INTRODUCTION

The majority of deaths in patients with type 2 diabetes mellitus (T2DM) result from accelerated cardiovascular arteriosclerosis. The mortality attributable to cardiovascular disease (CVD) is increased 3.2-fold in men and 8.5-fold in women with T2DM compared to that in people not affected by the disease. Macrovascular disease is associated with lower degrees of hyperglycaemia than microvascular disease. The heightened risk for CVD extends to individuals with impaired glucose tolerance (IGT). Both IGT and impaired fasting glucose (IFG) are intermediate states in glucose metabolism and associated with increased CVD risk.

Abdul-Ghani et al showed conversion rates to T2DM at 2.4%, 5.1%, 11.5% and 13.5% for individuals with normal glucose tolerance (NGT), IFG, IGT or combined glucose tolerance (CGI), respectively, over a 7- to 8-year follow-up period. Moreover, they divided NGT
and IFG subjects into four groups (I–IV), based upon the time (30, 60, or 120 minutes or never) at which their plasma glucose concentrations declined below the fasting glucose concentration after the oral glucose load. In NGT subjects, the incidence rate for the development of T2DM was 0% in group I and increased progressively to 1.8%, 2.1% and 2.9% in groups II, III and IV, respectively.5

Analyses from the DECODE data set have demonstrated that the hazard ratios for all-cause mortality in patients with IFG and IGT compared with those with normal fasting and 2-h glucose tolerance were 1.20 and 1.50, respectively.6 A number of studies comparing IGT to IFG seem to point to IGT as being the better predictor of future T2DM development.7–9 However, one study demonstrated both of them are equivalent.10

Reliable models for the identification of individuals at high-risk of T2DM are essential to improve strategies for the prevention of the disease. The oral glucose tolerance test (OGTT) is commonly used to identify high-risk individuals.11 The OGTT is a useful examination tool, not only for diagnosis of T2DM, but also for estimation of insulin secretion. However, due to the need for frequent blood samplings, few studies have been done in young subjects who are commonly healthy.

In this study, we obtained OGTT results from university students, analysed their glucose curves and insulin secretion, and divided the subjects into four groups, according to a published protocol.5 We also studied lipid profiles and compared with the indices of insulin sensitivity and insulin secretion, since glucose intolerance is associated with dyslipidaemia.12

2 | SUBJECTS AND METHODS

2.1 | Study population

All the participants signed informed consent forms, and the Gunma University Ethical Review Board for Medical Research Involving Human Subjects approved the study protocol. Participants were volunteers purely.

The participants were 595 medical candidates who practised at the Gunma University Hospital between May 2010 and July 2016. No subjects were diagnosed as having T2DM or received any medication. As part of their medical practice, they all underwent a comprehensive medical examination, including an OGTT (75 g dextrose monohydrate in 250 mL water) after an overnight fast.
2.2 | Study design

The OGTTs were performed after 10-hour fasts with 0-, 30-, 60- and 120-min samplings to establish plasma glucose and insulin levels, and at the preload time, serum high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), haemoglobin A1c (HbA1c) and glycoalbumin (GA) were measured. Excluded from this study were subjects who were over 30 years of age.

We classified 575 subjects into the NGT group, 19 into the IGT group, and 1 into the IFG group. We removed the subjects with IGT and IFG from further analyses in this analysis. We found no subjects with apparent T2DM.

We measured height and weight and calculated BMIs (weight [kg]/height [m$^2$]). We used enzymatic methods to measure serum HDL-C, LDL-C, TG and GA concentrations, with an automatic analyser (LABOSPECT 008; Hitachi). Serum insulin concentrations were measured by chemiluminescence immunoassay using an automatic analyser (AIA-2000 LA; Tosoh). Plasma glucose concentrations were measured using a hexokinase method, and HbA1c levels were measured by high-performance liquid chromatography, using automatic analysers (ADAMS Glucose GA-1170 and ADAMS A1c HA8180, respectively; Arkray).

2.3 | Grouping

We divided the subjects with NGT into four groups (I-IV), based upon the time (30, 60, or 120 minutes or never) and showed the sequential changes in plasma glucose (Figure 1A) and insulin (Figure 1B) at which their plasma glucose concentration during the OGTT declined below the fasting glucose concentration, following a published protocol. Groups I, II and III included subjects whose plasma glucose concentration fell below the PG0 at 30, 60 and 120 minutes, respectively. Subjects whose plasma glucose never fell below the PG0 at any time during OGTT were defined as group IV.

2.4 | Statistical methods

We calculated areas under the glucose or insulin curves (AUCg and AUCi) based on the trapezoid rule. We also calculated the homeostasis model assessment of insulin resistance (HOMA-IR, fasting plasma glucose [PG0] (mg/dL) × IR0 (μU/mL)/405), $\beta$-cell function (HOMA-$\beta$, IR0 (μU/mL) × 360/PG0 (mg/dL) − 63))$^{13}$ and Matsuda index of insulin sensitivity (10,000/square root of [fasting glucose (mg/dL) × fasting insulin (μU/mL)] × [mean glucose (mg/dL) × mean insulin (μU/mL) during OGTT]$^{14}$ as reported. We calculated the insulinogenic index by dividing the increment in serum insulin (μU/mL) by the increment in plasma glucose (mg/dL) during the 0- to 30-min time periods of the OGTT.$^{15}$ The insulin secretion/insulin resistance (disposition) index was calculated as insulinogenic index × Matsuda index.$^{16}$

The SPSS version 25 statistical software package was used to perform the statistical analyses. The data were expressed as the mean ± standard deviation. We compared the continuous variables across the glucose tolerance groups using one-way ANOVA followed by Tukey’s post hoc tests. We used ROC curves to discriminate between group II and group III + IV by some indices.

3 | RESULTS

3.1 | Clinical characteristics

The average age of the 575 NGT subjects was 23.7 ± 1.7 years, and the average BMI was 21.2 kg/m$^2$ (Table 1). Of these, 28 subjects (4.9%), 120 subjects (20.9%), 143 subjects (24.9%), and 284 subjects (49.4%) were classified into groups I, II, III and IV, respectively (Table 2).

3.2 | Sequential changes in plasma glucose and insulin

Figure 1 and Table 2 show the sequential changes in plasma glucose and insulin for each group during OGTT. We observed significant differences among the groups, especially in the plasma glucose values. The 30-minute postload plasma glucose (PG30) in group II was significantly higher than that in group I, and that in groups III and IV was significantly higher than that in groups I and II. Regarding the 60-minute postload plasma glucose (PG60), the values in groups III and IV were significantly higher than those in groups I and II. The mean level of the 120-minute postload plasma glucose (PG120) in group IV was significantly higher than that in the other groups.

Regarding plasma insulin, we observed no significant differences in the fasting plasma insulin (IRI0) among groups. For the mean 30-minute postload plasma insulin (IRI30), those in groups II, III and IV were significantly higher than the average in group I. The mean 60-minute postload plasma insulin (IRI60) was significantly higher in groups III and IV than in groups I and II. The mean 120-minute postload plasma insulin (IRI120) of group IV was significantly higher than that in the other groups. Although we divided subjects according
3.3 | Lipid profiles

In the analyses of lipid profiles, the HDL-C levels in groups III and IV were significantly lower than those in group II. The LDL-C levels in group IV were significantly higher than those in group III, and we found no significant differences in the TG levels among the groups (Table 2).

3.4 | AUC of glucose and insulin

The incremental areas under both the glucose and insulin curves (AUCg and AUCi) increased progressively from groups II to IV (Table 2). In contrast, we found no significant differences in the ratio of AUCi/AUCg among the groups.

3.5 | Glucose metabolism indices

We found no significant differences in the HbA1c levels among groups. Likewise, the mean insulin resistance index, HOMA-IR, showed no significant group differences. Similarly, there were no significant differences in mean HOMA-β and indicator of insulin secretion potential. However, the Matsuda index, an indicator of whole-body insulin sensitivity, declined progressively from group I to IV, and the insulinogenic index, calculated by (IRI30-IRI0)/(PG30-PG0), was less than 0 in group I, a higher value in group II, a lower value in group III and an even lower value in group IV. The
disposition index (the product of Matsuda index and insulinogenic index), reflected the combination of insulin secretion and insulin sensitivity, also showed a similar trend (less than 0 in group I, the highest value in group II, and progressively lower values in groups III and IV).

3.6 | ROC curves of identifying the prolonged glucose elevation group

In the past study, Abdul-Ghani et al defined group I + II as low-risk group for T2DM, and group III + IV as high-risk group for T2DM.5 Low-risk group showed rapid glucose lowering, and high-risk group showed prolonged glucose elevation. Therefore, we described ROC curves of indices between groups identifying the high-risk group (group III + IV) from the low-risk group (group I + II). The Matsuda, insulinogenic and disposition indices were compared. Between groups I + II and III + IV, the AUCs of ROC of the disposition, the insulinogenic and Matsuda indices were not good (AUC = 0.706, 0.652 and 0.629, respectively) (Figure 2A). Since PG30 is smaller than PG0 in group I, insulinogenic and disposition indices were calculated into negative value in group I. Negative value misled to be poor insulin secretion potential. Therefore, we excluded the group I from ROC analysis. As a result, good AUCs were obtained in disposition index (AUC = 0.847) better than the insulinogenic and Matsuda indices (AUC = 0.786, 0.616, respectively; Figure 2B).

4 | DISCUSSION

In this cross-sectional study, we found that healthy young Japanese individuals within physiological range of glycaemic control accompanied the sequential decreases in insulin sensitivity and secretion. We showed the differences in the OGTT-derived indices of insulin sensitivity and insulin secretion among 4 groups, according to a previous study.3 The insulin sensitivity, calculated using the Matsuda index, decreased progressively in subjects from groups I to IV as with the study.5 In addition, the insulin secretion assessed using the insulinogenic index decreased in the subjects from groups II to IV (the value of this index in group I was less than zero by definition) along with the study.5

Although we tried to classify participants by insulin secretion pattern according to the previous study,17 we could not find a significant difference among groups. We also tried to apply other indices such as the QUICKI (quantitative insulin sensitivity check index),18 the McAuley (an index of insulin resistance)19 and the fasting Belfiore (fasting insulin resistance index)20; however, we found no significant differences between the groups (data not shown).

In terms of glucose tolerance, our results showed similar insulin sensitivities or secretion levels to the levels in the above-mentioned study.11 Even though the mean age of subjects in our study at 23.7 was 30 years younger than the mean age of subjects in the prior study at 54.1,5 our results suggest that Japanese young individuals and Finnish middle-aged individuals share similar glucose metabolism. This is similar to a publication, suggesting that the insulin response in Asian Americans was lower than that in other ethnic groups such as Hispanic American, Caucasians and African Americans.21

Among the Japanese population, a study on OGTTs among 2157 middle-aged Japanese individuals showed that only 1125 (52.1%) had NGT, while the others had IFG (525 [24.3%]), IGT (159 [7.3%]), IFG + IGT (263 [12.2%]) and diabetes (85 [3.9%]).22 The mean age of that study was 52.6 years, which was similar to that in the study by Abdul-Ghani et al.5,22 Young normoglycaemic children of Indian parents with diabetes mellitus showed higher plasma insulin levels, and lower insulin sensitivity and β-cell compensation than subjects without parents with T2DM.23 The difference may have been caused by the different ethnicities, dietary habits or family histories.
The IR10, an index that can identify the future risk for T2DM, has been associated with insulin resistance. A comparison between the OGTT and the euglycaemic hyperinsulinaemic clamp technique suggested that the fasting insulin level should be a marker of insulin resistance. In addition, an insulin suppression test concluded that fasting plasma insulin and HOMA-IR were highly correlated in nondiabetic individuals. However, in the present study, we found no significant difference in IR10 among the study groups.

Some subjects showed lower glucose values at PG30 than at PG0. In addition, about 5% of the subjects in group I had negative insulinogenic index values, a percentage close to that in the literature, where the majority of individuals with low glucose and increased insulin values belonged to the NGT group. In the present study, all the subjects in group I showed similar changes in glucose and insulin. Although we tried to draw the ROC curves classifying the prolonged glucose elevation group (group III + IV) from the rapid glucose lowering group (group I + II), the specificities of insulinogenic and disposition indices were poor (Figure 2A). These results were explained as follows: they were calculated into negative value in group I. In general, a higher insulinogenic index shows better insulin secretion. Although subjects in group I were thought to have good insulin secretion potential, negative value misled to be poor insulin secretion potential. Therefore, we excluded the group I from ROC analysis. As a result, good AUCs were obtained in disposition index (Figure 2B).

Among the glucose and insulin indices, the most significant differences were observed in the AUCg among the groups. However, this was an inevitable result given that we divided the subjects into four groups according to their glucose values.

The definition of the disposition index varies among the researchers. While Weiss et al determined it as the product of the insulinogenic index and the Matsuda index, Asano et al calculated it as the quotient of the insulinogenic index divided by the HOMA-IR, and Retnakaran et al calculated it as the product of the Matsuda index and the AUCi/AUCg. We used the product of the insulinogenic index and the Matsuda index because we found no significant differences in HOMA-IR and AUCi/AUCg, among the study groups. Our analyses with the AUCi/AUCg x Matsuda index showed differences among groups similar to those of the Matsuda index alone (data not shown). For the product of the insulinogenic index and the Matsuda index, we found more significant differences among groups than insulinogenic index as with the study by Abdul-Ghani et al.

We compared the ROC curves of indices between groups distinguishing the prolonged glucose elevation group (group III + IV) from the rapid glucose lowering group (group II). Between group II and group III + IV, disposition index showed good AUC of ROC compared to insulinogenic and Matsuda indices (Figure 2B). The Matsuda index is a good indicator of whole-body insulin sensitivity. On the other hand, in Japanese, IGT is associated with reduction of insulin secretion. These data suggested low early secretion of insulin has larger impact on glucose intolerance of Japanese than low peripheral insulin sensitivity. Further, disposition index, an index that indicates the composition of insulin secretion and sensitivity, may be a good indicator of glucose tolerance.

CONFLICT OF INTEREST
There are no conflicts of interest to declare.

AUTHORS’ CONTRIBUTIONS
TK and MM designed the study. OA, TO, KT, YS, HI and MN have contributed to data collection. AY contributed to data interpretation and wrote the initial draft of the manuscript. TK contributed to analysis and interpretation of data, and assisted in the preparation of the manuscript. MM critically reviewed the manuscript and finally approved of the article. All authors approved the final version of the
manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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How to cite this article: Ishigaki H, Yoshida A, Araki O, et al. Prolonged plasma glucose elevation on oral glucose tolerance test in young healthy Japanese individuals. Endocrinol Diab Metab. 2020;3:e00098. https://doi.org/10.1002/edm2.98