Arterial Wall Thickness is Associated with Insulin Resistance in Type 2 Diabetic Patients

Shigehiko Fujiwara, Masanori Emoto, Miyoko Komatsu, Koka Motoyama, Tomoaki Morioka, Hidenori Koyama, Tetsuo Shoji, Masaaki Inaba, and Yoshiki Nishizawa

Metabolism, Endocrinology and Molecular Medicine, Department of Internal Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan

The aim of the present study was to investigate the independent association of the intimal-medial thickness of carotid and femoral arteries (CA-IMT and FA-IMT), a marker of atherosclerosis, with insulin resistance in type 2 diabetic patients. We evaluated CA-IMT and FA-IMT by high-resolution ultrasonography and insulin resistance determined by euglycemic hyperinsulinemic clamp in 119 type 2 diabetic subjects, 71 males and 48 females (age, 54 ± 12 (SD) years). In simple regression analyses, CA-IMT and FA-IMT were significantly inversely correlated with insulin sensitivity index (CA-IMT, \( r = -0.225, p = 0.010 \); FA-IMT, \( r = -0.186, p = 0.043 \), respectively). Multiple regression analysis was performed with the logarithm of CA-IMT or FA-IMT as a dependent variable and insulin sensitivity index as an independent variable along with known clinical risk factors. Insulin sensitivity index exhibited a significant independent contribution to log (CA-IMT) (\( \beta = -0.204, p = 0.033 \)) and to log (FA-IMT) (\( \beta = -0.237, p = 0.010 \)) in these models (CA-IMT, \( R^2 = 0.347, p < 0.0001 \); FA-IMT, \( R^2 = 0.398, p < 0.0001 \), respectively). In conclusion, insulin resistance is associated with both CA-IMT and FA-IMT in type 2 diabetic patients, suggesting that it is an independent risk factor for the development of atherosclerosis in type 2 diabetes. J Atheroscler Thromb, 2003; 10: 246–252.

Key Words: Diabetes, Atherosclerosis, Insulin resistance, Clinical research

Introduction

Insulin resistance has been proposed to play a crucial role in the development and progression of atherosclerotic cardiovascular disease, the most common cause of death in diabetic patients (1, 2). Insulin resistance is linked to established risk factors for atherosclerosis such as hypertension, hyperlipidemia, and obesity, which subsequently accelerate the development and progression of atherosclerosis. However, it is still unclear whether insulin resistance per se is linked to atherosclerosis independent of these major risk factors.

Arterial wall intimal-medial thickness (IMT) has been established by many studies to be an early marker and predictor of atherosclerotic disease (3–5). The study by Laakso et al. was the first to show reduced insulin sensitivity by euglycemic hyperinsulinemic clamp in nonobese, nondiabetic subjects with asymptomatic atherosclerosis, along with increased intimal-medial thickness (IMT), compared with control subjects (6). Subsequent studies mainly in non-diabetic subjects including those with hypertension or angina demonstrated complex outcomes concerning the association of IMT of the carotid artery with insulin resistance; significant (7–10), partially significant, that is, significant in univariate but not in multivariate analysis (11–15), or nonsignificant (16, 17). In type 2 diabetic patients, two studies found an inverse association of IMT of carotid artery with insulin resistance (18, 19), but another did not (20). Only two studies reported
no association of IMT of the femoral artery with insulin resistance in healthy subjects (13, 21). This discrepancy in findings may be due to differences in ethnicity, study size, and other clinical characteristics of subjects. However, the most important issue is that these studies used different surrogate measures of insulin resistance, such as fasting insulin, homeostasis model assessment (HOMA-IR), insulin tolerance test ($K_{TT}$), steady-state plasma glucose determined by the modified insulin suppression test (SSPG), and frequently-sampled intravenous glucose tests ($S_{c}$, FSIVGTT). Although these surrogate indices are correlated with the index assessed by euglycemic hyperinsulinemic clamp, the current gold standard technique, each index has at least one or more intrinsic problem, especially in application to type 2 diabetic patients (22). Among these previous studies, only two used the euglycemic hyperinsulinemic clamp to evaluate insulin resistance in healthy (13) or type 2 diabetic patients (19).

The aim of the present study was to investigate the association of IMT of both carotid and femoral arteries (CA-IMT and FA-IMT) with insulin resistance assessed by euglycemic hyperinsulinemic clamp and to explore the direct impact of insulin resistance on early atherosclerotic change in type 2 diabetic patients.

Materials and methods

Subjects
One hundred and nineteen type 2 diabetic subjects participating in diabetes educational programs were selected for the present study from among type 2 diabetic patients attending our diabetic out-patient clinic at Osaka City University Hospital. The diagnosis of diabetes mellitus was based on a previous history of diabetes or the criteria of the American Diabetes Association (23). The mean age of the subjects was 53.7 ± 11.9 years (SD), and ranged from 15 to 74 years. The known duration of diabetes ranged from 0 to 30 years, with a mean of 8.5 ± 7.2 years (SD). Hypertension was defined as blood pressure above 140/90 mmHg, or the use of known agents for the treatment of hypertension; hyperlipidemia was defined as total cholesterol above 5.17 mmol/l (200 mg/dl), or LDL cholesterol 3.36 mmol/l (130 mg/dl), or use of known agents for the treatment of hyperlipidemia. The percentages of subjects with hypertension, hyperlipidemia and of current smokers were 34.2%, 30.8% and 58.3%, respectively. Sixty-two subjects were treated with oral hypoglycemic agents, 25 subjects with insulin therapy and 32 subjects with medical nutritional therapy alone. Twelve subjects with hypertension were treated with calcium channel blockers and 6 subjects with angiotensin-converting enzyme (ACE) inhibitors. Fifteen subjects with hyperlipidemia were treated with β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) reductase inhibitors and 4 subjects with clofibrates. Subjects who underwent dialysis therapy, or had other endocrinopathy, malignancies, infections or other active diseases were excluded. Informed consent for participating in this study was obtained from all type 2 diabetic subjects, and the overall protocol of the study was approved by Local Ethics Committee.

Study protocol
The type 2 diabetes subjects were admitted to our diabetes ward one week before the glucose clamp study. During admission, medical nutritional therapy (30 kcal/kg body weight/day, 50% carbohydrates, 30% fat, and 20% protein) was performed for all subjects. Blood pressure was measured three times using a mercury sphygmomanometer on the right arm after a 15-min rest in the supine position before ultrasonographic examinations.

Ultrasonography
Prior to the glucose clamp study, ultrasonographic examinations for atherosclerosis of the common carotid and femoral arteries were performed by the same examiner in the supine position with slight hyperextension of the neck, using an ultrasonic phase-locked echo-tracking system, which was equipped with a high-resolution realtime 7.5 MHz linear scanner (SSD 610, Aloka Co. Ltd, Tokyo, Japan) (24, 25). The scan examination included approximately 4 cm of the common carotid artery and the carotid bulb for the carotid artery and approximately 4 cm of the femoral artery and the bifurcation between the profound and superficial femoral artery. These regions were scanned bilaterally in the longitudinal and transverse projections. The image was focused on the far wall of the artery. The intimal-medial thickness (IMT) was measured at the site of the most advanced atherosclerotic lesion which exhibited the greatest distance between the lumen-intimal interface and the media-adventitia interface of the far wall in both carotid and both femoral arteries. We used the greatest IMT including plaques as a marker of atherosclerotic change in each artery of the subject (CA-IMT, FA-IMT) (24, 25).

All measurements were made under blind conditions. To assess intraobserver variability, 40 subjects (20 patients and 20 control subjects) were examined on two different occasions. The coefficient of variation for IMT was 3.2% for the carotid artery and 3.0% for the femoral artery for subjects (25, 26).

Euglycemic hyperinsulinemic clamp
Ten to 12 hours after an overnight fast, euglycemic hyperinsulinemic clamp (clamp study) was performed in type 2 diabetic subjects using an STG 22 artificial pancreas model (Nikkiso Co. Ltd., Tokyo) according to the method of De Fronzo et al. (27) as described elsewhere (28–31). In brief, after baseline blood sampling, insulin
(Humulin®, Eli Lilly & Company, Indianapolis, IN) was infused in a continuous fashion at a rate of 1.25 mU/kg\textsuperscript{-1}/min\textsuperscript{-1} after the priming insulin infusion during the first 10 minutes of the clamp at the same doses as previously reported (18). Blood glucose levels were determined every 5 min during the 120-min clamp study, and euglycemia (5.0 mmol/l) was maintained by infusion of variable amounts of 20% glucose solution. Steady-state blood glucose level during the last 30 minutes of the clamp was kept constant throughout at 5.0 mmol/l (4.99 ± 0.03 mmol/l), and the mean coefficient of variance was 1.29%, with a range of 0.4 to 2.9%. The total-body glucose disposal rate was evaluated as the mean of the glucose infusion rate (GIR) during the last 30 min of clamping. We adopted as the insulin sensitivity index the value obtained by dividing the mean GIR by the steady-state plasma insulin (SSPI) levels during the last 30 min of the clamp, multiplied by 100 for convenience.

Biochemical analysis
Plasma glucose levels were measured by the glucose oxidase method, and glycated hemoglobin A1c (HbA1c) by high-pressure liquid chromatography (normal range: 4.0 – 5.8%). Serum creatinine, serum total cholesterol, triglyceride, and HDL-cholesterol levels were measured by enzymatic methods adapted to an autoanalyzer (Hitachi 7450, Hitachi Co. Ltd., Tokyo, Japan). LDL-cholesterol was estimated by the equation of Friedewald et al. (32).

Statistical analyses
Statistical analysis was performed with the Stat View V system (Abacus Concepts Inc., Berkely, CA) for Windows OS. All results are expressed as means ± SD, unless otherwise indicated. Simple linear regression analysis, non-parametric analysis and multiple regression analysis were performed to evaluate the relations among IMT and various clinical factors as appropriate. 
P values less than 0.05 were considered significant.

Results

Clinical characteristics, IMT and insulin sensitivity index (Table 1)
The clinical characteristics of all type 2 diabetic subjects are shown in Table 1. The mean of CA-IMT was 0.88 ± 0.41 mm, with a range of 0.30 to 2.54, and that of FA-IMT was 1.13 ± 0.71, with a range of 0.28 to 4.00 mm. Since both CA-IMT and FA-IMT exhibited skewed distributions, the logarithmic transformations of CA-IMT and FA-IMT (log(CA-IMT) and log(FA-IMT)), which exhibited normal distributions, were also used for subsequent analyses. GIR and SSPI during the last 30 minutes of the clamp were 4.9 ± 1.8 mg kg\textsuperscript{-1} min\textsuperscript{-1} and 630 ± 179 pmol/l, respectively. The insulin sensitivity index was 5.2 ± 2.5 mg kg\textsuperscript{-1} min\textsuperscript{-1} mU\textsuperscript{-1} l\textsuperscript{-1}, with a range of 1.0 to 12.7.

Associations of IMT with insulin resistance (Tables 2 and 3, Figs. 1)
Table 2 shows the correlation coefficients of possible

| Table 2. Correlation coefficients determined by simple regression analysis between CA-IMT or FA-IMT and other clinical factors possibly affecting IMT in type 2 diabetic subjects. |
|-----------------|-----------|-----------|
|                | CA-IMT    | FA-IMT    |
| Age            | 0.397     | < 0.0001  |
| Duration of diabetes | 0.074     | 0.4523    |
| BMI            | – 0.024   | 0.7919    |
| Systolic blood pressure | 0.264     | 0.0037    |
| HbA1c (%)      | – 0.169   | 0.0669    |
| LDL cholesterol | 0.095     | 0.3018    |
| Creatinine     | – 0.058   | 0.5293    |

| Table 3. Multiple regression analysis of clinical factors possibly affecting Log(CA-IMT) or Log(FA-IMT) in type 2 diabetic subjects. |
|-----------------|-----------|-----------|
|                | Log(CA-IMT) | Log(FA-IMT) |
| Age            | 0.446      | < 0.0001  |
| Gender         | 0.119      | 0.2736    |
| Duration of diabetes | 0.150     | 0.0909    |
| Smoker or not  | 0.162      | 0.1085    |
| BMI            | – 0.102    | 0.2738    |
| Systolic blood pressure | 0.284     | 0.0016    |
| HbA1c (%)      | – 0.021    | 0.8133    |
| LDL cholesterol | 0.039      | 0.6451    |
| Insulin Sensitivity Index | – 0.204 | 0.0334    |

All values represent n, mean ± SD (range). OHA, oral hypoglycemic agents.
risk factors with CA-IMT and FA-IMT in all diabetic subjects. CA-IMT was correlated with age and systolic blood pressure, and FA-IMT with age and duration of diabetes. Both CA-IMT and FA-IMT were inversely correlated with insulin sensitivity indices, as were their logarithmic transformations (Fig. 1) (CA-IMT, \( r = -0.225 \), \( p = 0.014 \); FA-IMT, \( r = -0.186 \), \( p = 0.043 \); log(CA-IMT), \( r = -0.223 \), \( p = 0.015 \); log(FA-IMT), \( r = -0.225 \), \( p = 0.014 \)). Since these clinical factors are expected to interfere with each other, multiple regression analysis were performed to explore the independent impact of insulin resistance on IMT. In these analyses, log (CA-IMT) or log (FA-IMT) was adopted as a dependent variable and insulin sensitivity index as an independent variable, along with age, gender (male as 1 and female as 0), duration of diabetes, smoking or not, BMI, systolic blood pressure, HbA1c, and LDL cholesterol (Table 3). Insulin sensitivity index was found to be an independent contributor to log(CA-IMT) secondary to age and systolic blood pressure, and to log(FA-IMT) secondary to age, smoker or not, and BMI. The determination coefficients of this model for log(CA-IMT) and log(FA-IMT) were 0.347 and 0.398, respectively, indicating that this model accounted for 34.7% and 39.8% of the variability of log(CA-IMT) and log(FA-IMT), respectively.

**Discussion**

The findings of present study demonstrated that the IMTs of both the carotid and femoral arteries were correlated with insulin resistance in type 2 diabetic patients, and that these correlations remained significant after adjustment for other established risk factors for atherosclerosis. In comparison with findings of previous studies, the present observations are characterized by the following three points; first, we used euglycemic hyperinsulinemic clamp, the current gold standard technique, to accurately measure insulin sensitivity. Second, we demonstrated a direct association of insulin resistance with IMT not only for the carotid artery but also for the femoral artery. Third, the present subjects were type 2 diabetic patients with various clinical profiles. The present cross-sectional study provided reliable, conclusive findings concerning the association of IMT with insulin resistance in type 2 diabetes.

Previous studies reported inconsistent findings concerning the relation between IMT and insulin resistance. In non-diabetic healthy subjects, CA-IMT was reported to be inversely correlated with insulin sensitivity assessed by KITT (8), euglycemic clamp (9), and SSPG (10) in univariate and multivariate analyses. Several studies found that the inverse correlation of CA-IMT with insulin resistance was no longer significant after adjustment for established risk factors in multivariate analysis in studies using the index of fasting proinsulin (12), HOMA-IR (15), KITT (14), FSIVGTT (11), or euglycemic clamp (13) as indices. Recent studies failed to find significant associations of CA-IMT with insulin resistance assessed by HOMA-IR (16) or SSPG (17). To our knowledge, only two studies have found a lack of a significant association of FA-IMT with insulin resistance assessed by FSIVGTT (21) or euglycemic clamp (13) in healthy subjects, and there are no such findings for type 2 diabetes. In type 2 diabetic patients, Bonora et al. and Watarai et al. demonstrated the independent contribution of insulin resistance assessed by KITT (18) or euglycemic clamp (19) to CA-IMT in multivariate analysis even after adjustment for established risk factors.

These inconsistent results may be due, at least in part, to the use of different methods for measurements of insulin resistance. These studies employed fasting insulin, HOMA-IR, KITT, SSPG, FSIVGTT. These surrogate indices were reported to be highly correlated with the index obtained by euglycemic hyperinsulinemic clamp in many studies including our study, and have been widely used in many epidemiological and clinical studies (30, 33–36). However, the limitations of their application to diabetes are also well-documented (22). For example, fasting IRI and HOMA-IR may be affected by the crossreaction of insulin assay with proinsulin, proinsulin split products, and prevailing plasma glucose levels, and sampling in the fasting state, in which glucose levels are mainly determined by hepatic glucose production or insulin resistance. Since fasting insulin and HOMA-IR exhibit a hyperbolic relation with the clamp-based index of insulin resistance, logarithmic or reciprocal transformation is needed in order to more accurately predict insulin resistance (30, 37, 38). The insulin tolerance test is a direct and simple method of measuring the insulin effect derived from the rate of fall of plasma glucose level after intravenous bolus injection. This method may be confounded by the neuro-endocrine response to hypogly-
cemia, and does not permit quantitative measurement of insulin-mediated glucose metabolism. To date, only a few studies examined the validity of KITT in application to type 2 diabetes (39). Insulin-modified FSIVGTT, which is improved for application to diabetic patients with a decreased insulin secretion capacity, still features intrinsic systematic errors (40–44). These problems in methodology of quantitating insulin sensitivity may have made the relation between IMT and insulin resistance less reliable.

To avoid these methodological issues in measuring insulin resistance in type 2 diabetic patients, we evaluated insulin resistance by euglycemic hyperinsulinemic clamp in 119 type 2 diabetic patients. The number of subjects in the present study was large enough to compare the findings obtained with those in previous studies other than large epidemiological ones (11, 12, 15). The coefficients of correlations between CA-IMT or FA-IMT and insulin resistance were not strong but were significant and comparable with those in previous studies on diabetic patients (18, 19). As expected, in univariate analysis, known risk factors for atherosclerosis, age, blood pressure, and duration of diabetes were found to be correlated with CA-IMT or FA-IMT. After adjustment for these risk factors, insulin resistance was found to be a significant contributor to not only CA-IMT but also FA-IMT in the present diabetic patients.

Another explanation of the association between IMT and insulin resistance may be differences in the populations studied, diabetic or non-diabetic. Bonora et al. also demonstrated an independent contribution of insulin resistance assessed by KITT to CA-IMT in diabetic patients but not in healthy subjects in a multivariate analysis (18). In contrast to the previous findings for healthy subjects that the association between IMT and insulin resistance did not remain significant after adjustment for other established risk factors (13, 18), we found that the association between IMT and insulin resistance remained significant in a multivariate analysis in the present diabetic patients. Type 2 diabetic patients exhibit a more atherogenic internal environment than do healthy subjects, including lipoprotein abnormality, oxidative stress, hypercoagulative state and other factors. Thus, it may be that the independent effect of insulin resistance on arterial walls is more evident in type 2 diabetes than in the non-diabetic state. Recently, insulin sensitizers including thiazolidine derivatives have been available in the treatment of diabetes. Taken together with our findings, these drugs may exert an anti-atherogenic effect in addition to a hypoglycemic effect (45).

In conclusion, the present study confirmed the close association of insulin resistance with increased IMT of both the carotid and femoral arteries in type 2 diabetic patients and provides reliable evidence that insulin resistance per se, independent of other established risk factors, contributes to the development of subclinical atherosclerosis, at least in type 2 diabetes mellitus.

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References


