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Impact of one-hour postchallenge glucose on the relationship between insulin sensitivity and secretion

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Abstract. The impact of postchallenge glucose on the relationship between insulin sensitivity (SI) and secretion (β) is unknown. We analyzed data from 2,264 health examinees (male/female 1,524/740, median age 54 yrs) with normal glucose tolerance (NGT, *n* = 1,623), non-diabetic hyperglycemia (NDH, *n* = 555), or diabetes (DM, *n* = 86) using OGTT-derived indices of SI (insulin sensitivity index [ISI]-Matsuda, 1/HOMA-IR, and 1/fasting IRI) and β ($\delta IRI_{0\text{-}30}/\delta PG_{0\text{-}30}$, and Stumvoll 1st [Stumvoll-1] and 2nd [Stumvoll-2] phases). The combination of 1/HOMA-IR and Stumvoll-1 recapitulated the hyperbolic SI-β relationship with the slope of the fitted line –1.000 in NGT subjects, and therefore it was utilized in the following analysis of the SI-β correlation. In multiple regression analysis of the relationship between SI and β, an independent correlation was found for 1 h-plasma glucose (PG; PG_{60}) but not for 2 h-PG. When the NGT subjects were grouped by PG_{60} quartile (Q), the fitted line was flat in Q1 but progressively steeper from Q2 to Q4, with a slope (95%CI) of –0.663 (–0.726~–0.605), –0.680 (–0.745~–0.622), –0.847 (–0.922~–0.779), and –1.259 (–1.370~–1.158) (*P* for trend < 0.05). The fitted line steepened further in the NDH and DM groups, with a slope of –1.545 and –1.915, respectively (*P* < 0.01 for the difference). The intercept of the fitted line for SI-β correlation was also progressively lower across the PG_{60} Q for NGT, NDH, and DM. In conclusion, using the 1/HOMA-IR-Stumvoll-1 pair for an analysis of the SI-β relationship, elevated PG_{60} was associated with steepening and downward shifting of the fitted line for the SI-β correlation. The finding suggests impaired beta cell function.

Key words: Insulin sensitivity, Insulin secretion, OGTT, Postchallenge glucose

IN SUBJECTS with normal glucose metabolism, attenuation of insulin sensitivity (SI) is counterbalanced by increased insulin secretion (β) so that glucose homeostasis is maintained. A curvilinear relationship between SI and β was found in an apparently healthy population by Bergman *et al*. [1]. Subsequently, Kahn *et al*. [2] demonstrated that the function between SI and β was hyperbolic by determining SI with glucose clamp and β with acute insulin response (AIR) on intravenous glucose injection. It should be noted that subjects who, according to current criteria, would be defined as impaired glucose tolerance (IGT), impaired fasting glycemia (IFG) and 'diabetes diagnosed by plasma glucose (PG) at 2h on the oral glucose tolerance test (OGTT) (2hPG)' were not necessarily excluded in the earlier studies [1-3]. Among OGTT-derived indices, Retnakaran *et al*. [4] found hyperbolic function between Matsuda index and the 'ratio of total area-under the-insulin-curve to area-under-the-glucose-curve ($AUC_{inc/glu}$)' but not between other SI-β combinations in subjects with normal glucose tolerance (NGT). In other studies with NGT subjects, a hyperbolic relationship between the clamp-based whole body SI and iv glucose-induced AIR [5] and between indices of hepatic SI and insulinogenic index ($\delta IRI_{0\text{-}30}/\delta PG_{0\text{-}30}$) [6] have been found. Quasi-hyperbolic function between indices of whole body SI and insulinogenic index has also been reported in NGT subjects [7, 8].

A direct comparison of SI-β pairs using a variety of OGTT-derived indices of SI and β [9, 10] has rarely been carried out [4]. Consequently, it has not as yet been possible to establish which combination of OGTT-derived indices of SI and β faithfully reca-

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pitulates the hyperbolic function demonstrated by the direct glucose infusion technique [1-3, 5, 10, 11]. Nor has the influence of glycemia [1, 5, 7] on the SI-β relationship been fully elucidated.

Against this background, we analyzed the relationship between SI and β using the data from 2,264 middle-aged Japanese comprising subjects with NGT, IFG, IGT, and newly diagnosed diabetes. We employed three OGTT-derived indices of SI, *i.e.*, $ISI_{Matsuda}$ (index of whole body SI) [12, 13], 1/fasting immunoreactive insulin (FIRI) [14] and 1/HOMA-IR [15, 16] (indices of hepatic SI). At the same time, three measures of β, insulinogenic index ($\delta IRI_{0\text{-}30}/\delta PG_{0\text{-}30}$) (an index of early phase glucose-stimulated insulin secretion (GSIS)) [17] and Stumvoll 1st phase (index of AIR) and 2nd phase (Stumvoll-2) [18] of GSIS were calculated. Firstly, we determined which SI-β relationship best recapitulated the hyperbolic function. The data from a totally independent group of NGT subjects [7] were used to validate the results of this part of the study. Secondly, we investigated how the relationship between SI and β was influenced by the level of post-challenge glucose.

Materials and Methods

Diagnosis of glucose tolerance

Diagnosis of glucose tolerance was made on the basis of WHO criteria [19]. Namely, NGT was defined as fasting plasma glucose (FPG) <6.1 mmol/L and 2hPG <7.8 mmol/L; IFG as FPG ≥6.1 mmol/L but <7.0 mmol/L and 2hPG <7.8 mmol/L; IGT as FPG <7.0 mmol/L and 2hPG ≥7.8 mmol/L but <11.1 mmol/L; and diabetes mellitus (DM) as FPG ≥7.0 mmol/L and/or 2hPG ≥11.1 mmol/L.

Study subjects

The analysis was conducted using two datasets. One was provided by the Health Service Department of Hokuriku Central Hospital, where public school employees receive annual medical checkups. The subjects were defined as Hokuriku Cohort hereafter. The study group consisted of 2,264 consecutive individuals receiving the checkup, which included a 75 g OGTT with IRI measurement, between April 2006 and March 2010. None had a history of DM or gastrectomy, and nor were any of the subjects taking glucocorticoid or anticancer drugs [20]. One thousand six hundred and twenty-three were NGT, 555 had non-diabetic hyperglycemia (NDH) and 86 DM. The other dataset was from Chikuma Central Hospital [7] health examinees, which is defined as Chikuma Cohort hereafter. Only NGT subjects of Chikuma Cohort were analyzed in this study. All of them were Japanese. Signed informed consent was obtained from all subjects, and the hospital review board of Aizawa Hospital approved the study protocol.

Measurements and calculation of indices

For Hokuriku Cohort, PG was analyzed by the glucose oxidase method (Automatic Glucose Analyzer ADAMS Glucose GA-1160, Arkray, Kyoto) and IRI by the chemiluminescence method (ADVIA Centaur, Siemens Medical Solution) [21]. IRI was lower than the assay limit, 0.4 μU/mL (2.8 pmol/L) in 32 samples (28, 3 and 1 at fasting, 30 and 120 min during OGTT, respectively) and 0.2 μU/mL (1.4 pmol/L) was used as a substitute for these values. During 75 g OGTT, PG was measured at 0, 30, 60 and 120 min and IRI at 0, 30 and 120 min: they were abbreviated as FPG, PG_{30}, PG_{60} and 2hPG, and FIRI, IRI_{30} and 2hIRI. Detail of the measurement in Chikuma Cohort was described elsewhere [7].

The following indices were utilized in this study. $ISI_{Matsuda}$, an index of whole body SI, was calculated using the fasting and 2-h blood samples at OGTT: $ISI_{Matsuda}$ = 10,000/[sqrt(FPG·2hPG·FIRI·2hIRI)] [12, 13]. 1/FIRI [14] and 1/HOMA-IR [15, 16], which are indices of primarily hepatic SI, were also employed. HOMA-IR was calculated as [FPG·FIRI]/405 [15]. As an index of early phase GSIS, insulinogenic index [17] was utilized. For $ISI_{Matsuda}$, 1/FIRI and insulinogenic index, the units used for PG and IRI were mg/dl and μU/mL, respectively [12, 13]. Stumvoll-1 and Stumvoll-2 were used as indices of AIR and 2nd phase GSIS, respectively: Stumvoll-1 = $1283 + 1.829 \cdot IRI_{30} - 138.7 \cdot PG_{30} + 3.772 \cdot FIRI$ and Stumvoll-2 = $287 + 0.4164 \cdot IRI_{30} - 26.07 \cdot PG_{30} + 0.9226 \cdot FIRI$, the units adopted for IRI and PG being pmol/L and mmol/L, respectively [18]. Negative or unusable values for insulinogenic index and negative values for Stumvoll-1 and Stumvoll-2 were obtained in 111 (5%), 98 (4%) and 14 (1%) subjects, respectively, and the values for these results were assumed absent.

Analysis of the SI-β relationship

Three indices for SI ($ISI_{Matsuda}$, 1/FIRI and 1/HOMA-IR) and three indices for β (insulinogenic

index, Stumvoll-1 and Stumvoll-2) were employed in order to evaluate the SI-β relationships for the nine pairs of SI and β. The best-fit regression line for SI and β was obtained after $\log_{10}$ transformation of the data: $\log_{10}(\beta) = a \cdot \log_{10}(SI) + b$, which is equivalent to $\beta = 10^b \cdot (SI)^a$. Because there were measurement errors for both the *x* and *y* axis, fitting was performed by standardized major axis regression (SMA) [22], using SMATR version 2.0 [23]. This enabled us to make a pair-wise statistical comparison of the slope values for multiple groups by using the likelihood ratio test [24]. Because the program does not provide *p* values for the difference of intercept, *P* was judged to be <0.05 and <0.01 if 95%CI and 99%CI, respectively, did not overlap.

To examine the effects of possible confounding factors on the SI-β relationship, a multiple regression analysis was performed by taking $\log_{10}$(Stumvoll-1) as a dependent variable and $\log_{10}$(1/HOMA-IR) as an explanatory variable, and gender, BMI, FPG, PG_{30}, PG_{60}, 2hPG and HbA1c as covariates.

Statistics

Kruskal-Wallis test, Spearman's rank correlation, Mann-Whitney U test, chi square test, Steel-Dwass tests and multiple regression analysis were used as needed. SMA was performed by using SMATR version 2.0 [23]. $P < 0.05$ was considered significant except for multiple comparisons in which the Holm correction of *P* value was used. SPSS version 21.0 and JMP version 11.2 were used for analysis.

Results

Characteristics of the study population

The characteristics of Hokuriku Cohort are shown in Table 1. As a group, the participants were middle-aged, non-obese and male-dominant. The vast majority were NGT. The characteristics of Chikuma Cohort were reported elsewhere [7]. As a group, they were also middle-aged, non-obese and male-dominant.

Table 1 Characteristics of study subjects in Hokuriku Cohort

Variable	NGT	Non-diabetic hyperglycemia			DM
		iIFG	iIGT	IFG/IGT	
n	1,623	123	319	113	86
Age, yr	53(47-59)	54(48-59)	55(50-59)	56(50-59)	53(50-59)
Male/Female	1,061/562	105/18	210/109	86/27	62/24
Family Hx of diabetes, +/-	295/1328	39/84	74/245	32/81	26/60
BMI, kg/m^2	23.2(21.5-25.2)	24.3(22.3-25.9)	24.4(22.3-26.6)	24.7(23.1-27.5)	25.2(22.5-27.5)
FPG, mmol/L	5.3(5.0-5.6)	6.3(6.2-6.5)	5.5(5.2-5.8)	6.4(6.2-6.6)	6.3(5.8-7.0)
PG_{60}, mmol/L	7.4(6.0-8.9)	10.2(8.3-11.4)	10.2(9.0-11.4)	11.9(10.4-12.9)	13.5(12.3-14.6)
2hPG, mmol/L	5.9(5.2-6.7)	6.5(5.4-7.0)	8.6(8.1-9.2)	8.9(8.3-10.0)	11.8(11.3-12.9)
FIRI, pmol/L	26(19-35)	29(22-37)	29(21-42)	28(22-50)	33(21-46)
2hIRI, pmol/L	149(97-228)	148(105-242)	272(172-427)	258(159-417)	302(222-490)
HbA1c, %	5.6(5.4-5.8)	5.9(5.6-6.1)	5.8(5.5-6.0)	6.0(5.7-6.2)	6.2(5.9-6.5)
$ISI_{Matsuda}$	11.5(7.9-16.6)	9.8(6.5-13.9)	6.5(4.2-9.2)	5.2(3.4-8.3)	4.6(2.9-6.3)
1/HOMA-IR	1.15(0.83-1.55)	0.84(0.66-1.14)	0.98(0.67-1.38)	0.77(0.44-1.09)	0.78(0.51-1.12)
1/FIRI	0.29(0.21-0.38)	0.24(0.19-0.32)	0.24(0.16-0.33)	0.21(0.12-0.31)	0.21(0.15-0.33)
Stumvoll-1	711.1(501.6-956.6) (*n* = 1,605)	346.4(169.3-638.4) (*n* = 105)	491.9(321.3-706.1) (*n* = 305)	336.9(170.4-644.8) (*n* = 93)	290.2(169.2-426.2) (*n* = 58)
Stumvoll-2	200.0(154.7-250.2) (*n* = 1,622)	122.0(77.1-167.4) (*n* = 120)	154.2(115.0-203.8) (*n* = 318)	115.6(68.0-181.9) (*n* = 110)	105.6(61.2-134.5) (*n* = 80)
Insulinogenic index	0.56(0.33-0.98) (*n* = 1,531)	0.28(0.16-0.52) (*n* = 117)	0.36(0.23-0.53) (*n* = 319)	0.27(0.17-0.49) (*n* = 109)	0.19(0.11-0.28) (*n* = 85)

Numerical data is the median (25-75 percentile). NGT, normal glucose tolerance; iIFG, isolated impaired fasting glucose; iIGT, isolated impaired glucose tolerance; IFG/IGT, IFG and IGT; DM, diabetes. FPG, fasting plasma glucose; PG_{60} and 2hPG, PG 1-h and 2-h, respectively, after oral intake of 75 g glucose; FIRI, fasting immunoreactive insulin; 2hIRI, IRI 2h after oral intake of 75 g glucose; HbA1c, glycohemoglobin; $ISI_{Matsuda}$, Matsuda insulin sensitivity index; Stumvoll-1, Stumvoll index of first-phase insulin secretion; Stumvoll-2, Stumvoll index of second-phase insulin secretion. Regarding HbA1c, the Japan Diabetes Society (JDS) value was converted to NGSP and IFCC values (http://www.ngsp.org/docs/IFCCstd.pdf). The number of subjects was smaller than the total number for Stumvoll-1, -2 and insulinogenic index due to minus values in some participants for these indices. All numerical and categorical variables were significantly different ($P < 0.01$) between the groups by Kruskal-Wallis or chi square tests. See Text for the detail.

Table 2 Characteristics of SI-β relationship, in Hokuriku Cohort, as indexed by Spearman's *rho* and slope and *R* values of the best-fit regression line obtained by standardized major axis regression

Secretion index, β	Sensitivity index, SI	Spearman's rank correlation		Standardized major axis regression				
		rho	*P*	Slope	95%CI		*R*	*P*
					Lower	Upper		
Stumvoll-1	1/HOMA-IR	-0.462	< 0.01	-1.000	-1.047	-0.955	-0.335	< 0.01
	$ISI_{Matsuda}$	-0.293	< 0.01	-0.995	-1.043	-0.949	-0.261	< 0.01
	1/FIRI	-0.412	< 0.01	-1.028	-1.075	-0.982	-0.370	< 0.01
Stumvoll-2	1/HOMA-IR	-0.505	< 0.01	-0.688	-0.719	-0.658	-0.394	< 0.01
	$ISI_{Matsuda}$	-0.343	< 0.01	-0.686	-0.718	-0.655	-0.322	< 0.01
	1/FIRI	-0.457	< 0.01	-0.707	-0.739	-0.677	-0.427	< 0.01
Insulinogenic index	1/HOMA-IR	-0.323	< 0.01	-1.476	-1.550	-1.406	-0.217	< 0.01
	$ISI_{Matsuda}$	-0.214	< 0.01	-1.483	-1.557	-1.412	-0.176	< 0.01
	1/FIRI	-0.290	< 0.01	-1.515	-1.591	-1.444	-0.237	< 0.01

Abbreviations are the same as in Table 1. See Text for the detail.

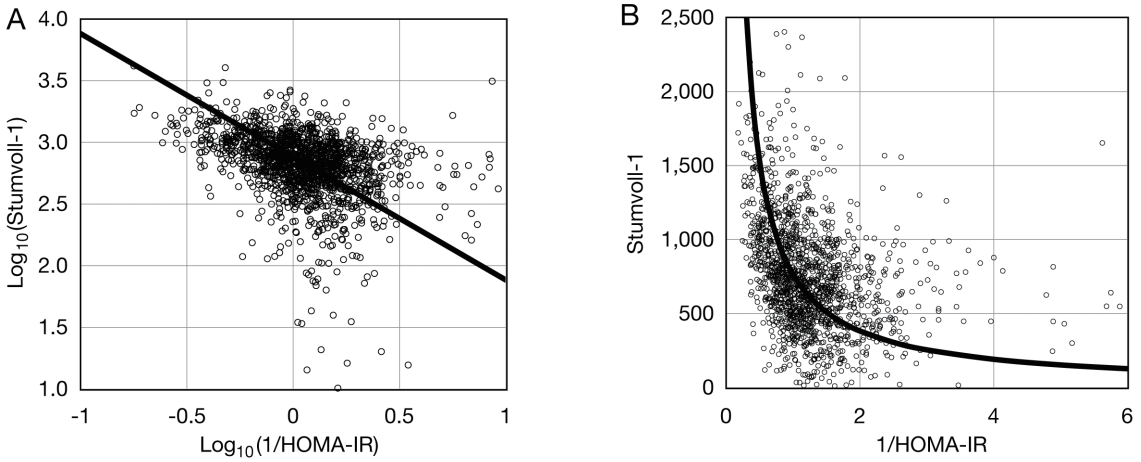

Fig. 1 Relationship of 1/HOMA-IR and Stumvoll-1 in subjects with normal glucose tolerance (n = 1,605). Individual plots and the best-fit regression lines are shown. The equation of the regression line was $\log_{10}(\text{Stumvoll-1}) = -1.000 \cdot \log_{10}(1/\text{HOMA-IR}) + 2.883$ for $\log_{10}$ transformed data (A, $R = -0.315$, $P < 0.01$) and equivalently, $\text{Stumvoll-1} = 10^{2.883} \cdot (1/\text{HOMA-IR})^{-1.000}$ for raw data (B). The ordinate and the abscissa were truncated so that 22 individuals (1.4%) in A and 24 individuals (1.5%) in B lie outside the figure. Note that the graph is not isometric in panel A.

Comparison of SI-β correlation in the nine SI and β pairs in subjects with NGT

Initially, the data from Hokuriku Cohort was analyzed. The bivariate correlation between the nine SI and β pairs was ascertained by Spearman's rank correlation (Table 2). There was significant inverse correlation between all pairs, with *rho* values being -0.214 to -0.505. The slope values of the best-fit regression lines for SI-β correlation obtained by SMA were variable between the nine pairs. For '1/HOMA-IR and Stumvoll-1', '$ISI_{Matsuda}$ and Stumvoll-1' and '1/FIRI and Stumvoll-1', the slope values did not differ significantly from -1 and therefore the SI-β relationship was considered to be hyperbolic. Whereas, the fitted lines for '$ISI_{Matsuda}$ and Stumvoll-2', '1/HOMA-IR and Stumvoll-2' and '1/FIRI and Stumvoll-2' were relatively flat and the slope values were significantly greater than -1. In contrast, the slope values were significantly smaller than -1 for '$ISI_{Matsuda}$ and insulinogenic index', '1/HOMA-IR and insulinogenic index' and '1/FIRI and insulinogenic index'. These findings indicated that the fitted regression lines were steeper than hyperbolic function for these combinations. In the next analysis in which the data from Chikuma Cohort was evaluated, the results were qualitatively the same as in Hokuriku Cohort (Supplemental Table).

The 1/HOMA-IR and Stumvoll-1 pair (Fig. 1) was chosen as the index of SI and β to be employed for

Table 3 Effect of possible confounders on the relationship between 1/HOMA-IR and Stumvoll-1 in subjects with NGT

Variable	Partial regression coefficient	95%CI		Standardized partial regression coefficient	*P*	VIF
		Lower	Higher			
Explanatory variable						
Log_{10}(1/HOMA-IR)	-0.375	-0.414	-0.336	-0.378	< 0.01	1.32
Covariates						
Male gender	-0.030	-0.051	-0.009	-0.054	< 0.01	1.18
BMI, kg/m^2	0.018	0.014	0.022	0.197	< 0.01	1.36
FPG, mmol/L	-0.090	-0.120	-0.060	-0.123	< 0.01	1.41
PG_{30}, mmol/L	-0.047	-0.055	-0.038	-0.274	< 0.01	2.15
PG_{60}, mmol/L	-0.048	-0.054	-0.041	-0.358	< 0.01	2.19
2hPG, mmol/L	0.008	-0.002	0.018	0.031	0.11	1.21
HbA1c, %	-0.022	-0.055	0.010	-0.026	0.17	1.21

Multiple regression analysis was performed with log_{10}(Stumvoll-1) as a dependent variable and log_{10}(1/HOMA-IR) as an explanatory variable. $PG_{30\ and}$ PG_{60}, plasma glucose at 30 and 60 min, respectively, during 75 g OGTT. VIF, variance inflation factor. Multiple collinearity was not a problem because VIF was not large. Regarding HbA1c, the Japan Diabetes Society (JDS) value was converted to an NGSP value (http://www.ngsp.org/docs/IFCCstd.pdf). See Text for the detail.

analysis because the slope value of the best-fit regression line between the two variables was -1, implying that the relationship was hyperbolic, and the correlation between the two variables was relatively strong among the nine pairs (Table 2). Nevertheless, qualitatively similar results were obtained by using $ISI_{Matsuda}$ in place of 1/HOMA-IR in all of the following analysis (data not shown).

Analysis of the impact of PG_{60} on the SI-β relationship

In multiple regression analysis between SI and β, 1h-PG (PG_{60}) showed an independent correlation after adjustment for gender and BMI (Table 3). On the other hand, 2hPG and HbA1c were not significant confounders. Accordingly, subjects with NGT were divided into 4 groups on the basis of the PG_{60} quartile (Q). The grouping disrupted the hyperbola, and the fitted line for SI-β correlation was very flat in Q1, and then progressively steeper in the group of subjects belonging to PG_{60} Q2 to Q4 (Table 4 and Fig. 2A). Actually, the SI-β relationship was not hyperbolic in any Q: the slope value of the best-fit regression line was significantly larger than -1 in Q1, Q2 and Q3, and significantly smaller than -1 in Q4. The intercept of the fitted line, median 1-HOMA-IR and Stumvoll-1 were all progressively lower from Q1 to Q4, with the lowering of intercept and Stumvoll-1 much pronounced compared to the lowering of 1/HOMA-IR, whereas, *R* was progressively larger from Q1 to Q4. Median FPG and HbA1c were slightly but progressively higher from Q1 to Q4: FPG 5.1, 5.2, 5.3 ad 5.4 mmol/L, and HbA1c 5.4%, 5.5%, 5.6% and 5.7%, respectively (P <0.01 for both).

The male/female ratio and BMI became progressively higher from Q1 to Q4 (Table 4). However, in the subgroup of subjects in Q4 who were gender- and BMI-matched to Q1 (n = 132, male/female 67/65, and median BMI and PG_{60}, 23.0 kg/m^2 and 10.1 mmol/L, respectively), the slope value and intercept of the fitted line were -1.319 and 456.0, respectively. These values were not significantly different from the corresponding values from the original Q4.

In the NDH and DM groups, the slope values of the fitted lines were significantly smaller than -1, indicating that the hyperbolic function between 1/HOMA-IR and Stumvoll-1 was not present (Table 4). The slope value in the DM group was significantly smaller than that for the NDH group. The intercept of the fitted line was also significantly lower in DM than in NDH. When subjects with NDH were dichotomized by median PG_{60}, the slope value was -1.194 and -1.653 (P <0.01) in those with lower and higher PG_{60}, respectively. The intercept was also significantly lower in those with higher PG_{60}. A conventional classification of subjects with NDH into iIFG, iIGT and IFG/IGT yielded no particular trend in PG_{60}, or in the slope values and intercepts of the regression lines between SI and β in the 3 subgroups (data not shown).

In the population as a whole, the best-fit regression line for the SI-β relationship became progressively steeper and the intercept of the fitted line pro-

Table 4 Impact of elevated PG_{60} on the relationship between insulin sensitivity (1/HOMA-IR) and secretion (Stumvoll-1)

Group	*n*	M/F	Slope[a] (95%CI)	Intercept[a] (95%CI)	R^a	*P*	1/HOMA-IR[a] (25-75 percentile)	Stumvoll-1[a] (25-75 percentile)	BMI[a] (25-75 percentile)
A. Entire NGT subjects Median PG_{60}, 7.4 mmol/L	1,605	1,046/559	-1.000 (-1.047 to -0.955)	764.4 (737.6 - 792.3)	-0.315	<0.01	1.15 (0.83-1.55)	711.1 (501.6-956.6)	23.3 (21.5-25.2)
Q1, PG_{60} <6.0 mmol/L Median PG_{60}, 5.2 mmol/L	409	207/202[b]	-0.663[c] (-0.726 to -0.605)	1099.0[d] (1052.0 - 1148.2)	-0.345	<0.01	1.25 (0.94-1.61)	945.2 (754.3-1160.0)	22.3 (20.7-23.9)
Q2, PG_{60} 6.0-7.3 mmol/L Median PG_{60}, 6.8 mmol/L	400	250/150	-0.680 (-0.745 to -0.622)	837.5 (798.0 - 881.0)	-0.399	<0.01	1.17 (0.86-1.60)	745.2 (574.7-949.6)	23.4 (21.5-24.9)
Q3, PG_{60} 7.4-8.9 mmol/L Median PG_{60}, 8.1 mmol/L	398	277/121	-0.847 (-0.922 to -0.779)	696.6 (662.2 - 732.8)	-0.522	<0.01	1.15 (0.78-1.54)	639.4 (495.3-828.1)	23.6 (21.7-25.4)
Q4, PG_{60} ≥9.0 mmol/L Median PG_{60}, 10.1 mmol/L	398	312/89	-1.259 (-1.370 to -1.158)	438.5 (405.5 - 473.2)	-0.524	<0.01	1.02 (0.74-1.40)	466.0 (317.0-687.3)	24.1 (22.4-26.1)
B. Entire NDH subjects Median PG_{60} 10.4 mmol/L	503	363/140	-1.545[e] (-1.677 to -1.424)	295.1[e] (266.7 - 325.8)	-0.124[e]	<0.01	0.91[e] (0.61-1.25)	431.4[e] (241.8-679.3)	24.4 (22.4-26.5)
PG_{60Low}, <10.4 mmol/L Median PG_{60} 9.0 mmol/L	253	171/82[f]	-1.194[g] (-1.337 to -1.066)	472.1[g] (426.6 - 521.2)	-0.412	<0.01	0.95[f] (0.68-1.34)	540.9[g] (370.1-783.8)	24.0[g] (22.1-26.0)
PG_{60High}, ≥10.4 mmol/L Median PG_{60} 11.7 mmol/L	250	192/58	-1.653 (-1.850 to -1.477)	182.8 (157.4 - 212.8)	-0.431	<0.01	0.88 (0.54-1.22)	313.0 (161.2-522.3)	25.0 (23.0-27.3)
C. DM Median PG_{60} 13.1 mmol/L	58	42/16	-1.915 (-2.414 to -1.520)	108.1 (77.6 - 150.7)	-0.491	<0.01	0.71 (0.46-0.99)	290.1 (169.2-426.1)	25.7 (23.8-28.3)

M/F, male/female ratio; NDH, non-diabetic hyperglycemia; DM, diabetes. Subjects with NGT were divided into 4 groups on the basis of quartile (Q) of PG at 60 min (PG_{60}). Subjects with NDH were subdivided into 2 groups on the basis of median PG_{60}. In NGT subjects, Stumvoll-1 values were significantly different between all quartiles. BMI was significantly different between all quartiles except for Q2 and Q3. [a], *p* for trend between the NGT quartiles < 0.05; [b], $P < 0.01$ between the NGT quartiles; [c], $P < 0.01$ compared to the values in Q3 and Q4; [d], $P < 0.01$ compared to the values in Q2, Q3 and Q4. 1/HOMA-IR values were significantly different between Q1 and Q3 or Q4, and between Q2 and Q4. In NDH subjects, [e], $P < 0.01$ compared to the corresponding values in the DM group; [f] and [g], $P < 0.05$ and < 0.01 (between the two NDH groups), respectively. For the sake of clarity, as the intercept value, corresponding Stumvoll-1 value at HOMA-IR = 1, which is 10^b, are shown: *b* denotes the intercept value in the equation for $\log_{10}$ transformed data. See Text for the detail.

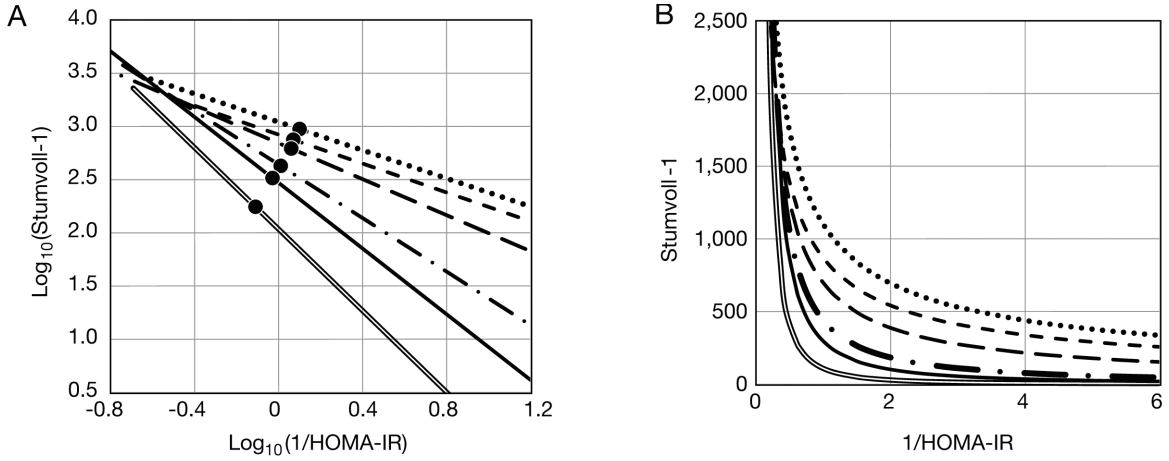

Fig. 2 The best-fit regression lines for SI (1/HOMA-IR) - β (Stumvoll-1) correlation in subjects with normal glucose tolerance (NGT) divided by 1-h PG (PG_{60}) quartile, non-diabetic hyperglycemia (NDH) and diabetes (DM). Regression lines for $\log_{10}$ transformed data (A) and raw data (B) are shown. Closed circles in panel A indicate median values for each group. Note that the graph is not isometric in panel A. ·····, NGTQ1; – – –, NGTQ2; — —, NGTQ3; · — ·, NGTQ4; ———, NDH; ═══, DM.

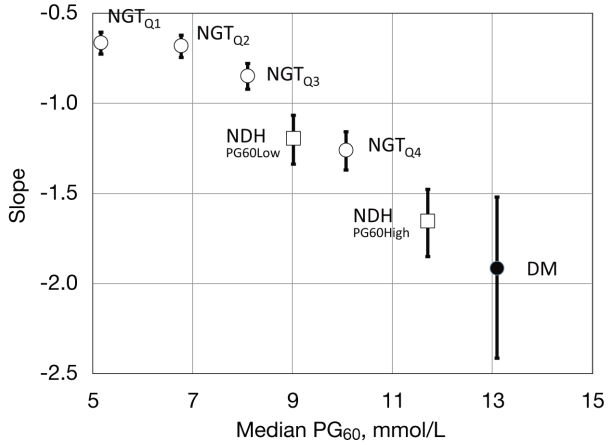

Fig. 3 Correlation between plasma glucose at 60 min (PG_{60}) for each group and slope values of the best-fit regression line. *P* for trend < 0.01. Variations are 95%CI.

gressively lower in the group with higher median PG_{60} (Fig. 3, Fig. 2A). PG_{60} was correlated with attenuation of Stumvoll-1 (Spearman's *rho* -0.572, n = 2,166, P <0.01) and lowering of 1/HOMA-IR (Spearman's *rho* -0.213, n = 2,264, P <0.01) so that the 1/HOMA-R - Stumvoll-1 regression line became progressively closer to the origin in the group with higher PG_{60} (Fig. 2B).

Discussion

To identify the SI-β pair best recapitulates the hyperbolic function, we systematically analyzed the relationship between the nine pairs of SI and β in a large number of subjects with NGT by SMA [23]. Hyperbolic function between the indices of SI and β was present only when Stumvoll-1 (index of AIR), but not insulinogenic index (index of early phase GSIS) or Stumvoll-2 (index of 2nd phase GSIS), was employed as an index of β. Hyperbolic function between SI and Stumvoll-1 was found irrespective of whether $ISI_{Matsuda}$ (index of whole body SI), 1/HOMA-IR (index of hepatic SI) or 1/FIRI (index of hepatic SI) was employed as the measure of SI. Importantly, the fact was confirmed in two independent group of subjects, *i.e.*, in Hokuriku and Chikuma cohorts. AIR was the measure of β used in the original study [2], and while the correlation between AIR and Stumvoll-1 [18] was linear, that between AIR and insulinogenic index was log-linear [17]. Stumvoll-2 was an index of 2nd phase GSIS, not that of AIR [18]. The absence of hyperbolic function between SI and insulinogenic index or Stumvoll-2 may be in part due to these characteristics of insulinogenic index and Stumvoll-2. The differences between our results and those of the previous studies [4, 6] might be due to differences in the study populations.

On the basis of all these results [4, 6, current study], one should not a priori expect the presence of hyperbolic function between a given pair of SI and β. Obviously, if the hyperbolic function does not exist, the adoption of a product of SI and β as a disposition index is an egregious error and should be avoided, as previous studies have argued [6, 10]. For example, it is obvious that the product of Si and $\beta^{1.00}$ (hyperbolic with a slope value of -1.00) is not equal to the product of Si and $\beta^{1.50}$ (non-hyperbolic with a slope value of -1.50). The discrepancy occurs regardless of the level of PG.

In the next step, we found that the relationship between SI and β was altered by the levels of PG_{60} even in the NGT range. The higher the PG_{60} of the group, the steeper the regression line and the lower the intercept. The effect of gender and BMI on the shifting of the fitted line was negligible because the steepening and downward shifting in Q4 was little affected by gender- and BMI-matching. Thus, the subjects with NGT were not homogeneous, *i.e.*, they were composed of groups with diverse SI-β relationships, none of which were necessarily hyperbolic. The steepening and the downward shift of the fitted line along with the elevation of PG_{60} was unequivocally demonstrated beyond NGT as well, *i.e.*, in NDH and diabetes (Fig. 2). Utzschneider *et al*. reported a hyperbolic SI-β relationship in Japanese-Americans with NDH and DM despite depressed SI and β [6]. The reason(s) for the discrepancy between their results and ours is not apparent. At least, FPG and 2hPG were significantly higher for the patients with diabetes in their study than for the DM group in our study, indicating that their patients were evaluated at a more advanced stage of diabetes than ours. In the "closed" data-set, the relation of parameters within the same data-set may have a given correlation which is specific to the population. Thus the result may be different between different study populations. Dissimilar regression between insulin sensitivity and secretion in groups of subjects with different ranges of glucose tolerance is a confirmation of the previously described idea [25] and data [6, 7]. Of note, the shift of correlation between "1/FIRI and insulinogenic index" with worsening of glucose metabolism in our population was qualitatively similar to it in the previous study (Supplemental Fig. 1) [6].

Beta cell senses the degree of SI in the insulin-target

tissues and adjusts the secretion inversely to the level of SI, giving rise to a hyperbolic function between SI and β [2]. In other words, attenuation of SI acts as a stimulus for the beta cell although the molecular nature of this effect remains to be identified [26]. In the group of subjects with elevated PG_{60}, the regression line for SI and β shifted downward and, therefore, the intercept was lowered. This means that insulin secretion was insufficient for a given level of SI. The steepening of the regression line might be the result of stronger beta cell stimulation from greater attenuation of SI. The previously known beta cell abnormality associated with elevated PG_{60} is diminished insulin secretion [27], a quantitative change. In addition, we found significant downward shifting and steepening of the insulin response curve in subjects with elevated PG_{60}, suggesting a qualitative change in beta cell stimulus-secretion coupling. This might be a *primary* beta cell dysfunction [28] and/or beta cell insult resulting from low level hyperglycemia. Because steepening and downward shifting of the regression line was observed also with elevation of $\Sigma PG_{0\text{-}120}$ (Supplemental Fig. 2), PG_{60} was not a peculiar variable to be associated with the shifting.

It is intriguing that 8.6 mmol/L has been proposed as a PG_{60} cut-off value for the prediction of diabetes [29] and that the corresponding value for impaired beta cell function was 8.95 mmol/L [27], values which are very close to the cut-off for PG_{60} NGTQ4 (9.0 mmol/L) in our study (Table 4). Notably, while PG_{30} and PG_{60} were strong confounders of the SI-β correlation (Table 3), HbA1c and 2hPG were not. Thus, the 'inability to control early glucose peak at OGTT' is a unique marker of glucose dysregulation in subjects with prediabetes and mild diabetes.

Among the NGT subjects, SI-β correlation was significantly weaker in the group of individuals with lower PG_{60}. Auxiliary glucose regulating systems such as incretin [30, 31], glucose effectiveness [8, 32, 33] and input from the central nervous system [33, 34] may be relatively robust in subjects with lower PG_{60}, resulting in weaker SI-β correlation. Weak SI-β correlation in subjects with lower PG_{60} was not due to larger measurement variations in 1/HOMA-IR and/or Stumvoll-1 because the coefficient of variation for 1/HOMA-IR and Stumvoll-1 was no larger in those with lower PG_{60} (data not shown).

There were several limitations in this study. First, the participants were school teachers who might be expected to be health conscious in comparison with the population at large. This would limit the general applicability of the findings. The validity of Stumvoll indices in subjects with DM has not been established [18]. Updated HOMA-S [35] could not be calculated because IRI was <20 pmol/L in a substantial number (627, 28%) of the participants. Due to the cross-sectional nature of the study, a cause-result relationship could not be firmly established. Our study is depend on the credibility of the indices obtained from an oral glucose tolerance test, which is not completely assured because the target glucose tolerance levels were included in the same oral glucose tolerance test. Insulin secretion and insulin sensitivity should be independently assessed to answer the question. The limitation from this issue should also be appreciated.

In conclusion, hyperbolic function between a given pair of SI and β was not always present, but was found in three pairs of indices: '1/HOMA-IR and Stumvoll-1'; '$ISI_{Matsuda}$ and Stumvoll-1'; and '1/FIRI and Stumvoll-1'. The correlation between 1/HOMA-IR and Stumvoll-1 was relatively strong and judged to be the pair most suited for analysis of SI-β relationship. The best-fit regression line for SI and β shifted progressively downward and steepened in the group of subjects with higher PG_{60}, over the entire range of the glucose tolerance category. This finding suggested an abnormality in beta cell stimulus-secretion coupling in association with an elevation of post-challenge glucose.

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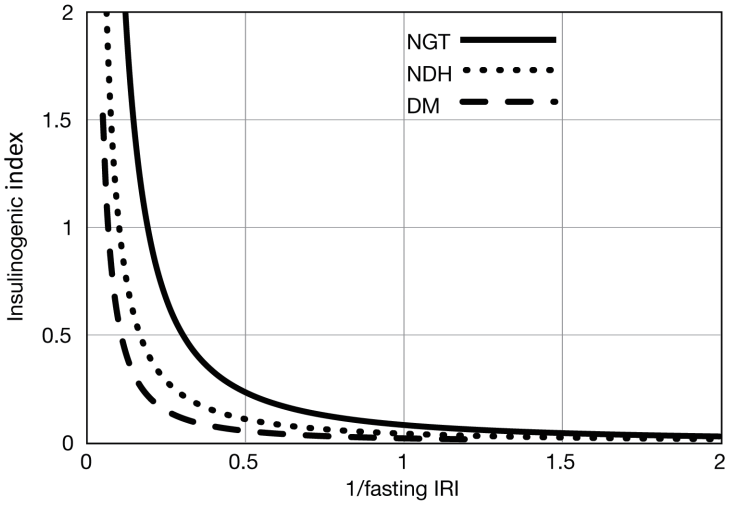
Disclosure

None of the authors have any potential conflicts of interest associated with this research.

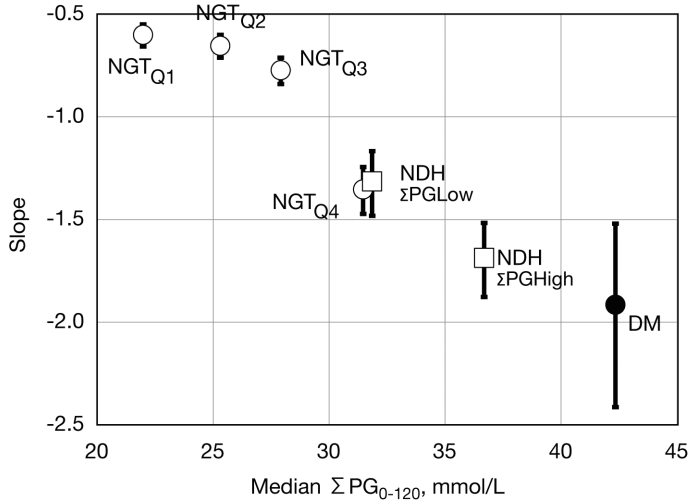
Supplemental Table Characteristics of SI-β relationship, in Chikuma Cohort, as indexed by Spearman's *rho* and slope and *R* values of the best-fit regression line obtained by standardized major axis regression

Secretion index, β	Sensitivity index, SI	Spearman's rank correlation		Standardized major axis regression				
				Slope	95%CI		*R*	*P*
		rho	*P*		Lower	Upper		
Stumvoll-1	1/HOMA-IR	-0.508	< 0.01	-0.994	-1.097	-0.900	-0.455	< 0.01
	$ISI_{Matsuda}$	-0.407	< 0.01	-1.004	-1.113	-0.906	-0.367	< 0.01
	1/FIRI	-0.559	< 0.01	-1.020	-1.123	-0.926	-0.499	< 0.01
Stumvoll-2	1/HOMA-IR	-0.559	< 0.01	-0.676	-0.743	-0.614	-0.507	< 0.01
	$ISI_{Matsuda}$	-0.458	< 0.01	-0.682	-0.755	-0.617	-0.414	< 0.01
	1/FIRI	-0.607	< 0.01	-0.693	-0.760	-0.632	-0.550	< 0.01
Insulinogenic index	1/HOMA-IR	-0.257	< 0.01	-1.197	-1.332	-1.076	-0.257	< 0.01
	$ISI_{Matsuda}$	-0.201	< 0.01	-1.209	-1.347	-1.085	-0.197	< 0.01
	1/FIRI	-0.294	< 0.01	-1.228	-1.364	-1.104	-0.292	< 0.01

Abbreviations are the same as in Table 1. See Text for the detail.



Supplemental Fig. 1 Regression line for 1/fasting IRI and insulinogenic index in subjects with NGT, NDH and DM



Supplemental Fig. 2 Correlation between $\Sigma PG_{0\text{-}120}$ for each group and slope values of the best-fit regression line. *P* for trend < 0.01. Variations are 95%CI. In this analysis, subjects with NGT were divided by quartile of $\Sigma PG_{0\text{-}120}$, and those with NDH by dichotomy of $\Sigma PG_{0\text{-}120}$. The Stumvoll-1 value corresponding to the intercept was 1,111.7 in NGT_{Q1}, and progressively smaller to 108.1 in DM.

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