Identification of Insulin Resistance in Subjects with Normal Glucose Tolerance

Jiunn Diann Lin, MD, Jin Biou Chang, MD, Chung Ze Wu, MD, Dee Pei, MD, Chang Hsun Hsieh, MD, An Tsz Hsieh, MD, Yen Lin Chen, MD, Chun Hsien Hsu, MD, Chuan Chieh Liu, MD

Abstract

Introduction: Decreased insulin action (insulin resistance) is crucial in the pathogenesis of type 2 diabetes. Decreased insulin action can even be found in normoglycaemic patients, and they still bear increased risks for cardiovascular disease. In this study, we built models using data from metabolic syndrome (Mets) components and the oral glucose tolerance test (OGTT) to detect insulin resistance in subjects with normal glucose tolerance (NGT).

Materials and Methods: In total, 292 participants with NGT were enrolled. Both an insulin suppression test (IST) and a 75-g OGTT were administered. The steady-state plasma glucose (SSPG) level derived from the IST was the measurement of insulin action. Participants in the highest tertile were defined as insulin-resistant. Five models were built: (i) Model 0: body mass index (BMI); (ii) Model 1: BMI, systolic and diastolic blood pressure, triglyceride; (iii) Model 2: Model 1 + fasting plasma insulin (FPI); (iv) Model 3: Model 2 + plasma glucose level at 120 minutes of the OGTT; and (v) Model 4: Model 3 + plasma insulin level at 120 min of the OGTT. Results: The area under the receiver operating characteristic curve (aROC curve) was observed to determine the predictive power of these models. BMI demonstrated the greatest aROC curve (71.6%) of Mets components. The aROC curves of Models 2, 3, and 4 were all substantially greater than that of BMI (77.1%, 80.1%, and 85.1%, respectively). Conclusion: A prediction equation using Mets components and FPI can be used to predict insulin resistance in a Chinese population with NGT. Further research is required to test the utility of the equation in other populations and its prediction of cardiovascular disease or diabetes mellitus.

Key words: Area under the receiver operating characteristic curve, Body mass index, Insulin resistance, Metabolic syndrome, Oral glucose tolerance test

Introduction

Currently, type 2 diabetes is a leading cause of death in Taiwan, as well as in many other countries. It places a tremendous burden not only on patients themselves but also on patients’ families and society. Although which process occurs first remains controversial, both insulin resistance and impaired insulin secretion are the 2 principal causes of type 2 diabetes. According to the Insulin Resistance Atherosclerosis Study, approximately 85.7% to 93.2% of type 2 diabetes patients have insulin resistance. In addition, Reaven et al found that approximately 30% of normoglycaemic patients can be classified as insulin-resistant. Insulin-resistant patients were proven to have a higher cardiovascular risk than those without. Therefore, it is crucial to identify insulin resistance in normoglycaemic patients. Preventive interventions, such as lifestyle modification, can be suggested to this apparently healthy cohort.
Insulin resistance and insulin sensitivity are conceptually reciprocal. In other words, a high level of insulin resistance corresponds to a low level of insulin sensitivity. Numerous methods have been developed for measuring insulin action (insulin-mediated glucose disposal). Among these methods, the glucose clamp technique is considered the “gold standard” for measuring insulin sensitivity, but because it is both time- and labour-consuming, it is not suitable for most hospitals and research institutes. Another sophisticated method, the insulin suppression test (IST), provides an accurate measurement of insulin resistance found to be closely correlated with the insulin sensitivity derived from the clamp technique. Because the oral glucose tolerance test (OGTT) is a simple method, it is a widely used test. Many surrogate tests used for measuring insulin action were derived from the OGTT, such as the Matsuda and Stumvoll Index. The homeostasis model assessment of insulin resistance (HOMA-IR) is the easiest method to conduct. However, it is less accurate than the mentioned tests, and can only be used for large cohorts.

Metabolic syndrome (Mets), the clustering of glucose intolerance, hypertension, obesity, and dyslipidaemia, was found to be associated with insulin resistance and an increased risk of cardiovascular disease. Moreover, all Mets components have found to be associated with insulin resistance. Combining these biological variables to identify insulin-resistant people has been assessed by several studies. For instance, Stern et al used routine clinical measurements to build a tree model that predicted insulin resistance with 78.7% sensitivity and 79.6% specificity in non-diabetic participants. In addition, McAuley et al developed an equation for the same purpose. It is crucial to identify insulin-resistant normoglycaemic patients. Both Mets components and data from the OGTT were used in predictive models for this purpose. However, no known study has considered OGTT data, which could further increase the accuracy of predictive models.

In this study, we developed a simple but accurate multivariable risk score model to predict insulin resistance in patients with normal glucose tolerance (NGT) using both Mets components and data from the OGTT.

Materials and methods

Subjects

A total of 513 participants were enrolled and received the standard 75-g OGTT in Cardinal Tien Hospital. Participants were either self-referred or referred by health professionals who recommended screening for diabetes. The participants had no history of diabetes. After excluding frank diabetes, impaired fasting glucose, and impaired glucose tolerance (IGT), only 292 persons with NGT were included. They were defined to be “normal” according to the criteria published by the American Diabetes Association in 2003. None of the participants had a notable medical or surgical history. Before the study, they were instructed by physicians and dietitians to avoid any medication known to affect glucose or lipid metabolism and to maintain a stable diet for at least 1 week before the study. On the day of the first study, a complete routine examination was administered to exclude the presence of cardiovascular, endocrine, renal, hepatic, and respiratory disorders. The study protocol had been approved by the hospital’s institutional review board and ethics committee, and all participants had provided written informed consent prior to participation.

Study Protocol

Both tests were performed in the Clinical Research Center. On the day of the OGTT, after a 10-hour overnight fast, a standard 75-g OGTT was performed at 8:00 am. Blood was drawn before the glucose load and at 30, 60, 90, and 120 minutes after the glucose load for the measurements of plasma glucose and insulin. The blood sample collected before the OGTT was also used to determine lipid profiles. At least 1 week from the first study visit, the IST for estimating insulin action (glucose-mediated glucose disposal) was performed. After a 10-hour overnight fast, an intravenous catheter was set in a forearm vein for administration of somatostatin (250 μg/h, preceded by a 125-μg bolus), insulin (25 mU/m²/min), and glucose (240 mg/m²/min) (m² refers to body surface area). A second intravenous catheter was placed in the contralateral forearm vein for blood collection. Blood was sampled at 30-minute intervals during the initial 150-minute period, and then at 10-minute intervals between 150 and 180 minutes of the infusion. The mean calculated from the last 4 measurements of glucose and insulin was used to determine the steady-state plasma glucose (SSPG) and the steady-state plasma insulin (SSPI) values. During this test, because the SSPI concentration was similar in all participants, the SSPG concentration was used as a direct measure of the ability of insulin to mediate glucose disposal; a high SSPG value indicated a high level of insulin resistance. According to the levels of SSPG, study participants were divided into tertiles. Participants in the highest tertile were defined as insulin-resistant. The remaining two-thirds of the participants were considered not insulin-resistant. The HOMA-IR was determined by the formula of fasting plasma insulin (FPI): (mU/L) X (fasting plasma glucose, FPG)(mmol/L)/22.5.

Laboratory Measurements

Plasma was separated within 1 hour of blood withdrawal and stored at −30°C until the time of analysis. Plasma glucose was measured using the oxidase method by employing a glucose analyzer (YSI Model 203, Scientific Division,
Statistical Analysis

Data were shown as mean ± standard deviation. One-way ANOVA was used to evaluate the demographic data, clinical characteristics, and parameters derived from the tests of the 3 groups. The Bonferroni test was used for post hoc examination.

All of the variables of interest were first evaluated for their predictive performance by logistic regression. The receiver operating characteristic (ROC) curve of each variable (or model) was then plotted as the sensitivity (true-positive rate, y-axis) against the 1-specificity (false-positive rate, x-axis). The area under the ROC curve (aROC curve) was calculated by the trapezoidal rule, and used to determine the predictive accuracy of the models. In general, a large area corresponds to a high predictive accuracy of the variable (or model). 24

Five binary logistic models were built. Insulin resistance was the dependent variable (i.e. 0 or 1 in statistical analysis), and all other baseline clinical and metabolic variables, including BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), TG, HDL-C, FPG, FPI, plasma glucose level at 120 minutes of OGTT (OGTT120g), and plasma insulin level at 120 minutes of OGTT (OGTT120i), were taken as the independent variables. Models were built to predict the occurrence of insulin resistance in the order of the simplest and most clinically available datum to the most complex datum that requires 2-hour OGTT results. The 5 models are as follows:

Model 0: BMI
Model 1: BMI + SBP + DBP + TG (Mets model)
Model 2: Model 1 + FPI
Model 3: Model 2 + OGTT120g
Model 4: Model 3 + OGTT120i (full model)

A model was selected using the enter method in binary logistic regression. The Hosmer-Lemeshow test was used to assess the goodness of fit of these models. A comparison of the aROC curves of the models was performed using the method developed by Hanley et al. 24 From binary logistic regression, equations were built for each model. After inputting all of the factors into the model, optimal cut-point values were derived, and the point with the highest sensitivity and specificity was selected. In other words, if the value derived from the equation is higher than the specified cutoff point, the chance of having insulin resistance is high.

For the sensitivity test (i.e. external validation), we divided the participants into 2 groups. To build the model and equation, three-fourths of the study participants (n = 219) were randomly selected. The remaining one-fourth (n = 73) were considered the external validation group. This procedure was repeated with Model 4 with these 219 participants only because it was the most accurate model. Because the study group was only three-fourth of the original Model 4 group, the equation derived from Model 4 to calculate the probability (P) of having insulin resistance was expected to contain the same factors but different coefficients. This equation was then used to calculate the P value of having insulin resistance in the external validation group. The sensitivity and specificity of this equation in the external validation group were evaluated.

To verify its accuracy, we not only compared our models with HOMA-IR but also the McAuley’s equation. In order to do this, ROC curve was drawn by using McAuley’s equation and HOMA-IR and their aROC curves were calculated in the study cohort.

All statistical analyses were performed using the SPSS software system, version 13.0 (SPSS Inc., Chicago, IL, USA). P values less than .05 were considered statistically significant.

Results

In total, 292 participants with NGT were enrolled and divided into 3 groups based on SSPG values. Group 1 represented the lowest tertile of insulin resistance, whereas Group 3 represented the highest. The demographic data of these 3 groups are shown in Table 1. Participants in Group 3 exhibited the highest BMI. After adjusting for age and BMI, Group 3 also had higher FPG, OGTT120g, OGTT120i, and HOMA-IR values than the other 2 groups. However, the TG level of Group 3 was only higher than that of Group 1. There were no statistically significant differences in age, sex, SBP, DBP, HDL-C, and FPG among these 3 groups.

The aROC curves of individual parameters and models, their Hosmer-Lemeshow goodness-of-fit tests, and tests of statistical difference between the models are shown in Table 2 and Figure 1, respectively. SBP, DBP, TG, and BMI had larger aROC curves than the diagonal reference line. This indicates that the prediction of insulin resistance can be improved by these parameters, but not by FPG or HDL-C. BMI demonstrated the greatest aROC curve (71.6%). Moreover, the aROC curves of FPI, OGTT120g,
Table 1. Baseline Demographic and Metabolic Characteristics of Study Participants Categorised by the Degree of Insulin Resistance

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (n = 97)</th>
<th>Group 2 (n = 98)</th>
<th>Group 3 (n = 97)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>45.6 ± 10.3</td>
<td>44.9 ± 11.7</td>
<td>46.4 ± 11.4</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>57/40</td>
<td>53/45</td>
<td>55/42</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.5 ± 2.1‡</td>
<td>22.8 ± 3.2‡</td>
<td>25.0 ± 3.3*†</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>114.7 ± 13.7</td>
<td>113.7 ± 14.0</td>
<td>119.1 ± 14.5</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>74.6 ± 7.9</td>
<td>74.3 ± 8.9</td>
<td>78.1 ± 9.5</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.0 ± 0.4†</td>
<td>1.1 ± 0.6</td>
<td>1.1 ± 0.51</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.2 ± 0.4</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.7 ± 0.9†</td>
<td>1.9 ± 0.7†</td>
<td>3.5 ± 3.2‡†</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>5.1 ± 0.3</td>
<td>5.1 ± 0.4</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td>OGTT120g (mmol/L)</td>
<td>6.1 ± 1.1‡</td>
<td>6.5 ± 1.2‡</td>
<td>7.4 ± 1.6*†</td>
</tr>
<tr>
<td>FPI (pmol/L)</td>
<td>55.6 ± 21.1‡</td>
<td>64.3 ± 28.2‡</td>
<td>125.4 ± 263.8**†</td>
</tr>
<tr>
<td>OGTT120i (pmol/L)</td>
<td>334.0 ± 198.4‡</td>
<td>443.5 ± 238.2‡</td>
<td>765.9 ± 408.0*†</td>
</tr>
<tr>
<td>SSPG (mmol/L)</td>
<td>4.6 ± 0.9‡†</td>
<td>7.9 ± 1.2*†</td>
<td>12.9 ± 2.0*†</td>
</tr>
<tr>
<td>SSPI (pmol/L)</td>
<td>429.4 ± 77.3</td>
<td>453.4 ± 97.2</td>
<td>462.3 ± 104.2</td>
</tr>
</tbody>
</table>

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; FPG: Fasting plasma glucose; FPI: Fasting plasma insulin; HOMA-IR: Homeostasis model assessment of insulin resistance; OGTT120g: Plasma glucose level at 120 min of the 75-g OGTT; OGTT120i: Plasma insulin level at 120 min of the 75-g OGTT; SSPG: Steady-state plasma glucose; SSPI: Steady-state plasma insulin. Data are expressed as (mean ± SD).

*P < 0.05 against Group 1
†P < 0.05 against Group 2
‡P < 0.05 against Group 3

<table>
<thead>
<tr>
<th>Models</th>
<th>aROC curve ± SE (95% CI)</th>
<th>*P value (Hosmer-Lemeshow)</th>
<th>†P value for omnibus test</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (Model 0)</td>
<td>0.716 ± 0.032 (0.653 – 0.778)</td>
<td>0.691</td>
<td>0.000</td>
</tr>
<tr>
<td>FPG</td>
<td>0.555 ± 0.036 (0.485 – 0.625)</td>
<td>0.340</td>
<td>Non-significant</td>
</tr>
<tr>
<td>SBP</td>
<td>0.614 ± 0.036 (0.534 – 0.675)</td>
<td>0.682</td>
<td>0.002</td>
</tr>
<tr>
<td>DBP</td>
<td>0.640 ± 0.036 (0.570 – 0.710)</td>
<td>0.027</td>
<td>0.000</td>
</tr>
<tr>
<td>logTG</td>
<td>0.618± 0.035 (0.549 – 0.686)</td>
<td>0.738</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.556 ± 0.035 (0.487 – 0.626)</td>
<td>0.830</td>
<td>Non-significant</td>
</tr>
<tr>
<td>FPI</td>
<td>0.710 ± 0.035 (0.641 – 0.780)</td>
<td>0.079</td>
<td>0.000</td>
</tr>
<tr>
<td>OGTT120g</td>
<td>0.714 ± 0.032 (0.651 – 0.777)</td>
<td>0.742</td>
<td>0.000</td>
</tr>
<tr>
<td>OGTT120i</td>
<td>0.828± 0.027 (0.776 – 0.881)</td>
<td>0.236</td>
<td>0.000</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.705 ± 0.034 (0.649 – 0.757)</td>
<td>0.100</td>
<td>0.000</td>
</tr>
<tr>
<td>McAuley’ index</td>
<td>0.728 ± 0.032 (0.665 – 0.790)</td>
<td>0.086</td>
<td>0.000</td>
</tr>
<tr>
<td>Model 1</td>
<td>0.729 ± 0.031 (0.668 – 0.791)</td>
<td>0.665</td>
<td>0.000</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.771 ± 0.030 (0.713 – 0.830)</td>
<td>0.500</td>
<td>0.000</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.801 ± 0.029 (0.745 – 0.857)</td>
<td>0.297</td>
<td>0.000</td>
</tr>
<tr>
<td>Model 4</td>
<td>0.851 ± 0.025 (0.802 – 0.900)</td>
<td>0.201</td>
<td>0.000</td>
</tr>
</tbody>
</table>

aROC curve: Area under the receiver operating characteristic curve; Model 0: BMI; Model 1: BMI+ SBP; Model 2: BMI+ FPG; Model 3: BMI+ SBP+ FPG; Model 4: BMI+ SBP+ FPG+ OGTT120g; Model 5: BMI+ SBP+ FPG+ OGTT120i; BMI: Body mass index; SBP: Systolic blood pressure; FPG: Fasting plasma glucose; OGTT120g: Plasma glucose level at 120 min of the 75-g OGTT; OGTT120i: Plasma insulin level at 120 min of the 75-g OGTT; HOMA-IR: Homeostasis model assessment of insulin resistance; McAuley’ index, exp [2.63 – 0.28ln (FPI) – 0.31ln(TG)]
*P values calculated using the Hosmer-Lemeshow goodness-of-fit test.
†P value calculated using an omnibus test.
and OGTT120i were similar to or greater than that of BMI (71.0%, 71.4%, and 82.8%, respectively).

Compared to Model 0, Models 2, 3, and 4 had significantly larger aROC curves and improved predictive accuracy (Table 2 and Fig. 1). Model 4 exhibited the largest aROC curve of the models and the optimal ability to predict insulin resistance. In addition to the observation of aROC curves, the probability of having insulin resistance was also obtained from logistic regression. The basic equation for all models was the same: \( P = \frac{e^x}{1+e^x} \). However, each model exhibited unique parameters for calculating probability. For instance, the equation of Model 4 for 293 participants is shown as follows:

\[
x = -9.653 + 0.183(BMI) - 0.005(SBP) + 0.023(DBP) + 0.947(\log TG) + 0.007(FPI) + 0.159(OGTT120g) + 0.03(OGTT120i).
\]

If the calculated \( P \) value is higher than the cut-off point (provided in the next paragraph), the value indicates a high probability of having insulin resistance. In the meanwhile, the aROC curves of the McAuley’s index and HOMA-IR were also calculated and it could be noted that the aROC curves of Model 2, 3 and 4 were greater than those of McAuley’s index and HOMA-IR.

Figure 1 shows the ROC curves of the 5 models. For Model 2, a cutoff of 0.30 provided the highest sum of sensitivity (70.1%) and specificity (70.3%). For Model 4, a cutoff of 0.29 provided the highest sum of sensitivity (76.0%) and specificity (77.1%).

Although the factors in the sensitivity test were the same, a reduction in sensitivity occurred compared to the equation built from the whole study cohort. With this equation of Model 4, the \( P \) value was also calculated for the external validation group. Participants were classified as having or lacking insulin resistance according to the \( P \) value. These results were then compared to the classification of measured insulin resistance. The comparison yielded 68.0% sensitivity and 85.4% specificity.

**Discussion**

Several past studies have been conducted to identify non-diabetic patients with insulin resistance. Stern et al developed a tree model using routine clinical measurements in non-diabetic patients. The most accurate tree model for predicting insulin resistance demonstrated an 85.0% aROC curve, 78.7% sensitivity, and 70.6% specificity. In addition, McAuley et al used product-moment correlations to build an equation for predicting insulin sensitivity in patients. They found that the variables with the most accurate predicting power were FPI and fasting TG. In the present study, we also built models to predict the occurrence of insulin resistance in non-diabetic patients. However, we added the results of the OGTT to the model rather than using only routine laboratory measurements. We intended to increase the aROC curve to enhance the sensitivity and specificity of the model.

Previous research has established that a high BMI corresponds to high insulin resistance. Our study results showed that BMI was the central component in predicting insulin resistance (Table 2). This is in agreement with Stern et al, who consistently used BMI to identify insulin...
resistance in the first 2 layers of their 3 different tree models. However, the average BMI in the Stern et al study was much higher than that of our study. This is a common occurrence when similar studies are conducted between Caucasian and Chinese populations. In the McAuley et al study, only insulin and TG were important predictors. However, the BMI of the participants in the McAuley et al study was higher than that of our study (27.5 ± 5.3 kg/m² versus 23.3 ± 3.1 kg/m²). In addition, the strong correlation between BMI and TG might have also reduced the significance of BMI in their study. Nevertheless, BMI is a crucial factor in Chinese populations.

The association between insulin levels and insulin resistance in normoglycaemic patients has been widely explored, and it has been established that the insulin level is increased in patients with insulin resistance.²⁶,²⁷ In agreement with McAuley et al, McLaughlin et al reported that FPI was a valuable predictor of insulin resistance in normoglycemic patients.²⁰,²¹ In the tree models proposed by Stern et al, the aROC curve increased from 85.1% to 90.0% after conducting HOMA-IR, which is a product of FPG and FPI. In the present study, we found that the aROC curve of FPI is similar to that of BMI (71.0% and 71.6%, respectively). After adding FPI to Model 1, the aROC curve of Model 2 significantly increased from 72.9% to 77.1%. This indicates that BMI and FPI contribute to insulin resistance using different pathways.

Postprandial hyperglycemia is one of the earliest glucose dysregulations detected in patients with type 2 diabetes and IGT.²⁸,²⁹ This metabolic abnormality initiates before the onset of clinical diabetes because of the loss of first-phase insulin secretion and increased insulin resistance.³⁰,³¹ Therefore, measuring the postprandial plasma glucose and insulin levels can facilitate recognition of early metabolic derangements. Thus, the role of the OGTT is vital because it can provide changes in plasma glucose and insulin levels after glucose challenges. The OGTT is not only a standard method for diagnosing glucose intolerance but also a tool for assessing insulin resistance in population studies.²¹,³² In our study, Model 3 was built by adding OGTT120g to Model 2, forming a larger aROC curve (80.1%) that provided a better predictive accuracy (P = 0.006, P < 0.001, and P < 0.001, respectively). Finally, since McAuley also built an equation for estimating IR, we also compared his equation with our models. It could be shown that the aROC was 72.8%. From this curve, the best specificity and sensitivity of the arbitrary cut-off point (0.34) were 67.0% and 66.7% respectively, which is less accurate than our proposed models. In conclusion, considering the economic aspects of health screening, Model 2 is more appropriate and practical than Model 4. Moreover, Model 2 also showed greater predictive accuracy than the available equations.

In our study, Model 4 was tested again for sensitivity with three-quarters of the participants. The results of the comparison showed high sensitivity and specificity. However, we are aware of the limitations of the study. First, this is a cross-sectional study. A longitudinal study may yield more conclusive results. Second, we did not consider the effects of exercise or smoking, which are all known to be related to insulin resistance. However, despite these limitations, our results can be used easily and extensively in clinical practice.

Conclusion

In conclusion, we demonstrated that FPI, OGTT120g, and OGTT120i are independently related to insulin resistance and can further improve the aROC curves of predictive models. In practice, insulin resistance can be accurately predicted in NGT patients with a sensitivity of 70.1% and a specificity of 70.3% using Mets components and FPI. In contrast, Model 4 provides the optimal prediction model for insulin resistance, but lacks practicality because OGTT data are required.

Acknowledgments

The authors thank all the participants of this study. The present study was supported, in part, by a research grant of Cardinal Tien Hospital (CTH-101-2-5-028).
REFERENCES


