Decreased Glucose Effectiveness But Not Insulin Resistance in Glucose-Tolerant Offspring of Japanese Non–Insulin-Dependent Diabetic Patients: A Minimal-Model Analysis

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The aim of the study was to estimate insulin sensitivity (SI), insulin secretion, and glucose effectiveness (SG) in 10 subjects with normal glucose tolerance (eight men and two women) with a family history of non–insulin-dependent diabetes mellitus (NIDDM offspring). Ten glucose-tolerant subjects (eight men and two women) without a family history of NIDDM served as control subjects. All subjects were Japanese. They underwent a modified frequently sampled intravenous glucose tolerance test (FSIGT): glucose (300 mg/kg body weight) was administered, and insulin (20 mU/kg over 5 minutes) was infused from 20 to 25 minutes after glucose. SI and SG were estimated by Bergman's minimal-model method. No significant difference was observed in body mass index (22.6 ± 1.5 *v* 21.5 ± 0.6 kg/m^2) and fasting glucose (5.1 ± 0.1 *v* 5.2 ± 0.1 mol/L) and insulin (40.7 ± 6.3 *v* 42.6 ± 6.7 pmol/L). SI was not different between the two groups (0.83 ± 0.11 *v* 0.94 ± 0.15 × 10^{-1} · min^{-1} · pmol/L^{-1}, $P > .05$). The acute insulin response to glucose ($AIR_{glucose}$) estimated by intravenous glucose tolerance testing was significantly lower in the offspring than in the normal controls (2,139 ± 265 *v* 3,438 ± 318 pmol/L · min, $P < .05$). The glucose disappearance rate (KG) and SG were significantly diminished in the offspring versus normal controls (KG, 1.50 ± 0.22 *v* 2.10 ± 0.15 min^{-1}, $P < .05$; SG, 0.016 ± 0.003 *v* 0.023 ± 0.002 min^{-1}, $P < .05$). Thus, glucose-tolerant Japanese NIDDM offspring with normal insulin sensitivity are characterized by a reduced $AIR_{glucose}$ and diminished SG. This is the first report that glucose resistance but not insulin resistance already exists in glucose-tolerant Japanese NIDDM offspring.

MANY PREVIOUS STUDIES have demonstrated that glucose-tolerant relatives of patients with non–insulin-dependent diabetes mellitus (NIDDM) manifest severe insulin resistance.[1-4] This finding favors the idea that one of the genetic factors responsible for the evolution of NIDDM is insulin resistance. However, not all NIDDM patients are insulin-resistant. Banerji and Lebovitz[5] and our group[6] previously reported NIDDM patients with normal insulin sensitivity $C_{(SI)}$ in African-American and Japanese subjects, respectively. Thus, there is the possibility that factors other than insulin resistance contribute to the evolution of NIDDM, especially when SI is normal. This idea is supported by our recent study[7] that SI in Japanese subjects with impaired glucose tolerance (IGT) is heterogenous in nature, not in agreement with reports by Reaven et al[8] and DeFronzo[9] that IGT subjects are insulin-resistant.

One way to find the genetic markers responsible for the evolution of NIDDM is to study glucose-tolerant relatives of NIDDM patients. It is well established that the degree of overweight, hyperglycemia, or hyperinsulinemia per se impairs SI, thus making the pathogenesis complex.[10] We therefore recruited normoglycemic relatives of NIDDM patients carefully matched for body mass index and fasting glucose and insulin to normoglycemic subjects without any family history of NIDDM. In this experiment, we used the minimal-model approach used by Bergman,[11] since this method enables us to estimate not only SI but also insulin secretion and glucose effectiveness (SG), and since this approach has not been applied to Japanese NIDDM offspring.

SUBJECTS AND METHODS

Ten NIDDM offspring (eight men and two women) and 10 sex- and age-matched normals were studied (Table 1). All subjects were diagnosed to have normal glucose tolerance following World Health Organization criteria.[12] Fasting and 2-hour glucose levels in offspring and normal controls were 4.9 ± 0.1 and 5.1 ± 0.1 mmol/L and 6.3 ± 0.4 and 6.1 ± 0.4 mmol/L, respectively. A positive family history of NIDDM was observed in all offspring studied. Four subjects had two parents with NIDDM, and the other had one parent with NIDDM. Normal subjects had no first-degree relatives with NIDDM. All subjects were normotensive and had normal renal, hepatic, and thyroid function and no family history of hypertension. They did not take any medication known to alter carbohydrate metabolism. None of the participants in this study consumed alcohol or performed heavy exercise for at least 1 week before the test. Their body weight and daily diet were stable for at least 3 days before the test. Women were studied in the follicular phase of the menstrual cycle. Before participation, the nature, purpose, and risks of the study were explained to all subjects and informed written consent was obtained.

After an overnight fast, butterfly needles were inserted into the antecubital vein and maintained by a slow drip of physiological saline. Subjects were allowed to rest quietly for at least 15 minutes before blood sampling was begun. Baseline samples for glucose and insulin were obtained at −20, −10, and −3 minutes. Glucose (300 mg/kg body weight) was administered intravenously within 2 minutes, and subsequent samples were obtained from the contralateral antecubital vein at frequent intervals for 180 minutes as previously described.[6,7] Plasma was frozen and stored at −20°C for subsequent analysis. Insulin (20 mU/kg over 5 minutes) was infused into an antecubital vein from 20 to 25 minutes after administration of glucose.[6,7] The plasma glucose level was measured in duplicate with an automatic analyzer (Kyoto-Daiichi-Kagaka, Japan) by the glucose oxidase method. The measurement error for glucose was assumed to show a normal distribution of zero mean,

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Submitted August 10, 1996; accepted February 19, 1997.

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0026-0495/97/4608-0005$03.00/0

Table 1. Physical Characteristics and Metabolic Parameters of NIDDM Offspring and Normal Controls

Variable	NIDDM Offspring (n = 10)	Normal Controls (n = 10)	P
Physical characteristics			
Age (yr)	25.1 ± 1.7	22.1 ± 0.4	NS
BMI (kg/m²)	22.6 ± 1.5	21.5 ± 0.6	NS
Metabolic parameters			
Fasting glucose (mmol/L)	5.1 ± 0.1	5.2 ± 0.1	NS
Fasting insulin (pmol/L)	40.7 ± 6.3	42.6 ± 6.7	NS
KG (min^{-1})	1.50 ± 0.22	2.10 ± 0.15	<.05
SI ($\times 10^{-4} \cdot min^{-1} \cdot$ pmol/L)	0.83 ± 0.11	0.94 ± 0.15	NS
$AIR_{glucose}$ (pmol/L · min)	2,139 ± 265	3,438 ± 318	<.05
SG (min^{-1})	0.016 ± 0.003	0.023 ± 0.002	<.05

Abbreviation: BMI, body mass index.

with a coefficient of variation of 1.5%. Immunoreactive insulin was assayed in duplicate using the Phadeseph insulin radioimmunoassay kit (Shionogi, Osaka, Japan). Coefficients of variation were 4% for insulin levels higher than 180 pmol/L and 7% for insulin levels less than 180 pmol/L, respectively. The glucose disappearance rate (KG) was calculated as the slope of the least-square regression line relating the natural logarithm of the glucose concentration to time from more than four samples drawn between 10 and 19 minutes.

SI and SG were estimated by the minimal-model approach.[6,7,11,13] In this analysis, fluctuations in circulating glucose levels over time are described by the differential equations, $dG(t)/dt = -p1[G(t) - Gb] - X(t)G(t)$ and $dX(t)/dt = -p2X(t) + p3[I(t) - Ib]$, where G(t) is the plasma glucose, I(t) is plasma insulin, and Gb and Ib are baseline concentrations. X(t) represents the time course of the peripheral insulin effect. Parameter p1 represents the effect of glucose per se at basal insulin to normalize its own concentration in plasma independently of increased insulin. This parameter is known as glucose effectiveness (SG) and has been verified through comparison to studies in which the insulin secretory response was suppressed.[14] The ratio between p3 and p2 defines SI, the insulin sensitivity index, which represents the insulin-dependent increase in the net glucose disappearance rate. Index SI has been validated by comparison to a direct measure of insulin sensitivity from glucose clamp experiments in humans.[15,16]

The minimal-model program was written in Pascal (Borland International, Scotts Valley, CA) on a Macintosh IIcx (Apple Computer, Cupertino, CA) as described previously.[6,7] Precision of parameter estimates was evaluated using a covariance matrix as previously described[6,7] and expressed as the fractional standard deviation. The mean ± SEM values of the fractional standard deviations of p1, p2, and p3 in 20 subjects were comparable to published results.[6,7]

The acute insulin response to glucose ($AIR_{glucose}$) was expressed as the area under the insulin curve between 0 and 10 minutes after intravenous glucose. The integrated area of plasma insulin above basal was calculated using the trapezoid method.[17]

The data are expressed as the mean ± SEM. To evaluate differences between NIDDM offspring and control subjects, data were analyzed by Mann-Whitney *U* test, with *P* less than .05 as significant.[18]

RESULTS

NIDDM offspring had a similar age and body mass index compared with the normal controls (Table 1).

Plasma glucose and insulin concentrations during the modified frequently sampled intravenous glucose tolerance test (FSIGT) are charted in Fig 1. Basal glucose and insulin concentrations were similar between the two groups. The peak glucose level achieved during FSIGT in the offspring and normals was 53.6 ± 6.5 and 54.5 ± 6.8 mmol/L, respectively, but was not statistically significant. From 10 to 19 minutes KG was significantly lower in offspring than in normal controls (1.50 ± 0.22 *v* 2.10 ± 0.15 min^{-1}, $P < .05$; Table 1). Plasma insulin in both groups increased immediately after glucose administration. During the first 20 minutes the peak insulin level was significantly lower in offspring than in normal controls (276.9 ± 69.4 *v* 615.3 ± 113.7 pmol/L, $P < .05$). $AIR_{glucose}$ expressed as the integrated area of plasma insulin above basal during the FSIGT was significantly lower in offspring than in normal controls (2,139 ± 265 *v* 3,438 ± 318 pmol/L · min. $P < .05$, Table 1). From 20 to 180 minutes plasma insulin was similar between the two groups. SI was similar between the two groups, but SG was significantly lower in NIDDM offspring than in normal controls (0.016 ± 0.003 *v* 0.023 ± 0.002 min^{-1}, $P < .05$; Table 1).

DISCUSSION

Glucose tolerance was determined from World Health Organization criteria.[12] KG, an estimate of glucose tolerance during the intravenous glucose tolerance test, was significantly lower in NIDDM offspring than in normal controls. We analyzed the time course of plasma glucose and insulin concentrations during the intravenous glucose tolerance test using a minimal-model approach. This is a simple, noninvasive, and reliable method to assess in vivo SI, SG, and insulin secretion that has been applied

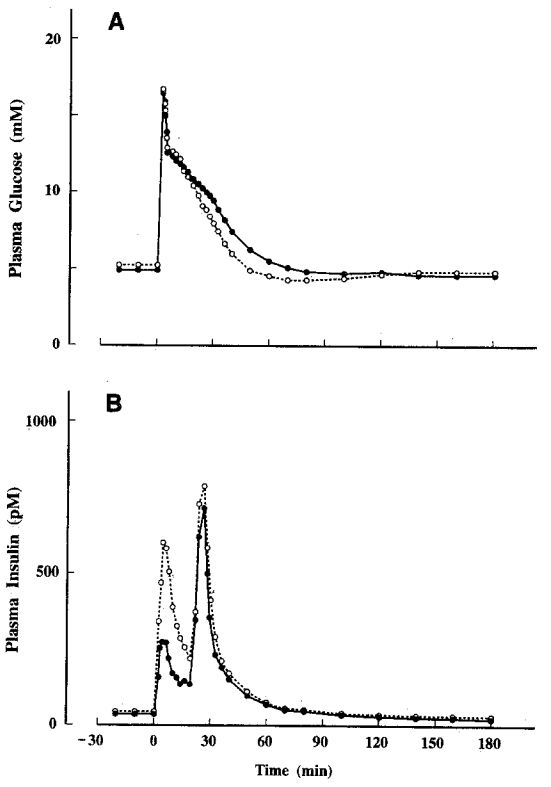

Fig 1. Time course of mean plasma glucose (A) and insulin (B) concentrations during FSIGT: (●) offspring, (○) controls.

to various pathologic states.[3,4,6,7,11,13-16] Using this technique, we studied glucose-tolerant NIDDM offspring. The offspring were unique in that they had no severe impairment in SI and had similar body mass index, and fasting glucose and insulin levels compared with normal control subjects. The suggestion that not all NIDDM offspring may have insulin resistance can be inferred from the data shown by Martin et al[19] and Johnston et al,[20] who found normal SI in NIDDM offspring. Banerji et al[5] and our group[6] reported NIDDM patients who had normal SI. Thus, it is conceivable that factors other than insulin resistance contribute to the evolution of NIDDM with normal SI.

The minimal-model technique enables us to estimate not only SI but also SG simultaneously. The present study showed that SG was significantly decreased in NIDDM offspring. This reduction was as severe as that seen in NIDDM or IGT subjects.[6,21] In our previous study, SG in NIDDM and IGT subjects was reduced to 0.011 ± 0.002 and 0.012 ± 0.002 min^{-1}, respectively.[6,21] Interestingly, our NIDDM and IGT subjects had normal SI. Therefore, it may be suggested that decreased SG is one of the predictors for the evolution of NIDDM with normal SI.

Controversial results exist with regard to SG in glucose-tolerant relatives.[3,4] Osei et al[3] confirmed that 10 relatives (whites and blacks) had normal SG but lower SI, and concluded that reduced SG succeeds the reduction in sensitivity to insulin in the pathogenesis of NIDDM. Henriksen et al[4] studied Danish NIDDM offspring and suggested that greater SG may be a compensatory mechanism to maintain normal glucose tolerance. The reason for the discrepant results for SI and SG between their data and ours remains unclear, but it may be due to the racial difference, the different clinical status of the subjects studied, or the different dietary intake.[22-25] Osei et al[3] and Henriksen et al[4] recruited glucose-tolerant relatives, but their relatives had a significantly higher fasting glucose concentration than the normoglycemic control subjects. It is well known that hyperglycemia per se worsens SI.[10] Lovejoy and DiGirolamo[22] studied healthy lean and obese subjects and showed that habitually low dietary fiber intake along with elevated dietary fat correlates with diminished SI. High-fat diets have been shown to cause insulin resistance in rats.[23] It is reported that low-carbohydrate or very–low-calorie diets (420 kcal) decrease SG in humans.[24,25]

Irrespective of the discrepant results, these studies are important to clarify the pathogenesis of NIDDM, but they are cross-sectional and small population studies. Thus, longitudinal studies in many populations are needed to determine predictors of the future development of NIDDM.

In this regard, the finding presented by the Joslin investigators is more impressive. They disclosed that a low glucose disappearance rate (KG) and high serum insulin levels independently increased the risk for developing diabetes in white relatives during a follow-up study.[26] Osei et al[3] and Henriksen et al[4] reported NIDDM offspring with normal KG, reduced SI, and normal to high insulin levels. We have presented herein NIDDM offspring with low KG, low SG, normal SI, and normal fasting insulin level. Although further study should be undertaken to clarify the hypothesis that Japanese NIDDM offspring with both low KG and low SG eventually develop NIDDM, the longitudinal study by Martin et al[19] showing that offspring with low SG in the presence or absence of coexisting insulin resistance developed diabetes supports this idea. Further study should be undertaken to determine whether a reduced SG could predict the future development of NIDDM in Japanese populations.

Another interesting finding is that our NIDDM offspring also had diminished $AIR_{glucose}$. Rojas et al[27] also reported NIDDM offspring with a reduced first-phase insulin response to glucose. Compared with NIDDM patients with normal SI reported by us,[6] the offspring reported herein had similar SG (0.016 ± 0.003 *v* 0.011 ± 0.002 min^{-1}, $P > .05$) but higher $AIR_{glucose}$ ($2,139 \pm 265$ *v* 15.7 ± 5.7 pmol/L · min, $P < .01$), indicating that impairments in $AIR_{glucose}$ but not derangements in SG worsen glucose tolerance. One might argue that $AIR_{glucose}$ per se affects SG in the present study, since it is suggested that the estimate of SG is dependent on $AIR_{glucose}$.[28] However, it seems unlikely, since $AIR_{glucose}$ was not correlated with SG in the present study. We found very recently that bulimic patients had reduced SG but normal $AIR_{glucose}$.[29] Welch et al[13] also reported NIDDM patients who had similar SG but unmeasurable to normal endogenous insulin secretion to glucose.

In summary, we first reported glucose-tolerant Japanese NIDDM offspring who had normal SI, impaired $AIR_{glucose}$, and diminished SG. Compared with NIDDM offspring, NIDDM patients reported in our study[6] had severe impairments in $AIR_{glucose}$ but had similar SG. Therefore, it appears that an impairment in the insulin response to glucose is the factor responsible for tipping the balance toward development of NIDDM, and that decreased SG may be an additional candidate for the evolution of NIDDM.

ACKNOWLEDGMENT

We are grateful to Dr Akira Kiyonaga and Dr Munehiko Shindo, Department of Exercise Physiology, School of Physical Education, Fukuoka University, Fukuoka, and the Department of Biochemistry, Kansai Denryoku Hospital and Shionogi Biomedical Laboratory, Osaka, Japan, for encouraging this study.

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