Vascular Inflammation in Patients With Impaired Glucose Tolerance and Type 2 Diabetes
Analysis With 18F-Fluorodeoxyglucose Positron Emission Tomography

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Background—Type 2 diabetes mellitus (T2DM) is associated with an increased risk of atherosclerotic cardiovascular disease. Vascular inflammation is a key factor in both the pathogenesis and outcome of atherosclerosis. 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) is a promising tool for identifying and quantifying vascular inflammation within atherosclerotic plaques. This study was designed to examine the vascular inflammation measured using FDG-PET in patients with impaired glucose tolerance and T2DM, in comparison with age- and sex-matched control subjects with normal glucose tolerance.

Methods and Results—We investigated vascular inflammation using FDG-PET in 90 age- and sex-matched subjects with different glucose tolerance (30 normal glucose tolerance subjects, 30 impaired glucose tolerance subjects, and 30 T2DM subjects). Vascular 18F-FDG uptake was measured as both the mean and maximum blood-normalized standardized uptake value, known as the target-to-background ratio (TBR). Both mean and maximum TBR measurements were significantly different, based on glucose tolerance, although the carotid intima-media thickness measurements were not significantly different. The maximum TBR values in patients with impaired glucose tolerance and T2DM were significantly increased compared with the normal subjects. In addition, subjects with metabolic syndrome had increased maximum TBR values compared with those without metabolic syndrome. Age-, sex-, and body mass index–adjusted maximum TBR levels were positively correlated with triglyceride, hemoglobin A1c, insulin resistance, high-sensitivity C-reactive protein, and Framingham risk score and were negatively correlated with high-density lipoprotein cholesterol and adiponectin levels.

Conclusions—the results of the present study suggest that impaired glucose tolerance and T2DM are associated with vascular inflammation in carotid atherosclerosis detected by FDG-PET. (Circ Cardiovasc Imaging. 2010;3:142-148.)

Key Words: atherosclerosis ■ inflammation ■ positron emission tomography ■ impaired glucose tolerance ■ type 2 diabetes

Atherosclerotic cardiovascular diseases are major causes of morbidity and mortality in the diabetic population. Although the exact mechanisms underlying the association between diabetes and atherosclerotic disease are not known, inflammation has been suggested to play a central role in the development of atherosclerosis. Epidemiological studies have demonstrated that type 2 diabetes mellitus (T2DM) is not only an independent risk factor for atherosclerotic cardiovascular disease but is also associated with increased levels of inflammatory markers. Atherosclerosis is an insidious disorder, and many patients already have atherosclerotic disease before they are diagnosed with diabetes. Impaired glucose tolerance (IGT) is associated with an increased risk of diabetes and has been officially a “prediabetes” state. Many studies have suggested that IGT is associated with an increased risk of atherosclerosis.

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Atherosclerosis is now recognized as an inflammatory disorder. The inflammatory state and composition of atherosclerotic plaques are considered the main contributing factors responsible for acute cardiovascular events, rather than the degree of stenosis. Angiography, the current gold standard imaging modality, is invasive and cannot identify inflamed plaques or nonstenotic plaques, which may be tend to rupture. Therefore, effective techniques for the detection of inflamed vulnerable plaques are critically needed. Recently, positron emission tomography (PET) with fluorodeoxyglucose (FDG) has become one of the best imaging
techniques for the detection of vulnerable atherosclerotic plaques. Ogawa et al reported that macrophages are responsible for the accumulation of FDG in atherosclerotic lesions using PET imaging in a rabbit model of atherosclerosis. Moreover, Tawakol et al reported a significant correlation between FDG-PET signal from carotid plaques and macrophage staining from the corresponding histological sections of specimens removed after endarterectomy. Recently, Tahara et al reported that simvastatin treatment for 3 months attenuates plaque inflammation visualized using FDG-PET and FDG uptake in carotid atherosclerosis was higher in proportion to the accumulation of the components of the metabolic syndrome (MetS). These studies suggest that FDG-PET imaging may help with measurement of plaque burden, may reflect plaque inflammatory activity, and may be useful in the study of the vascular inflammation response to medical treatment. However, the relationship between the vascular inflammation assessed by FDG-PET and glucose tolerance status has not been studied.

In the present study, we examined the difference in vascular inflammation measured using FDG-PET in patients with normal glucose tolerance (NGT), IGT, and T2DM. We also compared the findings of FDG-PET/CT with the carotid intima-media thickness (IMT), a measure used to quantify atherosclerosis. Furthermore, we evaluated the association between vascular inflammation, predictor of coronary heart disease such as Framingham risk score (FRS), high-sensitivity C-reactive protein (hsCRP), and adiponectin, an anti-inflammatory adipokine.

Methods

Study Design and Subjects

We enrolled 30 age- and sex-matched patients with known T2DM (16 men, 14 women; 55.6±8.6 years of age) and 30 IGT subjects (16 men, 14 women; 54.7±9.4 years of age) using predefined inclusion and exclusion criteria at the Korea University Diabetes Center. The diagnosis of IGT was based on a 75-g oral glucose tolerance test, and exclusion criteria at the Korea University Diabetes Center. The mean duration of diabetes in the diabetic subjects was 5.3 years.

Clinical and Laboratory Assessments

Body mass index (BMI) was calculated as the weight/height² (kg/m²), and the waist circumference was measured at the midpoint between the lower border of the rib cage and the iliac crest. All blood samples were obtained in the morning after a 12-hour overnight fast and were immediately stored at –80°C for subsequent assays. Serum triglycerides and high-density lipoprotein (HDL) cholesterol levels were determined enzymatically using a chemistry analyzer (Hitachi 747, Tokyo, Japan). The low-density lipoprotein (LDL) cholesterol concentration was estimated using the Friedewald formula. A glucose oxidase method was used to measure plasma glucose, and an electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, Ind) was used to measure insulin levels. Insulin resistance was calculated by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). Hemoglobin A1c (HbA1c) was measured using high-performance liquid chromatography (Bio-Rad Variant II, Hercules, Calif). MetS was defined according to the criteria established by the National Cholesterol Education Program Adult Treatment Panel III using the adjusted waist circumference for Asians. An individual 10-year coronary heart disease risk was estimated using the Framingham Heart Study prediction score sheet and used to classify subjects as low, intermediate, or high risk (<10%, 10% to 20%, and >20%, respectively). hSCRP levels were measured using a chemiluminescence immunoassay (Beckman, Coulter). Serum adiponectin was measured using an enzyme-linked immunosorbent assay (Mesdia, Seoul, Korea). The intra-assay and interassay variances of adiponectin levels were 4.1% to 5.9% and 3.9% to 6.3%, respectively.

Measurement of the Carotid IMTs

The IMT of the common carotid artery was determined using high-resolution B-mode ultrasonography (EnVisor, Philips Medical Systems, Andover, Mass) with a 5- to 12-MHz transducer. Measurements of the carotid IMT were made using IMT measurement software, Intimascope (Media Co, Tokyo, Japan) at 3 levels of the lateral and medial walls, 1 to 3 cm proximal to the carotid bifurcation. The average IMT was the mean value of 99 computer-based points in the region, and the maximal IMT was the IMT value at the maximal point of the region. All measurements were recorded by a single trained technician who was blinded to the subject’s clinical history.

FDG-PET/CT Imaging

PET/CT was performed using the Gemini TF 16 Slice PET/CT scanner (Philips Medical Systems, Cleveland, Ohio), with a 18-cm field of view. TF scanner is a high-performance, time-of-flight capable, fully 3D PET scanner using lutetium-yttrium oxyorthosilicate crystals. 18F-FDG (370 to 550 MBq) was injected intravenously, and patients rested in a quiet room for 60 minutes. The whole-body PET image (below cerebellum to inguinal) was acquired for 1 hour (1 bed cycle). PET image analysis was performed on a dedicated workstation (Extended Brilliance Work-space 3.5 with PET/CT viewer for automated image registration, Philips). Right carotid FDG uptake was measured along the length of the right carotid vessel, starting at the bifurcation and extending inferiorly and superiority every 4 mm. Arterial FDG uptake was quantified by drawing a region of interest around each artery on every slice of the coregistered transaxial PET/CT images, the region of interest was fitted to the artery wall on each axial slice, and coronal and sagittal views were used to ensure that the FDG uptake was from the artery. The standardized uptake value (SUV) is the decay-corrected tissue concentration of FDG (in kBq/mL) divided by the injected dose per body weight (kBq/g). The mean artery SUV value was normalized to the blood pool SUV value measured from the jugular vein (standardized circular regions of interest; right carotid artery, area = 77.9±3.4 mm², 9 pixels; right jugular vein, area = 95.0±12.7 mm², 9 pixels). Afterward, a target-to-background ratio (TBR) was calculated as right carotid vessel plaque SUV divided by venous blood SUV, and a mean and maximum value of TBR was calculated for each patient. To determine the variability of the mean and maximum TBR measurements, images from 20 subjects were twice analyzed, several weeks apart, by 2 readers. The intraobserver and interobserver correlation coefficient values of mean and maximum TBR measurements were >0.8.
The clinical and biochemical characteristics of the study subjects are presented in Table 1. No significant differences in age, sex distribution, waist circumference, mean and maximum carotid IMT, LDL cholesterol, hsCRP, percentage of smokers, or antihypertensive treatment were found among the groups. BMI, HDL cholesterol, triglyceride, glucose, insulin resistance, HbA1c, and adiponectin levels were significantly different, based on glucose tolerance status. The mean SUV and TBR levels were significantly different according to the glucose tolerance. Both the SUV and TBR levels were significantly different, based on glucose tolerance.

Table 2 shows the SUV and TBR measurements by glucose tolerance. Both the SUV and TBR levels were significantly different according to the glucose tolerance status. The mean SUV and TBR levels were significantly higher in patients with T2DM compared with subjects with NGT or IGT.
NGT or IGT. Furthermore, the maximum SUV and TBR levels were increased in patients with IGT and T2DM compared with normal subjects (Figure 1). In addition, subjects with MetS showed increased mean TBR (1.5 \pm 0.4 versus 1.2 \pm 0.3, \( P < 0.003 \)) and maximum TBR levels (1.9 \pm 0.6 versus 1.4 \pm 0.4, \( P < 0.001 \)) compared with those without MetS.

The partial correlation analysis, adjusted for age and sex, showed that the mean TBR measurements were significantly positively associated with triglyceride (\( r = 0.24, P = 0.013 \)), HOMA-IR (\( r = 0.52, P < 0.001 \)), and the number of MetS components (\( r = 0.31, P = 0.006 \)) and were negatively associated with the HDL cholesterol (\( r = 0.23, P = 0.044 \)) and adiponectin levels (\( r = 0.23, P = 0.042 \)) (Table 3). The results using maximum TBR measurements were similar to those using the mean TBR measurements. Furthermore, mean and maximum TBR levels were significantly correlated with the FRS (\( r = 0.57, P < 0.001 \)) (Table 3). Subjects with intermediate risk (10\% to 20\%) based on the FRS, as well as those with a high risk (>20\%), showed significantly increased mean and maximum TBR levels compared with those with a low-risk (<10\%) (\( P < 0.001 \)) (Figure 2).

Table 4 shows the clinical and biochemical characteristics stratified by tertiles of maximum TBR levels. According to increments of maximum TBR levels, triglyceride, HOMA-IR, HbA1c, and hsCRP levels were increased. By contrast, HDL cholesterol and adiponectin levels were decreased. Furthermore, the prevalence of the MetS, number of MetS components and the FRS were significantly increased by increments of the maximum TBR levels.

**Discussion**

The results of the present study showed that patients with T2DM had increased vascular inflammation in areas of carotid atherosclerosis measured using FDG-PET/CT. Moreover, patients with IGT had increased maximum TBR levels compared with NGT subjects even though there was no significant difference in the carotid IMT.

Inflammation is emphasized as a critical factor in the pathogenesis and destabilization of atherosclerotic lesions. Circulating inflammatory markers, such as hsCRP, are known to be associated with cardiovascular disease and are useful for risk stratification of patients with cardiovascular disease. However, circulating inflammatory markers cannot provide information on the vascular inflammation of local individual vascular lesions. Recent animal and clinical studies have shown that FDG-PET might be an accurate and reliable tool for the measurement of vascular inflammation in humans. FDG-PET imaging for vascular inflammation is based on the glycolytic activity of macrophages, which is typically higher than in background tissues. Rudd et al. showed that \(^{18}\)F-FDG-PET detected inflammatory atherosclerotic plaques in 8 patients who first underwent PET imaging and then
carotid endarterectomy. Towakol et al\textsuperscript{11} suggested that there was a significant correlation between the PET signal from the carotid plaques and macrophage staining from corresponding histological sections ($r=0.70$; $P<0.0001$). These studies suggest that FDG-PET imaging can be used for noninvasive identification and quantification of inflammation of atherosclerotic plaques, especially macrophage-related vascular inflammation.\textsuperscript{27} Moreover, the reproducibility of FDG uptake measurements is encouraging with correlation coefficients of $\geq 0.8$ on repeat studies, and both mean and maximum TBR measurements were equally reproducible.\textsuperscript{22} In the present study using FDG-PET/CT, the maximum TBR levels increased across the category of worsening glucose tolerance. The T2DM subjects exhibited higher maximum TBR levels than the IGT and NGT subjects, and the maximum TBR levels of IGT subjects was significantly higher than the NGT subjects. Rudd et al\textsuperscript{22} suggested that the mean TBR might be used for tracking systemic arterial therapies, whereas the maximum TBR might be an optimal measure used for detecting and monitoring local, plaque-based therapy.

Carotid artery IMT has been associated with cardiovascular disease and related risk factors in cross-sectional studies\textsuperscript{28} and as a predictor of cardiovascular disease in prospective studies.\textsuperscript{29} In a recent meta-analysis, the IGT subjects had a significantly greater carotid artery IMT than subjects in the control groups in 3 of 9 studies.\textsuperscript{30} Although the carotid IMT determined using high-resolution ultrasonography is one of the best methods for evaluation of atherosclerosis, IMT provides no information on the inflammation associated with atherosclerosis.\textsuperscript{31} In the present study, IGT patients did not show a higher carotid IMT, although the maximum TBR values detected by FDG-PET were significantly higher than in the control group. In addition, carotid IMT measurements, in patients with T2DM, also were not different compared with the normal and IGT subjects in the present study. Although the explanation for these findings is not clear, the results might have been affected by the short duration (5.3±5.9 years) of T2DM among the enrolled patients in this study, with relatively good glucose control (HbA1c=$7.1±1.0\%$). Moreover, we excluded patients with a history of cardiovascular disease in the present study. However, diabetic patients showed an increased in both the mean and maximum TBR levels compared with the normal and IGT subjects. These results suggest that the vascular inflammation detected by FDG-PET might reflect earlier stage dynamic changes of atherosclerosis than the atherosclerotic burden detected by ultrasonography. Tahara et al\textsuperscript{12} reported that FDG-PET showed significantly decreased FDG uptake in the atherosclerotic plaques in a simvastatin treatment group after 3 months. These results suggest that FDG-PET can identify a decrease in plaque inflammation much earlier than any structural changes detected by MRI.\textsuperscript{32} Dyslipidemia is one of the major risk factors for cardiovascular disease in patients with diabetic mellitus. T2DM patients with higher triglyceride levels might not have substantially higher concentrations of LDL cholesterol than matched individuals without diabetes, but for any LDL cholesterol concentration, those with diabetes generally have

<table>
<thead>
<tr>
<th>1st Tertile (n=30)</th>
<th>2nd Tertile (n=30)</th>
<th>3rd Tertile (n=30)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose status, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2DM</td>
<td>0</td>
<td>6.7</td>
<td>93.3</td>
</tr>
<tr>
<td>IGT</td>
<td>32.3</td>
<td>61.3</td>
<td>6.5</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>80.5±6.8</td>
<td>82.2±8.0</td>
<td>83.4±7.6</td>
</tr>
<tr>
<td>Mean carotid IMT, mm</td>
<td>0.66±0.14</td>
<td>0.67±0.11</td>
<td>0.68±0.17</td>
</tr>
<tr>
<td>Max carotid IMT, mm</td>
<td>0.80±0.18</td>
<td>0.81±0.14</td>
<td>0.84±0.24</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>124.0±15.7</td>
<td>120.5±16.0</td>
<td>128.0±14.3</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>79.3±10.2</td>
<td>78.1±9.2</td>
<td>80.8±11.3</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.1±0.8</td>
<td>3.9±1.0</td>
<td>4.0±0.9</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.3±0.6</td>
<td>2.2±0.7</td>
<td>2.1±0.9</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.2±0.4†</td>
<td>1.1±0.3‡</td>
<td>1.0±0.2‡</td>
</tr>
<tr>
<td>Triglycerides, mmol/L*</td>
<td>0.9 (0.7, 1.1)†</td>
<td>1.2 (0.6, 2.1)‡†</td>
<td>1.2 (1.0, 2.0)‡</td>
</tr>
<tr>
<td>HOMA-IR*</td>
<td>0.3 (0.1, 0.6)†</td>
<td>0.9 (0.3, 1.3)†</td>
<td>1.2 (0.5, 1.6)‡</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.6±0.5†</td>
<td>5.8±0.6†</td>
<td>7.0±1.1‡</td>
</tr>
<tr>
<td>Adiponectin, µg/mL*</td>
<td>2.2 (0.9, 4.8)††</td>
<td>3.0 (2.0, 4.8)††</td>
<td>1.0 (0.4, 2.4)‡</td>
</tr>
<tr>
<td>hsCRP, mg/L*</td>
<td>0.4 (0.2, 0.8)††</td>
<td>0.3 (0.2, 0.9)††</td>
<td>0.6 (0.2, 1.3)‡</td>
</tr>
<tr>
<td>No. of MetS components*</td>
<td>1.0 (0.5, 2.0)†</td>
<td>2.0 (1.0, 3.0)‡†</td>
<td>3.0 (2.0, 3.0)‡</td>
</tr>
<tr>
<td>Framingham risk score*</td>
<td>5.5 (4.0, 7.0)†</td>
<td>6.0 (4.0, 8.0)†</td>
<td>15.0 (7.5, 22.0)‡</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD, median (interquartile range), or n (%). $P$ values represent overall differences across groups as determined by ANCOVA adjusted for age, sex, and BMI. Matching symbols (†, ‡) indicate no statistical significance based on the Bonferroni multiple comparison. Logarithmic transformed data were used.
an increase in LDL particles because small, dense, lipid-poor LDL particles accumulate in the circulation.\textsuperscript{33} It was reported that antioxidant and anti-inflammatory characteristics of HDL cholesterol may be as pivotal as its cholesterol efflux function in terms of protecting against the development of atherosclerosis.\textsuperscript{34} Moreover, monocyte-derived macrophages isolated from individuals with low HDL cholesterol concentrations manifest a proinflammatory phenotype.\textsuperscript{35} Therefore, mean and maximum TBR may be associated with low HDL cholesterol and increased small, dense LDL particles rather than circulating LDL cholesterol level. The results of this study also showed that hyperglycemia-related variables, such as the fasting plasma glucose and HbA\textsubscript{1c}, were significantly associated with the vascular inflammation measured by FDG-PET.

Adiponectin is a well-known adipokine that has been associated with protective effects in the vasculature and myocardium.\textsuperscript{36} Adiponectin plays a key role as an antiabetic, insulin-sensitizing factor that has antiatherogenic and anti-inflammatory properties in the vasculature.\textsuperscript{36} Epidemiological studies have shown that reduced adiponectin levels are correlated with an increased risk of cardiovascular disease in patients with diabetes and hyperglycemia.\textsuperscript{37} In the present study, both the mean and maximum TBR levels were negatively correlated with adiponectin levels and positively correlated with insulin resistance.

Some limitations are observed in our study. First, the principal limitation of this case-control study is the cross-sectional design. Therefore, we cannot definitely identify whether increased SUV and TBR measured by FDG-PET were due to worsening glucose tolerance status. However, the present study had several advantages such as the prospective recruitment of subjects with predefined inclusion and exclusion criteria compared with several previous retrospective studies that enrolled patients who had FDG-PET studies for cancer follow-up. Furthermore, we compared the TBR values among groups matched for age and sex because previous studies have reported that the SUV and TBR values differ according to age and sex.\textsuperscript{38} Second, circulating insulin levels might alter the expression of glucose transporter (GLUT) isotypes. The most important pathway for FDG to enter the cell body of almost all human cells is mediated by facultative GLUT.\textsuperscript{39} Accordingly, the presence of T2DM and IGT might limit the observed difference of SUV between groups. The relationship between the expression of GLUT isotypes and FDG accumulation in atherosclerotic macrophages may be an important area for further investigation.

Conclusion

The results of the present study using FDG-PET/CT showed that patients with IGT, as well as T2DM, had higher maximum TBR levels reflecting vascular inflammation than subjects in the control group. Furthermore, TBR levels were significantly and positively associated with components of the MetS and the FRS and negatively associated with adiponectin levels. These results suggest that FDG-PET/CT might be useful for early diagnosis of vascular inflammation and vulnerable plaques and for estimating the risk stratification of patients with abnormal glucose metabolism.

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Disclosures

None.

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Inflammatory atherosclerotic plaque has been established as a main contributing factor responsible for cardiovascular disease. Type 2 diabetes mellitus is not only an independent risk factor for atherosclerotic cardiovascular disease but is also associated with increased levels of inflammatory markers. There is growing, consistent evidence that quantitative positron emission tomography with $^{18}$F-deoxyglucose can provide a noninvasive measure of vascular inflammation. In this study, we found increased $^{18}$F-deoxyglucose uptake in patients with impaired glucose tolerance and type 2 diabetes mellitus compared with the healthy subjects, even though there were no significant differences in the carotid intima-media thickness across the groups. This result suggests that vascular inflammation detected by positron emission tomography with $^{18}$F-deoxyglucose may reflect an earlier stage in atherogenesis that is manifested before anatomic changes can be detected by ultrasonography.
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