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Insulin and Proinsulin Dynamics Progressively Deteriorate From Within the Normal Range Toward Impaired Glucose Tolerance

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Context: Slight elevations in plasma glucose (PG) manifest in advance of diabetes onset, but abnormalities in immunoreactive insulin (IRI), proinsulin (Pro), and adiponectin dynamics during this stage remain poorly understood.

Objective: The objective of this work is to investigate whether IRI and Pro dynamics become abnormal as glucose tolerance deteriorates from within the normal range toward impaired glucose tolerance (IGT), as well as the relationship between PG, and these dynamics and serum adiponectin levels.

Design: A cross-sectional study was designed.

Setting: This study took place at Jichi Medical University in Japan.

Participants and Measurements: PG, IRI, and Pro levels were determined in 1311 young Japanese individuals (age < 40 years) with normal or IGT before and at 30, 60, and 120 minutes during a 75-g oral glucose tolerance test. Participants were assigned to 4 groups according to glucose tolerance, and then background factors, adiponectin levels, insulin sensitivity (SI), and insulin secretion (β) indexes were determined.

Results: PG levels as well as IRI and Pro levels 60 and 120 minutes after glucose-loading increased incrementally with deteriorating glucose tolerance. All measures of β and the SI measure index of insulin sensitivity (ISI)-Matsuda decreased incrementally. Serum adiponectin levels were not significantly different among the glucose tolerance groups, but were independently and negatively correlated with fasting glucose.

Conclusions: Early β decreased and postloading Pro levels became excessive in a progressive manner as glucose tolerance deteriorated from within the normal range toward IGT.

Abbreviations: β, insulin secretion; BMI, body mass index; bpm, beats per minute; DBP, diastolic blood pressure; DM, diabetes mellitus; FH, family history of diabetes (first and/or second degree); FH1, family history of diabetes (first degree); FIRI, fasting immunoreactive insulin; GSIS, glucose-stimulated insulin secretion; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; HR, heart rate; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IRI, immunoreactive insulin; ISI, index of insulin sensitivity; LDL, low-density lipoprotein; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PG, plasma glucose; P/I, proinsulin to insulin ratio; Pro, proinsulin; SBP, systolic blood pressure; SI, insulin sensitivity; TC, total cholesterol; TG, triglyceride.

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Key Words: glucose tolerance, insulin sensitivity, insulin secretion, proinsulin, adiponectin

Glucose intolerance develops as a consequence of β-cell dysfunction and/or insulin resistance. Glucose tolerance is classified as normal glucose tolerance (NGT), impaired glucose tolerance (IGT), impaired fasting glucose (IFG), and diabetes mellitus (DM) according to plasma glucose (PG) levels during an oral glucose tolerance test (OGTT) [1]. Impaired glucose-stimulated insulin secretion (GSIS) is an indicator of β-cell dysfunction in the prediabetic categories of IGT and IFG [2, 3]. High triglyceride (TG) levels, hypertension, and high body mass index (BMI) are also risk factors for prediabetes and diabetes, even in individuals with NGT [4]. We previously revealed that the disposition index, which is the product of insulin sensitivity (SI) and insulin secretion (β), decreases with increasing BMI in young Japanese individuals with NGT, and that β-cell dysfunction can manifest with increasing BMI even when glucose tolerance is normal [5]. However, in a population of mainly NGT individuals, whether SI and β deteriorate with worsening glucose tolerance has been poorly elucidated.

Slight elevations in PG manifest in advance of glucose intolerance [6], but the understanding of abnormalities in immunoreactive insulin (IRI) and proinsulin (Pro) dynamics in this stage remains insufficient. A recent study in Chinese individuals with NGT or IGT (but not IFG or DM) showed a delayed peak in Pro level with deteriorated glucose tolerance despite no abnormalities in insulin dynamic patterns, even in those with NGT following a 75-g OGTT [7]. However, that study had a small sample size and did not seem to sufficiently characterize the relationship between glucose tolerance and insulin and Pro dynamics during OGTT. Differences in Pro assay procedures and background factors, including age and ethnicity, can also affect this relationship.

Adiponectin is an insulin-sensitizing hormone secreted by adipocytes. High circulating levels of adiponectin reduce the risk of prediabetes and diabetes [8, 9]. Serum adiponectin levels are low in individuals with visceral fat accumulation, insulin resistance, and diabetes [10, 11]. Hypoadiponectinemia was also found to be related to impaired β and diabetes onset in the participants, including those with prediabetes [12, 13]. It is unclear, however, whether serum adiponectin levels are related to PG, β, and SI in people with NGT.

In the present study, we used data from a large number of young Japanese individuals to test the hypothesis regarding whether insulin and Pro dynamics progressively become abnormal as glucose tolerance deteriorates from within the normal range toward IGT. We also investigated the relationship of serum adiponectin levels to PG, β, SI, and other parameters in this population.

1. Materials and Methods

A. Diagnosis of Glucose Tolerance

The criteria of the Japan Diabetes Society define NGT as a fasting PG of less than 110 mg/dL and a 120-minute value of less than 140 mg/dL, IGT as a fasting PG of less than 110 mg/dL and a 120-minute value greater than or equal to 140 mg/dL to less than 200 mg/dL, IFG as a fasting PG of greater than or equal to 110 mg/dL to less than 126 mg/dL, and DM as a fasting PG of greater than or equal to 126 mg/dL or a 120-minute value of greater than or equal to 200 mg/dL during a 75-g OGTT [1].

B. Participants

The participants were 1311 medical students of Jichi Medical University who were younger than 40 years and had NGT or IGT (but not IFG or DM) among the about 1400 medical students who underwent a 75-g OGTT from December 2002 to April 2015. The present study was approved by the ethics committee of Jichi Medical University (EKI 09-45). The participants were fully informed of the purpose of the study and gave written consent to participate. The participants were assigned into 4 groups according to their glucose tolerance (OGTT 120-minute value; PG120) (NGT0: PG120 < 100, n = 864; NGT1: 100 ≤ PG120 < 120, n = 331; NGT2: 120 ≤ PG120 < 140, n = 87, IGT: 140 ≤ PG120 < 200, n = 29, in mg/dL). Background factors, PG, insulin, SI, β, Pro, and adiponectin were determined.

C. Measurements and Calculation of Indices

PG was determined using a glucose oxidase assay, and insulin using an immunoradiometric assay for IRI (Insulin RIA Beads II; Yamasa), as described previously [5]. The manufacturer claims that there is little cross-reactivity with Pro in the immunoradiometric assay for IRI. Proinsulin was determined as intact Pro with the Intact-Proinsulin Assay (MLT Research, Ltd), a chemiluminescent immunoassay procedure, as described previously [14]. Interassay and intra-assay variability for insulin and intact Pro were less than 5% and 10%, respectively. Samples for insulin and intact-Pro analysis were frozen until immunoassays were performed at about 6-month intervals.

In the 75-g OGTT, PG, IRI, and proinsulin were measured under fasting conditions (preloading) and at 30, 60, and 120 minutes after glucose loading, and are abbreviated as PG0, PG30, PG60, and PG120 (PG), fasting immunoreactive insulin (FIRI), IRI30, IRI60, and IRI120 (IRI), and Pro0, Pro30, Pro60, and Pro120. The molar ratio of Pro to insulin (P/I) was calculated. P/I values are abbreviated as P/I0, P/I30, P/I60, and P/I120.

As in a previous study by the authors [5], the following measures of SI and β were used in the present study. Index of insulin sensitivity (ISI)-Matsuda, a measure of systemic SI, was calculated as follows: ISI-Matsuda = 10 000/[square root (PG0·PG120·FIRI·IRI120)] [15]. In addition, 1/FIRI and 1/homeostasis model assessment of insulin resistance (HOMA-IR) were used primarily as measures of hepatic SI [16, 17]. HOMA-IR was calculated as [PG0·FIRI]/405 [16]. The insulinogenic index was used as a measure of early GSIS [18, 19]: Insulinogenic index = (IRI30 – FIRI)/(PG30 – PG0). For ISI-Matsuda, 1/FIRI, HOMA-IR, and the insulinogenic index, the units of PG and IRI were milligram per deciliter and microunits per milliliter, respectively. Stumvoll 1 and Stumvoll 2 indexes were used as early- and second-phase GSIS, respectively: Stumvoll 1 = 1283 + 1.829·IRI30 – 13 8.7·PG30 + 3.772·FIRI; Stumvoll 2 = 287 + 0.4164·IRI30 – 26.07·PG30 + 0.9226·FIRI [20]. Here, the units for PG and IRI were millimole per liter and picomole per liter, respectively. Negative or unusable insulinogenic index values (PG30 = PG0) and negative Stumvoll 1 and Stumvoll 2 values were treated as missing.

Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL), and adiponectin levels of the participants were determined using serum collected under fasting conditions. Total adiponectin concentrations were determined using an enzyme-linked immunosorbent assay kit (Otsuka Pharmaceutical). Sitting heart rate and blood pressure were measured after the participants had been at rest for 5 minutes.

D. Statistical Analysis

JMP version 5.1 (SAS Institute Inc) was used to conduct the statistical analyses. Most variables lacked a normal distribution. Values are shown as median (25th-75th percentile). The Wilcoxon or chi-square test was used to test for differences among the 4 groups. Because serum adiponectin levels are susceptible to the effects of sex [21], the participants

were classified into the 4 groups according to glucose tolerance described earlier and further classified according to sex when evaluating adiponectin.

Multiple regression analysis was used to calculate regressions of the background factors for PG120. When conducting multiple regression analysis, factors potentially contributing to PG120 other than PG, insulin, SI, and β—that is, age, sex, BMI, blood pressure, heart rate, lipids, and family history of diabetes—were selected as explanatory factors. At this time, pairs of variables with a variation inflation factor greater than 2 (systolic and diastolic blood pressure) were considered to have multicollinearity, and the variable with a lower coefficient of correlation with PG120 (systolic blood pressure) was excluded.

PG, insulin, Pro, SI, β, and P/I were considered to constitute glucose-insulin-Pro profiles, and their correlation with serum adiponectin levels was tested using the Spearman rank correlation for bivariate analysis. Factors for which a significant correlation was identified were analyzed with multiple regression analysis after adjustment for background factors (age, sex, BMI, diastolic blood pressure, heart rate, lipids, family history of diabetes). In all statistical tests, a *P* value less than .05 was taken to indicate statistical significance.

2. Results

A. Characteristics of Participants' Background Factors According to Glucose Tolerance

The participants' background factors according to glucose tolerance are shown in Table 1. The participants were young, and no significant differences in age, BMI, high-density lipoprotein cholesterol, or TG were seen among the glucose tolerance groups. Blood pressure and TC increased from NGT0 to NGT2, but tended to decrease in IGT. As glucose tolerance deteriorated, the proportion of men decreased (NGT0: 79%, NGT1: 73%, NGT2: 69%, IGT: 59%), heart rate increased (NGT0: 62 beats per minute [bpm], NGT1: 64 bpm, NGT2: 67 bpm, IGT: 69 bpm), and the proportion of participants with a family history of diabetes (in a first- or second-degree relative) increased (NGT0: 32%, NGT1: 39%, NGT2: 46%, IGT: 59%). No significant difference among the glucose tolerance groups was seen in the proportion with a family history of diabetes in a first-degree relative.

B. Glucose, Insulin, Proinsulin, Insulin Sensitivity, and Insulin Secretion in Participants According to Glucose Tolerance

PG, IRI, Pro, and P/I levels in the participants according to glucose tolerance are shown in Fig. 1. IRI30 (Fig. 1B), Pro0, Pro30 (Fig. 1C), P/I30, and P/I60 (Fig. 1D) were not significantly different among the glucose tolerance groups. FIRI (Fig. 1B) showed an increasing tendency and P/I0 (Fig. 1D) showed a decreasing tendency from NGT0 to NGT1. PG0, PG30, PG60, and PG120 (Fig. 1A), IRI60 and IRI120 (Fig. 1B), and Pro60 and Pro120 (Fig. 1C) increased incrementally with deteriorating glucose tolerance. A clearly delayed peak in IRI and excessive postloading Pro levels were observed in the participants with IGT. P/I120 (Fig. 1D) decreased with deteriorating glucose tolerance and was significantly lower for NGT1, NGT2, and IGT than for NGT0.

The SI and β values of the participants according to glucose tolerance are shown in Fig. 2. The SI measure ISI-Matsuda (Fig. 2B) and all β measures (Fig. 2D-2F) incrementally decreased with deteriorating glucose tolerance. Few significant differences in 1/HOMA-IR (Fig. 2A) and 1/FIRI (Fig. 2C) were seen among the glucose tolerance groups.

C. Relationship Between Background Factors and 120-Minute Plasma Glucose Value

The results of multiple regression analysis of the background factors for PG120 are shown in Table 2. Multiple regression analysis revealed negative correlations of PG120 with the proportion of men and TG, and positive correlations with BMI, heart rate, TC, and family history of diabetes.

Table 1. Background factors of participants according to glucose tolerance

	NGT0 (n = 864)	NGT1 (n = 331)	NGT2 (n = 87)	IGT (n = 29)	*P*
Age, y	23 (22-23)	23 (22-24)	23 (22-24)	22 (22-23)	.58
Male, %	684 (79%)	243 (73%)	60 (69%)	17 (59%)	< .01[a]
BMI, kg/m^2	21 (20-23)	22 (20-23)	22 (20-25)	22 (19-24)	.10
SBP, mm Hg	118 (111-126)	119 (111-126)	123 (116-130)	119 (107-124)	< .05
DBP, mm Hg	66 (62-71)	67 (62-73)	70 (63-75)	67 (63-70)	< .05
HR, bpm	62 (56-69)	64 (58-71)	67 (60-76)	69 (61-77)	< .0001[a]
HDL, mg/dL	61 (53-70)	60 (53-70)	61 (54-69)	62 (56-69)	.98
TG, mg/dL	58 (45-79)	62 (47-85)	63 (48-82)	53 (33-84)	.19
TC, mg/dL	165 (148-183)	169 (154-187)	180 (160-202)	174 (150-191)	< .0001
Positive FH No., %	278 (32%)	129 (39%)	40 (46%)	17 (59%)	< .001[a]
Positive FH1 No., %	102 (12%)	47 (14%)	11 (13%)	7 (24%)	.19

Data are shown as median (25th percentile to 75th percentile). *P* values for the continuous variables and categorical variables were determined by the Wilcoxon and chi-square tests, respectively.

Abbreviations: BMI, body mass index; bpm, beats per minute; DBP, diastolic blood pressure; FH, family history of diabetes (first and/or second degree); FH1, family history of diabetes (first degree); HDL, high-density lipoprotein; HR, heart rate; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

[a]The association between the 4 groups is linear.

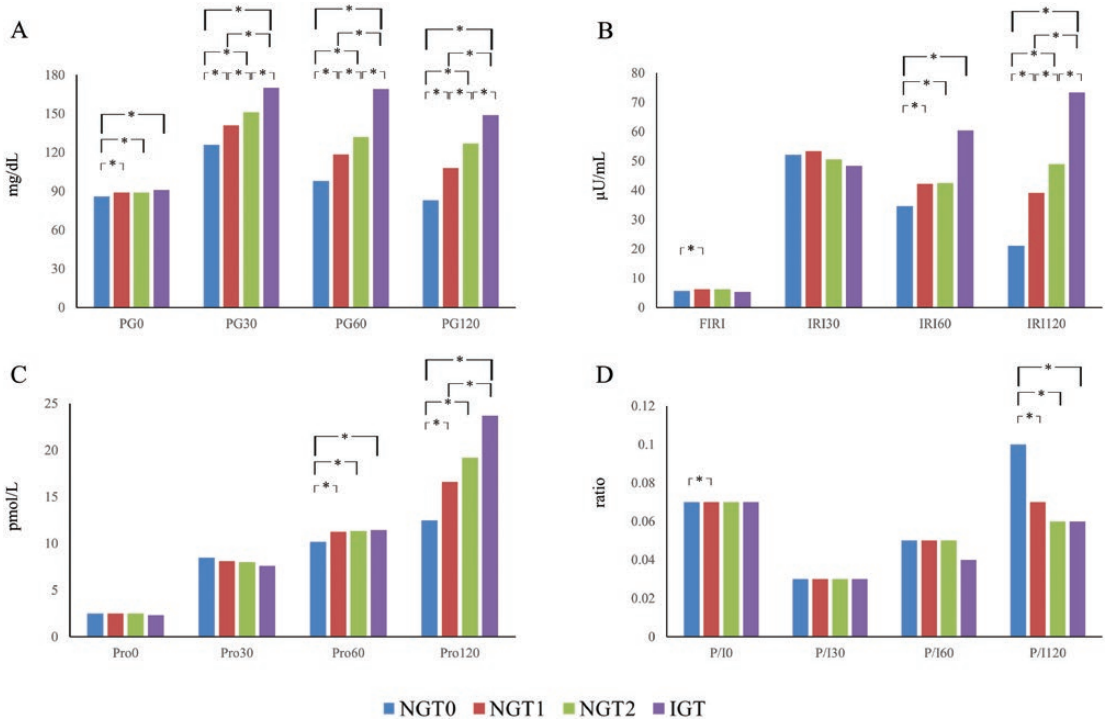

Figure 1. Glucose, insulin, proinsulin, and the proinsulin/insulin ratio according to glucose tolerance. Median bar graphs are shown for A, glucose; B, insulin; C, proinsulin; and D, the proinsulin/insulin ratio according to glucose tolerance. FIRI, fasting IRI; IGT, impaired glucose tolerance; IRI, immunoreactive insulin; NGT, normal glucose tolerance; NGT0, PG120 < 100; NGT1, 100 ≤ PG120 < 120; NGT2, 120 ≤ PG120 < 140, in mg/dL; PG, plasma glucose; P/I, proinsulin/insulin ratio; Pro, proinsulin. *P* values for the variables were determined by paired Wilcoxon test. **P* is less than .05.

D. Serum Adiponectin Levels According to Glucose Tolerance and Sex

Serum adiponectin levels according to glucose tolerance in all participants, women and men, are shown in Fig. 3. Serum adiponectin levels in the participants with NGT were significantly higher in women than in men ($P < .0001$ for NGT0, $P < .0001$ for NGT1, $P < .001$ for NGT2). Serum adiponectin levels in the participants with IGT tended to be higher in women than in men ($P = .058$). Serum adiponectin levels in all participants, women and men, were not significantly different among the glucose tolerance groups.

E. Relationship Between Serum Adiponectin Levels and Glucose-Insulin-Proinsulin Profiles

The results of the Spearman rank correlation analysis of serum adiponectin levels to glucose-insulin-Pro profiles and multiple regression analysis following adjustment for background factors are shown in Table 3. In the Spearman rank correlation analysis, serum adiponectin levels were negatively correlated with some PG, IRI, Pro, and SI measures in OGTT, but not with measures of β. Serum adiponectin levels were independently and negatively correlated with PG0 in the multiple regression analysis. However, the other measures of PG, IRI, Pro, P/I, and SI, as well as β, were not independently correlated with serum adiponectin levels.

3. Discussion

Slight elevations in PG in NGT and prediabetes have been reported to be associated with the long-term risk of diabetes onset [6, 22, 23]. Elucidating the relationship between slight

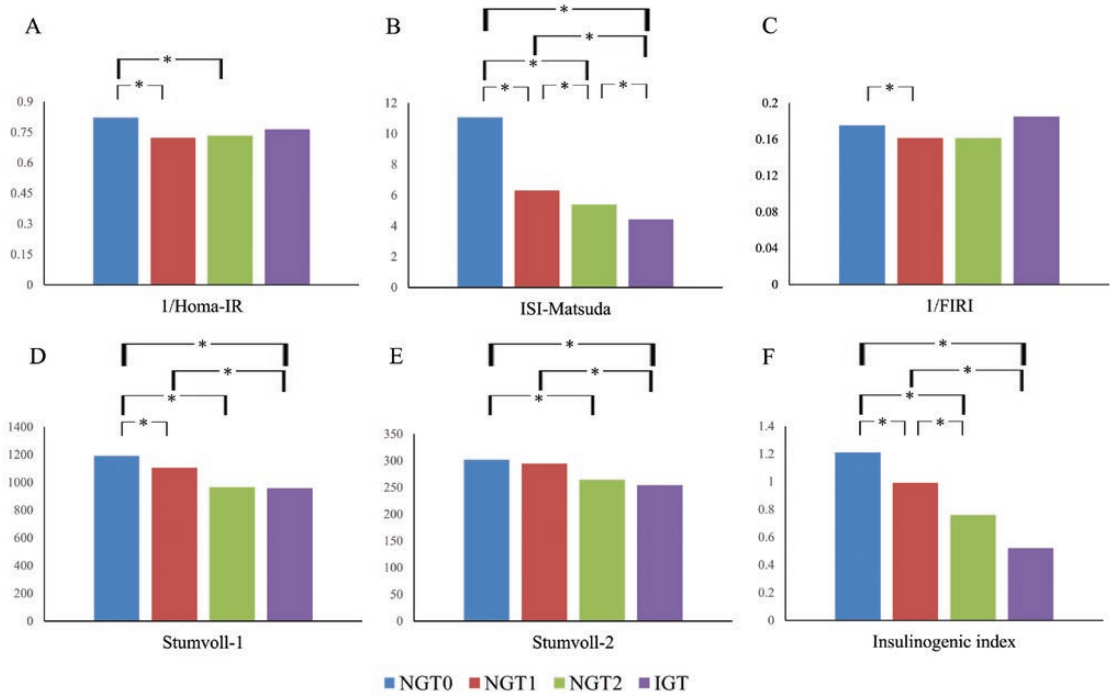

Figure 2. Insulin sensitivity (SI) and β according to glucose tolerance. Median bar graphs are shown for A to C, SI indices, and D to F, β indices, according to glucose tolerance. Abbreviations are the same as those in Fig. 1 FIRI, fasting IRI; IGT, impaired glucose tolerance; IRI, immunoreactive insulin; NGT, normal glucose tolerance; NGT0, PG120 < 100; NGT1, 100 ≤ PG120 < 120; NGT2, 120 ≤ PG120 < 140, in mg/dL. *P* values for the variables were determined by paired Wilcoxon test. **P* is less than .05.

Table 2. Multiple regression analysis for PG120 in all participants

	t	*P*
Age, y	0.49	.62
Male, %	–2.73	< .01
BMI, kg/m^2	3.01	< .01
DBP, mm Hg	0.31	.76
HR, bpm	3.82	< .001
HDL, mg/dL	–1.86	.063
TG, mg/dL	–2.07	< .05
TC, mg/dL	2.94	< .01
FH, %	2.62	< .01

Abbreviations: BMI, body mass index; bpm, beats per minute; DBP, diastolic blood pressure; FH, family history of diabetes (first and/or second degree); HDL, high-density lipoprotein; HR, heart rate; PG, plasma glucose; TC, total cholesterol; TG, triglycerides.

elevations in PG and β-cell function in NGT and prediabetes is therefore important to clarify pathogenic factors for progression from NGT to diabetes. However, it is not well known whether slight elevations in PG are correlated with β-cell function or markers related to β-cell function in individuals with NGT. The present study showed that in a large sample of young Japanese participants, early β progressively decreases, and postloading Pro levels become excessive as glucose tolerance deteriorates from within the normal range toward IGT. Serum adiponectin levels were inversely correlated with fasting glucose.

Chinese individuals with NGT or IGT showed no abnormalities in insulin dynamic patterns across the NGT categories of glucose tolerance, but the peak of Pro level was delayed [7]. In the present study, even participants with NGT had delayed and/or excessive

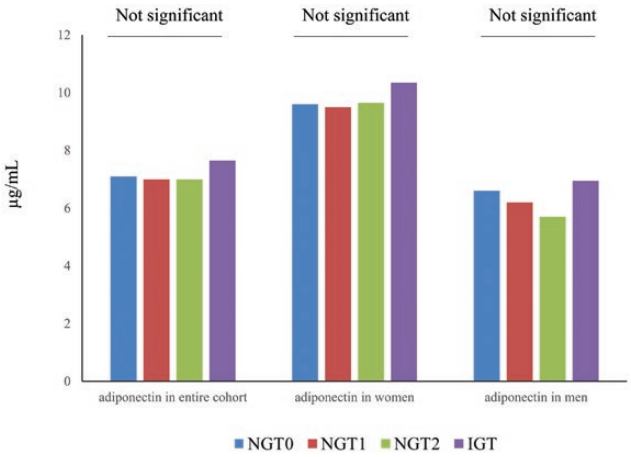

Figure 3. Serum adiponectin levels according to glucose tolerance and sexual status. Median bar graphs are shown for serum adiponectin levels according to glucose tolerance and sex. Abbreviations are the same as those in Fig. 1 IGT, impaired glucose tolerance; NGT, normal glucose tolerance; NGT0, PG120 < 100; NGT1, 100 ≤ PG120 < 120; NGT2, 120 ≤ PG120 < 140, in mg/dL. No differences in any comparison were significant. *P* values for the variables were determined by paired Wilcoxon test.

Table 3. Relationship between serum adiponectin levels and glucose-insulin-proinsulin profiles in all participants

	Nonparametric Spearman rank coefficient		Multiple regression analysis after adjusting for background factors	
	ρ	*P*	*t*	*P*
PG0	–0.0868	< .01	–2.13	< .05
PG30	–0.1298	< .0001	–0.76	.45
PG60	–0.0486	.098		
PG120	–0.0236	.42		
FIRI	–0.1078	< .001	0.18	.86
IRI30	–0.07	< .05	–0.20	.84
IRI60	–0.0699	< .05	0.07	.94
IRI120	0.0034	.91		
Pro0	–0.134	< .0001	–0.98	.33
Pro30	–0.0773	<0.01	–0.97	.33
Pro60	–0.0948	< .01	–1.05	.29
Pro120	–0.0511	.081		
P/I0	0.0164	.58		
P/I30	–0.0047	.87		
P/I60	–0.0075	.80		
P/I120	–0.0694	< .05	–0.89	.38
1/HOMA-IR	0.1183	< .0001	–0.49	.63
ISI-Matsuda	0.0458	.12		
1/FIRI	0.1078	< .001	–0.71	.48
Stumvoll-1	–0.0321	.27		
Stumvoll-2	–0.0419	.15		
Insulinogenic index	0.0276	.36		

Abbreviations: FIRI, fasting immunoreactive insulin; HOMA-IR, homeostasis model assessment of insulin resistance; IRI, immunoreactive insulin; ISI, index of insulin sensitivity; PG, plasma glucose; P/I, proinsulin/insulin ratio; Pro, proinsulin.

insulin and Pro levels as glucose tolerance deteriorated, but the peak of the Pro level was not delayed (Fig. 1C). In the previously mentioned study [7], those with NGT (OGTT 120-min value < 120 mg/dL; equivalent to NGT0 or NGT1 in the present study) had a Pro peak at 60 minutes after glucose loading, but intact Pro levels in the present study continued to increase through 120 minutes after glucose loading, even in the NGT0 and NGT1 categories (Fig. 1C). Differences in the Pro assay procedures, age, or sample size may explain this disparity. In the present study, SI was used to evaluate systemic and hepatic insulin sensitivity, and β was used to evaluate GSIS. ISI-Matsuda and all β measures decreased with deteriorating glucose tolerance. It cannot be concluded from the earlier mentioned study [7] that β abnormalities did not manifest over the glucose tolerance categories of NGT because that study did not evaluate SI and β in detail, as in the present study.

Other studies have found that in people with prediabetes or diabetes, Pro levels increase as glucose tolerance deteriorates, and that high Pro levels precede diabetes onset [24-26]. Those studies, however, did not evaluate insulin and Pro dynamics in different glucose-tolerance categories within NGT. Fasting Pro and the P/I ratio did not essentially differ across the NGT categories and IGT in the present study (Fig. 1C and 1D). Postloading insulin and Pro levels became delayed and/or excessive as the NGT categories approached IGT (Fig. 1B and 1C). P/I120 values from NGT1 to IGT were lower than that of NGT0 (Fig. 1D), whose PG120 level had returned to baseline (Fig. 1A). This is consistent with the results that P/I120 values were lower in IFG/IGT and diabetic participants than those in NGT [26]. These findings suggest that β exceeds Pro secretion in response to sustained postloading elevated glycemia in different NGT categories or in IGT, which seems to be a plausible response [27].

We also investigated the relationship of various clinical background factors to glucose tolerance (OGTT 120-minute value; PG120). As glucose tolerance deteriorated, the proportion of men decreased, and the proportion of participants with a family history of diabetes and resting heart rate increased. Multivariable analysis showed these factors to be independently correlated with PG120. Studies have associated sex and a family history of diabetes with glucose intolerance in participants, including those with IGT [28, 29]. In those studies, IGT was more prevalent in women and those with a family history of diabetes, consistent with our findings. One possible explanation for the preponderance of women with IGT is assumed to be lower body mass and skeletal muscle mass in female participants [30]. A recent genome-wide assessment of the relationship between resting heart rate and the risk of diabetes onset found resting heart rate to be strongly associated with the onset of type 2 diabetes [31]. Consistent with that study, increased resting heart rate was associated with deteriorated glucose tolerance in the present study. Elevated resting heart rate in young Japanese individuals with NGT could therefore be a risk factor for future glucose intolerance.

We also investigated whether serum adiponectin levels were associated with glucose-insulin-Pro profiles in our mainly NGT participants. As in previous studies, serum adiponectin levels were higher in women, although serum adiponectin levels across the glucose-tolerance categories showed no differences in all participants or in women or men. In bivariate analysis with glucose-insulin-Pro profiles and multivariable analysis adjusted for background factors, serum adiponectin levels were independently and negatively correlated with fasting glucose. This correlation between serum adiponectin levels and glucose-insulin-Pro profiles has often been examined in people with prediabetes or diabetes, but less often in those with NGT [32-34]. A study in a non-DM population found low serum adiponectin levels to be associated with IFG [33]. Consistent with that study, our findings suggest that low serum adiponectin levels could be associated with moderately elevated fasting glucose in non-IFG individuals consisting mainly of those with NGT. Other studies have associated hypoadiponectinemia with impaired β [12, 13], but the present study found no association between serum adiponectin levels and impaired β in the stages of NGT or IGT.

Several limitations must be noted. Owing to the cross-sectional nature of this study, a direct causal relationship of β-cell function and Pro dynamics affecting glucose tolerance

was not proven. However, the strength of the present study is that β-cell function and Pro dynamics were examined using a large sample of participants with common characteristics (mainly NGT and young). Abnormalities in insulin and Pro dynamics have been associated with aging [35], but no age differences were observed in this study across the different glucose-tolerance categories. Because we measured peripheral venous levels of insulin and Pro and the half-lives of those in serum are different, serum levels may not accurately reflect secretion rates. However, comparison of those levels at the same time during OGTT could allow us to estimate secretion abnormalities [17-20]. Because measurements were sparse and restricted within 120 minutes after glucose loading, the true insulin and Pro peak by differences in glucose tolerance may not be captured. Some of the participants in the present study could possibly experience glucose intolerance and develop diabetes in the future, and a comparative study between this study and disease onset may be of potential interest.

In conclusion, all measures of β and ISI-Matsuda decreased with deteriorating glucose tolerance, even within individuals with NGT. Delayed and/or excessive Pro and insulin levels and a decrease in the P/I ratio with deteriorating glucose tolerance are plausible responses to postloading elevated glycemia. The proportion of women, proportion of participants with a family history of diabetes, and elevated resting heart rate were associated with deteriorated glucose tolerance (ie, high PG120), and low serum adiponectin levels were associated with elevated fasting glucose. Insulin and Pro dynamics progressively became abnormal as glucose tolerance deteriorated incrementally from within the normal range toward IGT.

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Disclosure Summary: The authors have nothing to disclose.

Data Availability: The data sets generated and/or analyzed during the present study are not publicly available, but are available from the corresponding author on reasonable request.

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