Endocrine Journal 2014, **61** (1), 91-99

ORIGINAL

The evolution of non-diabetic hyperglycemia: a longitudinal study

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Abstract. The risk factors for impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) have yet to be established. Our aim was to elucidate the predisposing factors for IFG and IGT in Japanese subjects with normal glucose tolerance (NGT). Using a 75 g oral glucose tolerance test (OGTT), we analyzed 604 adults with the ADA-defined NGT. Follow-up glucose tolerance status was determined by 75 g OGTT performed 3.7 yrs later. Glucose-stimulated insulin secretion (GSIS), whole body insulin sensitivity (SI) and beta cell function (BCF) were estimated by Stumvoll indices, $ISI_{Matsuda,}$ and a product of Stumvoll 1st and $ISI_{Matsuda}$, respectively, and hepatic SI by quantitative insulin sensitivity check index. Logistic regression analysis revealed that attenuated BCF due to low GSIS was an independent risk factor for IFG. Low whole body SI was an additional risk for IGT. Male gender and high BMI were independently related to the progression to both IFG and IGT, whereas a positive diabetes family history was independently related to IGT. The worsening of glucose tolerance at large was predicted with 66% sensitivity by risk engine with GSIS, whole body SI, gender, BMI and glucose. This finding may help when implementing early intervention strategies for diabetes.

Key words: Normal glucose tolerance, Impaired fasting glucose, Impaired glucose tolerance, OGTT

BETA CELL dysfunction and low insulin sensitivity (SI) are causally related to the development of diabetes mellitus (DM). The existence of this relationship has been established by a number of cross-sectional and longitudinal studies [1-5]. However, the predisposing factors for non-diabetic hyperglycemia (NDH) have not been established. More precisely, the significance of attenuated glucose-stimulated insulin secretion (GSIS), SI and beta cell function (BCF) for the worsening of glucose metabolism from normal glucose tolerance (NGT) to impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) has not been fully clarified [5-9]. Especially, a longitudinal analysis in Japanese subjects aiming at clarification of the predisposing factors for NDH has been lacking. The elucidation of critical factors in the beginning phase of glucose dysregulation is important for an understanding of the pathophysiology of diabetes evolution. It has been established that lifestyle modification is an effective means of preventing the development of DM in subjects with IGT [10-12], but no attempt has yet been made to intervene at an earlier stage to prevent the development of NDH. Establishing a scientific basis for such intervention, which would be a crucial first step in the fight against the diabetes pandemic [13], would require clarification of the risk factors for the worsening of glucose regulation in subjects with NGT.

In this study, we performed a longitudinal analysis of data from a large number of middle-aged Japanese school teachers with NGT. The analysis was performed using an insulin assay devoid of cross-reactivity with proinsulin. This enabled us to completely exclude any effects due to hyperproinsulinemia commonly found in subjects with NDH and in those with NGT predisposed to NDH [14-17].

Submitted Aug. 31, 2013; Accepted Oct. 4, 2013 as EJ13-0359
Released online in J-STAGE as advance publication Oct. 22, 2013

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Materials and Methods

Study sample

A retrospective observational study was conducted using a dataset provided by the Health Service Department of Hokuriku Central Hospital, where public school employees receive annual medical checkups. Data from consecutive 2,264 individuals who received the checkup including a 75 g OGTT with immunoreactive insulin (IRI) measurement between April 2006 and March 2010 [18] without a history of DM or gastrectomy and not taking glucocorticoid or anticancer drugs were used. Among them, the number of subjects with the ADA-defined NGT [19] was 1,209. Out of the 1,209 subjects, 604 who received a subsequent 75 g OGTT by March 2012 were analyzed in this study. The median (25-75 percentile) follow-up period was 3.7 (2.7-4.8) yrs. All of them were Japanese. Signed informed consent was obtained from all subjects, and the hospital review board approved the study protocol. Family history of diabetes was assessed using a questionnaire. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Measurement of plasma glucose and insulin, and diagnosis of glucose tolerance

After an overnight fast, a standard 75 g OGTT was performed as previously described [18]. Plasma glucose (PG) was analyzed by the glucose oxidase method (Automatic Glucose Analyzer ADAMS Glucose GA-1160, Arkray, Kyoto) and immunoreactive insulin (IRI) by the chemiluminescence method (ADVIA Centaur, Siemens Medical Solutions). The IRI assay does not cross react with proinsulin [20]. IRI was lower than the detection limit of the assay, 0.4 μU/mL, in 13 samples (12 fasting samples and one 2-h sample) and the values for these samples were assumed to be a half of the assay limit, 0.2 μU/mL. The diagnosis of glucose tolerance category was made according to the 2003 American Diabetes Association (ADA) criteria [19]: NGT as fasting PG (FPG) <100 mg/dL and 2-hour PG <140 mg/dL; IFG as FPG ≥100 mg/dL but <126 mg/dL and 2-hour PG <140 mg/dL; IGT as FPG <126 mg/dL and 2-hour PG ≥140 mg/dL but <200 mg/dL; DM as FPG ≥126 mg/dL and/or 2-hour PG ≥200 mg/dL.

Calculations

PG and IRI were measured using 0, 30, 60 and 120 min- and at 0, 30 and 120 min-samples at OGTT, respectively, which are abbreviated as FPG, PG_{30}, PG_{60} and 2hPG, and FIRI, IRI_{30} and 2hIRI, hereafter in this communication. $ISI_{Matsuda}$ [21] was calculated as an index of whole body SI using the fasting and 2-h blood samples: $ISI_{Matsuda} = 10{,}000/[\mathrm{sqrt}(FPG \cdot 2hPG \cdot FIRI \cdot 2hIRI)]$ [22]. The quantitative insulin sensitivity check index (QUICKI) [23] was calculated as an index of hepatic SI [24]: $QUICKI = 1/[\log(FPG) + \log(FIRI)]$. For $ISI_{Matsuda}$ and QUICKI, the unit of PG and IRI was mg/dL and μU/mL, respectively [22, 23]. As indices of early and late phase GSIS, Stumvoll 1st (Stumvoll-1) and 2nd phase (Stumvoll-2) indices, respectively, were used: $\text{Stumvoll-1} = 1283 + 1.829 \cdot IRI_{30} - 138.7 \cdot PG_{30} + 3.772 \cdot FIRI$ and $\text{Stumvoll-2} = 287 + 0.4164 \cdot IRI_{30} - 26.07 \cdot PG_{30} + 0.9226 \cdot FIRI$, in which the unit of IRI and PG was pmol/L and mmol/L, respectively [25]. Minus values for Stumvoll-1 and -2 were obtained in 4 and 1, respectively, and it was assumed that the values for these subjects were absent. There was a highly significant, strong linear correlation between Stumvoll-1 and Stumvoll-2: $\text{Stumvoll-1} = 4.47 \cdot [\text{Sumvoll-2}] - 192.5$, $r = 0.996$, $P < 0.01$. A product of $ISI_{Matsuda}$ and Stumvoll-1, oral disposition index (DIo), was used as a measure of BCF: the slope of the regression between $ISI_{Matsuda}$ and Stumvoll-1 in the 1,202 subjects with NGT was -0.89 (Fig. 1). Homeostasis model assessment-2 (HOMA2) indices of insulin secretion and insulin sensitivity [26] were not employed because FIRI was lower than 3.0 μU/ml, which was the lower limit for the calculation of HOMA2 in 189 (31%) of the participants. Instead, indices derived from the original HOMA [27] were calculated as, $\text{HOMA-IR} = (FPG \cdot FIRI)/405$, and $\text{HOMA-beta} = (FIRI \cdot 360)/(FPG - 63)$, where the unit of PG and IRI was mg/dL and μU/mL, respectively. Numerical data are expressed as median (25-75 percentile).

Statistics

Logistic regression analysis was performed to know the relation of variables to worsening of glucose tolerance. Age, gender, BMI, FPG, 2hPG, family history of DM, Stumvoll-1, Stumvoll-2, $ISI_{Matsuda}$, QUICKI, and/or DIo were included as independent explanatory variables. SPSS version 21.0 was used for statistical analysis. Mann-Whitney U test, Wilcoxon's signed-rank test, Fisher's exact test, one way analysis of variance and Steel-Dwass test were also used as needed, and $P < 0.05$ was considered significant. The values for event per variable (EPV) in logistic regression analysis was 9.6, 15.3, and 29.0 (see below) so that the

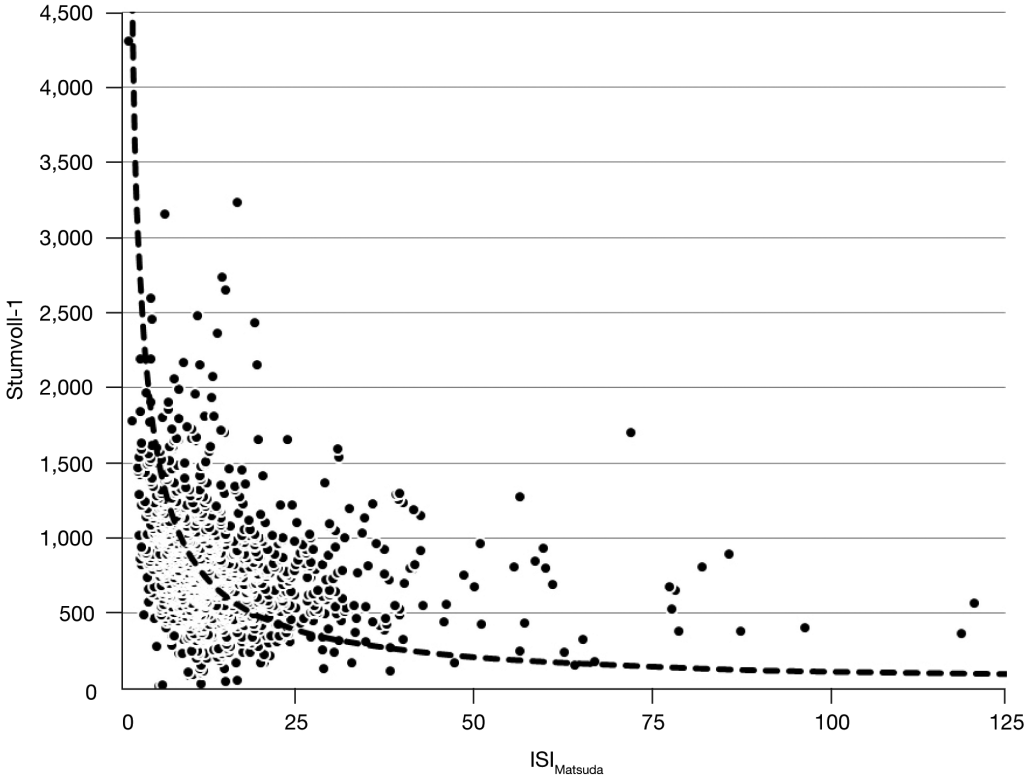

Fig. 1 Correlation between $ISI_{Matsuda}$ and Stumvoll-1 in 1,202 subjects with normal glucose tolerance. Equation for the regression was, Stumvoll-1 = 6,699· $[ISI_{Matuda}]^{-0.89}$ (r = -0.29, P <0.01), with the 95%CI for the slope and intercept being -0.84~-0.93 and 5,902~7,603, respectively. Standardized major axis regression [32-34] was performed because there were measurement errors for both x and y.

results were mathematically reliable [28]. Values for $ISI_{Matsuda}$, Stumvoll-2 and DIo were log-transformed for the logistic regression analysis because fitting was better with log-transformed values for these variables. Comparison of area under the curve of the receiver operating characteristic (ROC) curve was performed by using MedCalc (http://www.medcalc.org/manual/comparison_of_roc_curves.php).

Results

The baseline characteristics of the 604 subjects analyzed in this study are shown in Table 1. There was a modest male dominance. All participants were normoglycemic by definition and they were non-obese as a group. Fifty-nine (10%) and 36 (6%) were receiving antihypertensive and lipid lowering agents, respectively. There was little difference in the baseline characteristics between the 604 subjects and the rest of the NGT subjects (N = 605) who did not receive a follow-up OGTT (Supplemental Table 1).

At the end of the follow-up, 430 participants (71%) remained in the NGT category and the rest had progressed to NDH or DM. Accordingly, the former and the latter were designated non-progressors and progressors, respectively (Table 1). The progressors were divided into 3 subgroups according to whether they had progressed to IFG (N = 102), IGT (N = 67) or DM (N = 5) (Table 1). The IFG group consisted of those progressed to isolated IFG and IGT group included those progressed to isolated IGT and IFG/IGT. Only 5 subjects developed diabetes (in 3 cases according to both FPG and 2hPG criteria and in the other 2 according to 2hPG criterion).

Comparison of the baseline characteristics between Non-progressors, IFG-progressors, IGT-progressors and DM-progressors revealed that male/female ratio, FPG, 2hPG, FIRI, 2hIRI, BMI, HbA1C, $ISI_{Matsuda}$ and DIo were significantly different (Table 1). Compared to Non-progressors, male/female ratio, FPG, 2hPG, BMI and HbA1c were significantly elevated whereas DI_O was significantly lower in IFG-progressors and

Table 1 Baseline characteristics of the NGT subjects analyzed in this study

Variable	All	Non-progressors NGT*	Progressors					*P* value†
			IFG*	IGT*			DM*	
				Total	Subgroup			
					iIGT*	IFG/IGT*		
N	604	430	102	67	39	28	5	
Age (yr)	53 (47-59)	53 (47-59)	53 (49-58)	53 (48-59)	53 (50-57)	54 (48-61)	52 (43-62)	0.96
Male/Female	402/202	262/168	81/21§	55/12§	32/7	23/5	4/1	<0.01
PG (mg/dL)								
FPG	93 (90-97)	92 (89-96)	95 (93-97)§	94 (91-98)‡	92 (89-96)	96 (95-97)	93 (88-99)	<0.01
2hPG	105 (92-118)	102 (90-114)	108 (94-122)‡	114 (105-124)§	116 (108-124)	110 (97-124)	124 (116-132)§	<0.01
IRI (μU/mL)								
FIRI	3.6 (2.5-4.8)	3.5 (2.4-4.6)	3.6 (2.5-4.8)	4.3 (2.8-5.8)‡	4.3 (2.9-5.7)	4.1 (2.4-5.9)	4.3 (2.0-6.6)	0.04
2hIRI	20.4 (11.4-29.4)	19.5 (11.4-27.7)	21.7 (12.0-31.5)	28.7 (17.6-39.8)‡	30.5 (20.6-40.4)	21.8 (9.0-34.7)	27.8 (8.5-47.2)	<0.01
BMI (kg/m^2)	23.4 (21.7-25.2)	23.0 (21.2-24.9)	24.1 (22.5-25.7)§	24.7 (23.4-26.0)§	24.9 (23.9-26.0)	24.5 (23.1-26.0)	22.6 (19.3-26.0)	<0.01
HbA1C (%)	5.5 (5.3-5.8)	5.5 (5.3-5.8)	5.6 (5.4-5.8)‡	5.6 (5.4-5.8)‡	5.6 (5.5-5.8)	5.7 (5.6-5.9)	5.9 (5.8-6.1)	<0.01
Family Hx +/-	97/507	63/367	17/85	17/50	7/32	10/18	0/5	0.19
Stumvoll-1	734.7 (516.9-952.5)	742.7 (534.8-950.6)	702.9 (505.1-900.7)	674.8 (431.4-918.2)	696.2 (530.2-862.2)	623.3 (322.6-924.0)	520.9 (106.2-935.7)	0.09
Stumvoll-2	205.9 (159.4-252.4)	207.1 (163.4-250.9)	196.4 (152.9-239.9)	196.6 (144.5-248.8)	199.6 (160.7-238.5)	178.3 (160.2-196.5)	162.1 (71.2-253.1)	0.12
$ISI_{Matsuda}$	12.1 (7.5-16.7)	12.5 (7.8-17.2)	11.5 (7.0-16.1)	8.8 (5.7-11.9)§	8.5 (6.1-11.0)	9.5 (5.3-13.7)	9.2 (3.2-15.3)	<0.01
QUICKI	0.40 (0.38-0.42)	0.40 (0.38-0.42)	0.39 (0.37-0.41)	0.39 (0.37-0.41)	0.39 (0.37-0.41)	0.38 (0.35-0.41)	0.39 (0.36-0.42)	0.27
1/HOMA-IR	1.20 (0.83-1.58)	1.25 (0.86-1.64)	1.18 (0.84-1.52)	1.05 (0.72-1.39)	1.07 (0.74-1.40)	1.00 (0.55-1.45)	1.26 (0.21-2.32)	0.75
HOMA-beta	44.5 (30.0-59.1)	44.7 (30.4-59.0)	41.7 (29.5-53.9)	51.9 (32.5-71.4)§	57.3 (33.2-81.5)	45.2 (28.5-62.0)	55.5 (28.8-82.2)	0.16
DIo	8,685 (4,989-12,381)	9,449 (5,376-13,522)	6,971 (3,464-10,478)‡	5,825 (3,779-7,871)‡	5,993 (3,951-8,036)	5,746 (4,324-7,168)	4,067 (1,540-6,595)§	<0.01
Follow-up period (yr)	3.7 (2.7-4.8)	3.7 (2.7-4.7)	4.1 (3.0-5.2)	3.3 (2.1-4.5)	3.7 (2.6-4.9)	3.1 (1.8-4.5)	2.6 (1.1-4.2)	0.07

Numerical data are median (25-75 percentile) for the cohort. iIGT, isolated IGT; IFG/IGT, combined IFG and IGT; PG, plasma glucose; IRI, immunoreactive insulin; FPG and FIRI, fasting PG and IRI, respectively; 2hPG and 2hIRI, PG and IRI at 120 min during 75 g OGTT, respectively; BMI, body mass index; Stumvoll-1 and -2, Stumvoll's 1st and 2nd phase indices of insulin secretion, respectively; QUICKI, quantitative insulin sensitivity check index: DIo, oral disposition index. *, status of glucose tolerance upon follow-up; †, overall *P* values for difference between the four groups, i.e., Non-progressors, IFG-progressors, total IGT-progressors (isolated IGT and IFG/IGT combined) and DM-progressors, determined by one-way analysis of variance for numerical variables and Fisher's exact test for categorical variables; ‡ and §, *P* <0.05 and <0.01, respectively, compared to the corresponding values in Non-progressors (Steel-Dwass test or Fisher's exact test). Values from the total IGT-progressors (isolated IGT and IFG/IGT combined), not the subgroups, were adopted for the statistical analyses.

IGT-progressors. Additionally, FIRI and 2hIRI were significantly elevated in IGT-progressors compared to Non-progressors. None of the differences between the non-progressors and DM-progressors were statistically significant except for elevated 2hPG and depressed DI_O: this was likely due to the small number of subjects who progressed to DM.

Firstly, predisposing factors for worsening of glucose tolerance, i.e., IFG, IGT and DM combined, were searched for by binomial logistic regression analysis (Supplemental Table 2). In this analysis, male gender, high BMI, high FPG, positive family history, low Stumvoll-2 and $ISI_{Matsuda}$ were significantly and independently related to the worsening. When DI_O was taken in place of Stumvoll-2 and $ISI_{Matsuda}$, attenuation of it also significantly related to worsening of glucose tolerance. High 2hPG was significantly related to the worsening in univariate analysis but not in multivariate analysis. Age and QUICKI were not significantly related to worsening even in univariate analysis.

Secondly, the relation of the baseline variables to progression to each of IFG and IGT were analyzed (Table 2). Those progressed to diabetes were excluded in this analysis because the number of such individuals

Table 2 Relation of the baseline variables to progression from NGT to IFG and IGT

	Multinominal-univariate logistic regression		Multinominal-multiple logistic regression			
			Model-1		Model-2	
Progression to IFG						
Variable	OR(95%CI)	*P*	OR(95%CI)	*P*	OR(95%CI)	*P*
Gender (female 0, male 1)	2.47(1.47-4.15)	<0.01	1.91(1.10-3.33)	0.02	2.07(1.20-3.57)	<0.01
BMI (kg/m^2)	1.13(1.05-1.22)	<0.01	1.10(1.00-1.20)	0.05	1.06(0.97-1.15)	0.18
FPG (mg/dL)	1.18(1.11-1.25)	<0.01	1.15(1.08-1.22)	<0.01	1.14(1.08-1.22)	<0.01
2hPG (mg/dL)	1.02(1.01-1.03)	<0.01	1.00(0.99-1.02)	0.63	1.00(0.99-1.02)	0.87
Family history (absent 0, present 1)	1.17(0.65-2.09)	0.61	1.30(0.69-2.44)	0.41	1.30(0.70-2.44)	0.41
Stumvoll-1	0.999(0.998-1.000)	0.01				
Stumvoll-2	0.15(0.04-0.56)	<0.01	0.10(0.02-0.55)	<0.01		
$ISI_{Matsuda}$	0.40(0.18-0.92)	0.03	0.52(0.16-1.71)	0.28		
DIo	0.15(0.07-0.33)	<0.01			0.32(0.12-0.80)	0.02
Progression to IGT						
Variable	OR(95%CI)	*P*	OR(95%CI)	*P*	OR(95%CI)	*P*
Gender (female 0, male 1)	2.94(1.53-5.65)	<0.01	2.66(1.31-5.39)	<0.01	2.68(1.33-5.37)	<0.01
BMI (kg/m^2)	1.23(1.12-1.34)	<0.01	1.16(1.04-1.29)	<0.01	1.15(1.05-1.27)	<0.01
FPG (mg/dL)	1.07(1.01-1.13)	0.03	1.01(0.94-1.07)	0.87	1.00(0.94-1.07)	0.90
2hPG (mg/dL)	1.04(1.02-1.06)	<0.01	1.02(1.00-1.04)	0.02	1.03(1.01-1.05)	<0.01
Family history (absent 0, present 1)	1.98(1.07-3.65)	0.03	2.67(1.37-5.20)	<0.01	2.68(1.37-5.22)	<0.01
Stumvoll-1	1.00(0.999-1.00)	0.88				
Stumvoll-2	0.50(0.10-2.51)	0.40	0.11(0.01-0.84)	0.03		
$ISI_{Matsuda}$	0.10(0.04-0.29)	<0.01	0.21(0.05-0.94)	0.04		
DIo	0.10(0.04-0.26)	<0.01			0.29(0.10-0.88)	0.03

OR, odds ratio; CI, confidence interval. Other abbreviations are the same as in Table 1. Non-progression, i.e., being NGT both at baseline and upon follow-up, was taken as a reference category. Stumvoll-1 was not included as an independent variable in Model-1 because of strong correlation with Stumvoll-2 (r = 0.996). DIo was a product of $ISI_{Matsuda}$ and Stumvoll-1, so that $ISI_{Matsuda}$ and Stumvoll-1 were not adopted as independent variables in Model-2. Data for age and QUICKI were unlisted because they were not significantly related to progression to IFG and IGT in univariate analysis. Although $ISI_{Matsuda}$ and Stumvoll-2 were entered as explanatory variables in Model 1, correlation between the two was not strong (r = -0.386) and variance inflation factor was 1.0 so that multicollinearity was not a problem. 'Progression to IGT' included progression to isolated IGT and IFG/IGT.

was only 5. In Model 1 (Table 2), low Stumvoll-2, male gender, increased BMI and FPG; in Model 2 (Table 2), male gender, elevated FPG and low DIo were independently and significantly related to progression to IFG. On the other hand, low Stumvoll-2, low $ISI_{Matsuda}$, male gender, increased BMI and 2hPG, and positive family history were independently and significantly related to progression to IGT. The relation of the variables to progression to IGT in Model 2 was qualitatively the same as in Model 1 except that DIo, instead of Stmvoll-2 and $ISI_{Matsuda}$, was a significant risk factor (Table 2). The HOMA-derived indices were not adopted as independent variables in logistic regression analysis. Although $ISI_{Matsuda}$ and Stumvoll-2 were entered as explanatory variables in Model 1, correlation between the two was not strong (r = -0.386) and variance inflation factor was 1.0 so that multicollinearity was not a problem.

Lastly, worsening of glucose regulation in NGT subjects was predicted on the basis of logistic regression analysis, in which development of IFG, IGT and DM was inclusively defined as 'worsening'. Because univariate analysis revealed that gender, BMI, FPG, 2hPG, Stumvoll-2 and $ISI_{Matsuda}$ were significantly related to the worsening (Supplemental Table 2), these variables were adopted as predictors in the equation below. The probability of worsening was calculated to be, $P = 1/[1+\exp^{-(-6.396 + 0.708\cdot gender + 0.105\cdot BMI + 0.081\cdot FPG + 0.012\cdot 2hPG - 2.285\cdot Stumvoll\text{-}2 - 1.013\cdot ISIMatsuda)}]$. Analysis by the receiver operating characteristic curve revealed that a P value of 0.30 was the best cutoff with a specificity of 69%, sensitivity of 66% and a positive predictive value of 44%. The area under the curve of ROC plot was greater (0.737, 95%CI 0.693-0.780) than the prediction of it by gender, BMI and baseline FPG (0.696, 95%CI 0.651-0.741) or baseline FPG and HbA1c (0.694, 95%CI 0.647-0.740). The difference between

the first value (AUC 0.737) and the third value (AUC 0.694) was statistically significant ($P < 0.01$).

Discussion

In this study, we systematically analyzed subjects with NGT diagnosed by the ADA criteria, which is a more stringent definition of normal glucose metabolism [19] than the definition proposed by the WHO [29]. We were the first to examine risk factor profiles for IFG and IGT in ADA-defined subjects with NGT. By doing so, we aimed to clarify the risk factors for IFG and IGT, i.e., the earliest stages of diabetes evolution. Our study utilized indices of whole body SI ($ISI_{Matsuda}$), hepatic SI (QUICKI), GSIS (Stumvoll-1 and -2) and BCF (DIo).

Here, we discuss the risk factors on the basis of the results of multiple logistic regression analysis. The most important discovery was that the differential impact of attenuated BCF and low whole body SI on progression from NGT to IFG and IGT. Namely, attenuated BCF due to low GSIS, and not due to low whole body SI, was a significant independent risk for IFG. Low whole body SI was an additional significant risk for IGT, and attenuated BCF was also a risk factor for IGT. In this case, it was attenuation of BCF with low GSIS *and* low whole body SI. Positive family history was a strong risk only for IGT, whereas male gender, and elevated BMI and PG (FPG or 2hPG) were common risk factors for both IGT and IFG. Significance of attenuated BCF and SI for NDH in Japanese subjects, which has been speculated in cross sectional analysis, was proved by us in a longitudinal analysis. Our data is compatible with the notion that IFG and IGT differ in their site of insulin resistance, and the latter is associated with predominantly muscle rather than hepatic insulin resistance [30]. On the basis of these observations, attenuated BCF as well as male gender, and elevated body weight and glucose can be categorized as common predisposing factors for IFG and IGT, and low SI and positive family history as additional predisposing factors for IGT.

Our data is in part agreement with the data obtained in the previous longitudinal studies in which the WHO-defined NGT subjects had been analyzed [5-9]. Weyer *et al*. reported that low GSIS and low SI but not male gender and increased BMI were risk factors for development of IGT in Pima Indians who were obese [5]. Also, male gender, increased BMI and positive family history were risk factors for worsening of glucose tolerance in mildly overweight Europids in one study but not in the other [8, 9]. Indices of insulin sensitivity and insulin secretion derived from the original HOMA [27], 1/HOMA-IR and HOMA-beta were not significantly related to worsening of glucose tolerance ($P = 0.341$ for 1/HOMA-IR and 0.445 for HOMA-beta) in this study.

Accurate prediction of the worsening of glucose regulation in subjects with NGT is mandatory for intervention aiming at halting the development of NDH. This is because the number of subjects with NGT is so large that it is absolutely necessary to segregate an appropriate target population for such intervention. Accordingly, we attempted to predict worsening of glucose regulation in NGT subjects. Here, worsening denotes the development of IFG, IGT and DM inclusively. Performance of the prediction compares well with that made in a study of diabetes development in a non-diabetic population (NDH and NGT combined) [31]. At any rate, "being slender" may be important in seeking to prevent progression from NGT to NDH even in non-obese populations such as the Japanese because high BMI was significantly related to both IFG and IGT development.

The study had several limitations. The follow-up period was not very long and the eventual disposition of the participants was largely unknown. The relatively small number of subjects who progressed to IGT compromised the statistical power of the study. SI, GSIS and BCF were estimated by using data from 75 g OGTT, not by a direct glucose infusion technique such as a glucose clamp or a frequently sampled intravenous glucose tolerance test.

In conclusion, in the middle-aged Japanese subjects, attenuated BCF due to low GSIS was a predisposing factor for IFG. Low SI was an additional predisposing factor for IGT, so that attenuated BCF with reduced GSIS *and* SI was a risk for IGT. Male gender and increased BMI were independently related to both IFG and IGT development, whereas a positive family history of diabetes was significantly related only for IGT. Understanding the multifaceted nature of the risk factors for IFG and IGT may help when implementing early intervention strategies for diabetes.

Disclosures

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

Supplemental Table 1 Baseline characteristics of entire NGT subjects

Variable	Group		
	A. Entire NGT population	B. Follow-up OGTT (-)	C. Follow-up OGTT (+)
N	1,209	605	604
Age (yr)	53(47-59)	53(47-59)	53(47-59)
Male/Female	734/475	332/273	402/202*
PG (mg/dL)			
FPG	93(90-97)	92(89-96)	93(90-97)
2hPG	104(91-117)	103(90-116)	105(92-118)
IRI (μU/mL)			
FIRI	3.6(2.5-4.7)	3.6(2.6-4.7)	3.6(2.5-4.8)
2hIRI	20.4(11.3-29.5)	20.4(10.8-30.0)	20.4(11.4-29.4)
BMI (kg/m^2)	23.1(21.3-24.9)	22.7(21.0-24.5)	23.4(21.7-25.2)
HbA1C (%)	5.5(5.3-5.7)	5.5(5.3-5.8)	5.5(5.3-5.8)
Family Hx +/-	203/1006	106/499	97/507
Stumvoll-1	757.5(528.3-986.7)	791.7(555.7-1027.7)	734.7(516.9-952.5)
Stumvoll-2	209.0(161.3-256.8)	213.2(163.2-263.3)	205.9(159.4-252.4)
$ISI_{Matsuda}$	12.2(7.5-16.9)	12.4(7.5-17.3)	12.1(7.5-16.7)
QUICKI	0.40(0.38-0.42)	0.40(0.38-0.42)	0.40(0.38-0.42)
DIo	8,801(5,145-12,456)	9,026(5,521-12,531)	8,685(4,989-12,381)

Numerical data are median (25-75 percentile) for the cohort. Abbreviations are the same as in Table 1.
*, $P < 0.05$ compared to the corresponding value in Group B.

Supplemental Table 2 Relation of the baseline variables to worsening of glucose tolerance in subjects with NGT

Variable	Univariate analysis		Multivariate analysis model-1		Multivariate analysis model-2	
	OR(95%CI)	*P*	OR(95%CI)	*P*	OR(95%CI)	*P*
Age (yr)	1.01 (0.98-1.03)	0.50				
Gender (female 0, male 1)	2.64 (1.73-4.03)	<0.01	2.15 (1.35-3.41)	<0.01	2.26 (1.44-3.57)	<0.01
BMI (kg/m^2)	1.16 (1.09-1.23)	<0.01	1.11 (1.03-1.20)	0.01	1.08 (1.01-1.16)	0.02
FPG (mg/dL)	1.12 (1.08-1.17)	<0.01	1.08 (1.03-1.13)	<0.01	1.08 (1.03-1.13)	<0.01
2hPG (mg/dL)	1.03 (1.02-1.04)	<0.01	1.01 (0.99-1.03)	0.07	1.01 (0.99-1.03)	0.07
Family history (absent 0, present 1)	1.42 (0.89-2.24)	0.14	1.69 (1.02-2.81)	0.04	1.71 (1.03-2.84)	0.04
Stumvoll-1	0.999 (0.999-0.9999)	0.045				
Stumvoll-2	0.22 (0.07-0.67)	<0.01	0.10 (00.24-0.43)	<0.01		
$ISI_{Matsuda}$	0.24 (0.12-0.49)	<0.01	0.36 (0.13-0.98)	0.04		
QUICKI	0.06 (0.01-1.60)	0.09				
DIo	0.12 (0.06-0.24)	<0.01			0.29 (0.13-0.66)	<0.01

OR, odds ratio; CI, confidence interval. Other abbreviations are the same as in Table 1. 'Worsening' denotes progression to IFG, IGT and DM inclusively in this analysis. Stumvoll-1 was not included as an independent variable in Model-1 because of strong correlation with Stumvoll-2 ($r = 0.996$). DIo was a product of $ISI_{Matsuda}$ and Stumvoll-1, so that $ISI_{Matsuda}$ and Stumvoll-1 were not adopted as independent variables in Model-2. Although $ISI_{Matsuda}$ and Stumvoll-2 were entered as explanatory variables in Model 1, correlation between the two was not strong ($r = -0.386$) and variance inflation factor was 1.0 so that multicollinearity was not a problem.

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