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Hyperbolic correlation between insulin sensitivity and insulin secretion fades away in lean subjects with superb glucose regulation

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Abstract. The relationship between insulin sensitivity (Si) and insulin secretion (β) was analyzed in 533 health examinees. The subjects underwent a 75 g oral glucose tolerance test, with plasma glucose (PG) and immunoreactive insulin (IRI) determined at fasting, 30 min and 120 min, and were classified according to the current criteria as normal glucose tolerance (NGT, n=328), non-diabetic hyperglycemia (NDH, n=113) including impaired fasting glucose and impaired glucose tolerance, and diabetes mellitus (DM, n=72). NGT was subdivided by fasting PG (FPG) tertile, ≤ 4.9 , 5.0-5.4 and 5.5-6.0 mM, into NGT_{FPG1}, NGT_{FPG2} and NGT_{FPG3}, or by body mass index (BMI) tertile, ≤ 21.8 , 21.9-24.4 and ≥ 24.5 kg/m², into NGT_{BMI1}, NGT_{BMI2} and NGT_{BMI3}. As an index of Si and β , Matsuda index=10,000/sqrt [FPG·FIRI·2hPG·2hIRI] and $\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}$, were employed respectively: FIRI, 2hPG and 2hIRI denote fasting IRI, 2h-post glucose PG and IRI, respectively. Correlation between Si and β was evaluated by Spearman's rank correlation and the parameters for $[\beta]=a\cdot[\text{Si}]^b$ were obtained by standardized major axis (SMA) regression. Si- β correlation was strongest in NDH (Spearman's rho=-0.546, SMA regression $r^2=0.277$), intermediate in DM (rho=-0.432, $r^2=0.193$) and weakest in NGT (rho=-0.201, $r^2=0.039$). Spearman's rho for the Si- β correlation was significantly lower in NGT than in NDH ($p=0.003$). Si- β correlation was significant in NGT_{FPG3}, NGT_{FPG2} and NGT_{BMI3}, but not in NGT_{FPG1}, NGT_{BMI2} and NGT_{BMI1}. The slope, b , was -1.184~-1.530 without significant differences between any groups. In conclusion, the hyperbolic Si- β correlation was weaker in NGT than in NDH and absent in NGT subjects belonging to the lowest FPG or BMI tertile.

Key words: Beta cell function, Insulin sensitivity, Hyperbolic law, Glucose tolerance

PLASMA GLUCOSE (PG) level is maintained within the normal or near normal range even if insulin sensitivity (Si) is diminished. A typical example is obesity. In many obese subjects, normoglycemia is maintained despite low Si. In this situation, insulin secretion (β) usually increases. The opposite holds true for athletes who are euglycemic with increased Si and lowered β . Accordingly, in apparently healthy population, there is an inverse correlation between Si and β , which is expressed as $[\beta]=a\cdot[\text{Si}]^b$ with the exponent b being not significantly different from -1 [1-4].

However, we consider that the characteristics of the

Si- β correlation in the general population have not yet been fully elucidated. The heterogeneity of normal subjects with respect to the relationship between insulin secretion and insulin sensitivity, i.e., poor inverse correlation between fasting plasma insulin concentration and Si, was briefly described in the original study [1]. More recently, we [5] and others [6-8] have definitively established the heterogeneity of insulin-glucose interplay in subjects with normal fasting glucose (NFG) or normal glucose tolerance (NGT). Nevertheless, the relationship between Si and β in apparently healthy subjects has been studied by treating NGT as a single population, and the general consensus is that there is a progressive left and downward shifting of the regression curve, $[\beta]=a\cdot[\text{Si}]^b$, with worsening of glucose tolerance [9-18]. In some studies, non-hyperbolic regression of Si to β was reported [4, 15, 16].

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We hypothesized that the hyperbolic regression between Si- β is not a priori, and therefore systematically analyzed data from apparently healthy subjects containing NGT, non-diabetic hyperglycemia (NDH) and mild untreated diabetes in order to obtain a comprehensive understanding of the characteristics of the Si- β correlation in the general population.

Materials and Methods

Study design

The study was approved by the Chikuma Central Hospital Review Board and the Aizawa Hospital Review Board. We analyzed data from health examinees. Those with renal failure (serum creatinine >145 μ M), liver cirrhosis, previous gastrointestinal operation or a known history of diabetes mellitus (DM) were excluded. Blood samples were obtained from 534 consecutive participants at 0, 30 and 120 min during a standard 75 g oral glucose tolerance test (OGTT) for PG and serum immunoreactive insulin (IRI) measure-

ments. In 1 subject, PG at 30 min was lower than FPG and therefore the index of β cannot be calculated (see below). Accordingly, this person was excluded and the data from 533 subjects (male/female 367/166, and the mean age, BMI and FPG, 52.8 years, 23.6 kg/m² and 5.6 mM, respectively) were used for the study (Table 1). They formed part of the subject group analyzed in our previous studies [5,19], and therefore this study is a re-analysis of previously obtained data. PG was determined by the hexokinase method and IRI by a specific enzyme immunoassay [20].

Classification of the participants

In Analysis I, the participants were classified according to the current diagnostic criteria of diabetes and other categories of hyperglycemia [21] into NGT, NDH including impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), and DM. The definition of each glucose tolerance category was as follows: NGT, FPG $\leq$ 6.0 mM and 2-h post glucose PG (2hPG) at 75g OGTT <7.8 mM; IFG, FPG between

Table 1 Characteristics of the participants and Si- β correlation and regression in Analysis I

Variable	All (n=533)	Group		
		NGT (n=328)	NDH (n=133)	DM (n=72)
M/F	367/166	227/101	92/41	48/24
Age, yr	52.8 $\pm$ 11.3	50.7 $\pm$ 11.1	55.3 $\pm$ 10.9	57.2 $\pm$ 10.6
BMI*, kg/m ²	23.6 $\pm$ 3.2 (n=502)	23.2 $\pm$ 3.0 (n=318)	24.0 $\pm$ 3.2 (n=118)	25.3 $\pm$ 3.5 (n=66)
FPG, mM	5.6 $\pm$ 0.9	5.2 $\pm$ 0.4	5.8 $\pm$ 0.6	7.2 $\pm$ 1.2
2hPG, mM	7.4 $\pm$ 3.1	5.8 $\pm$ 1.0	8.2 $\pm$ 1.6	13.7 $\pm$ 3.7
FIRI, pM	38.2 $\pm$ 25.7	34.8 $\pm$ 21.5	42.4 $\pm$ 27.1	47.2 $\pm$ 34.7
2hIRI, pM	244.5 $\pm$ 208.4	194.6 $\pm$ 153.6	330.8 $\pm$ 269.0	309.1 $\pm$ 232.7
δ IRI ₀₋₃₀ / δ PG ₀₋₃₀ [†]	0.62 $\pm$ 0.75	0.79 $\pm$ 0.87	0.45 $\pm$ 0.39	0.18 $\pm$ 0.17
ISI _{Matsuda} [‡]	11.4 $\pm$ 10.5	14.1 $\pm$ 11.4	8.1 $\pm$ 8.1	5.3 $\pm$ 4.1
DI _O	7.0 $\pm$ 11.0	10.1 $\pm$ 13.0	2.7 $\pm$ 2.4	0.7 $\pm$ 0.5
Speraman's rank correlation				
rho	-0.025	-0.201 $\ddagger$	-0.546	-0.432
p	0.569	<0.001	<0.001	0.003
Standardized major axis regression				
a (95%CI)	N.A.	9.991 (7.189~13.885)	2.996 (2.100~4.273)	1.065 (0.625~1.814)
b (95%CI)	N.A.	-1.205 (-1.340~-1.083)	-1.216 (-1.407~-1.050)	-1.530 (-1.892~-1.238)
r ²	N.A.	0.039	0.277	0.193
p	N.A.	<0.001	<0.001	<0.001

Plus-minus values are mean $\pm$ SD. M/F, male/female; BMI, body mass index; 2hPG, 2-h post glucose PG at 75 g oral glucose tolerance test (OGTT); FIRI, fasting immunoreactive insulin (IRI); 2hIRI, 2-h post glucose IRI at 75 g OGTT; DI_O, oral disposition index; N.A., not applicable; CI, confidence interval; *, BMI data is missing in 31 subjects so that the number of subjects with BMI value is indicated in the parentheses; [†], conventional units were used for δ IRI₀₋₃₀/ δ PG₀₋₃₀ and ISI_{Matsuda}, [μ U/mL]/[mg/100 mL] and [μ U/mL]⁻¹·[mg/100 mL]⁻¹, respectively, as in the original reports [22,24]; $\ddagger$, significantly different ($p=0.003$) from the corresponding value in NDH. By Kruskal-Wallis test, age, BMI, FIRI, 2hIRI, δ IRI₀₋₃₀/ δ PG₀₋₃₀, ISI_{Matsuda} and DI_O in NGT, NDH and DM were all significantly different ($p<0.001$ for all variables except for FIRI for which $p=0.001$).

≥ 6.1 mM and < 7.0 mM, and 2hPG < 7.8 mM); IGT, FPG < 7.0 mM and 2hPG between ≥ 7.8 and < 11.1 mM); DM, FPG ≥ 7.0 mM and/or 2hPG ≥ 11.1 mM. The number of subjects in each group was 328 in NGT, 133 in NDH and 72 in DM.

Si- β correlation was weakest in NGT group (see below). Therefore, factors associated with poor Si- β correlation were sought among subjects with NGT. Because low FPG and BMI were independently correlated with degree of deviation from the regression curve (see below at “Identification of variables related to deviation from the hyperbolic regression in subjects with NGT”), subjects with NGT were subdivided on the basis of FPG tertile and BMI tertile. Namely, NGT_{FPG1} (n=103), NGT_{FPG2} (n=118) and NGT_{FPG3} (n=107) by FPG tertile (≤ 4.9 , 5.0–5.4 and 5.5–6.0 mM), or NGT_{BMI1} (n=106), NGT_{BMI2} (n=103) and NGT_{BMI3} (n=109) by BMI tertile (≤ 21.8 , 21.9–24.4 and ≥ 24.5 kg/m²). FPG tertile and BMI tertile were selected as the closest to the equal 3 division of the subjects. BMI values were missing in 10 subjects, so that the number of subjects analyzed by BMI tertile was 318.

Parameters

As a measure of Si, the insulin sensitivity index proposed by Matsuda [22] (ISI_{Matsuda}) was calculated by using fasting and 2-h post glucose blood samples during 75 g OGTT [23], i.e., $ISI_{Matsuda} = 10,000 / \sqrt{FPG \cdot FIRI \cdot 2hPG \cdot 2hIRI}$, where FIRI was fasting IRI and 2hIRI was 2-h post glucose IRI. Correlation between clamp-based Si and ISI_{Matsuda} obtained by a modified method [23] was reportedly slightly stronger than that between clamp-based Si and ISI_{Matsuda} in the original study [22]. As an index of β , $\delta IRI_{0-30} / \delta PG_{0-30}$ during 75 g OGTT [24] was adopted. As another index of β , the Stumvoll index (first phase) [25] was used in some analyses. The equation for the index was, $1283 + 1.829 \cdot [IRI_{30}] - 138.7 \cdot [PG_{30}] + 3.772 \cdot [FIRI]$, where IRI₃₀ and PG₃₀ were 30-min post glucose IRI and PG during 75g OGTT, respectively [25]. Analysis using the Stumvoll index was performed in the groups of subjects with NDH and NGT, and in the NGT subgroups, because the index was formulated using data from non-diabetic subjects [25]. Strong linear correlations were established between insulin sensitivity measured by euglycemic clamp and ISI_{Matsuda} ($r=0.73$) [22], between acute insulin release (AIR) upon intravenous glucose injection and $\delta IRI_{0-30} / \delta PG_{0-30}$ ($r=0.88$) [26], and between AIR and the Stumvoll index ($r=0.79$)

[25]. A product of ISI_{Matsuda} and $\delta IRI_{0-30} / \delta PG_{0-30}$ was obtained as an oral disposition index DI (DI_O) [18, 27], a measure of beta cell function with the degree of insulin sensitivity being taken into account.

Statistics

The bivariate correlation between Si and β was evaluated by Spearman's rank correlation. The parameters of the power regression, $[\delta IRI_{0-30} / \delta PG_{0-30}] = a \cdot [ISI_{Matsuda}]^b$, were obtained by standardized major axis (SMA) regression [28]. We ascertained that the distribution of log-transformed ISI_{Matsuda} and $\delta IRI_{0-30} / \delta PG_{0-30}$ in each group was not significantly different from the normal distribution. Accordingly, using log-transformed ISI_{Matsuda} and log-transformed $\delta IRI_{0-30} / \delta PG_{0-30}$, SMA regression was applied, i.e., $\log[\delta IRI_{0-30} / \delta PG_{0-30}] = \log(a) + b \cdot \log[ISI_{Matsuda}]$ was calculated. SMA regression was performed using SMATR ver 2.0 [29]. The SMA regression slope parameters, b , for 2 or 3 groups were compared by using a likelihood ratio test [30]. Because there were no significant differences between the slopes, b , for NGT, NDH and DM, or between those for NGT_{FPG2} and NGT_{FPG3}, a common slope was calculated for NGT, NDH and DM, or NGT_{FPG2} and NGT_{FPG3} [29, 30]. Subsequently, intercept, a , with “the common slope” was compared across the groups by using all-pair comparisons as an ad hoc method of analysis. Adjustment of multiplicity was performed by the Holm method [31]. The orthogonal distance from the regression line to each data point was calculated by the following formula.

$$\sqrt{\left[x_i - \frac{\left[\frac{x_i}{b} + y_i \right] - \log(a)}{b + \frac{1}{b}} \right]^2 + \left[y_i - \frac{x_i + by_i + b \cdot \log(a)}{b + \frac{1}{b}} + \log(a) \right]^2}$$

where x_i , y_i , b and $\log(a)$ were $\log[ISI_{Matsuda}]$, $\log[\delta IRI_{0-30} / \delta PG_{0-30}]$, the slope and intercept of SMA regression, respectively.

Other statistical analyses were performed using StatView (SAS®) and $p < 0.05$ was considered significant. Statistical comparison of correlation coefficients was performed after Fisher's Z transformation.

Results

Analysis I

Characteristics of subjects with NGT, NDH and DM

The mean FPG and 2hPG were lowest in NGT and progressively higher in NDH and DM as defined (Table

1). On the contrary, the mean values for $\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}$, $\text{ISI}_{\text{Matsuda}}$ and DI_O were highest in NGT and progressively lower with worsening of glucose tolerance. The mean age and BMI were lowest in NGT and progressively higher in NDH and DM. The difference was statistically significant for all of these variables (Table 1).

Correlation between Si and β

According to Spearman's rank correlation, significant correlation between $\text{ISI}_{\text{Matsuda}}$ and $\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}$ was present in NGT, NDH and DM (Table 1). Rather unexpectedly, correlation between $\text{ISI}_{\text{Matsuda}}$ and $\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}$ was strongest (Spearman's $\rho=-0.546$) in NDH, intermediate (-0.432) in DM, and weakest (-0.201) in NGT, with the difference of ρ between NGT and NDH being highly significant ($p=0.003$). Correlation between $\text{ISI}_{\text{Matsuda}}$ and the Stumvoll index was also stronger in NDH (Spearman's $\rho=-0.614$) than in NGT (-0.408). The ρ values were significantly different ($p=0.039$).

SMA regression showed that regression was significant in all three groups: it was strongest (r^2 for SMA regression= 0.277) in NDH, intermediate ($r^2=0.193$) in DM, and weak ($r^2=0.039$) in NGT. The parameters of regression for $\text{ISI}_{\text{Matsuda}}$ to $\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}$, $\log[\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}]=\log(a)+b\cdot\log[\text{ISI}_{\text{Matsuda}}]$, are shown in Table 1. b was -1.205 , -1.530 , and -1.216 , in NGT, NDH, and DM respectively, and the values were not significantly different from each other: the slope common to the three groups was $b=-1.250$. a obtained by SMA regression was 9.991 , 2.996 and 1.065 , in NGT, NDH, and DM respectively. a with the common slope, $b=-1.250$, was 11.145 , 3.190 and 0.712 in NGT, NDH and DM respectively, and the values were significantly different from each other ($p<0.001$). The finding was compatible with reduced DI_O in NDH and DM compared to NGT (Table 1). As shown in Fig. 1, the best-fit regression line for $[\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}]=a\cdot[\text{ISI}_{\text{Matsuda}}]^b$ was progressively closer to the origin of the graph from NGT to DM.

Identification of variables related to deviation from the hyperbolic regression in subjects with NGT

FPG, 2hPG and BMI but not age showed significant inverse correlation with orthogonal distance from the regression line obtained by SMA regression. However, the variability of 2hPG during 75 g OGTT is substantially greater than that of FPG [32], so that 2hPG was considered to be unsuitable as a variable to classify subjects with NGT. In multivariate analysis, both FPG

Fig. 1 Correlation between Si and β in each group in Analysis I. The curvilinear element is the best-fit regression line for $[\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}]=a\cdot[\text{ISI}_{\text{Matsuda}}]^b$ obtained from all values in each group by standardized major axis regression. To facilitate visual comparison across the groups, graphs with the same y (0-4) and x (0-70) axes for all groups are shown. Accordingly, symbols for the individuals with $\beta>4.0$ and/or $\text{Si}>70$ in metric units ($n=6$; 5 in NGT and 1 in NDH) are not shown.

Fig. 2 Distance from the regression line in NGT subgroups. Values are mean+SD. Orthogonal distance from the regression line to each data point was calculated by the following formula.

$$\sqrt{\left[\frac{x_i - \left(\frac{x_i + y_i}{b} \right) - \log(a)}{b + \frac{1}{b}} \right]^2 + \left[\frac{y_i - \frac{x_i + by_i + b \cdot \log(a)}{b + \frac{1}{b}} + \log(a)}{b + \frac{1}{b}} \right]^2}$$

where x_i , y_i , b and $\log(a)$ were $\log[\text{ISI}_{\text{Matsuda}}]$, $\log[\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}]$, the slope and intercept of SMA regression, respectively. A. FPG tertile; FPG1, NGT_{FPG1}; FPG2, NGT_{FPG2}; FPG3, NGT_{FPG3}. B. BMI tertile; BMI1, NGT_{BMI1}; BMI2, NGT_{BMI2}; BMI3, NGT_{BMI3}. *, significantly different from FPG2 and FPG3, and †, significantly different from BMI3 (ANOVA followed by Scheffe test).

($p < 0.001$) and BMI ($p < 0.001$) were independently correlated with distance from the regression line. On the basis of this finding, subjects with NGT were subdivided by FPG or BMI tertile. As expected, the distance from the regression line in the lowest tertile was significantly larger than in the higher tertiles in the case of both FPG and BMI (Fig. 2).

Analysis II

Characteristics of subjects in NGT subgroups

The mean 2hPG was lowest in NGT_{FPG1} and progressively higher in NGT_{FPG2} and NGT_{FPG3} (Table 2). On the contrary, the mean values for $\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}$, $\text{ISI}_{\text{Matsuda}}$ and DI_O were highest in NGT_{FPG1} and progressively lower with elevation of FPG. The mean age and BMI were lowest in NGT_{FPG1} and progressively higher in NGT_{FPG2} and NGT_{FPG3}. The difference was statistically significant except for age (Table 2).

Both FPG and 2hPG were progressively higher from NGT_{BMI1} to NGT_{BMI3}. $\text{ISI}_{\text{Matsuda}}$ and DI_O were highest in NGT_{BMI1} and progressively lower in NGT_{BMI2} and NGT_{BMI3}. The differences in these variables across the 3 groups were statistically significant (Table 2). But there were no significant differences in age or $\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}$.

The larger coefficient of variation (SD/mean) for DI_O in the lowest FPG and BMI tertile compared with the respective higher tertiles indicates successful segregation of subjects with greater deviation from the regression by FPG and BMI tertiles (Table 2).

Correlation between Si and β

Statistically significant bivariate correlation between $\text{ISI}_{\text{Matsuda}}$ and $\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}$ was present in NGT_{FPG3} and NGT_{FPG2} but not in NGT_{FPG1} (Table 2). The correlation between the two variables was statistically significant in NGT_{BMI3} but not in NGT_{BMI2} or NGT_{BMI1} (Table 2). The correlation between $\text{ISI}_{\text{Matsuda}}$ and the Stumvoll index was significant ($p < 0.001$ for all groups) in all FPG tertiles with Spearman's rho being smallest in NGT_{FPG1}, i.e., -0.534 in NGT_{FPG3}, -0.606 in NGT_{FPG2} and -0.451 in NGT_{FPG1}. The correlation between $\text{ISI}_{\text{Matsuda}}$ and the Stumvoll index was significant in NGT_{BMI3} ($\rho = -0.493$, $p < 0.001$) and NGT_{BMI2} ($\rho = -0.450$, $p < 0.001$) but not in NGT_{BMI1} ($\rho = -0.163$, $p = 0.094$). Bivariate correlation between $\text{ISI}_{\text{Matsuda}}$ and $\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}$ was stronger in the 3rd FPG and BMI tertiles than in the entire NGT population as indicated by the greater rho in the 3rd tertiles: the difference was not statistically significant though.

Using SMA regression, statistically significant regression was obtained in NGT_{FPG3}, NGT_{FPG2} and NGT_{BMI3} (Table 2). The parameters of SMA regression for $\text{ISI}_{\text{Matsuda}}$ to $\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}$, $\log[\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}] = \log[(a) + b \cdot \log[\text{ISI}_{\text{Matsuda}}]]$, were as follows (Table 2). b was -1.184 in NGT_{FPG2} and -1.334 in NGT_{FPG3}, values which were not significantly different, and the common slope was $b = -1.253$. a by respective SMA regres-

Table 2 Characteristics of the participants and Si- β correlation and regression in Analysis II (NGT subgroup analysis)

Variable	FPG tertiles			BMI tertiles		
	NGT _{FPG1} (n=103)	NGT _{FPG2} (n=118)	NGT _{FPG3} (n=107)	NGT _{BMI1} (n=106)	NGT _{BMI2} (n=103)	NGT _{BMI3} (n=109)
M/F	56/47	88/30	83/24	68/38	68/35	85/24
Age, yr	49.3 $\pm$ 11.7	50.6 $\pm$ 10.5	52.3 $\pm$ 11.0	50.3 $\pm$ 12.0	51.5 $\pm$ 10.1	50.3 $\pm$ 10.6
BMI*, kg/m ²	22.3 $\pm$ 2.9 (n=100)	23.2 $\pm$ 2.9 (n=116)	24.0 $\pm$ 3.0 (n=102)	20.0 $\pm$ 1.4	23.0 $\pm$ 0.7	26.4 $\pm$ 1.8
FPG, mM	4.7 $\pm$ 0.2	5.2 $\pm$ 0.1	5.7 $\pm$ 0.2	5.1 $\pm$ 0.4	5.2 $\pm$ 0.4	5.3 $\pm$ 0.4
2hPG, mM	5.4 $\pm$ 1.1	5.8 $\pm$ 1.0	6.1 $\pm$ 0.9	5.5 $\pm$ 1.0	5.9 $\pm$ 1.0	6.0 $\pm$ 1.0
FIRI, pM	31.3 $\pm$ 18.1	34.0 $\pm$ 22.9	37.5 $\pm$ 23.6	22.5 $\pm$ 13.0	35.4 $\pm$ 18.5	45.2 $\pm$ 25.6
2hIRI, pM	163.9 $\pm$ 139.6	197.9 $\pm$ 148.6	221.5 $\pm$ 166.7	123.1 $\pm$ 72.6	205.7 $\pm$ 151.6	258.1 $\pm$ 185.0
δ IRI ₀₋₃₀ / δ PG ₀₋₃₀ [†]	1.00 $\pm$ 1.07	0.76 $\pm$ 0.60	0.62 $\pm$ 0.86	0.75 $\pm$ 0.75	0.74 $\pm$ 0.61	0.89 $\pm$ 1.16
ISI _{Matsuda} [†]	17.3 $\pm$ 12.1	14.4 $\pm$ 13.3	10.6 $\pm$ 6.7	20.4 $\pm$ 14.7	12.1 $\pm$ 7.7	9.5 $\pm$ 7.0
DI _O	15.9 $\pm$ 18.5	9.1 $\pm$ 9.2	5.6 $\pm$ 7.0	14.7 $\pm$ 17.5	8.6 $\pm$ 10.0	7.2 $\pm$ 9.2
Spearman's rank correlation						
rho	-0.129	-0.383	-0.344	-0.044	-0.170	-0.313
p	0.1926	<0.001	<0.001	0.654	0.086	0.001
Standardized major axis regression						
a (95%CI)	N.A.	9.557 (5.686~16.131)	7.845 (4.494~13.693)	N.A.	N.A.	11.112 (6.315~19.531)
b (95%CI)	N.A.	-1.184 (-1.408~-0.997)	-1.334 (-1.599~-1.114)	N.A.	N.A.	-1.426 (-1.707~-1.191)
r ²	N.A.	0.118	0.119	N.A.	N.A.	0.108
p	N.A.	<0.001	<0.001	N.A.	N.A.	<0.001

Plus-minus values are mean $\pm$ SD. Abbreviations are the same as in Table 1. *, BMI data is missing in 10 subjects so that the number of subjects with BMI value is indicated in the parentheses for FPG tertiles; [†], conventional units were used for δ IRI₀₋₃₀/ δ PG₀₋₃₀ and ISI_{Matsuda}, [μ U/mL]/[mg/100 mL] and [μ U/mL]⁻¹·[mg/100 mL]⁻¹, respectively, as in the original reports [22, 24]. In FPG tertiles, by Kruskal-Wallis test, BMI, 2hIRI, δ IRI₀₋₃₀/ δ PG₀₋₃₀, ISI_{Matsuda} and DI_O in FPG tertiles were all significantly different (p <0.001 for all variables except for 2hPG for which p =0.003). In BMI tertiles, FPG, 2hPG, FIRI, 2hIRI, ISI_{Matsuda} and DI_O were significantly different (p <0.001 for all variables).

sion was 9.557 in NGT_{FPG2} and 7.845 in NGT_{FPG3}. a with the common slope, b =-1.253, was 11.314 and 6.573 for NGT_{FPG2} and NGT_{FPG3} respectively, and the values were significantly different (p <0.001). The finding is compatible with DI_O being lower in the latter than in the former (Table 2). The parameters of regression in NGT_{BMI3} were as follows: b =-1.426 and a =11.112. As shown in Fig. 3A, the best-fit regression line for [δ IRI₀₋₃₀/ δ PG₀₋₃₀]= a ·[ISI_{Matsuda}] ^{b} in NGT_{BMI3} lies relatively close to the origin (Fig. 3).

Discussion

In this study, we confirmed the fundamental characteristic of the Si- β correlation across glucose tolerance categories, i.e., progressive left and downward shifting of the hyperbolic regression curve and lowering of DI with worsening of glucose tolerance [1-4]. The novel findings in this study are as follows. Firstly, Si- β inverse correlation was strongest in NDH, intermediate in DM and weakest in NGT. The weak Si- β correlation in DM was to be expected because both insulin sensitivity and beta cell function deteriorate to vary-

ing degrees in patients with diabetes. However, the significant weakening of the Si- β correlation in NGT compared to NDH was unexpected because beta cell adaptation to altered insulin sensitivity is thought to be most robust in subjects with NGT. This was the case not only for the relationship between ISI_{Matsuda} and δ IRI₀₋₃₀/ δ PG₀₋₃₀, but also for the relationship between ISI_{Matsuda} and the Stumvoll index. If distribution of PG values, FPG and 2hPG, in subjects with NGT had been significantly wider than the distribution of the corresponding values in NDH, it would have implied that the subjects in NGT were more heterogeneous than those in NDH with respect to glucose regulation. Then, the Si- β correlation in NGT would have been weaker than that in NDH. However, the coefficient of variation for FPG and 2hPG was consistently lower in NGT than in NDH, which ruled out any such possibility (Table 1).

Secondly, we hypothesized that the Si- β correlation is very poor or insignificant in a certain group of subjects with NGT, thereby making the correlation in the entire NGT group weak. Using orthogonal distance from the regression line as a quantitative index of deviation, we found that lower BMI and FPG were inde-

Fig. 3 Correlation between Si and β in each group in Analysis II (NGT subgroup analysis).

A, FPG tertile; B, BMI tertile. The curvilinear element is the best-fit regression line for $[\delta IRI_{0-30}/\delta PG_{0-30}] = a \cdot [ISI_{Matsuda}]^b$ obtained from all values in each group by standardized major axis regression. There was no significant bivariate correlation between Si and β in NGT_{FPG1} , NGT_{BMI1} and NGT_{BMI2} . To facilitate visual comparison across the groups, graphs with the same y (0–1.2) and x (0–70) axes for all groups are shown. Accordingly, symbols for the individuals with $\beta > 1.2$ and/or $Si > 70$ in metric units are not shown: 23 in NGT_{FPG1} , 20 in NGT_{FPG2} and 9 in NGT_{FPG3} , and 19 in NGT_{BMI1} , 14 in NGT_{BMI2} and 19 in NGT_{BMI3} .

pendently associated with the deviation. Moreover, any significant inverse Si- β correlation in the lowest BMI tertile was found to have disappeared. This was also the case not only for the relationship between $ISI_{Matsuda}$ and $\delta IRI_{0-30}/\delta PG_{0-30}$, but also for the relationship between $ISI_{Matsuda}$ and the Stumvoll index.

$ISI_{Matsuda}$ contains $1/\sqrt{FIRI}$, $1/\sqrt{2hPG}$ and $1/\sqrt{2hIRI}$, so that it is not surprising that it correlates more robustly with the Stumvoll index than $\delta IRI_{0-30}/\delta PG_{0-30}$. The fading of any significant Si- β inverse correlation in the lowest FPG tertile was not as clear as that in the lowest BMI tertile because the correla-

tion was significant when the Stumvoll index was used in the place of $\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}$. Lower BMI in lower NGT tertiles may be an important factor in the weakening of the Si- β inverse correlation. Nevertheless, it is of note that the Si- β correlation in NDH subjects ($n=49$) with BMI values matched to those in NGT_{BMI1} was highly significant ($p=0.003$), indicating that lowest BMI tertile and NGT are required for diminution of the Si- β correlation. In other words, for increased insulin secretion to occur, relatively high BMI or relatively high glycemia may be needed.

Lastly, in all groups with significant inverse correlation between Si and β , 95% CI of the slope b of $[\beta]=a\cdot[\text{Si}]^b$ exceeded -1, except for NGT_{FPG2} in which -1 was included in the 95% CI of b . We consider that the Si- β regression is generally not significantly different from the hyperbola because the upper limit of 95% CI did not exceed -2.

The weak Si- β correlation in NGT subjects may be due to the presence of a confounding factor or factors which favorably affect glucose metabolism. Glucose effectiveness (Sg) and the incretin effect may be such factors [1]. Sg is the mass action of glucose itself [1, 33], i.e., insulin-independent, glucose-mediated, glucose disposal. Among subjects with NGT, stronger Sg is associated with lower glycemia, larger β , and higher Si [34]. With stronger Sg, PG is lower at a given level of IRI, and overestimation of Si would ensue. Incretins are gut-derived peptides such as glucagon-like peptide-1, which augment insulin secretion in a glucose-dependent manner [35]. The incretin effect is progressively stronger with lower FPG in subjects with NGT [36]. A stronger incretin effect may result in overestimation of β . Notably, incretin enhances Sg [37], and therefore the two are synergistic. Taken together, both Sg and the incretin effect may be strong in a certain number of subjects with NGT, so that the statistical significance of the Si- β correlation in NGT as a whole was weak.

There are several limitations in this study. Validation of the current finding by direct measurement of Si

and β with intravenous glucose injection [33] is critically needed because use of $[\text{ISI}_{\text{Matsuda}}]$ and $[\delta\text{IRI}_{0-30}/\delta\text{PG}_{0-30}]$ for analysis of the hyperbolic law has not been performed. The correlation of the indices of Si and β employed in this study, which were obtained by OGTT, with the actual values obtained by direct insulin clamp technique and intravenous glucose tolerance test, respectively, may not be very strong in the individuals with extraordinarily high Si and β . Ethnic diversity may play a role with Japanese subjects being leaner and more insulin sensitive [5, 15, 19, 38, 39 and current data] than subjects in Caucasian [6-8, 10-14, 17, 18] at a comparable level of glucose regulation. In previous studies of non-diabetic subjects, the number of participants with a mean BMI similar to the value in NGT_{BMI1} in this study might have been too small to form a single group. At any rate, similar analyses in other ethnic groups are warranted. Also, analysis on the larger number of subjects is needed to definitively establish the current findings.

In conclusion, we found that the hyperbolic correlation between Si and β is weaker in NGT than in NDH, and that it fades away in NGT subjects belonging to the lowest FPG or BMI tertile. The finding strongly suggests that a certain factor, such as Sg, may be a more dominant determinant of glucose metabolism rather than insulin secretion and action, and diminution of Sg might be the most proximal event in diabetes evolution.

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Disclosures

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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