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Diabetic family history in young Japanese persons with normal glucose tolerance associates with k-means clustering of glucose response to oral glucose load, insulinogenic index and Matsuda index

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ABSTRACT

Aims: The present study aimed to clarify the relationships between diabetic family history (FH), and dysglycemic response to the oral glucose tolerance test (OGTT), insulin secretion, and insulin sensitivity in young Japanese persons with normal glucose tolerance (NGT).
Methods: We measured plasma glucose (PG) and immunoreactive insulin levels in 1,309 young Japanese persons (age <40 years) with NGT before and at 30, 60, and 120 min during a 75-g OGTT. Dysglycemia during OGTT was analyzed by k-means clustering analysis. Body mass index (BMI), blood pressure (BP), and lipids were measured. Insulin secretion and sensitivity indices were calculated.
Results: PG levels during OGTT were classified by k-means clustering analysis into three groups with stepwise decreases in glucose tolerance even among individuals with NGT. In these clusters, proportion of males, BMI, BP and frequency of FH were higher, and lipid levels were worse, together with decreasing glucose tolerance. Subjects with a diabetic FH showed increases in PG after glucose loading and decreases in insulinogenic index and Matsuda index.
Conclusions: Dysglycemic response to OGTT by k-means clustering analysis was associated with FH in young Japanese persons with NGT. FH was also associated with post-loading glucose, insulinogenic index, and Matsuda index.

1. Introduction

Diabetes mellitus develops is a disease group in which hyperglycemia occurs due to insufficient sensitivity and/or secretion of insulin [1]. To clarify risk factors for of developing diabetes, aspects of the shape of the glucose response in the oral glucose tolerance test (OGTT shape) has been analyzed [2]. Such aspects include the timing of the appearance of the plasma glucose (PG) peak, when the post-loading PG level becomes lower than the pre-loading (fasting) level, and the timing of a second rise after declining from the peak (biphasic) [3–8]. Hulman et al. recently reported a method to classify OGTT shape into clusters based on machine learning [9–11]. In those reports, a latent class mixed model was used, and a high PG level 30 min after glucose loading predicted the risk of developing diabetes in the future. K-means clustering analysis has been adopted as another type of cluster analysis [12]. In obese patients, among clusters classified according to insulin secretion or sensitivity, PG levels, degree of obesity, age, and other factors reportedly differed [13]. To the best of our knowledge, however, no reports have classified OGTT shape using k-means clustering analysis.

A family history (FH) of diabetes or a first-degree relative with diabetes (FH1) are risk factors for developing non-diabetic hyperglycemia or diabetes [14–16], and the risk is reportedly higher for among

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individuals with FH1 than among those with a second-degree relative with diabetes (FH2) [17]. The risk is also reportedly higher for among individuals with a mother who has diabetes than for among those with a father who has diabetes [18–20]. PG levels after glucose loading are higher, and insulin secretion and sensitivity indices are lower among individuals with more than one FH1 or FH2 [21]. Such findings reports suggest that an FH involving more than one relative is associated with lower insulin secretion and sensitivity and raises the risk of for diabetes.

In contrast, dyslipidemia is more frequent among children with FH and in non-obese, non-diabetic individuals with FH [22,23]. FH1-positive (FH1+) patients with normal glucose tolerance (NGT) are reported to show decreased insulin secretion and sensitivity with as increases in blood pressure, lipid levels, and degree of obesity [24,25]. Females with FH appear more susceptible to diabetes than males with FH [26]. The effect of FH differs by sex and whether the individual is obese, and also affects lipid levels Consideration of subject characteristics is thus important when investigating the relationship between FH and PG or insulin levels. Among the many investigations of OGTTs in young persons with NGT, none appear to have examined show the relationships between FH details and abnormal OGTT shape or insulin secretion and sensitivity indices in individuals with homogeneous features.

We hypothesized that even in young persons with NGT, the presence or absence of FH would be related to abnormal OGTT shape and decreased insulin secretion and sensitivity. To test this hypothesis, we first classified OGTT shape by k-means clustering analysis of PG levels during OGTT from a large number of young persons with NGT and compared various background factors, including FH, among the clusters. We also compared background factors, PG levels, insulin secretion and sensitivity indices in OGTT in subjects with and without FH and evaluated the relationship between FH and insulin secretion and sensitivity indices.

2. Subjects, materials and methods

2.1. Participants

Study participants comprised 1,309 medical students at Jichi Medical University (age <40 years) who had NGT, from among about 1,400 students who had undergone a 75-g OGTT between December 2002 and April 2015. NGT was defined based on Japan Diabetes Society criteria (fasting PG <110 mg/dL and 120-min value <140 mg/dL) [27]. Because a high frequency of non-diabetic hyperglycemia (impaired fasting glucose and/or impaired glucose tolerance) was seen among individuals with FH+, and these individuals were excluded to maintain subject uniformity. The present study was approved by the ethics committee at Jichi Medical University (approval no. EKI 09–45). Written consent was obtained from all participants after providing full information on the purposes of the study. Background factors, PG, insulin, and proinsulin levels were investigated in all participants.

2.2. Measurements and calculation of indices (glucose-insulin-proinsulin profiles)

We measured PG concentration using a glucose oxidase assay, and insulin using an immunoradiometric assay for immunoreactive insulin (IRI) (Insulin RIA Beads II; Yamasa, Tokyo, Japan), as described previously [28]. The manufacturer claims that there is little cross-reactivity with proinsulin in the immunoradiometric assay for IRI. Proinsulin (Pro) levels were was determined with the Intact-Proinsulin Assay (MLT Research, Cardiff, UK), a chemiluminescent immunoassay procedure, as described previously [29]. Inter- and intra-assay variabilities for IRI and Pro were less than 5% and 10%, respectively. Samples for IRI and Pro analyses were frozen until immunoassays were performed at about 6-month intervals. In the 75-g OGTT, PG, IRI, and Pro levels were measured under fasting conditions (preloading) and at 30, 60, and 120 min after glucose loading; these are abbreviated as PG0, PG30, PG60, and PG120, FIRI, IRI30, IRI60, and IRI120, and Pro0, Pro30, Pro60, and Pro120, respectively. The molar ratio of Pro to IRI (P/I) was calculated. P/I values are abbreviated as P/I0, P/I30, P/I60, and P/I120.

Similar to our previous studies [28,29], we used the following measures. Systemic insulin sensitivity (SI) as determined by the Matsuda index (ISI-Matsuda) was calculated as: ISI-Matsuda = 10,000/[sqrt (PG0 × PG120 × FIRI × IRI120)] [30,31]. In addition, 1/FIRI and 1/homeostasis model assessment of insulin resistance (HOMA-IR) were used primarily as measures of hepatic SI. HOMA-IR was calculated as [PG0·FIRI/405] [32]. The insulinogenic index, a measure of acute insulin response to glucose load, was calculated as follows: insulinogenic index = (IRI30 – FIRI)/(PG30 – PG0) [33,34]. The units for PG and IRI were milligrams per deciliter and microunits per milliliter for calculating ISI-Matsuda, 1/FIRI, HOMA-IR, and the insulinogenic index. Stumvoll-1 and Stumvoll-2 indices were used as first- and second-phase insulin responses to glucose load, respectively: Stumvoll-1 = 1283 + 1.829•IRI30–138.7•PG30 + 3.772•FIRI; and Stumvoll-2 = 287 + 0.4164•IRI30–26.07•PG30 + 0.9226•FIRI [35]. Here, the units for PG and IRI were millimoles per liter and picomoles per liter, respectively. We treated negative or unusable insulinogenic index values (PG30 = PG0) and negative Stumvoll-1 and Stumvoll-2 indices as missing.

2.3. Questionnaires and measurements of background factors

Data on age, sex, and FH were obtained through questionnaires. FH1+ was defined as a positive family history of any diabetes in a first-degree relative; that is, a parent (father or mother) or full siblings (brother or sister). FH2+ was defined as a positive family history in a second-degree relative; that is, a grandparent, aunt, uncle, niece, or nephew. Subjects with both FH1+ and FH2+ were designated included as FH1+.

High-density lipoprotein cholesterol (HDL), triglycerides (TG), and total cholesterol (TC) levels were measured using serum collected under fasting conditions. Low-density lipoprotein cholesterol (LDL) concentration was calculated using the Friedewald formula [36]. Sitting heart rate (HR) and blood pressure (BP) (systolic blood pressure [SBP] and diastolic blood pressure [DBP]) were measured after the participant had been seated at rest for 5 min. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared. Waist circumference (WC) was measured at the umbilical level with the subject standing [37].

2.4. Cluster analysis

K-means clustering analysis was performed to differentiate the OGTT shape in OGTT. PG levels in OGTT were was classified by k-means clustering analysis using JMP version 5.1 statistical software (SAS Institute Inc., Cary, NC, USA). The most suitable number of clusters was selected from among 3 to 6 based on the optimum cubic clustering criterion. Subjects lacking PG30 or PG60 (n = 14) were excluded from analysis.

2.5. Analysis of the relationship between SI and insulin secretion (β)

The three indices of SI (1/HOMA-IR, ISI-Matsuda, 1/FIRI) and three indices of β (Stumvoll-1, Stumvoll-2, insulinogenic index) were used in nine combinations. The regression line of best fit for SI and β was obtained by ($\log10(\beta) = a \cdot \log10(SI) + b$) after each logarithmic transformation. Because errors exist in x-axis and y-axis measurements, fitting was performed by standardized major axis (SMA) regression using SMATR version 2.0 statistical software [28]. With the SMA of this software, pairwise statistical comparison of the slope value can also be performed using the likelihood ratio test. When −1 is contained within the 95% confidence interval of the slope value of SI and β, the relationship between SI and β is thought to be hyperbolic, and the product of

the two becomes the disposition index (β corrected by SI).

2.6. Statistical analysis

JMP version 5.1 was used for all statistical analysis. Since almost none of the variables had a normal distribution, results are expressed as the median (25th percentile, 75th percentile). K-means clustering analysis was performed as described above. In comparing each of the clusters, glucose-insulin-proinsulin profiles were expressed with mean values, and analyses of variance were used in these comparisons. All values except glucose-insulin-proinsulin profiles are shown as median (25th percentile, 75th percentile) or number. In these comparisons, the Wilcoxon signed-rank test or chi-squared test was used to compare differences among the three groups. For comparisons of background factors and glucose-insulin-profiles according to FH positivity, the Wilcoxon signed-rank test or chi-squared test was used to compare differences between the two groups. For all statistical tests, values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Characteristics of the entire subject cohort

The median age of subjects was 23 (22, 23) years and the cohort included 1,002 men and 307 women. Median BMI was 21.3 (20.0, 23.0) kg/m^2. The number of FH+ subjects was 448 (34%). Among those who were FH+, 163 (36%) were FH1+ and 285 (64%) were FH2+. In FH1+ subjects, the FH of diabetes was the father only, mother only, and both parents in 124, 31, and 5 subjects, respectively. There was also 1 subject each with an FH from both the father and older sister, from the younger brother only, and from the older sister only.

3.2. Identification of OGTT shape clusters by k-means clustering analysis

The selected number of clusters was three based on the optimum cubic clustering criterion. The OGTT shape was divided into three groups with stepwise decreases in glucose tolerance (Fig. 1). The PG levels value rose from Cluster 1 through Cluster 3 both before and after loading. The IRI levels value tended to be higher in Cluster 2 than in Cluster 1 or 3 at 30 min after loading, but rose from Cluster 1 through Cluster 3 before loading and at 60 and 120 min after loading (Fig. 1).

The Pro levels value did not differ significantly among between groups before or at 30 min after loading, but rose from Cluster 1 through Cluster 3 at 60 and 120 min after loading. The P/I ratio, although showing no significant difference among between groups before loading, decreased from Cluster 1 through Cluster 3 after loading (Fig. 2).

All β indices decreased from Cluster 1 through Cluster 3. For SI indices, although no significant differences in 1/FIRI were seen evident among groups, 1/HOMA-IR and ISI-Matsuda decreased from Cluster 1 through Cluster 3 (Fig. 3).

3.3. Characteristics of participants by OGTT shape clusters

The background factors for each cluster are shown in Table 1. No significant differences in age were seen among clusters. The proportion of males, BMI, WC, HR, SBP and DBP, TG, TC, LDL, proportion of FH+, and proportion of FH1+ rose from Cluster 1 through Cluster 3. HDL decreased from Cluster 1 through Cluster 3.

3.4. Hyperbolic combinations of SI and β by SMATR

SMA was investigated with SMATR in all subjects and with and without FH. The slope of the regression lines of best fit with respect to the relationship between SI and β varied in the nine combinations obtained with SMA varied. In all subjects, no combination of SI and β showed a hyperbolic relationship. Likewise, with and without FH, no combination of SI and β became hyperbolic (data not shown). Consequently, disposition index was not calculated in the present study.

3.5. Characteristics of participants according to FH

Background factors and glucose-insulin-proinsulin profiles were compared between FH negative (FH-) and FH+, between FH- and FH1+, and between FH- and FH2+ (Table 2).

In the comparison of FH- and FH+, there were no significant differences were identified in age, BMI, WC, HR, SBP or DBP, lipids, PG0, FIRI, IRI30, Stumvoll-1, Stumvoll-2, 1/HOMA-IR, 1/FIRI, each Pro level value, or P/I ratio, except for P/I120. The proportion of males decreased with FH+. Post-loading PG, IRI60, and IRI120 levels rose with FH+. The Both insulinogenic index and ISI-Matsuda decreased with FH+. P/I120 decreased with FH+.

In the comparison of FH- and FH1+, there were no significant differences were evident in age, BMI, WC, HR, SBP or DBP, lipids, PG0, FIRI, IRI30, Stumvoll-1, Stumvoll-2, 1/HOMA-IR, 1/FIRI, Pro levels value except for Pro120, or each P/I ratio. The proportion of males decreased with FH1+. Post-loading PG, IRI60, and IRI120 levels rose with FH1+. The Both insulinogenic index and ISI-Matsuda decreased with FH1+. Pro120 levels rose with FH1+.

In the comparison of FH- and FH2+, no significant differences were seen in age, BMI, WC, HR, SBP or DBP, PG0, FIRI, IRI30, IRI60, Stumvoll-1, Stumvoll-2, insulinogenic index, 1/HOMA-IR, 1/FIRI, each Pro level, P/I0, or P/I60. Although a significant difference in P/I30 was seen, the median values were almost identical (0.0318 with FH- and 0.0316 with FH2+). TC levels also rose with FH2+, and no significant differences with other lipids were apparent. The proportion of males

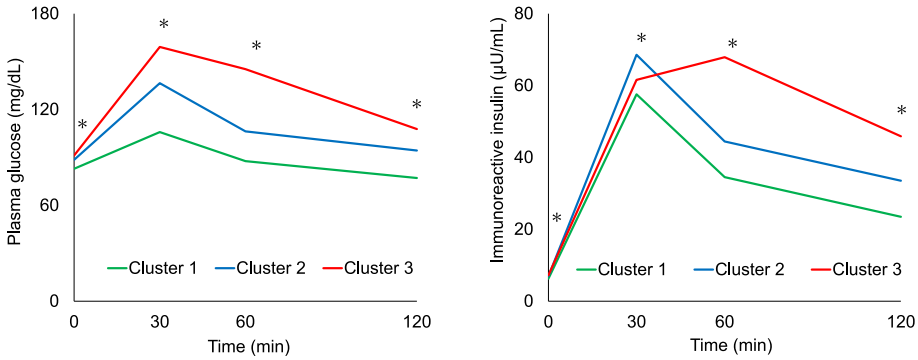

Fig. 1. OGTT shape clusters by k-means clustering analysis and corresponding immuno-reactive insulin levels.
Data are shown as means. Analysis of variance was used to compare differences among the three groups. *$P < 0.05$. Green lines are Cluster 1. Blue lines are Cluster 2. Red lines are Cluster 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

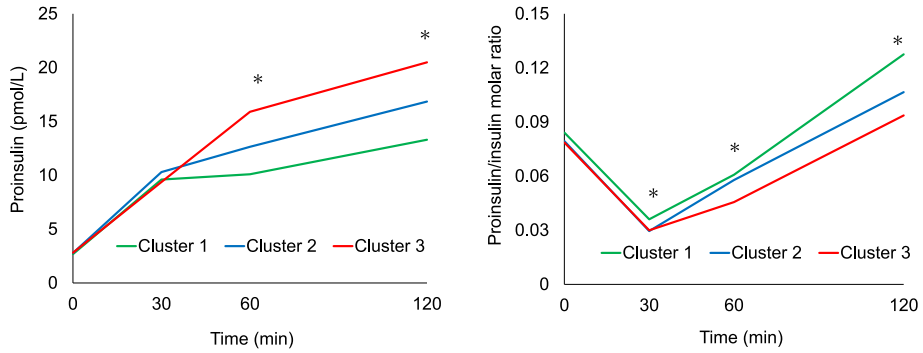

Fig. 2. Corresponding proinsulin levels and proinsulin/insulin molar ratio according to OGTT shape clusters.
Data are shown as means. Analysis of variance was used to compare differences among the three groups. *$P < 0.05$. Green lines are Cluster 1. Blue lines are Cluster 2. Red lines are Cluster 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

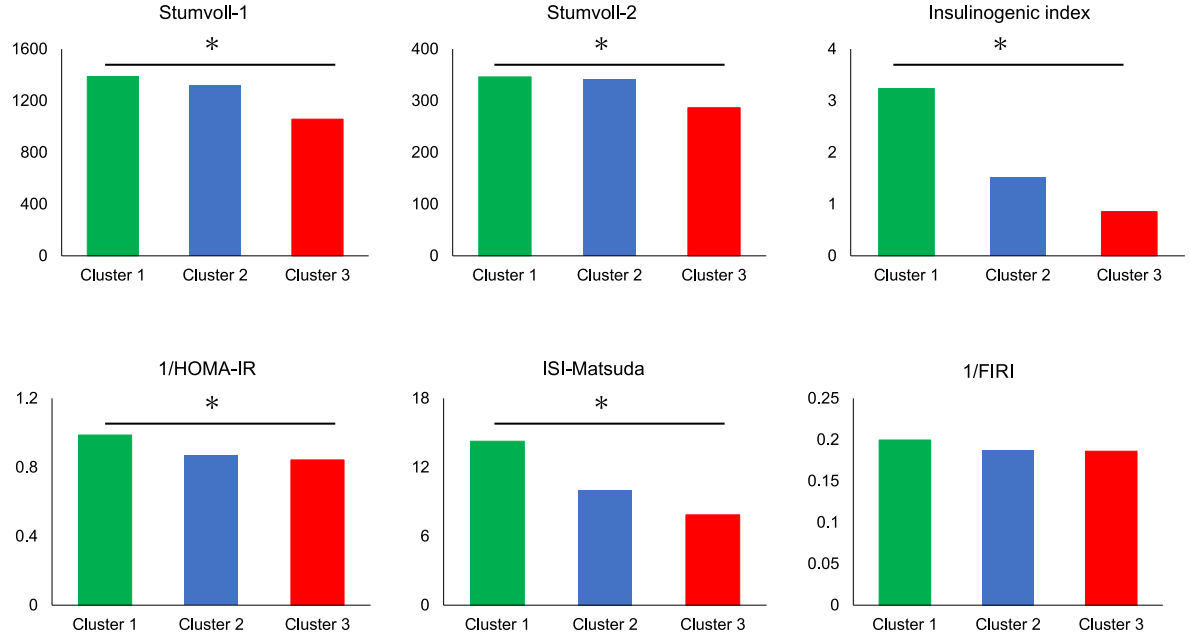

Fig. 3. Insulin secretion (upper panels) and insulin sensitivity (lower panels) indices according to OGTT shape clusters.
Data are shown as means. Analysis of variance was used to compare differences among the three groups. *$P < 0.05$. Green bars are Cluster 1. Blue bars are Cluster 2. Red bars are Cluster 3. Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; ISI-Matsuda, Matsuda index; FIRI, fasting immunoreactive insulin. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

decreased with FH2+. Post-loading PG and IRI120 levels rose with FH2+, ISI-Matsuda decreased with FH2+, and P/I120 decreased with FH2+.

A comparison of FH2 and FH1 found no significant differences in any indices (data not shown). Moreover, among FH1+ subjects, no factors showed a significant difference in a comparison of background factors and glucose-insulin-proinsulin profiles among subjects in whom the FH of diabetes was in the father, mother, or both parents.

4. Discussion

In the present this study, the OGTT shape of NGT subjects was classified by k-means clustering analysis of PG levels, and three groups were identified by differences in glucose tolerance. The proportion of males, BMI, and blood pressure and lipid levels rose from Cluster 1 (with good glucose tolerance) through Cluster 3 (with poor glucose tolerance). The FH+ rate became also increased. Meanwhile, in an analysis based on whether subjects had FH, an elevation in PG levels after glucose loading, higher IRI60 and IRI120 levels, and lower insulinogenic index and ISI-Matsuda were seen with FH+ in young NGT subjects. This phenotype was clearer with FH1+ than with FH2+. FH+ was related to abnormal OGTT shape and elevated PG levels after glucose loading even in the NGT category. The mechanism seems related to decreased insulinogenic index and ISI-Matsuda.

To identify OGTT shape, we performed classification with a k-means clustering analysis of OGTT PG levels and identified three clusters. PG levels rose progressively from Cluster 1 through Cluster 3. Higher IRI levels from Cluster 1 through Cluster 3 before loading and at 60 and 120 min after loading were seen. In Cluster 3, peak PG was at 30 min after loading, but the IRI peak was at 60 min after loading. In a report by Hulman et al., OGTT shape was classified into four patterns in a latent class mixed-effects analysis, and the cluster with a maximum PG at 30 min after loading was shown to carry a high risk of developing diabetes [10]. In that report, similar to the present study, insulin levels in OGTT showed a trend linked to PG. Peak insulin level occurred at 60 min after loading, which was delayed compared with the peak PG level. Likewise, in the cluster analysis of the present this study, peak IRI was behind the PG peak, particularly in Cluster 3, which displayed had the worst glucose tolerance.

Pro levels tended to be higher and P/I ratio tended to decline from

Table 1
Characteristics of backgrounds in all subjects by k-means clustering analysis of PG in OGTT.

	Cluster 1 (n=420)	Cluster 2 (n=585)	Cluster 3 (n=290)	P value
Age (years)	23 (22, 23)	23 (22, 23)	23 (22, 24)	0.45
Male/Female (n)	302/118	461/124	233/57	<0.05
BMI (kg/m^2)	21.1 (19.7, 22.5)	21.4 (20.1, 23.1)	21.6 (20.1, 23.7)	<0.01
WC (cm)	74 (70, 78)	76 (71, 80)	76 (71, 82)	<0.0001
HR (beats/min)	63 (56, 70)	63 (56, 69)	65 (58, 72)	<0.05
SBP (mmHg)	117 (109, 124)	119 (111, 126)	120 (113, 128)	<0.001
DBP (mmHg)	66 (62, 71)	66 (62, 72)	68 (63, 78)	<0.01
HDL (mg/dL)	63 (55, 72)	60 (53, 69)	59 (52, 68)	<0.01
TG (mg/dL)	58 (44, 76)	60 (45, 79)	64 (47, 91)	<0.05
TC (mg/dL)	165 (148, 189)	167 (150, 183)	172 (156, 190)	<0.001
LDL (mg/dL)	88 (73, 105)	92 (78, 105)	95 (83, 113)	<0.0001
FH-/FH+ (n)	310/110	368/217	171/119	<0.0001
FH-/FH2+/FH1+ (n)	310/74/36	368/136/81	171/73/46	<0.001

Date are shown as median (25th percentile, 75th percentile) or number.
The Wilcoxon signed-rank test or the chi-squared test was used to test for differences between the three groups.
Abbreviations: PG, plasma glucose; OGTT, oral glucose tolerance test; BMI, body mass index; WC, waist circumference; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; TG, triglycerides; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; FH, family history of diabetes; FH2+, positive family history of diabetes among second-degree relatives; FH1+, positive family history of diabetes among first-degree relatives.

Cluster 1 through Cluster 3 at 60 min and later after loading. From Cluster 1 through Cluster 3, all β indices (Stumvoll-1, Stumvoll-2, and insulinogenic index) decreased and two SI indices (1/HOMA-IR and ISI-Matsuda) also decreased. We have previously shown that, together with worsening glucose tolerance, Pro levels were higher and P/I ratio, all β indices, and a SI index (ISI-Matsuda) were lower in a population with NGT and impaired glucose tolerance, representing a similar result to the present findings [29].

In a large number of middle-aged people in Finland, the proportion of males and blood pressure reportedly rose and dyslipidemia worsened from NGT to non-diabetic hyperglycemia and then to new diabetes [38]. The present study further showed that the proportion of males, blood pressure, and lipid levels abnormalities rose as glucose tolerance became worsened from Cluster 1 through Cluster 3, even in young Japanese with NGT. FH+ and FH1+ rates rose from Cluster 1 through Cluster 3. FH+ showed a relationship with abnormal OGTT shape by k-means clustering analysis. From the perspective point view of FH, the proportion of females was high among subjects with FH+, consistent with a previous report [26], and subjects with FH+ displayed higher PG levels after loading. The reasons for this discrepancy are unclear.

In the comparisons of FH- and FH+, post-loading PG levels rose with FH+, while higher IRI60 and IRI120 levels were seen with FH+. Meanwhile, Pro120 levels rose with FH1+ and P/I120 decreased with FH+ and FH2+. With FH+, the slight elevation in PG levels after loading seemed to be sustained, insulin secretion was sustained, IRI60 and IRI120 levels were high, and P/I120 was low. These results are consistent with glucose-raising genetic risk factors that have been reported to result in beta cell dysfunction and insulin resistance [39–41].

Although Stumvoll-1 and Stumvoll-2 as β indices and 1/FIRI and 1/HOMA-IR as SI indices were unrelated to FH in the present study, the insulinogenic index and ISI-Matsuda decreased with FH+. Several reports have shown a relationship between FH and insulin secretion and sensitivity, but those reports were for populations with high PG levels or older high age groups [24,42]. The present results suggested that insulin secretion and sensitivity are related to FH even in a homogeneous

Table 2
Characteristics among all subjects by FH.

	FH-(n=861)	FH+ (n=448)	FH1+ (n=163)	FH2+ (n=285)
Age (years)	23 (22, 23)	23 (22, 23)	23 (22, 24)	23 (22, 23)
Male/Female (n)	685/176	317/131 ***	116/47 **	201/84 ***
BMI (kg/m^2)	21.3 (20.1, 23.0)	21.3 (19.9, 23.1)	21.4 (19.7, 23.0)	21.2 (20.0, 23.3)
WC (cm)	75 (71, 80)	75 (70, 80)	74 (69, 80)	75 (71, 81)
HR (beats/min)	63 (56, 70)	64 (57, 71)	65 (57, 72)	63 (56, 70)
SBP (mmHg)	118 (111, 125)	119 (111, 128)	120 (111, 128)	119 (110, 127)
DBP (mmHg)	66 (62, 72)	67 (62, 73)	67 (63, 73)	67 (62, 73)
HDL (mg/dL)	61 (53, 69)	61 (54, 70)	62 (54, 70)	61 (54, 70)
TG (mg/dL)	60 (46, 81)	59 (44, 79)	58 (45, 75)	60 (44, 82)
TC (mg/dL)	166 (150, 184)	169 (152, 189)	166 (149, 188)	170 (152, 189)*
LDL (mg/dL)	91 (77, 106)	93 (77, 109)	92 (77, 105)	94 (77, 112)
PG0 (mg/dL)	87 (81, 93)	88 (82, 93)	87 (82, 93)	88 (82, 94)
PG30 (mg/dL)	131 (111, 148)	135 (119, 153) ***	138 (122, 155) ***	134 (118, 153) **
PG60 (mg/dL)	103 (88, 123)	110 (94, 129) ****	113 (94, 131) ***	109 (93, 128) ***
PG120 (mg/dL)	90 (78, 102)	94 (82, 108) ****	94 (83, 110) **	92 (80, 108) ***
FIRI (μU/mL)	5.9 (4.3, 8.4)	5.7 (4.3, 8.1)	5.8 (4.2, 8.1)	5.7 (4.5, 8.1)
IRI30 (μU/mL)	53 (35, 77)	52 (36, 80)	51 (36, 79)	54 (35, 80)
IRI60 (μU/mL)	36 (24, 56)	40 (25, 63) *	42 (26, 66) *	39 (25, 60)
IRI120 (μU/mL)	25 (15, 41)	30 (18, 48) ***	30 (18, 48) **	29 (17, 48) **
Stumvoll-1	1177 (912, 1524)	1110 (882, 1517)	1110 (809, 1506)	1109 (911, 1526)
Stumvoll-2	302 (243, 382)	292 (239, 386)	286 (225, 381)	293 (243, 389)
Insulinogenic index	1.20 (0.69, 2.12)	1.08 (0.70, 1.65) *	1.00 (0.63, 1.50) *	1.13 (0.71, 1.70)
ISI-Matsuda	9.6 (6.3, 14.0)	8.6 (5.8, 12.5) **	8.6 (6.0, 11.6) *	8.6 (5.8, 12.8) *
1/HOMA-IR	0.79 (0.56, 1.13)	0.81 (0.57, 1.06)	0.80 (0.58, 1.08)	0.81 (0.57, 1.06)
1/FIRI	0.17 (0.12, 0.23)	0.18 (0.12, 0.23)	0.18 (0.12, 0.24)	0.18 (0.13, 0.23)
Pro0 (pmol/L)	2.6 (2.0, 3.2)	2.5 (2.0, 3.1)	2.5 (2.1, 3.1)	2.5 (2.0, 3.1)
Pro30 (pmol/L)	8.5 (6.0, 11.7)	8.1 (5.7, 11.6)	8.6 (6.4, 11.9)	8.0 (5.5, 11.5)
Pro60 (pmol/L)	10.3 (7.4, 14.9)	10.9 (7.5, 15.1)	11.4 (8.0, 16.1)	10.4 (7.1, 14.6)
Pro120 (pmol/L)	13.7 (9.2, 19.6)	14.6 (9.9, 21.7)	15.2 (10.2, 24.5) *	14.0 (9.6, 20.4)
P/I0	0.07 (0.05, 0.10)	0.07 (0.05, 0.10)	0.07 (0.05, 0.10)	0.07 (0.05, 0.10)
P/I30	0.03 (0.02, 0.04)	0.03 (0.02, 0.04)	0.03 (0.02, 0.04)	0.03 (0.02, 0.04) *
P/I60	0.05 (0.03, 0.07)	0.05 (0.03, 0.07)	0.05 (0.03, 0.07)	0.04 (0.03, 0.07)
P/I120	0.10 (0.06, 0.14)	0.08 (0.06, 0.13) **	0.09 (0.06, 0.13)	0.08 (0.06, 0.12) **

Date are shown as median (25th percentile, 75th percentile) or number.
The Wilcoxon signed-rank test or the chi-squared test was used to test for differences.
Abbreviations: FH, family history of diabetes; FH1+, positive family history of diabetes among first-degree relatives; FH2+, positive family history of diabetes among second-degree relatives; BMI, body mass index; WC, waist circumference; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; TG, triglycerides; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; PG, plasma glucose; FIRI, fasting IRI; IRI, immunoreactive insulin; ISI-Matsuda, Matsuda index; HOMA-IR, homeostasis model assessment of insulin resistance; Pro, proinsulin; P/I, proinsulin/insulin molar ratio.
*p <0.05 vs FH-; **p <0.01 vs FH-; ***p <0.001 vs FH-; ****p <0.0001 vs FH-.

population of young persons with NGT. Because insulin secretion is affected by decreased sensitivity, and so a disposition index that considers decreased sensitivity should preferably be used [28]. However, no hyperbolic correlations were observed between insulin secretion and SI, and we were thus unable to obtain a disposition index. With FH+, however, the indices of both insulin secretion and sensitivity decreased. We assumed that the post-loading elevation in PG levels was due to worsened glucose disposition.

Limitations of this study were that the subjects included a high proportion of males (76.5%), possibly resulting in some degree of statistical bias. Further, FH was determined from oral interviews, so accuracy is could not be guaranteed. However, nearly all studies that handle FH use FH obtained by oral interview [15–18,21,22,24,25]. While the majority of diabetes patients in FHs were without insulin therapy and had probably had type 2 diabetes, the type of diabetes in the very small number of patients receiving insulin therapy was uncertain. In addition, subjects were young and their parents were presumably not elderly. There is a possibility that subjects with parents who had not yet developed diabetes were judged as FH1-. However, the frequency of FH1+ individuals (12.5%) roughly agrees with the estimated prevalence of diabetes in the Japanese population ≥50 years of age [43]. Type 2 diabetes shows a multifactorial pattern of inheritance, and genetic risk scores for the likelihood of developing diabetes have been proposed [44]. The relationship between each cluster, FH, and genetic risk scores is interesting, but could not be investigated in this study. Further follow-up of glucose tolerance among subjects from this study would be interesting.

In conclusion, k-means clustering analysis revealed that dysglycemic response to OGTT was associated with FH in young Japanese persons with NGT. FH was also associated with higher post-loading glucose levels, and lower insulinogenic index and Matsuda index. Glucose and insulin responses to the OGTT and insulin secretion and sensitivity indices should be carefully interpreted, even in NGT persons with an FH of diabetes.

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Credit author statement

N Murai: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Project administration. N Saito: Investigation, Resources, Supervision. Nii S, Nishikawa Y, Suzuki A, E Kodama, T Iida, K Mikura, H Imai, M Hashizume, Y Kigawa, R Tadokoro, C Sugisawa, K Endo, T Iizaka, and F Otsuka: Validation, Formal analysis. S Ishibashi: Writing – review & editing, Supervision. S Nagasaka: Conceptualization, Methodology, Validation, Investigation, Resources, Writing – review & editing, Supervision, Project administration.

Data availability

The datasets generated and/or analyzed during the present study are not publicly available, but are available from the corresponding author on reasonable request.

Declaration of competing interest

None.

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