An inverse U-shaped association of late and peak insulin levels during an oral glucose load with glucose intolerance in a Japanese population: a cross-sectional study

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Abstract. The current study investigated the association of post-load insulin levels with glucose tolerance in a Japanese population. A total of 1450 Japanese employees who underwent a 75-g oral glucose tolerance test (OGTT) were included. Glucose tolerance was assessed by 120-min glucose levels during a 75-g OGTT. A penalized cubic regression spline model analysis revealed that the 60- and 120-min insulin levels, but not 0- or 30-min insulin levels, had an inverse U-shaped relationship to the 120-min glucose level. Furthermore, peak insulin level followed an inverse U shape in relation to the 120-min glucose level, whereas the peak of insulin appeared at a later point in time as the 120-min glucose level increased. These associations were similarly observed in both obese and non-obese subgroups, although obesity was associated with higher insulin levels. Peak insulin levels also demonstrated an inverse U shape in association with 0-min glucose levels and indices of β cell function, assessed by the disposition index and the β-cell function index. In conclusion, peak insulin levels followed an inverse U shape in relation to glucose intolerance in a Japanese population, whereas the impairment of glucose tolerance was associated with a delay in the time to reach peak insulin levels.

Key words: Japanese, Glucose tolerance, Post-load insulin level, Oral glucose tolerance test

EPIDEMIOLOGICAL studies have suggested that insulin levels after an oral glucose load is transiently increased during progression from normal glucose tolerance to diabetes mellitus in Caucasians [1], similar to observations in animal models of type 2 diabetes mellitus associated with obesity and insulin resistance [2]. However, it remains inconclusive whether the phenomenon is also the case for a Japanese population.

It has long been described that Japanese patients with type 2 diabetes mellitus were less obese and less insulin resistant compared to Caucasians [3] and predominantly had impaired insulin secretion [4]. Therefore, a Japanese population would possibly demonstrate a different pattern of insulin response; they might lack an observable transient increase of insulin secretion during the course of glucose intolerance. However, to date, few detailed data are available regarding the association between glucose tolerance and post-load insulin levels in a Japanese population. Although some previous studies investigated its association, they categorized their study population and just assessed the inter-category differences [5, 6]. A broad categorization would remove a considerable amount of information from the original data and could lead to biased findings [7, 8]. Indeed, the previous reports on insulin levels after an oral glucose load were somewhat controversial; some reports showed a progressive decline of insulin levels in subjects with normal glucose tolerance, dysglycemia and diabetes [5], whereas others indicated an increase of insulin levels in dysglycemia and diabetes compared to normal glucose tolerance [6], during the development from normal glucose tolerance to type 2 diabetes mellitus.

In the current study, we analyzed the continuous association between glucose tolerance and insulin levels after an oral glucose load in a Japanese population, to investigate whether an increase of insulin levels would be observed in relation to glucose intolerance in
a Japanese population.

Materials and Methods

Study population and definitions
We used a database of the Amagasaki Visceral Fat Study (UMIN000002391). The study was approved by the human ethics committee of Osaka University. Written informed consent was obtained from every participant. The current study included 1450 Japanese employees of the city office of Amagasaki, Hyogo, who underwent a 75-g oral glucose tolerance test (OGTT) in addition to an annual general health checkup between 2004 and 2006. None of the subjects had anti-diabetes treatment. An OGTT was performed based on the physician’s recommendation in health checkups, mainly in (1) subjects with suspected diabetes mellitus (fasting glucose ≥ 110 mg/dL, casual glucose ≥ 140 mg/dL, or hemoglobin A1c ≥ 6.0%), and (2) those with one or more of the following metabolic risk factors: elevated blood pressure (systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg), dyslipidemia (triglycerides ≥ 150 mg/dL, high-density lipoprotein cholesterol < 40 mg/dL, or low-density lipoprotein cholesterol ≥ 140 mg/dL), elevated uric acid levels (≥ 7.0 mg/dL), and increased waist circumference (≥ 85 cm in males and ≥ 90 cm in females). An OGTT was also recommended for other reasons based on the physicians’ discretion. Of a total of 1450 subjects, 466 subjects (32%) had suspected diabetes mellitus, and 929 (64%) had other metabolic risk factors without suspected diabetes mellitus, whereas the remaining 55 (4%) had none of these metabolic abnormalities. When subjects underwent a 75-g OGTT more than one time during the 3-year periods, the data of the first year were used for the analysis. Exclusion criteria were as follows: under treatment for renal or hepatic diseases, malignant neoplasms, estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m², and/or transaminase ≥ 100 IU/L. Hemoglobin A1c levels were converted to a National Glycohemoglobin Standardization Program equivalent value with the conversion equation reported by the Japan Diabetes Society [9].

During a 75-g OGTT, plasma glucose and insulin levels were measured at 0, 30, 60, and 120 min. Insulin levels were measured by immunoradiometric assay (IRMA) kits (Insulin RIA Beads II, Yamasa Co., Ltd.). The glycemia were categorized into the following three types according to the Japan Diabetes Society [10]: a diabetic type (0-min glucose ≥ 126 mg/dL or 120-min glucose ≥ 200 mg/dL), a normal type (0-min glucose < 110 mg/dL and 120-min glucose < 140 mg/dL), and a borderline type (neither diabetic nor normal type).

Statistical analysis
Data are given as means and standard deviations (SD) or medians and interquartile range (IQR, 25th percentile to 75th percentile) for continuous variables or as percentages for discrete variables. A p value of less than 0.05 was considered to be significant, and 95% confidence intervals (CI) were given when required. All statistical analyses were performed using R version 3.1.0 (R Development Core Team). Detailed analytic procedures are described below.

Association of glucose tolerance with glucose and insulin levels
The penalized cubic regression spline model was used to analyze the association between the independent variable (120-min glucose levels) and the dependent variables (glucose or insulin levels during an OGTT). Glucose and insulin levels had a right-skewed distribution and therefore their log-transformed variables were entered into the model. We also investigated the first derivative of the obtained regression function to determine its slope at any level of the log-transformed 120-min glucose levels. When the slope continues to be significantly positive (i.e. higher than zero), the curve will follow a monotonic increase within the range. In contrast, when the slope is significantly negative (i.e. lower than zero), the curve will follow a monotonic decrease. On the other hand, the curve will follow a U shape or an inverse U shape when the slope changes from negative to positive or from positive to negative, respectively. The slope was considered to be significantly positive at a certain level of the independent variable when the lower limit of the 95% CI was higher than zero. The slope was significantly negative when the upper limit of the 95% CI was lower than zero.

We subsequently divided the study population into obese and non-obese subjects, which were defined as body mass index (BMI) ≥ and < 25 kg/m², respectively. To estimate the difference in the dependent variable (i.e. log-transformed glucose or insulin levels) between the two subgroups, a dummy variable representing the presence of obesity and its interaction term with the log-transformed 120-min glucose levels were additionally
entered as independent variables into the original regression model. Based on this newly developed regression model, the difference in the dependent variable generated by the presence of obesity was evaluated at any level of the log-transformed 120-min glucose levels. Given that the dependent variable was log-transformed glucose or insulin levels, the exponential transformation of its estimated difference gave the fold difference in glucose or insulin levels. When the 95% CI of the fold difference did not include 1, the glucose or insulin levels were determined to be significantly different.

We similarly investigated the association of 120-min glucose levels with the peak glucose and insulin levels during an OGTT and their peak time, with the use of the penalized cubic regression spline model. The peak levels and the peak time in each subject were estimated using smoothing spline functions. In addition, the regression analysis was performed using fasting (0-min) glucose levels and two indices of β cell function, i.e., the disposition index [11, 12], and the β-cell function index [13], as the independent variable in substitution for 120-min glucose levels. The disposition index and the β-cell function index were determined as follows.

**Calculation of disposition index and β-cell function index**

According to previous reports [11, 12], the OGTT-derived disposition index was calculated as the product of the $AUC_{\text{Ins}}/AUC_{\text{Glu}}$ and the Matsuda index [14], where the $AUC_{\text{Ins}}/AUC_{\text{Glu}}$ represents the ratio of area under the curve of insulin levels to that of glucose levels during the 0- to 120-min time periods under the OGTT. They were calculated in accordance with the trapezoidal rule. In the subgroup with normal glucose tolerance in the current study population, the slope of the regression line between the log-transformed values of the $AUC_{\text{Ins}}/AUC_{\text{Glu}}$ and the Matsuda index was calculated to be -1.11 (95% CI: -1.16 to -1.07) by the orthogonal regression analysis with the total least squares (TLS) method. The analysis was performed after exclusion of bivariate outliers, which were detected using the modified Stahel-Donoho estimators for the Mahalanobis squared distance. The finding indicates that the relationship between the $AUC_{\text{Ins}}/AUC_{\text{Glu}}$ and the Matsuda index was close to but statistically different from the hyperbola. The β-cell function index was calculated as $(AUC_{\text{Ins}}/AUC_{\text{Glu}}) \times (\text{Matsuda index})^{1.11}$ [13].

Although the Pearson’s correlation coefficient between $\log(AUC_{\text{Ins}}/AUC_{\text{Glu}})$ and $\log(\text{Matsuda index})$ was as high as -0.81 (95% CI: -0.83 to -0.79), the repeated Fisher-Yates shuffle of 0-min insulin values brought a lower correlation coefficient (95% percentile interval: -0.62 to -0.56) and a different slope of the regression line (95% percentile interval: -0.84 to -0.75), indicating that the correlation was not just artrificial.

**Results**

The 1450 analyzed subjects had mean age 49 ± 9 years old and included 1266 males (87%) (Table 1). A total of 1031 subjects (71%) were categorized as having normal glycemia, whereas 304 (21%) and 115 (8%) were borderline and diabetic type, respectively. Among 304 subjects with borderline type, 174 subjects had 0-min glucose levels of less than 110 mg/dL and 120-min glucose levels of 140 to 199 mg/dL, 59 subjects had 0-min glucose levels of 110 to 125 mg/dL and 120-min glucose levels of less than 140 mg/dL, and the remaining 71 subjects had 0-min glucose levels of 110 to 126 mg/dL and 120-min glucose levels of 140 to 199 mg/dL. The median $AUC_{\text{Ins}}(\muU/h/mL)/AUC_{\text{Glu}}(mg\cdot h/dL)$ was 0.21 (IQR: 0.14 to 0.30), median Matsuda index was 7.0 (IQR: 4.6 to 10.7), median disposition index was 1.5 (IQR: 1.1 to 2.0), and median β-cell function index was 1.8 (IQR: 1.3 to 2.5). A total of 748 subjects (52%) were obese, with mean BMI 27.5 ± 2.4 kg/m², and the remaining 702 subjects (48%) were non-obese, with mean BMI 22.7 ± 1.7 kg/m².

Fig. 1 shows the association of 120-min glucose levels with glucose and insulin levels at each blood-sampling point during the OGTT. The 0- to 60-min glucose levels demonstrated a monotonic increase as 120-min glucose levels increased. On the other hand, the 60- and 120-min insulin levels, but not 0- or 30-min insulin levels, followed an inverse U shape. The subgroup analysis in obese and non-obese subjects is shown in Fig. 2. Obesity was associated with higher insulin levels, whereas glucose levels were similar between obese and non-obese subjects.

We subsequently investigated the association between 120-min glucose levels and the peak of glucose and insulin levels during an OGTT (Fig. 3). The peak insulin levels had an inverse U shape in relation to 120-min glucose levels, while the peak glucose levels and their peak time demonstrated a monotonic increase as 120-min glucose levels increased. As shown in Fig. 4, the peak insulin levels were higher in obese subjects than in non-obese subjects. The peak glucose levels,
Table 1  Clinical characteristics of study population

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<tr>
<td>N</td>
<td>1450</td>
</tr>
<tr>
<td>Male</td>
<td>1266  (87%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49 ± 9</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>25.2 ± 3.2</td>
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<tr>
<td>Waist circumference (cm): male / female</td>
<td>87 ± 8 / 85 ± 11</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>131 ± 18</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81 ± 12</td>
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<tr>
<td>Hemoglobin A1c (%)</td>
<td>5.6 ± 0.5</td>
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<tr>
<td>Triglycerides (mg/dL)</td>
<td>150 (IQR: 101 to 227)</td>
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<tr>
<td>High-density lipoprotein cholesterol (mg/dL)</td>
<td>56 ± 16</td>
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<tr>
<td>Low-density lipoprotein cholesterol (mg/dL)</td>
<td>117 ± 31</td>
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<tr>
<td>Uric acid (mg/dL)</td>
<td>6.0 ± 1.4</td>
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<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>23 (IQR: 16 to 34)</td>
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<tr>
<td>Aspartate aminotransferase (IU/L)</td>
<td>22 (IQR: 18 to 27)</td>
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<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>79 ± 14</td>
</tr>
<tr>
<td>Category of glycemia: normal / borderline / diabetic</td>
<td>1031 (71%) / 304 (21%) / 115 (8%)</td>
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<tr>
<td>0-min glucose (mg/dL)</td>
<td>95 (IQR: 89 to 103)</td>
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<td>30-min glucose (mg/dL)</td>
<td>157 (IQR: 134 to 183)</td>
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<tr>
<td>60-min glucose (mg/dL)</td>
<td>149 (IQR: 117 to 192)</td>
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<tr>
<td>120-min glucose (mg/dL)</td>
<td>113 (IQR: 94 to 138)</td>
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<tr>
<td>0-min insulin (μU/mL)</td>
<td>5.5 (IQR: 3.7 to 8.6)</td>
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<td>30-min insulin (μU/mL)</td>
<td>28.7 (IQR: 17.6 to 45.0)</td>
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<tr>
<td>60-min insulin (μU/mL)</td>
<td>33.9 (IQR: 21.8 to 52.0)</td>
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<tr>
<td>120-min insulin (μU/mL)</td>
<td>26.8 (IQR: 16.0 to 43.7)</td>
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Data are n (percentage), mean ± SD, or median (interquartile range (IQR)).

Fig. 1  Association of glucose tolerance with glucose (right panels) and insulin profiles (left panels) during a 75-g OGTT. The upper panels show glucose and insulin profiles estimated from the penalized cubic regression spline model in which their log-transformed values were entered as the dependent variable and the log-transformed 120-min glucose levels as the independent variable. The lower panels indicate the first derivative \( f'(x) \) (i.e. the slope) of the regression model. Data are represented as estimates (bold lines) and their 95% CI (dotted lines).
Insulin levels during OGTT in Japanese was associated with delayed peak time of insulin. The current findings suggest a temporal and compensatory increase of insulin response to an oral glucose load in subjects with glucose intolerance. The findings were consistent with some previous reports in a Japanese population [6], which demonstrated that the late-phase insulin level after a glucose load was increased in glucose intolerance. Previous studies in Caucasians demonstrated that early-phase insulin secretion after a glucose load was decreased in subjects at the stage of prediabetes whereas their total insulin secretion was increased [15]. In a Japanese population, it was reported that total insulin secretion was not so remarkably declined as early-phase insulin secretion was associated with delayed peak time of insulin.

Discussion

The current study investigated the continuous association of post-load insulin levels with glucose tolerance assessed by 120-min glucose levels in a Japanese population. Peak insulin levels after an oral glucose load had an inverse U shape in relation to glucose intolerance, whereas the impairment of glucose tolerance and the peak time of glucose and insulin, were similar between obese and non-obese subjects.

Peak insulin levels also demonstrated an inverse U shape in association with 0-min glucose levels, the disposition index, and the β-cell function (Fig. 5).

Fig. 2 Association of glucose tolerance with glucose (A) and insulin profiles (B) during a 75-g OGTT in non-obese and obese subjects. The upper panels show glucose and insulin profiles estimated from the penalized cubic regression spline model in which their log-transformed values were entered as the dependent variable and the log-transformed 120-min glucose levels as the independent variable. The middle panels indicate the first derivative $f'(x)$ (i.e. the slope) of the regression model. The lower panels indicate the fold difference $D$ generated by the presence of obesity, which was estimated from the penalized cubic regression spline model in which the presence of obesity and its interaction term with the log-transformed 120-min glucose levels were additionally entered as the independent variables. Data are represented as estimates (bold lines) and their 95% CI (dotted lines).
Takahara et al. did not, but rather demonstrated a gradual decrease in association with the impairment of glucose tolerance. This would be consistent with previous studies reporting that the early phase of insulin secretion was predominantly and progressively decreased in the course of the impairment of glucose tolerance [5, 6, 18, 19].

In the current study, an inverse U shape of peak insulin levels was also demonstrated in association with indices of the β cell function (Fig. 5). Given that a progressive decline of β cell function is a key pathophysiological feature in the impairment of glucose tolerance [20, 21], it would be no surprise that peak insulin levels follow a similar pattern in association with the β-cell function as glucose tolerance. However, there might remain a concern about the validity of the indices of the β cell function. In the current study, the disposition index was calculated as the product of the AUC_{Ins}/AUC_{Glu} and the Matsuda index [11, 12]. Although the relationship between the two parameters were very close to the hyperbola, the statistical procedure denied the null hypothesis that the relationship was hyperbolic.

Fig. 3 Association of glucose tolerance with peak glucose and insulin levels (left panel) and their peak time (right panel) during a 75-g OGTT. The upper panels show peak glucose and insulin levels (left panel) and their peak time (right panel) estimated from the penalized cubic regression spline model in which their log-transformed values were entered as the dependent variable and the log-transformed 120-min glucose levels as the independent variable. The lower panels indicate the first derivative f’(x) (i.e. the slope) of the regression model. Data are represented as estimates (bold lines) and their 95% CI (dotted lines).
Fig. 4 Association of glucose tolerance with the peak glucose and insulin levels (A and B) and their peak time (C and D) during a 75-g OGTT in non-obese and obese subjects.

The upper panels show peak glucose and insulin levels (left panel) and their peak time (right panel) estimated from the penalized cubic regression spline model in which their log-transformed values were entered as the dependent variable and the log-transformed 120-min glucose levels as the independent variable. The middle panels indicate the first derivative \( f'(x) \) (i.e., the slope) of the regression model. The lower panels indicate the fold difference \( D \) generated by the presence of obesity, which was estimated from the penalized cubic regression spline model in which the presence of obesity and its interaction term with the log-transformed 120-min glucose levels were additionally entered as the independent variables. Data are represented as estimates (bold lines) and their 95% CI (dotted lines).
Fig. 5 Association of 0-min glucose levels (A), disposition index (B), and β-cell function index (C) with peak glucose and insulin levels (left panel) and their peak time (right panel) during a 75-g OGTT. The disposition index was calculated as \(\frac{AUC_{Ins}}{AUC_{Glu}} \times \text{(Matsuda index)}\) \(^\text{1.11}\). The upper panels show peak glucose and insulin levels (left panel) and their peak time (right panel) estimated from the penalized cubic regression spline model in which their log-transformed values were entered as the dependent variable and the log-transformed 0-min glucose levels (A), the log-transformed disposition index (B), and the log-transformed β-cell function index (C) as the independent variable. The lower panels indicate the first derivative \(f'(x)\) (i.e., the slope) of the original regression model. Data are represented as estimates (bold lines) and their 95% CI (dotted lines).
As an alternative, we therefore additionally calculated the β-cell function index [13]. However, the correlation between the index and glucose tolerance was unlikely superior to that between the original disposition index and glucose tolerance (Pearson’s correlation coefficient $r = -0.770$ vs. -0.774). A hyperbolic relationship between the insulin secretion and sensitivity indices is a key feature in discussion of the disposition index [22]. Although the relationship has been often validated by a statistical manner in which 95% CI of the slope between the log-transformed indices includes -1 [23], such a statistical approach always accompanies an issue of the sample size. A larger sample size can more easily detect a small if any difference and deny a null hypothesis of interest. The statistical denial of the hyperbolic relationship in the current study might come from a larger sample size than previous reports. Whether the indices of the β cell function in the current study was permissibly valid remains inconclusive.

The current study had some limitations. First, it used a cross-sectional design and the time course of the association between glucose tolerance and insulin levels remains to be revealed. Future longitudinal studies will be needed to validate the current cross-sectional findings. Second, a 75-g OGTT was performed in health checkups, rather than for a specified research purpose. Consequently, glucose tolerance of the study populations was widely distributed. However, this wide distribution enabled us to assess the association between glucose tolerance and insulin levels. Third, the blood sampling after the oral glucose load was performed up to 120 min, and post-load levels of glucose and insulin after 120 min were not examined. Fourth, the current study had a predominance of males, with few females included. Future studies with a sufficient number of females will be needed to validate the current findings.

In conclusion, the current study investigated the continuous association of post-load insulin levels with glucose tolerance assessed by 120-min glucose levels in a Japanese population. Peak insulin levels had an inverse U shape in relation to glucose intolerance, whereas the impairment of glucose tolerance was associated with delayed peak time of insulin and glucose levels.

Appendix

There are no conflicts of interests associated with this manuscript.

References