Insulin resistance & secretion in subjects with normal fasting plasma glucose


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Background & objectives: Lowering the diagnostic threshold of normal fasting plasma glucose (FPG) from 6.1 to 5.56 mmol/l has been proposed by American Diabetes Association. As early detection of subjects with risk of diabetes is important, it is crucial to understand the relationship between different levels of FPG, insulin resistance (IR) and insulin secretion. We measured IR and insulin secretion in subjects with different levels of FPG to investigate the relationship between FPG and IR and insulin secretion, and to see whether lowering the cut-off improves the sensitivity of impaired fasting glucose to predict diabetes.

Methods: Apparently healthy subjects (294) were enrolled and divided into 3 groups according to their FPG levels; group 1 (<5.56 mmol/l, n=129), group 2 (5.56-6.09 mmol/l, n=99) and group 3 (6.1-6.9 mmol/l, n=66). Steady state plasma glucose (SSPG) derived from insulin suppression test was used to quantify insulin resistance and 30 min insulinogenic index during an oral glucose tolerance test to measure acute insulin secretion.

Results: The blood pressure was higher in group 3 than group 2 and fasting plasma insulin and triglyceride were higher in group 3 than that in group 1. The insulinogenic index was higher in the group 1 than group 3 and the SSPG was significantly higher in group 3 compared to group 1.

Interpretation & conclusion: Our study showed a trend of progressive deterioration of both insulin action and secretion with increasing FPG level. These are supportive evidences for lowering the FPG to 5.56 mmol/l as suggested by the ADA.

Key words Fasting plasma glucose - insulin resistance - insulin secretion - insulin suppression test - insulinogenic index - metabolic syndrome

A new category of impaired glucose homeostasis, impaired fasting glucose (IFG) (fasting plasma glucose, FPG, between 6.1-6.9 mmol/l), was introduced by the American Diabetes Association (ADA) in 19971. Originally, the level of 6.1 mmol/l was chosen arbitrarily to be the cut-off for normal FPG (NFG) level based on two reasons; first, the epidemiological data show that the risk of microvascular complications begin to increase2, and second, the pathophysiological data
show that the first phase insulin secretion is lost after this cut-off. Recently, further lowering the diagnostic threshold from 6.1 to 5.56 mmol/l was proposed by ADA. Indeed, lowering the cut-off level of NFG may improve the sensitivity of IFG to predict diabetes. However, some authors still had different opinions and considered that lowering the criterion for IFG may not provide clinical benefits, and the risks in these subjects could be partially corrected by adopting lifestyle modifications. At the same time, it was also suggested that the glycaemic level did not carry an impact on cardiovascular disorders after adjusting other cardiovascular risk factors.

Insulin resistance (IR) and insulin secretion are well known underlying pathophysiology of diabetes which could be measured with different methods. However, FPG could be available easily in a routine health check up. Since early detection of subjects with risks of diabetes and cardiovascular diseases is important, it is crucial to investigate the relationships between different levels of FPG, IR and insulin secretion. Very scanty information is available on these complicated relationships. In one recent study, Piche et al. observed that increased IR and impaired insulin secretion develop even when FPG level is as low as 4.9 mmol/l. However, they used oral glucose tolerance test (OGTT), which is a less accurate method to measure IR.

In this study, we used a more sophisticated method - the insulin suppression test (IST) and insulinogenic index to quantify IR and first phase insulin secretion respectively in subjects with different levels of FPG with the objective to study the relationships between FPG and both insulin action and insulin secretion and to see whether our findings support the rationale for decreasing of the cut-off from 6.1 to 5.6 mmol/l for NFG level.

Material & Methods

Subjects: The subjects in this study were enrolled from the outpatient department in Tri-Service General Hospital in Taipei from 1995 to 1999. These subjects responded to the advertisement for the study. A total of 352 subjects were enrolled in the beginning and 58 subjects were excluded because of diabetes, severe medical diseases or using medications that might affect glucose or lipid metabolism. Finally a total of 294 persons were consecutively selected (aged 20-75 (50.0 ± 1.0 yr), male/female 148/146). They were all apparently healthy after a thorough physical examination and routine laboratory tests. Subjects with previous histories of diabetes, hypertension, hyperlipidaemia, or other significant medical or surgical diseases were excluded. Subjects who were put on medications influenced on insulin sensitivity were also excluded from the study. Subjects were divided into 3 age- and body mass index (BMI)-matched groups according to their FPG levels, 129 were classified as group 1 (FPG less than 5.56 mmol/l), 99 as group 2 (FPG 5.56-6.09 mmol/l), and 66 as group 3 (FPG 6.1-6.9 mmol/l). After obtaining written informed consent, a series of tests were conducted to assess glucose tolerance, insulin action and insulin secretion. The study protocol was approved by the local hospital Ethic Committee.

All tests were performed at our Clinical Research Center. All subjects visited twice for their fasting glucose and insulin (FPI) concentration after 8 to 10 h overnight fasting. Fasting plasma glucose or insulin results were calculated by averaging the two values. And the inter-assay variations for both FPG and FPI were 9.6 and 74 per cent, respectively. Besides, the intra-assay variations for both FPG and FPI were 3.0 and 24 per cent, respectively. According to this average FPG levels, subjects were divided into three groups. On one of the two visits, a standard 75 g oral glucose tolerance test (OGTT) was performed and plasma glucose and insulin concentrations were measured before and at 30, 60, 90, 120, and 180 min after the glucose load.

On a separate date, IR was estimated by the IST. After an overnight fast, an intravenous catheter was placed in each of the patients’ arms. One arm was used to administer a 180 min infusion of somatostatin (250 µg/h), insulin (25 mU/m²/min), and glucose
(240 mg/m²/min) respectively, and the other arm was used for collecting blood samples. Blood was sampled every 30 min initially and then at 10 min intervals from 150 to 180 min of the infusion to determine the steady state plasma insulin (SSPI) and glucose (SSPG) concentrations for each individual. Because SSPI concentrations were similar for all subjects, the SSPG concentration provided a direct measure of the ability of insulin to mediate disposal of an infused glucose load; the higher the SSPG, the more insulin resistant the individual. The plasma was separated from blood within 1 h and stored at -30°C until analyzed.

Waist and thigh circumferences were measured at the umbilicus and the gluteal fold in the supine and standing position, respectively. Systolic and diastolic blood pressure (SBP and DBP) were measured according to the JNC VI (Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure)¹⁰. Insulin was measured by a commercial solid phase radioimmunoassay kit (Coat-A-Count insulin kit, Diagnostic Products Corporation, Los Angles, California, USA). The intra- and inter-assay coefficients of variance for insulin were 3.3 and 2.5 per cent, respectively. Plasma glucose was measured using a glucose oxidase method¹¹ (YSI 203 glucose analyzer, Scientific Division, Yellow Spring Instrument Company, Inc., Yellow Spring, Ohio, USA). Both serum levels of total cholesterol (TC) and triglyceride (TG) were also not different in the 3 groups. However, the SBP and DBP were higher in group 3.

### Table. The demographic data of subjects with different fasting plasma glucose levels

<table>
<thead>
<tr>
<th>Patients</th>
<th>Group 1 (N=129)</th>
<th>Group 2 (N=99)</th>
<th>Group 3 (N=66)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NGT/IGT/DM)</td>
<td>(69/54/5)</td>
<td>(44/50/5)</td>
<td>(18/37/11)</td>
</tr>
<tr>
<td>2-h PG (mmol/l)</td>
<td>6.52±0.09**</td>
<td>7.52±0.14*</td>
<td>8.84±0.28</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>51/77</td>
<td>43/56</td>
<td>34/32</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>49.1±0.8</td>
<td>49.5±1.0</td>
<td>50.4±1.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.8±0.2</td>
<td>24.8±0.2</td>
<td>25.2±0.4</td>
</tr>
<tr>
<td>WHR (%)</td>
<td>0.87±0.00</td>
<td>0.87±0.01</td>
<td>0.89±0.01</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>120.8±1.33</td>
<td>120.0±1.73</td>
<td>127.3±1.0</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78.3±0.8</td>
<td>76.3±1.03*</td>
<td>81.1±1.3</td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>5.17±0.021*</td>
<td>5.74±0.013*</td>
<td>6.44±0.04</td>
</tr>
<tr>
<td>FPI (pmol/l)</td>
<td>68.3±3.03*</td>
<td>74.9±4.33</td>
<td>97.8±12.5</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.60±0.08</td>
<td>4.50±0.10</td>
<td>4.45±0.13</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.54±0.06*</td>
<td>1.75±0.093</td>
<td>2.15±0.173</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.11±0.03</td>
<td>1.14±0.03</td>
<td>1.01±0.05</td>
</tr>
</tbody>
</table>

The data are shown as mean ± SEM, group 1 (fasting plasma glucose, FPG less than 5.56 mmol/l), group 2 (FPG 5.56-6.09 mmol/l), group 3 (FPG 6.1-6.9 mmol/l). NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetes mellitus based on the WHO criteria. 2h-PG, 2 h plasma glucose during oral glucose tolerance test; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WHR, waist/hip ratio; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; FPI, fasting plasma insulin. P<0.05 * compared to group 2; + compared to group 3.

### Results

The presence of glucose intolerance (IGT or diabetes) increased from the group 1 to group 3 (46, 55, and 73% respectively). Since the age and BMI were matched in the 3 groups, no differences could be noted between them. In the same time, TC and HDL-C were also not different in the 3 groups. However, the SBP and DBP were higher in group 3.
Fig. The plasma glucose (panel A) and insulin (panel B) concentrations during oral glucose test. All data are shown as mean ± SEM, group 1 (fasting plasma glucose, FPG less than 5.56 mmol/l), group 2 (FPG 5.56-6.09 mmol/l), and group 3 (FPG 6.1-6.9 mmol/l). 

$P<0.05$ *between each group, §group 1 vs 3, #group 2 vs 3.
than that of group 2. FPI and TG levels were higher in group 3 than that of group 1 and, finally, the 2 h plasma glucose levels after glucose load were highest in group 3 than group 1 and 2 (Table).

The results of the OGTT are shown in the Fig. It is not surprising that group 3 had the highest and group 1 had the lowest glucose levels during the 180 min OGTT (panel A). However, it could be noted the insulin responses 60 min after glucose load were highest in group 3 (panel B). On the contrary, the response of insulin secretion in group 3 was sluggish before 60 min. Early phase of beta cell function, evaluated by 30 min IGI, was significantly higher ($P<0.05$) in the group 1 ($30.8 \pm 2.7 \text{ mIU/mmol}$) than group 3 ($14.3 \pm 1.8 \text{ mIU/mmol}$) but without difference between group 1 and 2 ($15.4\pm1.6 \text{ mIU/mmol}$) or group 2 and 3.

We used the IST to evaluate the IR. Under the similar SSPI level ($455 \pm 10, 463 \pm 10$ and $468 \pm 18 \text{ pmol/l}$, respectively), the SSPG was significantly higher ($P<0.05$) in group 3 than group 1. However, there was no difference either between group 1 and group 2 ($8.91 \pm 0.34$ and $9.52 \pm 0.4 \text{ mmol/l}$, respectively) or group 2 to group 3 ($9.52 \pm 0.4$ and $10.79 \pm 0.55 \text{ mmol/l}$, respectively). In addition, positive correlations were obtained between FPG levels and both FPI and SSPG levels using the multiple regression after adjusted for age and BMI ($r=0.135$ and 0.106, $P=0.002$ and 0.017 respectively).

**Discussion**

The ADA had proposed to lower the FPG level for diabetes diagnosis from 7.77 to 6.99 mmol/l in 1997. The rationale was that this new cut-off was considered to be a more sensitive predictor for the risk of chronic complications$^1$. The evidence supporting this revision was mainly based on the fact that when the FPG was higher than the cut-off level, the risk of eye and kidney diseases increased. Different hypotheses had been proposed in the past. Stern$^{15}$ suggested that both hyperglycaemia and cardiovascular diseases may share common predisposing factors. His theory was supported by the two studies which showed that subjects with IFG shared similar cardiovascular risk factors with subjects having cardiovascular diseases$^{12,13}$. Accordingly, it is reasonable to postulate that subjects with so call ‘high normal’ FPG level might have higher risk factors of metabolic syndrome than ‘low normal FPG’ subjects. If this hypothesis is correct, than it implies that these subjects may have higher degree of IR, which is one of the central pathophysiology of metabolic syndrome.

The previous epidemiological studies showed that the mortality due to cardiovascular diseases in subjects with diabetes was associated with the degree of hyperglycaemia$^{8,19}$. Shaw et al$^{20}$ confirmed the relationships between FPG and cardiovascular disease even when the FPG was lower than 7.0 mmol/l, i.e., in subjects with IFG. Ohlson et al$^{21}$ and Coutinho et al$^{22}$ further extended this observation in non diabetic subjects. Although the results of these two studies were only borderline significant, in another prospective cohort study done by Wei et al$^{23}$, the potential hazard of blood glucose even when below 6.1 mmol/l was proved. A few other studies reported that cardiovascular risk factors start to increase in subjects with high but non diabetic FPG$^{16,17}$ or even at the low NFG level$^{9,22,24,25}$.

In our study, we compared the levels of metabolic syndrome components in subjects with different FPG levels. Although all findings were not statistically significant, it was found that these components became more severe when the FPG levels were higher. This could be noted even when the FPG levels were between 5.6 to 6.1 mmol/l, which confirmed aforementioned risks of ‘high normal’ FPG$^{9,22,24,25}$. However, there was no significant difference between groups 1 and 2. This is different from other studies. The reasons could be varied numbers of subjects in the subgroups, use of different levels of FPG for grouping criteria$^{25}$ and different ethnicities$^{26}$.

Impaired insulin action and defective insulin secretion are the major metabolic abnormalities of
type 2 diabetes\textsuperscript{27-29}. Therefore, to evaluate whether subjects with FPG between 5.56 to 6.09 mmol/l (group 2) and between 6.1 to 6.9 mmol/l (group 3) had defects in insulin action and secretion became more important. By using OGTT and homeostasis model assessment (HOMA), Piche \textit{et al}\textsuperscript{9} showed that insulin action decreased with increasing FPG levels. Although these findings were important, OGTT and HOMA are considered to be less accurate methods to quantify IR. In our study, we used the IST, which has a very high correlation ($r=0.93$) with the gold standard - hyperinsulinaemic euglycaemic clamp\textsuperscript{30}. Our findings showed that the insulin action was different between groups 1 and 3. Although there was no difference between groups 1 and 2 or groups 2 and 3, the trend that higher FPG was related to more severe IR was obvious. Positive relationships noted between FPG levels and both FPI and SSPG levels confirmed the relationship between FPG level and IR. These results were similar to those of Piche’s \textit{et al} who showed that this relationship could be found at FPG level of 4.9 mmol/l.

The IGI is one of the commonly used methods to evaluate first phase insulin secretion\textsuperscript{31,32}. Although there was no difference between groups 1 and 2 or groups 2 and 3, the decrease in IGI with an increase in FPG could be seen clearly. Godsland \textit{et al}\textsuperscript{33} demonstrated higher level of FPG associated with lower first phase insulin secretion. This relationship could be noted even when the FPG was as low as 5.0 to 5.4 mmol/l\textsuperscript{33}. Our data confirmed results of several other studies which showed that IGI decreased progressively when FPG was still considered to be within ‘normal range’\textsuperscript{4,9,34}. Since it is well known that measuring plasma insulin level has relatively wider confidence interval in general, this might help to explain the non significant differences between the IGI of groups 1 to 2 and groups 2 to 3.

We took only BMI and waist hip ratio into consideration in the definition of metabolic syndrome. However, there are evidences showing that subjects with normal BMI might also have IR and, interestingly, it is related to their family history of diabetes\textsuperscript{35,36}. In this regard, the definition of the metabolic syndrome might give us an underestimation, especially in those subjects who are ‘metabolic obese normal-weight subjects’.

In conclusion, our study findings provides support for lowering the cut-off of normal and IFG from 6.1 to 5.56 mmol/l suggested by ADA. Hopefully, this could help to detect more subjects with risk factors for diabetes and coronary artery disease.

\textbf{References}


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