DM/IPH/NFG

Seventeen subjects were classified DM/IFH/NPG and 50 subjects were classified DM/IPH/NFG. Table 2 shows a comparison of the DM/IFH/NPG and DM/IPH/NFG groups. There was no significant difference in mean age, BMI, or HbA_{1c} between the 2 groups. As shown in Fig. 1B, DM/IFH/NPG was characterized by increasingly impaired

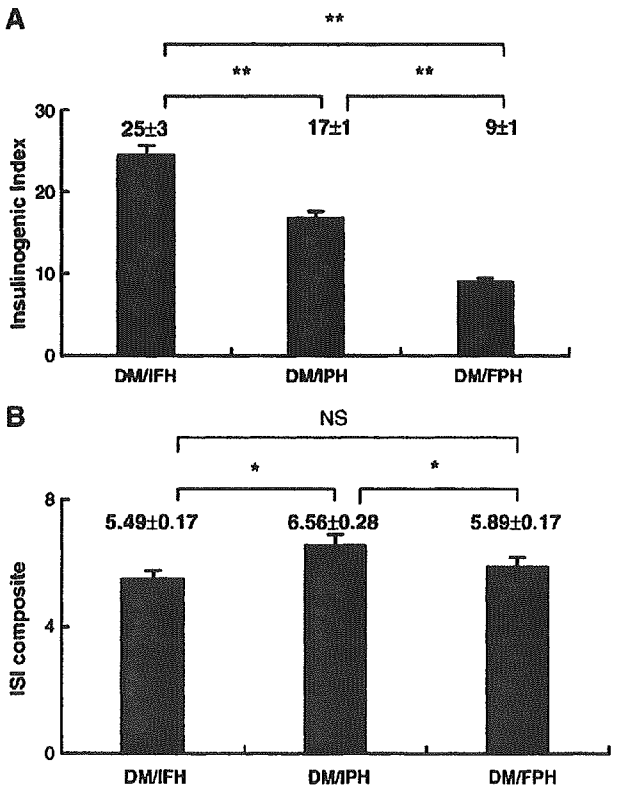

Fig. 2. Indexes of insulin secretion and sensitivity. A, Early-phase insulin secretion. Insulinogenic index in DM/IFH is highest. Insulinogenic index in DM/IPH is significantly higher than in DM/FPH. B, Insulin sensitivity. ISI composite in DM/IPH is significantly higher. *$P < .05$; **$P < .0001$; NS, not significant.

Table 2
Comparison of DM/IFH/NPG and DM/IPH/NFG

	DM/IFH/NPG	DM/IPH/NFG	P
n	17	50	
Age (y)	51.3 ± 2.7	54.8 ± 1.4	NS
BMI (kg/m^2)	23.6 ± 0.8	23.9 ± 0.3	NS
HbA_{1c} (%)	6.0 ± 0.2	5.9 ± 0.1	NS
Insulinogenic index	34 ± 8	17 ± 3	<.05
ISI composite	5.9 ± 0.82	8.33 ± 0.56	<.05

Data are mean ± SE. NS indicates not significant.

fasting glucose and 2-hour PG within normal limits, whereas DM/IPH/NFG was characterized by increasingly impaired 2-hour PG and fasting glucose within normal limits. The insulinogenic index in DM/IFH/NPG was significantly higher than in DM/IPH/NFG ($P < .05$). The ISI composite in DM/IPH/NFG was significantly higher than in DM/IFH/NPG ($P < .05$).

4. Discussion

In the present study, we evaluated the factors contributing to deterioration of glucose tolerance after the onset of type 2 diabetes in Japanese subjects. We previously reported that impaired early-phase insulin secretion plays an important role in the development from NGT via IGT to DM/IPH in Japanese subjects [6,17]. The present study reveals a reduction in the insulinogenic index, a measure of early-phase insulin secretion, in DM/FPH compared with DM/IPH (Fig. 2A). The ISI composite, an index of systemic insulin sensitivity, is also decreased in the deterioration from DM/IPH to DM/FPH (Fig. 2B). Although both impaired insulin secretion and insulin sensitivity are important factors in the deterioration from DM/IPH to DM/FPH, impaired early-phase insulin secretion is the more important factor in deterioration from DM/IFH to DM/FPH in Japanese subjects (Fig. 2A and B). We also classified the subjects into 3 groups based on American Diabetes Association classification: NGT, IFG, and DM. Thirty-two subjects were NGT, 117 were IFG, and 401 were DM. Of the 550 diabetic subjects judged only by the FPG level, 149 (27%) were NGT or IFG. Thus, FPG measurement as well as 2-hour PG measurement is important at the diagnosis of diabetes in Japanese subjects.

To clarify the factors involved in fasting and postchallenge hyperglycemia, we compared 2 subgroups of DM/IFH and DM/IPH: DM/IFH/NPG, DM/IFH/IGT, DM/IPH/NFG, and DM/IPH/IFG (Fig. 1B). DM/IFH/NPG is characterized by increasingly impaired fasting glucose and 2-hour PG within normal limits; DM/IPH/NFG is characterized by increasingly impaired 2-hour PG and fasting glucose within normal limits. In the present study, DM/IFH/NPG was associated with lower insulin sensitivity, and DM/IPH/NFG was associated with impaired early-phase insulin secretion as shown in Table 2. There was no significant difference in mean age, BMI, or HbA_{1c} between these 2 groups. Thus, although decreased insulin sensitivity plays the more important role in increasing FPG, reduced early-phase insulin secretion plays the more important role in increasing 2-hour PG in newly diagnosed Japanese type 2 diabetic subjects.

Glucotoxicity is induced by chronic hyperglycemia; a short time exposure to elevated glucose induces reversible glucose desensitization [18,19], whereas longer exposure causes irreversible beta-cell dysfunction, decreasing beta-cell mass by inducing apoptosis [20]. Immunohistochemical examination in autopsy cases of Japanese type 2 diabetes found that beta-cell mass was decreased because of oxidative stress [21]. Short-term glucotoxicity acts to reduce both glucose-induced insulin secretion and glucose uptake in skeletal muscle [19]. Both DM/IFH and DM/IPH showed normal PG levels in the postchallenge and the fasting state, respectively, suggesting that the subjects had been exposed to elevated glucose for a relatively short period. The simultaneously declining insulin secretion and the decreasing insulin sensitivity also implicate glucose desensitization in deterioration to DM/FPH in both groups. Deranged glucose metabolism induced by hyperglycemia per se was found in type 2 diabetic patients with a FPG level greater than 6.4 mmol/L in previous studies [22]. The mean fasting glucose level in DM/IPH of 6.4 mmol/L found in this study also suggests a role of glucotoxicity in the deteriorating glucose tolerance seen after onset of type 2 diabetes.

Type 2 diabetes is a disease of progressing glucose intolerance that frequently becomes more severe after onset. Previous large-scale studies comparing diet therapy to intensive therapy revealed that glucose tolerance continues to deteriorate even after treatment of diabetes has begun. For example, the United Kingdom Prospective Diabetes Study found that HbA_{1c} increased from 7.2% to 7.6% after 3 years and from 6.9% to 8.0% after 6 years among patients with type 2 diabetes on diet therapy [23,24]. In these studies, fasting glucose levels were increased from 8.3 to 9.0 mmol/L and 8.0 to 9.5 mmol/L. The Kissingen Diabetes Intervention Study found that both basal and reactive C-peptide levels continued to decrease 15 to 20 years after the diagnosis of type 2 diabetes and suggested a relationship between the decrease in C-peptide levels and the increase in HbA_1 levels [25]. Although there are few studies regarding deteriorating function after the development of type 2 diabetes, it is well known that decreasing beta-cell activity and increasing insulin resistance both play important roles in the increasing glucose intolerance [26]. Indeed, several studies have identified ethnic factors involved in the deteriorating glucose tolerance characteristic of the onset of type 2 diabetes. For example, increasing insulin resistance is the more important factor in Pima Indians, Mexican Americans, and Caucasians [9,10], whereas impaired insulin secretion is the more important factor in Japanese subjects, as reported previously [6,27,28].

We examined insulin sensitivity, glucose effectiveness, and endogenous glucose production using the stable-labeled minimal model approach in our previous study [29]. Despite the impairment in both glucose turnover rate and insulin secretion, the magnitude of the derangement in insulin secre-

tion is greater than in the glucose turnover rate in Japanese subjects. In addition, we have reported that the validity of the ISI composite and insulinogenic index is confirmed by insulin sensitivity index and insulin secretion capacity (acute insulin response) obtained from minimal model analysis, respectively [30]. Thus, similar conclusions were reached in different Japanese populations by different methods.

The reason for the increasing prevalence of type 2 diabetes in Japan is, at least in part, related to an increased prevalence of obesity due to lifestyle changes. However, obesity in Japan is less extreme, the average BMI of Japanese diabetic subjects having increased only slightly to 23 to 25 according to typical epidemiological study. It is also difficult to establish insulin resistance as the cause because the mean BMI of Japanese diabetic patients is less than 25 and the ISI composite is more than 5. In addition, glucose intolerance in Japanese subjects is well known to be dependent on poor reserve capacity of insulin secretion rather than insulin resistance [6,7,27,31]. Thus, Japanese subjects may develop glucose intolerance and diabetes because of only slight impairment of insulin sensitivity. Another factor may be that Japanese subjects are more readily susceptible to glucotoxicity and lipotoxicity due to slight impairments of carbohydrate and lipid metabolism [21,32,33].

In conclusion, although impaired early-phase insulin secretion plays a crucial role in the deterioration from DM/IFH to DM/FPH, impaired early-phase insulin secretion and decreased insulin sensitivity are both key factors in the deterioration from DM/IPH to DM/FPH. The simultaneous degradation of the various factors involved in the maintenance of PG after the development of type 2 diabetes suggests glucotoxicity. In addition, although decreased early-phase insulin secretion plays an important role in postchallenge hyperglycemia, decreased insulin sensitivity contributes to elevated FPG levels. The distinct pathophysiologies of type 2 diabetes could provide a basis for patient management. Treatment for DM/IFH might be targeted to impaired insulin secretion or to decreased insulin sensitivity, and treatment of DM/IPH might be targeted to impaired early-phase insulin secretion. These findings should be helpful clinically in stabilizing glucose levels in Japanese type 2 diabetic patients at onset of type 2 diabetes.

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Interleukin 6, adiponectin, leptin, and insulin resistance in nonobese Japanese type 2 diabetic patients

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Abstract

The aim of the present study was to investigate the relationships between interleukin 6 (IL-6) and insulin resistance, serum leptin, serum adiponectin, or serum lipids including triglycerides in 98 nonobese Japanese type 2 diabetic patients. Insulin resistance was estimated by the insulin resistance index of homeostasis model assessment (HOMA-IR). Serum IL-6 concentration was negatively correlated to high-density lipoprotein cholesterol ($r = -0.295$, $P = .004$), but was not associated with HOMA-IR ($r = 0.016$, $P = .871$), body mass index (BMI) ($r = 0.090$, $P = .375$), systolic ($r = 0.169$, $P = .116$) and diastolic ($r = -0.061$, $P = .570$) blood pressures, leptin ($r = 0.062$, $P = .544$), and adiponectin ($r = -0.020$, $P = .841$) in these patients. In contrast, serum leptin level was positively correlated to HOMA-IR ($r = 0.291$, $P = .004$), BMI ($r = 0.338$, $P < .001$), and systolic blood pressure ($r = 0.241$, $P = .025$). Serum adiponectin level was negatively correlated to HOMA-IR ($r = -0.288$, $P = .005$), BMI ($r = -0.308$, $P = .002$), diastolic blood pressure ($r = -0.269$, $P = .012$), and triglycerides ($r = -0.338$, $P < .001$), and positively correlated to high-density lipoprotein cholesterol ($r = 0.300$, $P = .003$) in our patients. From these results, it can be suggested that fasting serum IL-6 is not a major factor responsible for the evolution of insulin resistance in nonobese Japanese type 2 diabetic patients.

1. Introduction

Type 2 diabetes mellitus is a heterogeneous syndrome characterized by insulin resistance and/or defective insulin secretion [1]. The mechanisms underlying insulin resistance are not yet fully clarified. We previously demonstrated that body mass index (BMI) and serum triglycerides are the most important factors responsible for the evolution of insulin resistance in Japanese type 2 diabetic patients [2,3]. Thereafter, we showed that both leptin and adiponectin are correlated to insulin resistance in nonobese Japanese type 2 diabetic patients [4,5]. Serum triglyceride level is positively correlated with visceral fat area [6]. Serum leptin level is positively correlated to subcutaneous fat areas, whereas serum adiponectin level is negatively correlated to visceral fat areas [4,5]. Thus, the factors associated with insulin resistance in nonobese Japanese type 2 diabetic patients are hypothesized to be linked to adipose tissue–related insulin resistance.

Interleukin 6 (IL-6) is one of the candidates responsible for adipose tissue–related insulin resistance in man. Mohamed-Ali et al [7] are the first to show that a considerable portion of circulating IL-6 is derived from adipose tissue. Circulating levels of IL-6 have been reported to be high in obese people and in patients with type 2 diabetes mellitus [8-10]. Bastard et al [9] have shown that

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not only leptin but also IL-6 is associated with BMI and insulin resistance, and that IL-6 and leptin are interrelated in white obese type 2 diabetic patients. Haffner et al [10] demonstrated that serum levels of IL-6 were associated with BMI and insulin resistance in obese type 2 diabetic patients. However, obesity and insulin resistance are related to each other, and it remains to be elucidated whether the relationship between IL-6 and insulin resistance is independent of obesity in type 2 diabetic patients.

Nonobese Japanese type 2 diabetic patients are unique in that they are divided into 2 variants: one with insulin resistance and the other with normal insulin sensitivity [2,3,11,12]. Thus, the aim of the present study was to examine the relationship between fasting serum IL-6 level and insulin resistance in nonobese Japanese type 2 diabetic patients without confounding the effect of obesity.

2. Subjects and methods

Ninety-eight nonobese Japanese type 2 diabetic patients who visited Kansai-Denryoku Hospital were enrolled for the present study. Type 2 diabetes mellitus was diagnosed based on the World Health Organization criteria [13]. They had no evidence of current acute illness including clinically significant infectious diseases. The duration of diabetes was 11.1 ± 0.8 years (range, 1-35 years). Of 98 diabetic patients, 84 were taking sulfonylureas, and the rest were treated with diet alone. No patients had received insulin therapy. All subjects had ingested at least 150 g of carbohydrate for the 3 days preceding the study. None of the subjects had significant renal, hepatic, or cardiovascular disease. Patients did not consume alcohol or perform heavy exercises for at least 1 week before the study.

Blood was drawn in the morning after a 12-hour fast. Plasma glucose was measured with the glucose oxidase method. Triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL-C) were also measured. Serum insulin was measured using a 2-site immunoradiometric assay (Insulin Riabead II, Dainabot, Japan). Coefficients of variation were 4% for insulin higher than 25 μU/mL and 7% for insulin less than 25 μU/mL, respectively. Serum IL-6 was measured by enzyme-linked immunosorbent assay (Quantikine IL-6, R&D Systems, Oxford, UK). Serum leptin and adiponectin concentrations were measured with a radioimmunoassay kit (Linco Research, St Charles, MO) [4,5]. The intra- and interassay coefficients of variation were less than 5% for leptin and adiponectin. Samples for insulin, IL-6, leptin, and adiponectin were prepared, frozen, and stored at −70°C until the assay.

The estimate of insulin resistance by HOMA (HOMA-IR) was calculated with the formula: fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5 [14]. The HOMA-IR value (mean ± SD) of healthy tolerant subjects was 1.6 ± 0.9, and we defined the values higher than 2.5 as an insulin-resistant state and the values less than 2.5 as an insulin-sensitive state [2,3]. The threshold value for insulin resistance (2.5) in our study is similar to that (2.77) in nonobese subjects with no metabolic disorders, reported by Bonora et al [15]. It may be argued that the use of sulfonylureas in patients with diabetes might significantly affect the estimate of insulin resistance by HOMA, as these drugs are known to decrease fasting plasma glucose without substantially changing fasting plasma insulin [16]. It seems, however, unlikely because Bonora et al [17] and Emoto et al [18] showed that in the validation studies of HOMA, the correlation of insulin sensitivity estimated by such method and that measured by a glucose clamp was not substantially different in diet-treated and sulfonylurea-treated type 2 diabetes mellitus. Furthermore, no significant difference was observed in some variables including BMI, leptin, adiponectin, and IL-6 between diet-treated and sulfonylurea-treated diabetic patients (data not shown). Therefore, we estimated HOMA-IR in diet-treated and sulfonylurea-treated diabetic patients.

2.1. Data analysis

Data were presented as means ± SEM. Statistical analysis was conducted using the StatView 5 system (Statview, Berkeley, CA). The means of 2 groups were compared with Student *t* test. Spearman rank correlation coefficient analysis was performed to calculate a correlation. $P < .05$ was considered significant.

3. Results

The subjects studied were all Japanese type 2 diabetic patients (75 men and 23 women) with an age range of 41 to 84 years (62.7 ± 0.9 years) and a BMI of 17.9 to 26.7 kg/m^2

Table 1
Clinical characteristics in insulin-resistant and insulin-sensitive diabetic patients

	Insulin-resistant	Insulin-sensitive	*P*
No. of subjects	36	62	
Age (y)	61.9 ± 1.7	63.2 ± 1.0	.249
Male/female	29/7	46/16	.239
HOMA-IR	3.72 ± 0.24	1.65 ± 0.06	<.001
Diabetes duration (y)	10.8 ± 1.5	11.3 ± 0.9	.379
Smoking (none/previous/current)	12/15/9	23/19/20	.245
Sulfonylureas/diet	30/6	54/8	.306
BMI (kg/m^2)	23.8 ± 0.3	22.6 ± 0.3	.003
Systolic blood pressure (mm Hg)	140 ± 3	135 ± 3	.129
Diastolic blood pressure (mm Hg)	87 ± 2	80 ± 1	.004
Fasting glucose (mg/dL)	153 ± 3	138 ± 3	.003
Fasting insulin (μU/mL)	10.0 ± 0.7	4.9 ± 0.2	<.001
HbA_{1c} (%)	7.3 ± 0.2	6.9 ± 0.1	.014
Triglycerides (mg/dL)	168 ± 16	108 ± 5	<.001
Total cholesterol (mg/dL)	213 ± 6	197 ± 4	.019
HDL-C (mg/dL)	54 ± 2	59 ± 2	.034
Leptin (ng/mL)	6.6 ± 0.8	4.8 ± 0.4	.015
Adiponectin (μg/mL)	10.9 ± 1.0	13.2 ± 0.7	.027
IL-6 (pg/mL)	1.8 ± 0.2	2.1 ± 0.3	.217

Table 2
Correlation of IL-6, leptin, and adiponectin to measures of variables in diabetic patients

	IL-6		Leptin		Adiponectin	
	r	*P*	*r*	*P*	*r*	*P*
HOMA-IR	0.016	.871	0.291	.004	−0.288	.005
BMI	0.090	.375	0.338	<.001	−0.308	.002
Systolic blood pressure	0.169	.116	0.241	.025	−0.077	.472
Diastolic blood pressure	−0.061	.570	0.116	.281	−0.269	.012
HbA_{1c}	0.018	.861	−0.062	.543	−0.053	.605
Triglycerides	0.028	.785	0.093	.362	−0.338	<.001
Total cholesterol	−0.153	.133	0.184	.071	0.010	.922
HDL-C	−0.295	.004	0.106	.300	0.300	.003
Leptin	0.062	.544			0.002	.841
Adiponectin	−0.020	.841	0.002	.841		

(23.1 ± 0.2 kg/m^2). They were all nonobese [19]. The fasting plasma glucose was 141 ± 2 mg/dL, and glycosylated hemoglobin (HbA_{1c}) was 7.0% ± 0.1%. Fasting insulin level was 6.7 ± 0.4 μU/mL. Serum triglycerides, total cholesterol, and HDL-C levels were 130 ± 7, 203 ± 4, and 57 ± 1 mg/dL, respectively. Serum IL-6, leptin, and adiponectin concentrations were 1.99 ± 0.21 pg/mL (range, 0.5-19.3 pg/mL), 5.5 ± 0.4 ng/mL (range, 1.4-22.8 ng/mL), and 13.2 ± 0.7 μg/mL (range, 1.5-24.9 μg/mL), respectively. There was a wide variation in insulin resistance calculated from HOMA-IR in our diabetic patients (range, 0.51-8.55, 2.41 ± 0.14). Of 98 patients, 36 (37%) had HOMA-IR greater than 2.5, indicating that they are insulin resistant [2,3].

Table 1 shows the clinical profile between insulin-resistant and insulin-sensitive type 2 diabetic patients. Compared with insulin-sensitive type 2 diabetic patients, insulin-resistant patients had significantly higher levels of BMI, diastolic blood pressure, fasting glucose, fasting insulin, HbA_{1c}, triglycerides, total cholesterol, and leptin, and lower concentrations of HDL-C and adiponectin. No significant difference was observed in age, sex, diabetes duration, smoking status, status of medication for diabetes, systolic blood pressure, and IL-6 levels between the 2 groups.

The correlation between fasting IL-6 concentration and the factors associated with insulin resistance (HOMA-IR, BMI, systolic blood pressure, diastolic blood pressure, HbA_{1c}, triglycerides, total cholesterol, HDL-C, leptin, adiponectin) was investigated next (Table 2). The peripheral level of IL-6 was not associated with these variables in our patients. In contrast, serum leptin was positively correlated to HOMA-IR, BMI, and systolic blood pressure. Serum adiponectin was negatively correlated to HOMA-IR, BMI, diastolic blood pressure, and triglycerides, and positively correlated to HDL-C in our patients.

4. Discussion

Type 2 diabetes mellitus is a syndrome characterized by insulin resistance and/or defective insulin secretion [1]. There seems to be an ethnic difference in insulin resistance in type 2 diabetes mellitus. Haffner et al [20] surveyed the prevalence of type 2 diabetes mellitus in white patients and found that 92% of type 2 diabetic patients were insulin resistant. Chaiken et al [21] reported that 60% of type 2 diabetic patients with BMI of less than 30 kg/m^2 were insulin resistant in African American populations. We recently demonstrated that 40% of type 2 diabetic patients are insulin resistant in nonobese Japanese type 2 diabetic patients, indicating that they are divided into 2 variants: one with insulin resistance and the other with normal insulin sensitivity [2,3]. This finding was reconfirmed in the present study.

Japanese type 2 diabetic patients are unique in that they do not always manifest obesity. We previously showed that the mean BMI in representative epidemiological studies of Japanese type 2 diabetic patients is 23 to 25 kg/m^2, which is lower than that in studies of other ethnic populations such as whites [22]. Thus, this fascinating feature of Japanese type 2 diabetic patients, that they are not always obese, but are composed of individuals who are both insulin sensitive and insulin resistant, enables us to estimate the factors responsible for insulin resistance in type 2 diabetic patients without confounding the effects of obesity.

There are some factors associated with insulin resistance in nonobese Japanese type 2 diabetic patients [2-5]. Whereas BMI and triglycerides are considered to be the most important factors responsible for the evolution of insulin resistance, regional abdominal adipose tissue distribution per se contributes to insulin resistance in nonobese Japanese type 2 diabetic patients [6]. In distinct from white populations [23] and African American populations [24], subcutaneous and visceral fat areas are independently associated with insulin resistance in nonobese Japanese type 2 diabetic patients [6]. Not only serum triglyceride but also serum leptin and adiponectin levels are shown to be associated with insulin resistance in our populations [2-5]. Serum triglyceride level is positively correlated to visceral fat area [6]. Serum leptin level is positively correlated to subcutaneous fat areas, whereas serum adiponectin level is negatively correlated to visceral fat areas [4,5]. Thus, the factors associated with insulin resistance in nonobese Japanese type 2 diabetic patients are hypothesized to be linked to adipose tissue–related insulin resistance.

Interleukin-6 is another factor that is associated with adipose tissue–related insulin resistance in man. Human adipose tissue is shown to secrete IL-6, and this secretion is correlated with BMI in white healthy subjects [7]. In the present study, we first demonstrated that fasting serum IL-6 level is not responsible for insulin resistance, at least not in nonobese Japanese type 2 diabetic patients. This is a surprising finding because it is a commonly held belief that IL-6 has a key role in the assessment of insulin resistance in obese and type 2 diabetic patients [9,10]. Bastard et al [9] showed that not only leptin but also IL-6 is associated with BMI and insulin resistance, and that IL-6 and leptin are interrelated in white obese type 2 diabetic patients. Haffner

et al [10] demonstrated that the serum levels of IL-6 are associated with BMI and insulin resistance in obese type 2 diabetic patients.

Thus, the reason why serum IL-6 is not associated with BMI and insulin resistance in nonobese well-controlled unique Japanese type 2 diabetic patients is currently unknown. The possible explanation is the different clinical characteristics studied. The Japanese type 2 diabetic patients in our study were unique in that they were nonobese and were well controlled in terms of BMI, fasting glucose, and HbA_{1c}. Mean levels of BMI, fasting glucose, and HbA_{1c} were 23.1 kg/m^2, 141 mg/dL, and 7.0%, respectively. In contrast, the linkage of IL-6 to insulin resistance is confirmed in healthy obese subjects (mean BMI, 31.8 kg/m^2) and obese type 2 diabetic patients (mean BMI, 36.6 kg/m^2; mean fasting glucose, 11.1 mmol/L; mean BMI, 30.1 kg/m^2; mean fasting glucose, 213.4 mg/dL; mean HbA_{1c}, 8.7%) [7,9,10]. Thus, the degree of overweight or of hyperglycemia per se might lead to the close relationship between serum IL-6 and insulin resistance in man. In this respect, the recent study by Kern et al [25], which showed that although plasma IL-6 concentration is associated with insulin resistance, its release is greater in obese subjects especially when BMI exceeds 30 kg/m^2, is very interesting. Body mass index in our patients ranged from 16.0 to 26.8 kg/m^2, that is, nonobese [14]. Thus, it may be hypothesized that insulin resistance evoked by IL-6 is the result of elevated adiposity rather than IL-6 exerting a negative effect on insulin action. This hypothesis is supported by the study of Vozarova et al [26], which showed that although IL-6 was positively correlated to insulin resistance and adiposity, the relationship between IL-6 and insulin resistance disappeared after adjustment for adiposity in healthy nondiabetic populations. Using the euglycemic clamp technique, Carey et al [27] very recently demonstrated that plasma IL-6 concentrations are strongly related to fat mass, but are not indicative of insulin resistance in humans. Haffner et al [28] showed that proliferator-activated receptor γ agonist (rosiglitazone) has a beneficial effect on insulin resistance, but has no effect on serum concentrations of IL-6 in patients with type 2 diabetes mellitus. Steensberg et al [29] have shown that recombinant human IL-6 infusion into healthy humans does not result in impaired glucose disposal.

It may be argued that pancreatic β-cell function per se might affect HOMA-IR in Japanese type 2 diabetic patients because these patients have mild impairments in pancreatic β-cell function [30]. For that reason, we used serum levels of leptin and adiponectin as another index of insulin resistance and found that serum IL-6 level was not associated with serum levels of leptin and adiponectin in the present study.

One might argue that exercise per se affects the level of serum IL-6 in this study because Lyngs et al [31] recently demonstrated that IL-6 secretion by adipose tissue was totally suppressed during exercise. It seems unlikely, however, because our patients did not perform heavy exercises for at least 1 week before the study. Alternatively, the racial difference or the different genetic variation within the IL-6 gene might explain the discrepant results. Polymorphisms of the IL-6 gene are shown to influence insulin sensitivity in man [32].

Irrespective of this, adipose tissue may not play a major role in the determination of circulating IL-6 in our nonobese well-controlled unique Japanese type 2 diabetic patients. It is well known that IL-6 production by adipose tissue could explain only 10% to 30% of the whole circulating IL-6 concentration in humans [7]. Alternatively, adipose tissue–secreted IL-6 might function locally at the level of the adipocyte in a paracrine or autocrine fashion in the diabetic patients in our study. In this respect, our very recent study, which shows that tumor necrosis factor α system activity is not responsible for the evolution of insulin resistance in nonobese Japanese type 2 diabetic patients, is intriguing [33].

In summary, we demonstrated for the first time that although the number of patients with type 2 diabetes mellitus is limited, the peripheral level of IL-6 does not appear to be a major explanation of the mechanisms underlying insulin resistance, at least in nonobese well-controlled Japanese type 2 diabetic patients. Further studies should be undertaken to clarify whether other nonobese diabetic population would exhibit similar results.

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Insulin secretion and insulin sensitivity in Japanese subjects with impaired fasting glucose and isolated fasting hyperglycemia

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Abstract

Impaired fasting glucose (IFG) is a subgroup of impaired glucose regulation exhibiting an elevated fasting glucose levels without elevated 2-h glucose levels on oral glucose tolerance test (OGTT). Diabetes mellitus with isolated fasting hyperglycemia (DM/IFH) is a similar subgroup of diabetes having higher fasting glucose levels with 2-h glucose levels within the non-diabetic range. The aim of this study is to profile the characteristics of these subgroups to estimate the factors involved in the development from normal glucose tolerance (NGT) via IFG to DM/IFH. Five hundred and sixty seven Japanese males were classified on the basis of 75g OGTT into four groups, NGT, IFG, DM/IFH, and isolated impaired glucose tolerance (isolated IGT). Insulin secretion was evaluated by insulinogenic index, insulin sensitivity was evaluated by ISI composite, and insulin secretory patterns were compared additionally. IFG and DM/IFH subjects exhibited both lower insulin secretion and lower insulin sensitivity than NGT subjects. There was an insulin peak in NGT, IFG, and DM/IFH at 60 min, which did not occur in isolated IGT. Impaired early-phase and basal insulin secretion and decreased insulin sensitivity both are estimated as factors in progression from NGT via IFG to DM/IFH in these subjects. IFG and DM/IFH subjects have definite fasting hyperglycemia in contrast to isolated IGT subjects, 2-h glucose levels being maintained within the non-diabetic range partly by the insulin peak at 60 min.

Keywords: Impaired fasting glucose (IFG); Diabetes mellitus with isolated fasting hyperglycemia (DM/IFH); Isolated impaired glucose tolerance (isolated IGT); Insulinogenic index; ISI composite

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1. Introduction

The prediabetic state of development from normal glucose tolerance (NGT) to type 2 diabetes was defined as impaired glucose regulation by the criteria of the World Health Organization in 1998 [1]. The category includes impaired fasting glucose [IFG: fasting plasma glucose (FPG) level of 6.1–7.0 mmol/l and 2-h plasma glucose (PG) level <7.8 mmol/l, sometimes called isolated IFG], and impaired glucose tolerance (IGT: FPG level <7.0 mmol/l and 2-h PG level of 7.8–11.1 mmol/l). In the present study, we have focused on IFG because it is a critical prediabetic state with elevated fasting glucose levels and 2-h PG levels within the normal range and there are few analyses of insulin secretion and insulin sensitivity in IFG, especially in the Japanese population. We also evaluated a subgroup of diabetes with more strongly elevated fasting glucose levels and 2-h glucose levels within the non-diabetic range: diabetes mellitus with isolated fasting hyperglycemia (DM/IFH: FPG level ≥7.0 mmol/l and 2-h PG level <11.1 mmol/l).

Both IFG and IGT have clinical importance as risk categories for type 2 diabetes [2]. While IGT represents risk for cardiovascular diseases [3], it remains unclear if IFG is a risk category for atherosclerosis [2,4]. Thus, it is thought that IFG has characteristics and pathophysiology that differ from IGT. Indeed, fasting glucose levels and post-challenge glucose levels are regulated in part by different mechanisms [5]. Characterization of the insulin secretion and insulin sensitivity profiles of these IFG and DM/IFH subjects should clarify both the factors involved in progression from NGT via IFG to DM/IFH, as well as the mechanisms of fasting and postchallenge glucose regulation.

Glucose intolerance is caused by both impaired insulin secretion and decreased insulin sensitivity. The relevance of these factors in Japanese diabetic patients is known to differ from that in other ethnic populations [6–13]. In the present study, we analyzed the results of 75g oral glucose tolerance test (OGTT) in Japanese male subjects and calculated the insulinogenic index [14,15] as parameters of insulin secretion, and the ISI composite [16] as parameters of insulin sensitivity. We evaluated insulin secretion and insulin sensitivity in IFG and DM/IFH subjects to speculate the factors responsible in progression from NGT to type 2 diabetes in this population. In addition, we evaluated the insulin secretory pattern during OGTT to determine the cause of the absence of elevation of 2-h postchallenge glucose levels despite of the increasingly elevated fasting glucose levels in IFG and DM/IFH subjects.

2. Subjects and methods

2.1. Subjects

We recruited subjects undergoing 75g OGTT for closer evaluation because of positive urine glucose tests, >5.6 mmol/l FPG level, >5.0% HbA_{1c} level, or family history of diabetes at initial examination for regular medical check-up at Kyoto University Hospital, Ikeda Hospital, Kansai-Denryoku Hospital, Kansai Health Management Center and Kyoto Preventive Medical Center between 1994 and 2003. 75g OGTT was performed within 3 months of the initial examination and the metabolic characteristics of the subjects with NGT ($n = 319$: FPG level <6.1 mmol/l and 2-h PG level <7.8 mmol/l), IFG ($n = 76$), and DM/IFH ($n = 42$) were compared. In addition, subjects with isolated IGT ($n = 130$: FPG level <6.1 mmol/l and 2-h PG level of 7.8–11.1 mmol/l) were evaluated to compare insulin secretory patterns in IFG and DM/IFH. All subjects were Japanese males with no signs of hypertension, hepatic, renal, endocrine or malignant diseases. No subjects had gastrectomy or engaged in heavy exercise or taken medications known to affect the glucose metabolism before the study. The study was designed in compliance with the ethics regulations set out by the Helsinki Declaration.

Standard oral glucose tolerance test was administered according to the National Diabetes Data Group recommendations [17], which require subjects to fast overnight for 10–16 h. Blood samples for determination of blood glucose were drawn at 0, 30, 60, 90, and 120 min, and those for blood insulin was drawn at 0, 30, 60, and 120 min after oral administration of 75g glucose. Blood samples for measurements of HbA_{1c}, total cholesterol, HDL-cholesterol and triglycerides levels were drawn after an overnight fast.