One-hour post-load hyperglycemia by 75g oral glucose tolerance test as a novel risk factor of atherosclerosis

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Abstract. Postprandial hyperglycemia is considered as a risk factor of cardiovascular disease. We hypothesized that early post-load hyperglycemia might be more useful surrogate marker to assess atherosclerosis than plasma glucose (PG) level at 120 minutes because the peak of post-load glucose by 75gOGTT is usually shown at 60 minutes. 75gOGTT and carotid echography were investigated in 108 subjects who visited our hospital to examine impaired glucose tolerance. The association of post-load plasma glucose and insulin levels with intima-media thickness (IMT) was examined. Simple correlation analyses showed that fasting PG (FPG) (r=0.26, p=0.013), PG60 (r=0.40, p<0.001), PG90 (r=0.29, p=0.008), area under curve for PG (AUC-PG) (r=0.33, p=0.003), HbA1c (r=0.30, p=0.005), amount of PG increase at 60 minutes (PGA60) (r=0.39, p=0.002), and PGA60 (r=0.27, p=0.016), were significantly correlated with IMT. Multiple regression analysis using IMT as a dependent variable and PG60, FPG, HbA1c, and AUC-PG as independent variables showed that PG60 was only significantly and positively correlated with IMT (β=0.59, p=0.042). Moreover, PG60 and PGA60 were significantly and positively associated with IMT even after additional adjustment for classical atherosclerosis risk factors (β=0.30, p=0.005 and β=0.50, p=0.037, respectively). The cut-off values of PG60 and PGA60 to detect atherosclerosis (IMT > 1.1mm) were 188 and 101 mg/dL, respectively (p<0.01). These findings show that early post-load hyperglycemia, particularly PG60 is a novel risk factor of atherosclerosis and useful to assess it.

Key words: Post-load hyperglycemia, Atherosclerosis, Intima-media thickness, 75g oral glucose tolerance test

Type 2 diabetes mellitus (T2DM) is known as a risk factor of cardiovascular disease (CVD) [1]. Previous studies have shown that the CVD risk has already increased since the stage of impaired glucose tolerance (IGT) before the onset of T2DM [2, 3]. Postprandial hyperglycemia better predicts CVD morbidity and mortality compared with fasting hyperglycemia in both T2DM and normal glucose tolerance (NGT) individuals [4]. The DECODE STUDY GROUP showed that subjects with higher post-load PG at 120 minutes (PG120) by 75gOGTT had higher hazard ratio (HR) of CVD mortality, while the HR in IGT was higher than that in impaired fasting glucose [2]. Moreover, the STOP-NIDDM Trial showed that treatment of acarbose, an alpha-glucosidase inhibitor, for IGT patients prevented CVD event [5]. Recent studies demonstrated that postprandial hyperglycemia induced vascular endothelial dysfunction by increasing oxidative stress [6]. These findings indicate that postprandial hyperglycemia should be the target of treatment for preventing CVD event.

Because fasting plasma glucose (FPG) and PG120 by 75gOGTT are clinically used for diagnosis of IGT and T2DM, most epidemiological studies employed FPG and PG120 to examine the relationship between postprandial hyperglycemia and atherosclerosis. The incremental glucose peaks obtained at any point after the meal is shown at 60 minutes and they positively correlate with IMT [7]. The peak of post-load glucose by 75gOGTT is also shown at 60 minutes. Thus, postprandial hyperglycemia at early phase may be associated with atherosclerosis. However, the relationship between early post-load hyperglycemia by 75gOGTT and CVD remains unclear. We hypothesized that PG60 by 75gOGTT may be more useful to predict atherosclerosis than PG120. In this study, we examined the relationship between post-load glucose levels by 75gOGTT and intimal-medial thickness (IMT) in carotid artery, which is a surrogate marker of atherosclerosis because
increased IMT has shown to be associated with a risk of CVD event [8, 9].

**Material and Methods**

**Subjects and measures**

The subjects were 108 Japanese (age: 58.9 ± 12.6 years, male/female: 51/57) who visited Shimane university hospital to examine 75gOGTT and were never diagnosed as IGT or T2DM. Clinical characteristics of the subjects are shown in Table 1. The numbers of DM, IGT, and normal glucose tolerance (NGT) are 35, 44, and 29, respectively.

After overnight fasting, 75gOGTT examination was done in all subjects. PG and immunoreactive insulin (IRI) levels were measured before and after 75g glucose load at 30, 60, 90, and 120 minutes. The differences after glucose load (∆) were subtracted from FPG or fasting IRI. High density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and creatinine were evaluated using fasting blood samples. HbA1c was determined by high performance liquid chromatography. The value for HbA1c is estimated as an NGSP (National Glycohemoglobin Standardization Program) equivalent value calculated by the formula HbA1c (%) = HbA1c (JDS) (Japan Diabetes Society) (%) + 0.4 % [10]. To quantify carotid artery wall thickness, we used the maximum of IMT in the present study as previously described [11].

**Measurement of carotid IMT**

To measure the carotid IMT, B-mode ultrasonicographic imaging of the carotid artery was performed using HDI 5000 (Philips, Tokyo, Japan), a high-resolution, real-time ultrasonograph with a 7.5-MHz transducer as previously described [11]. All scans were performed by two trained sonographers who remained unaware of each other’s data. Briefly, the scanning of extracranial carotid arteries in the neck was conducted bilaterally at longitudinal projections and at the transverse projection for measurement of IMT. Each carotid wall was explored to identify the thickest intima-medial sites. IMT was measured as the distance between the lumen-intima interface and the media-adventitia interface on the B-mode image. The CV of measurements of IMT was 3.55%. To quantify carotid artery wall thickness, we used the maximum of IMT in the present study.

**Statistical analysis**

Data are expressed as mean ± SD. All analyses were carried out using statistical computer programs, StatView and IBM SPSS version 19. P<0.05 was considered to be significant.

**Ethic**

This study was cross-sectional and approved by the ethical review board of our institution and complied with the Helsinki declaration. All subjects agreed to participate in the study and gave informed consent.

**Results**

**Simple correlations of plasma glucose or insulin with IMT**

First, we investigated simple correlations of plasma glucose or insulin with IMT. Simple correlation analyses showed that FPG, PG60, PG90, area under curve for PG (AUC-PG), HbA1c, PG∆60, and PG∆90 were significantly and positively correlated with IMT (p<0.05),
while IRI30 and IRI∆30 were significantly and negatively correlated with it \((p<0.05)\) (Table 2).

**Multiple regression analysis between plasma glucose versus IMT**

Next, multiple regression analysis was then performed using IMT as a dependent variable and PG60, FPG, HbA1c, and AUC-PG as independent variables (Table 3). PG60, but not other parameters, was significantly and positively correlated with IMT \((p=0.042)\). This association was still significant when PG60 was substituted for PGA60 \((p=0.042)\). The association of PG60 or PGA60 with IMT was independent for IRI30 or IRI∆30 \((p=0.002\) and \(p=0.057\), respectively), while the association of IRI30 or IRI∆30 with IMT turned modest \((p=0.342\) and \(p=0.162\), respectively). Moreover, PG60 and PGA60 were significantly and positively associated with IMT even after additional adjustment for classical atherosclerosis risk factors such as age, BMI, sBP, LDL-C, HDL-C, and serum creatinine \((p=0.005\) and \(p=0.037\), respectively).

**Receiver operating characteristic analysis between PG60 or PGA60 versus IMT**

Finally, we performed receiver operating characteristic (ROC) analysis between PG60 or PGA60 versus IMT. ROC analysis showed that the predict values of PG60 and PGA60 with respect to IMT above 1.1 mm were 187.5 mg/dL \((p<0.001)\) and 101.0 mg/dL \((p<0.001)\), respectively, as well as to IMT above 1.5 mm were 199.5 mg/dL \((p<0.001)\) and 93.5 mg/dL \((p<0.001)\), respectively (Table 4 and Fig. 1).

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**Table 2** Simple correlations of plasma glucose or insulin with IMT

<table>
<thead>
<tr>
<th></th>
<th>IMT</th>
<th>IMT</th>
<th>IMT</th>
<th>IMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPG</td>
<td>0.26</td>
<td>0.013</td>
<td>0.26</td>
<td>0.085</td>
</tr>
<tr>
<td>PG30</td>
<td>0.40</td>
<td>&lt;0.001</td>
<td>0.29</td>
<td>0.008</td>
</tr>
<tr>
<td>PG60</td>
<td>0.29</td>
<td>0.003</td>
<td>0.29</td>
<td>0.008</td>
</tr>
<tr>
<td>PG90</td>
<td>0.21</td>
<td>0.003</td>
<td>0.33</td>
<td>0.003</td>
</tr>
<tr>
<td>AUC-PG</td>
<td>0.30</td>
<td>0.005</td>
<td>0.30</td>
<td>0.005</td>
</tr>
</tbody>
</table>

**Table 3** Multiple regression analysis between plasma glucose *versus* IMT

<table>
<thead>
<tr>
<th></th>
<th>PG60</th>
<th>FPG</th>
<th>HbA1c</th>
<th>AUC-PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMT</td>
<td>0.59</td>
<td>0.042</td>
<td>0.00</td>
<td>0.984</td>
</tr>
<tr>
<td></td>
<td>0.18</td>
<td>0.124</td>
<td>-0.19</td>
<td>0.560</td>
</tr>
<tr>
<td>PGA60</td>
<td>0.50</td>
<td>0.042</td>
<td>0.16</td>
<td>0.349</td>
</tr>
<tr>
<td></td>
<td>0.18</td>
<td>0.124</td>
<td>-0.19</td>
<td>0.560</td>
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</tbody>
</table>

**Table 4** ROC analysis between PG60 or PGA60 *versus* IMT

<table>
<thead>
<tr>
<th></th>
<th>cut-off value</th>
<th>sensitivity</th>
<th>specificity</th>
<th>AUC</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMT above 1.1 mm</td>
<td>PG60</td>
<td>187.5</td>
<td>0.75</td>
<td>0.41</td>
<td>0.78</td>
<td>0.68-0.88</td>
</tr>
<tr>
<td></td>
<td>PGA60</td>
<td>101.0</td>
<td>0.69</td>
<td>0.84</td>
<td>0.77</td>
<td>0.66-0.87</td>
</tr>
<tr>
<td>IMT above 1.5 mm</td>
<td>PG60</td>
<td>199.5</td>
<td>0.68</td>
<td>0.71</td>
<td>0.72</td>
<td>0.62-0.83</td>
</tr>
<tr>
<td></td>
<td>PGA60</td>
<td>93.5</td>
<td>0.84</td>
<td>0.52</td>
<td>0.72</td>
<td>0.61-0.83</td>
</tr>
</tbody>
</table>

95% CI, 95% confidence interval
The present study showed that PG60 was associated with increased IMT more strongly than PG120. A previous study showed that people with more than 1.18 mm of IMT in common carotid artery had 3.86 times-relative risk of incident stroke [8]. In this study, ROC analysis suggests that the split 1.1 mm point, which had the best prognostic potential on the risk of atherosclerosis, was around 190 mg/dL for PG60 and 100 mg/dL for PGΔ60. Thus, these data suggest that PG60 can be considered as a predictor of atherosclerosis when 75gOGTT is tested on general population.

Discussion

Not only T2DM but also IGT increase the risk of CVD, suggesting that temporary hyperglycemia after meal is a risk factor of atherosclerosis even if chronic hyperglycemia did not exist. The risk of CVD mortality is reported to be increased in subjects with IGT, which is defined by PG120 by 75gOGTT [2]. Indeed, previous studies have shown that suppression of glucose absorption by alpha-glucosidase inhibitors prevented CVD event [5]. Our present study supports the evidence that postprandial hyperglycemia is a risk factor of CVD. Since PG60 after glucose loading is usually higher than PG120 in general population, we hypothesized that PG60 might be a better predictor of CVD than PG at other time points. The present study showed that PG60 was associated with increased IMT more strongly than PG120. A previous study showed that people with more than 1.18 mm of IMT in common carotid artery had 3.86 times-relative risk of incident stroke [8]. In this study, ROC analysis suggests that the split 1.1 mm point, which had the best prognostic potential on the risk of atherosclerosis, was around 190 mg/dL for PG60 and 100 mg/dL for PGΔ60. Thus, these data suggest that PG60 can be considered as a predictor of atherosclerosis when 75gOGTT is tested on general population.

Esposito K et al. showed that the incremental glucose peaks obtained at any point after the meal is shown at 60 minutes and PG60 after meal was a good esti-
mating of increasing IMT [7]. However, in their study, the glucose peak was not examined by 75gOGTT and the subjects were T2DM patients with poor glycemic control. Moreover, the relationship between postprandial hyperinsulinemia and IMT was not investigated. It is better to assess postprandial hyperglycemia by 75gOGTT than meal, because glucose peak after meal is depend on the size and kinds of the meal. In addition, we found that PG60 was the glucose peak by 75gOGTT and associated with IMT independently of other glucose and insulin parameters as well as traditional risk factors of atherosclerosis among people who were never diagnosed as IGT or T2DM. Therefore, when PG60 by 75gOGTT is over 200 mg/dL, atherosclerosis parameters should be screened even if T2DM were not presented.

Previously, it was thought that high plasma insulin levels caused by insulin resistance might induce atherosclerosis [12, 13]. In contrast, Wingard et al. or McKeigue et al. showed that high plasma insulin did not always affect the incidence of cardiovascular disease [14, 15]. Nelson et al. showed that Pima Indians, an ethnic group with obesity, diabetes and high fasting insulin levels, had a low incidence of cardiovascular disease [16]. Thus, the effect of high plasma insulin on the atherosclerosis remains controversial. Previous studies showed that reactive oxygen species and chronic inflammation, which were caused by postprandial hyperglycemia, induced atherosclerosis [17, 18]. Our study showed that plasma glucose levels were associated with IMT, whereas insulin levels were not. Taken together, these findings suggest that postprandial hyperglycemia plays an important role in processing atherosclerosis rather than hyperinsulinemia. However, BMI in the present populations were lower than those observed in Western people. It is because the capacity of insulin secretion and the degree of obesity in Asian populations are known to be different from Western people. Therefore, further studies are needed to clarify the association between post-load hyperinsulinemia and atherosclerosis.

In the present study, we used IMT as a surrogate marker for CVD because IMT measurement is non-invasive and frequently examined at a clinical setting. However, we could not investigate whether PG60 is associated with CVD event. Moreover, we couldn’t examine the association between PG and IMT separately in subjects with and without antihypertensive and antilipemic drugs because the number of subjects was limited. Furthermore, we need to examine not only cross-sectional research but also longitudinal one to further understand the relationship between glucose parameters including PG60 and progression of atherosclerosis. Therefore, we are going to conduct a large scale longitudinal study to solve these issues in future.

In conclusion, the present study showed that early post-load hyperglycemia, most particularly PG60 is a novel risk factor of atherosclerosis. Although 75gOGTT is generally used to detect glucose intolerance, it is useful for screening atherosclerosis.

Disclosure

Nothing to declare.

References

nosed type 2 diabetes or impaired glucose regulation. *Endocrine* 37: 201-208.


