Pathophysiologic Phenotypes of Japanese Subjects With Varying Degrees of Glucose Tolerance: Using the Combination of C-Peptide Secretion Rate and Minimal Model Analysis

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We tried to characterize the clinical features associated with glucose metabolism in the development of diabetes. Study subjects were glucose-tolerant subjects without a family history of diabetes (normal glucose tolerance [NGT]1 group, n = 15) and with a first-degree diabetes relative (NGT2, n = 9), 12 subjects with impaired glucose tolerance (IGT), and 13 subjects with type 2 diabetes mellitus (DM). The first phase C-peptide secretion (CS1), insulin sensitivity (Si), and glucose effectiveness (Sg) were assessed by the combination of C-peptide 2-compartment model and minimal model analyses. Using these parameters, each group was characterized: CS1 was decreased in NGT2 and IGT compared with NGT1 and further decreased in DM; Si was not different among NGT1, NGT2, and IGT, whereas Si was decreased in DM; CS1 × Si value was decreased in NGT2 compared with NGT1 and decreased in IGT, DM, progressively; Sg was decreased in IGT and DM compared with NGT1 and NGT2. CS1 × Si and Sg values could segregate each group distinctively, although it had a large variety of phenotypes. CS1 × Si value and Sg are assumed to represent the contributions of insulin-dependent and independent mechanisms to glucose tolerance, respectively, and thus, both mechanisms should play an important role in the characterization of pathophysiologic phenotypes of the subjects with various degrees of glucose tolerance.

TYPE 2 DIABETES mellitus (DM) is pathophysiologically characterized by deterioration of β-cell function, hepatic glucose overproduction, and insulin resistance.[1] The extent to which each of these factors contributes to hyperglycemia is reported to vary depending on the race and ethnicity of the type 2 diabetic population. However, even in the same race, the mechanism of development of type 2 DM is complicated, and the clinical features of the patients are largely varied, because this disease is a heterogeneous syndrome and, in general, its genetic factors underlying the cause seem to be multiple.[2] To determine the detail phenotypes of pre and type 2 DM subjects is of specific importance in the search of the genetic basis of this disease and also for the determination of the therapeutic strategy.

Various approaches have been proposed to assess the β-cell function and insulin action. We previously reported that type 2 DM patients and nonobese impaired glucose tolerance (IGT) subjects showed a significant decrease in the first phase insulin secretion from the pancreas during intravenous glucose tolerance test (IVGTT)[3] using the method of 2-compartment model analysis of C-peptide kinetics derived from the original conception of Faber et al.[4-6]

For the measurement of insulin action, a glucose clamp technique has been assumed the best available standard.[7] The minimal model analysis proposed by Bergman et al[8] can, however, give us 2 valuable parameters, insulin sensitivity index (Si), and glucose-dependent (insulin-independent) glucose disposal (glucose effectiveness [Sg]) during frequently sampled intravenous glucose tolerance test (FSIGT). Finegood et al[9,10] developed an insulin-modified minimal model protocol for subjects with diminished insulin secretory function. This method was compared with the glucose clamp technique showing the significant correlation between the Si indices obtained by both methods.[11,12]

In this study, we propose the combined method of computer calculation of C-peptide secretion rate (CSR) and minimal model parameters Si and Sg during IVGTT, demonstrating that there are a large variety of phenotypes in various degrees of glucose tolerance by using all the indices obtained in this analysis.

SUBJECTS AND METHODS

Subjects

A total of 49 male subjects participated in this study. They included 24 healthy subjects with normal glucose tolerance (NGT). The NGT group was divided into 2 groups; normal control group (NGT1), which consisted of 15 subjects without any known family history of type 2 DM and the other group (NGT2), which consisted of 9 subjects with 1 of the first-degree relatives (parents) with type 2 DM. All the parents with diabetes had been treated with oral hypoglycemic agents or insulin. This study also included 12 subjects with IGT and 13 subjects with type 2 DM as defined by the World Health Organization (WHO) criteria.[13] All the subjects without a diagnosis of DM underwent oral glucose tolerance test (OGTT). All the diabetic patients (hemoglobin A_{1C} [HbA_{1c}], 7.78% ± 0.81%; duration of the disease, 3.58 ± 0.99 years) were treated with diet alone. None of the study participants without DM were taking any medication known to influence glucose homeostasis. All participants were asked not to take any drugs on the day of examination. The characteristics of the subjects are shown in Table 1. All participants gave their informed consent.

Study Protocol

After 10 to 12 hours overnight fast, intravenous cannulas ("heparin lock") were placed in both antecubital veins and kept patent with 0.9% normal saline infusion. A bolus of 50% glucose (25 g) was injected

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Table 1. Characteristics of the Study Subjects

	NGT1	NGT2	IGT	DM
No.	15	9	12	13
Age (yr)	34.7 ± 4.2	33.5 ± 4.2	47.3 ± 4.4	48.3 ± 5.4
BMI (kg/m^2)	24.1 ± 0.88	22.3 ± 0.88	25.7 ± 1.03	24.2 ± 1.26
75 g OGTT				
Fasting glucose (mg/dL)	91.1 ± 3.3	93.0 ± 2.8	107.5 ± 4.9	—
2-Hour glucose (mg/dL)	91.8 ± 6.6	103.1 ± 6.0	152.8 ± 8.1	—

NOTE. There are no significant differences among the groups in age ($P = .051$) and BMI ($P = .265$). Fasting and 2-hour glucose concentrations in IGT in 75 g OGTT were significantly greater than in NGT groups ($P = .0096$ and $P < .0001$, respectively).

over 1 minute at time zero. Regular human insulin (0.05 U/kg; Humalin R, Lilly, Indianapolis, IN) dissolved in 5 mL of 0.9% normal saline was infused over 30 seconds at time 20 minutes. Blood samples were collected at -5, 0, 2, 3, 5, 7, 10, 15, 20, 22, 23, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, and 150 minutes for the determination of plasma glucose and insulin concentrations. To calculate CSR after glucose injection, C-peptide concentrations were measured at -5, 0, 2, 3, 5, 7, 10, 15, and 20 minutes. For the NGT1 and NGT2 groups, C-peptide concentrations were measured at all the points when blood samples were collected to overview the time course of C-peptide profile.

Analytical Methods

Plasma glucose concentration was measured by the glucose oxidase method using a glucose autoanalyzer (Beckman, Fullerton, CA). Serum insulin and C-peptide levels of each individual were determined by a standard double antibody radioimmunoassay technique. The sensitivity of the insulin assay was 1.5 μU/mL. The intra- and interassay coefficients of variation (CVs) were 3.4% and 4.0%, respectively. The lower limit of C-peptide assay was 0.1 ng/mL. The intra- and interassay CVs were 4.0% and 7.5%, respectively. Insulin and C-peptide antibodies had cross-reactions to the human proinsulin by 15.3% and 30.6%, respectively, and insulin and C-peptide antibodies had no cross-reactions to the C-peptide and insulin, respectively.

Acute Insulin Response and Glucose Decay Constant

The basal plasma glucose and serum insulin concentrations were obtained as the mean values until time 0. Acute insulin response (AIR) was determined as the mean incremental insulin levels at 3, 5, 7, and 10 minutes after intravenous glucose administration. Area under the curve (AUC) for glucose, insulin, and C-peptide in the period 0 to 20 minutes were calculated from the incremental values above the baseline using the trapezoidal rule. Glucose decay constant (Kg) was calculated as the slope of the natural logarithm of glucose concentration in the time period 10 to 20 minutes.

CSR

CSR was mathematically estimated from serum C-peptide levels in the time period -5 to 20 minutes by deconvolution with a 2-compartment model for C-peptide disappearance kinetics.[4-6] The program for CSR was written in Basic language and run on a PC machine. This program was used in the previous work.[3] The standard kinetic parameters for C-peptide clearance described by Van Cauter et al[14] were used in the analysis. A least square cubic spline was used as the smooth analytical function approximating the discrete serum C-peptide data. The first phase C-peptide secretion (CS1) was calculated from the sum of CSR from 0 to 5 minutes.

Si and Sg

Parameters, Si and Sg, were calculated using glucose and insulin concentrations during the FSIGT by the minimal model software program, which we developed according to the algorithm described previously by Pacini and Bergman.[15] The minimal model program was written in C-language and run on a PC machine. The Sg at theoretical zero insulin concentration (GEZI) was also calculated as follows: GEZI = Sg - (Si × basal insulin).

Correlation Analysis

The CSR and minimal model parameters, Si and Sg, were examined to find a correlation with body mass index (BMI), fasting insulin level, fasting glucose concentration, and Kg. The CS1, Si, Sg and CS1 × Si were examined to correlate to each other. Because hyperglycemia itself can impair both insulin sensitivity and secretion, so-called glucose toxicity, a correlation analysis was performed for all the subjects without DM as a group.

Statistical Analysis

Data are expressed as mean ± SEM. Differences among the groups were analyzed by analysis of variance with Tukey adjustment for multiple comparisons. Correlation analyses were performed using Spearman rank sum correlation analysis. A P value less than .05 was considered statistically significant.

RESULTS

Characteristics of Subjects

There were no significant differences between the NGT1 and NGT2 groups in physical characteristics and fasting and 2-hour glucose concentrations after glucose load in OGTT. Age and BMI did not show any significant differences among the 4 groups.

Time Courses of Glucose, Insulin, and C-Peptide

Time courses of glucose concentration, insulin, and C-peptide levels after glucose administration at time 0 and insulin infusion at time 20 minutes are shown in Fig 1. The mean plasma glucose concentration at each point from 2 to 20 minutes after glucose load showed no significant differences among the groups. After 30 minutes, mean glucose concentrations were greatest in the DM group compared with the other groups. The mean insulin response after glucose bolus increased to a peak at 5 minutes in each group. The peak value of insulin in the NGT1 group was highest compared with the other groups. The mean insulin concentrations increased to peak values at 23 minutes (3 minutes after insulin infusion) and showed similar profiles from time 20 to 30 minutes in all groups. But the mean insulin concentrations after 40 minutes in the DM group were significantly higher compared with the NGT1 group. The mean C-peptide responses after glucose load

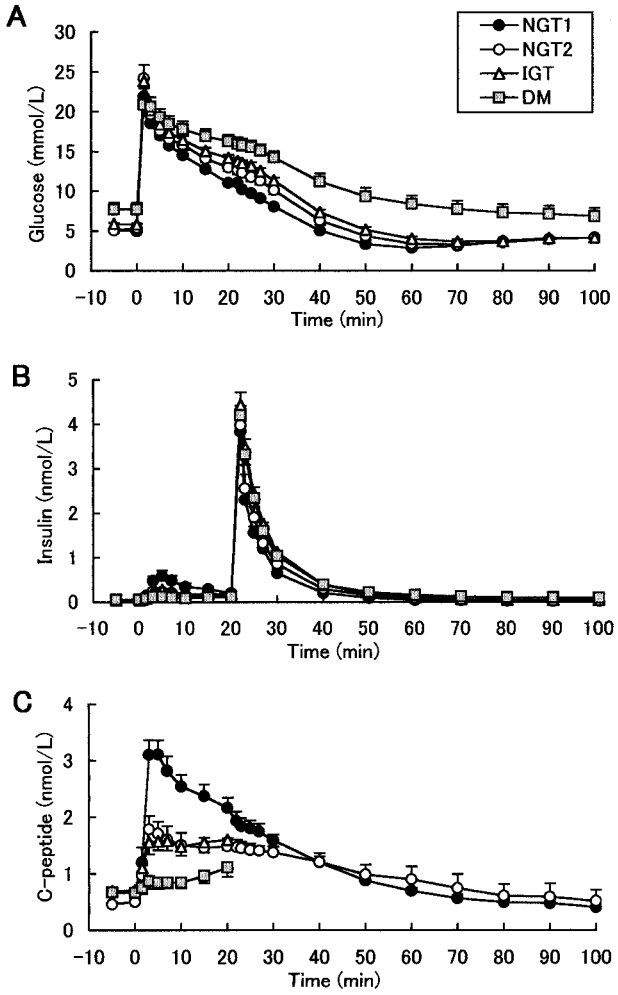

Fig 1. The mean ± SEM plasma glucose (A), insulin (B), and C-peptide (C) levels before and during the FSIGT in NGT1, NGT2, IGT, and DM groups. In the glucose concentration, there were significant differences among the groups at time -5 and 0 ($P = .004$) and at each time after 30 minutes ($P < .0001$). In the insulin level, differences among the groups were significant at time 5 and 10 minutes ($P = .002$) and at each time after 40 minutes ($P < .01$). In the C-peptide level, there were significant differences among the groups at time 3, 5, 10, and 15 ($P < .0001$) and 20 minutes ($P = .0005$).

had peak values at 3 or 5 minutes in the NGT1, NGT2, and IGT groups and at 20 minutes in the DM group. At each point from 3 to 15 minutes after glucose bolus, the mean C-peptide values were highest in the NGT1 group, intermediate in the NGT2 and IGT groups, and lowest in the DM group. The mean C-peptide value at 20 minutes in NGT1 was significantly greater compared with the DM group. There were significant differences in the AUC of insulin between the NGT1 and the other groups, as shown in Table 2. The AUC of C-peptide was significantly decreased in the NGT2 and IGT groups compared with the NGT1 group and further decreased in the DM group.

CSR Changes After Intravenous Glucose Administration

The changes in CSR after intravenous glucose administration were calculated from the C-peptide values and yielded 2 phase patterns (Fig 2). In each group, CSR rapidly increased after glucose injection, increased to peak at 2 minutes, immediately started to decrease, and reached the nadir at 5 minutes. The CSR values at 2 and 3 minutes in the NGT1 group were greater compared with the other groups. The sum of CSR from 1 to 5 minutes in the NGT1 group was greater compared with the other groups (Table 2). The second phase increase in C-peptide secretion occurred subsequently, but it was very low (negligible) compared with the first phase in each group. The sum of CSR from 0 to 5 minutes implies the first phase in C-peptide secretion, denoted CS1 in this report.

Comparison Between CS1 and AIR

Correlation between AIR and CS1 shown in Fig 3 revealed that AIR had good positive correlation to CS1 in normal subjects only when CS1 exceeded ≈ 1.5 nmol/L ($y = 195.6x - 272.2$, $P < .001$).

Minimal Model Analysis

Minimal model parameters were obtained from the analysis of overall glucose and insulin profiles during the FSIGT protocol. As shown in Table 2, the mean Si did not show the significant differences among the NGT1, NGT2, and IGT groups. The mean Si value decreased in the DM group compared with the NGT1 and NGT2 groups. The mean Sg value significantly decreased in the IGT and DM groups compared with the NGT1 and NGT2 groups. GEZI followed the similar pattern as Sg.

Relationship Among Si, CS1, and Sg

The individual values of Si and CS1 were plotted on the Si - CS1 plane and 1/Si - CS1 plane (Fig 4A and B) to examine the relationship between insulin sensitivity and β-cell function. The mean values of CS1 × Si were decreased according to the impairment of glucose tolerance (Table 2), being greatest in the NGT1 group, second in the NGT2 group, third in the IGT group, and least in the DM group. As shown in Fig 4A and B in the NGT1 and NGT2 groups, individual values of Si and CS1 were distributed around the hyperbolic function CS1 × Si = K-constant. Separate regression analysis was used between 1/Si and CS1 to examine whether a hyperbolic relationship between Si and CS1 existed in each group. There were significant positive correlations between CS1 and 1/Si in the NGT1 and NGT2 groups ($r = .70$, $P = .0035$; $r = .69$, $P = .038$, respectively). The IGT and DM groups showed the positive correlations, but did not attain statistical significance ($r = .47$, $P = .11$; $r = .45$, $P = .12$, respectively). Because there were some IGT subjects with relatively high CS1 × Si values, which were comparable to the NGT1 and NGT2 groups, the relationship between CS1 × Si and Sg was examined in each group (Fig 4C), showing that Sg values in the IGT subjects were lower compared with the NGT subjects with the same levels of CS1 × Si values. Using these parameters, each group was clearly segregated.

Correlation Between Parameters

In the subjects without diabetes, correlation coefficients between the parameters are shown in Table 3. BMI had no

Table 2. AUC for Glucose, Insulin and C-Peptide, AIR, Calculated CSR, and Minimal Model Parameters

	NGT1	NGT2	IGT	DM
AUC (glucose), mmol/L × 20 min	189.6 ± 6.47*	211.2 ± 14.0*	213.2 ± 6.57*	198.6 ± 8.89*
AUC (insulin), nmol/L × 20 min	6.00 ± 1.26*	2.54 ± 0.57†	2.78 ± 0.24†	0.85 ± 0.26†
AUC (C-peptide), nmol/L × 20 min	34.7 ± 3.25*	19.4 ± 3.34†	15.5 ± 2.43†	4.51 ± 1.00‡
AIR, pmol/L	447.8 ± 86.0*	178.4 ± 45.8†	175.8 ± 35.1†	49.5 ± 16.5†
CS1, nmol · L^{-1} · (5 min)$^{-1}$	3.58 ± 0.34*	1.95 ± 0.32†	1.45 ± 0.27†‡	0.51 ± 0.10‡
Si, 10^{-4} min^{-1} · (pmol/L)$^{-1}$	0.76 ± 0.08*	0.79 ± 0.09*	0.56 ± 0.07*†	0.36 ± 0.07†
Sg, 10^{-2}/min	2.80 ± 0.25*	2.78 ± 0.14*	1.60 ± 0.16†	1.39 ± 0.17†
GEZI, 10^{-2}/min	2.56 ± 0.24*	2.54 ± 0.16*	1.37 ± 0.17†	1.27 ± 0.18†
CS1 × Si, 10^{-4}	2.50 ± 0.17*	1.39 ± 0.18†	0.74 ± 0.15‡	0.16 ± 0.04§
Kg, 10^{-2}/min	2.70 ± 0.16*	1.97 ± 0.24†	1.34 ± 0.08‡	0.93 ± 0.13‡

NOTE. There was no significant difference among the groups in AUC (glucose) (P = .236). Differences among the groups were significant in AUC (insulin) (P = .0002), Si (P = .0007), and in other parameters (P < .0001). Different symbols (*,†,‡,§) indicate significant differences from each other.

Abbreviations: AIR, acute insulin response; GEZI, glucose effectiveness at theoretical zero insulin concentration; Kg, glucose decay constant.

significant correlation to CS1, Si, and CS1 × Si. Fasting glucose concentration had a positive correlation to fasting insulin level and significant negative correlation to Kg, CS1, and Sg. A significant negative correlation was observed between fasting insulin level and Si. Significant positive correlations were observed between Kg and CS1, Kg and Sg, and CS1 and Sg.

DISCUSSION

For the assessment of β-cell function, we used the CSR calculated based on the kinetic equation of 2-compartment model of C-peptide, which was originally introduced by Eaton et al. The time course of CSR showed that most of the insulin secretion after intravenous glucose administration was actually determined by the first phase, ie, 0 to 5 minutes, in insulin secretion, as shown in Fig 2. Thus, we adopted the sum of the CSR as an index of the β-cell function. In most studies, so far, using minimal model analysis, AIR has often been used to assess β-cell function.[16-18] Peripheral insulin level, however, does not represent β-cell function sufficiently, because plasma insulin is a result of insulin secretion from the pancreatic islets, the first-pass hepatic insulin extraction, and the overall rate of catabolism of insulin by all tissues. In fact, it is only when CS1 is over 1.5 nmol/L that AIR has good correlation to CS1 as shown in Fig 3. Because the values of CS1 are under 1.5 nmol/L in most of the Japanese subjects with IGT or DM as demonstrated in this study, it seems impossible in these cases to evaluate the β-cell function by AIR. This study demonstrated the significant reduction in the first phase of insulin secretion in the subjects of the NGT2 and IGT groups and the remarkable reduction in the subjects of the DM group being compatible with the previous reports.[19-22]

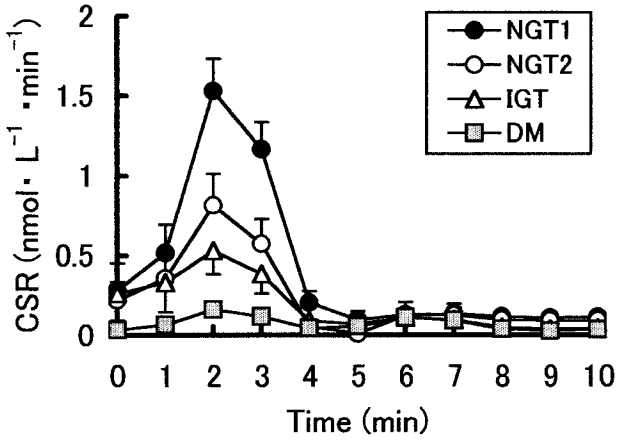

Fig 2. The changes in CSR after intravenous glucose administration. CSR was calculated from the C-peptide values during FSIGT using the 2-compartment model for C-peptide distribution and degradation. In this study, the kinetic parameters of C-peptide clearance were obtained according to the procedure described by Van Cauter et al.[14] Because the unit of CSR is represented by nmol · L^{-1} · min^{-1}, the total amount of C-peptide production can be calculated from the CSR values multiplied by the plasma volume (≈ 41.3 mL/kg body weight [BW]).[5] There were significant differences in CSR values among the groups at 2 and 3 minutes after intravenous glucose administration (P < .0001).

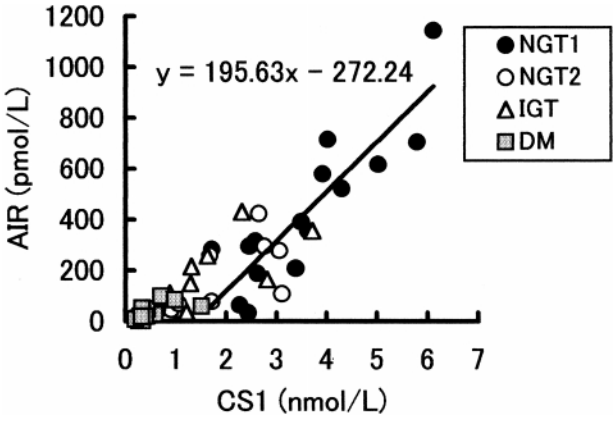

Fig 3. Relationship between AIR and CS1. AIR was calculated as the mean increment above basal of insulin values measured at 3, 5, 7, and 10 minutes after intravenous glucose administration. CS1 was calculated as the sum of CSR from 0 to 5 minutes after intravenous glucose administration indicating the first phase secretion of C-peptide. Note that in normal subjects, AIR had good positive correlation to CS1 (y = 195.6 × -272.2, P < .001).

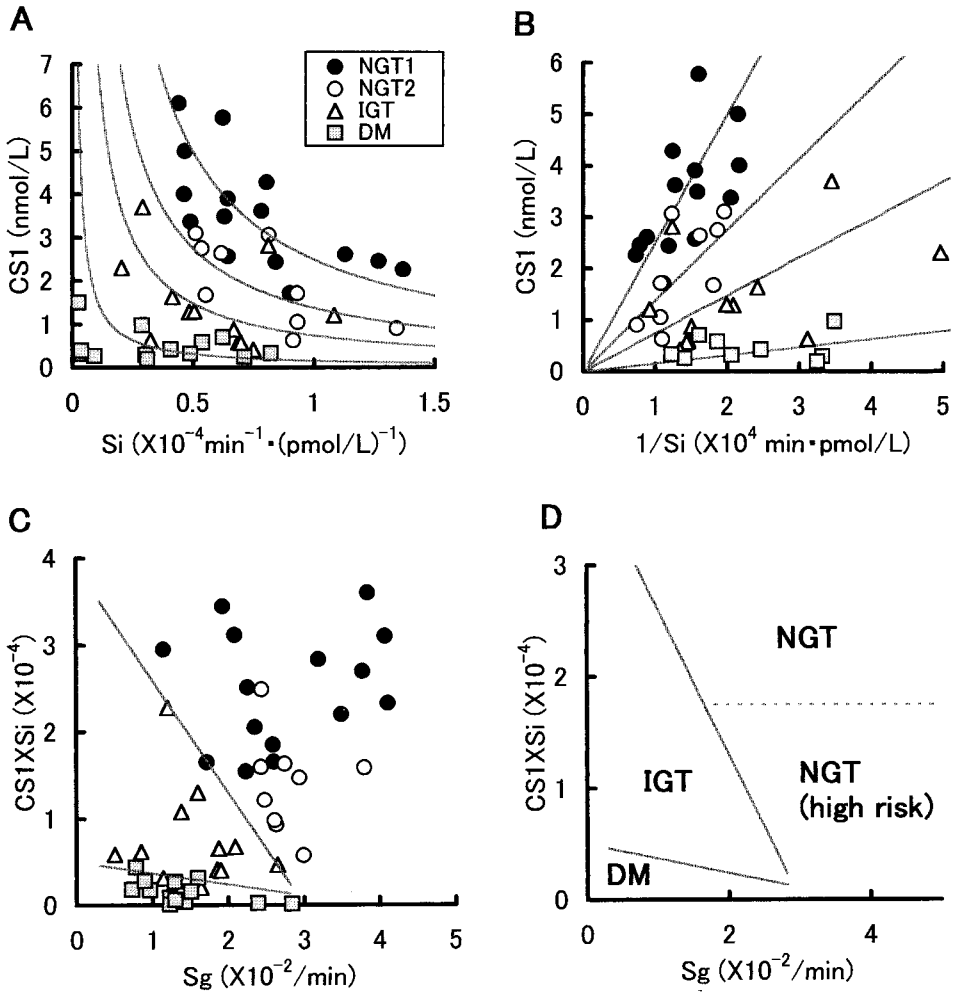

Fig 4. Relationship between Si and CS1 (A), 1/Si and CS1 (B), and Sg and CS1 × Si (C and D). Each line in (A) and (B) shows the best fit hyperbolic relationship described by CS1 × Si = K-const; K-const = 2.50 × 10^{-4} (NGT1), 1.39 × 10^{-4} (NGT2), 0.74 × 10^{-4} (IGT), 0.16 × 10^{-4} (DM). K-const shows the mean value of CS1 × Si in each group. Note that there are some subjects with IGT with relatively high CS1 × Si values (more than 1.0 × 10^{-4}) who had lower Sg compared with NGT2 subjects as shown in (C). The hypothetical borderlines among the groups with different glucose tolerance by OGTT are also drawn in (C). The hypothetical distribution area in CS1 × Si and Sg is shown in (D). Individuals with low CS1 × Si values in NGT groups are assumed to have a high risk of IGT or DM and thus indicated by NGT (high risk) in (D).

We used insulin modified minimal model approach during the FSIGT to evaluate Si and Sg and demonstrated that Sg, not Si, decreased in Japanese subjects with IGT, and both Sg and Si decreased in subjects with diabetes. No significant reduction in both Si and Sg was observed in the NGT2 group in this study. Decreased Sg has been demonstrated in subjects with IGT and type 2 DM in Japanese[23,24] and Caucasian populations.[25,26] However, in African Americans, IGT and type 2 DM, Si was reduced, while Sg remained intact,[27] suggesting that there are possibly racial and ethnic differences in the Si and Sg changes during the process to diabetes.

Kahn et al[16] and Bergman et al[28,29] showed the hyperbolic relationship between the β-cell function and insulin sensitivity in normal subjects. The present study also showed that a hyperbolic relationship exists in both NGT1 and NGT2 groups by using separate regression analysis of CS1 versus 1/Si. The mean CS1 × Si values were significantly decreased in NGT2, IGT, and DM groups progressively compared with the NGT1

Table 3. Correlation Coefficients Between Parameters

	FG	FI	Kg	CS1	Si	Sg	CS1 × Si
BMI	$r = .106$	$r = .355$	$r = .069$	$r = .35$	$r = -.309$	$r = .080$	$r = .103$
FG		$r = .405$*	$r = -.658$*	$r = -.569$*	$r = .039$	$r = -.522$*	$r = -.554$*
FI			$r = -.242$	$r = .154$	$r = -.567$*	$r = -.267$	$r = -.326$
Kg				$r = .720$*	$r = -.089$	$r = .561$*	$r = .674$*
CS1					$r = -.330$	$r = .546$*	$r = .716$*
Si						$r = .084$	$r = .355$
Sg							$r = .515$*

Abbreviations: FG, fasting glucose; FI, fasting insulin; Kg, glucose decay constant.

* $P < .05$ significant correlation between the parameters.

group. These findings are consistent with the previous report that the Si × Phi1 index was lower in normal glucose-tolerant individuals with first-degree type 2 DM relatives[30] and also with the recent longitudinal study in Pima Indians that showed AIR was already low relative to the degree of insulin resistance in the progressors to DM while they still have NGT, and both insulin action and insulin secretion deteriorate further during transition from NGT to IGT.[31]

Theoretically, the CS1 × Si value is considered an index that represents overall insulin-dependent factors in glucose metabolism, because insulin-dependent glucose disposal is determined by the β-cell function (CS1) and Si.[32] However, as shown in Fig 4A and B, because the distributions of subjects in NGT2 and IGT are closely overlapped on the Si-CS1 plane, the difference in glucose tolerance by WHO criteria based on OGTT cannot be explained by only the β-cell function (CS1) and the insulin action (Si). Thus, as an insulin-independent factor, Sg was taken into account to understand the precise clinical features of subjects with various glucose tolerance. Figure 4C exhibits the degree of contribution of insulin-dependent and independent components of glucose tolerance in each subject. Interestingly, each group in this study is almost segregated by CS1 × Si and Sg values as shown in Fig 4C. The NGT1 group has high CS1×Si and a broad range of Sg values. The CS1 × Si values were overlapped in the NGT2 and IGT groups. However, when comparing the subjects with the same levels of CS1 × Si, the Sg values in the NGT2 subjects were always greater than in the IGT subjects. This suggests that Sg may serve as a compensatory mechanism of glucose tolerance in NGT2 group as previously reported.[30]

Although substantial components that contribute to Sg are still unclear, Sg was reported to increase with physical training.[33-36] Recently, Wojtaszewski et al[37] observed that exercise modulated postreceptor insulin signaling and glucose transport in muscle-specific insulin receptor knockout mice, suggesting that normal expression of muscle insulin receptors is not needed for the exercise-mediated increase in glucose uptake and glycogen synthase activity in vivo. Further studies on Sg will be needed to elucidate the roles of insulin-independent mechanisms for glucose homeostasis in human subjects.

As mentioned above, clinical phenotype associated with glucose tolerance should be understood from the point of insulin-dependent and independent mechanisms, and also the insulin-dependent mechanism should be understood as the combination of β-cell function and insulin sensitivity. The subclassification of pretype 2 DM phenotype using CS1, Si, and Sg would bear an important meaning in the search for the genetic basis, which underlies the onset and progression of type 2 DM, because the combination of these 3 parameters should be, to some extent, derived from the genetic backgrounds that vary widely among races and individuals.[22,38] The specific phenotypes of individuals are expressed by the position in this 3-dimensional space and may be classified into subgroups by the spatial distribution of the parameters. The individuals with far distance in this space should be in different subgroups, even if they are classified in the same glucose tolerance by OGTT.

Most of the subjects in this study with low CS1 × Si values in the NGT groups belonged to the NGT2 group, suggesting that CS1 × Si values should be genetically determined. They are located closer to the IGT area in the Sg-CS1 × Si plane (Fig 4C), and they seem to go easily into the IGT area and furthermore to the DM area by the decrease of Sg or CS1 × Si values. This may be the pathophysiologic reason why the subjects with a family history of DM have a high risk of this disease. We speculate that the subjects with intrinsic low CS1 × Si values are susceptible to type 2 DM, although longitudinal follow-up study would be needed to answer this question.

In conclusion, this study demonstrated that there are significant alterations in CS1, Si, and Sg in the pathogenesis of type 2 DM and heterogeneous clinical phenotypes of subjects with varying degrees of glucose tolerance. This study also showed the possibility of subclassification of these subjects by determining the contribution of the insulin-dependent and independent mechanism, which were assessed by IVGTT-based C-peptide secretion analysis and minimal model approach. Because this approach can be easily performed even in the outpatient setting, using the same protocol from normal subjects to diabetic patients, this method is expected to be widely used for further understanding of the pathophysiology of type 2 DM for the determination of treatment strategy and for research of the underlying molecular causality of this disease.

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