Insulin Resistance Contributes to Carotid Arterial Wall Thickness in Patients with Non-Insulin-Dependent-Diabetes Mellitus

TAKAO WATARAI, YOSHIMITSU YAMASAKI, MASAHIKO IKEDA, MINORU KUBOTA, MINEO KODAMA, TAKAFUMI TSUJINO, MICHISHIRO KISHIMOTO, RUZUO KAWAMORI* AND MASATSUGU HORI

Department of Internal Medicine and Therapeutics, Osaka University School of Medicine
*Department of Medicine, Metabolism and Endocrinology, Juntendo University School of Medicine

Abstract. The aim of this study was to clarify whether insulin resistance contributes to atherosclerosis in patients with non-insulin-dependent diabetes mellitus (NIDDM). Fifty-three NIDDM patients (36 males and 17 females, 53 ± 10 years old (mean ± SD)) were studied. As an index of atherosclerosis, we measured the average thickness (IMT) as well as basal thickness excluding the maximum thickness and the height of the maximum thickness of the carotid artery wall. Euglycemic hyperinsulinemic glucose clamp was conducted for 90 min to evaluate average glucose infusion rate (GIR) as an index of insulin sensitivity in the peripheral tissues. For another 180 min after intake of oral glucose load with 0.3 g/kg, the euglycemic hyperinsulinemic clamp was continued to measure ratio of splanchnic glucose uptake (SGU) as an index of insulin sensitivity of the liver. The patients were separated into three activity groups according to the grade of their leisure-time physical activity. GIR (r = -0.32, p < 0.05) but not SGU (r = 0.139) showed a significant inverse relationship with IMT. Multivariant regression analysis indicated that age and total cholesterol remain as independent risk factors for basal thickness and GIR as only independent risk factor for the height of the maximum thickness. Paralleling the degrees of habitual exercise (low, moderate, and high active group), GIR was higher (6.19 ± 1.02, 6.38 ± 1.38, 7.44 ± 1.80, respectively) and IMT was lower (1.34 ± 0.33 mm, 1.20 ± 0.31 mm, and 1.12 ± 0.29 mm, respectively) in male NIDDM as well as in female NIDDM. These data suggest that insulin resistance in the peripheral tissues but not the splanchnic tissues may independently contribute to carotid arterial wall thickness and especially to plaque lesion, and that habitual exercise might reduce insulin resistance leading to attenuation of atherosclerosis.

Key words: Glucose clamp, Peripheral glucose uptake, Splanchnic glucose uptake, Plaque.

MACROVASCULAR diseases caused by atherosclerosis is the major cause of mortality in subjects with non-insulin dependent diabetes mellitus (NIDDM). Atherosclerosis accelerates with accompanying hypertension or dyslipidemia in NIDDM [1]. Insulin resistance is known to be an underlying factor in essential hypertension, dyslipidemia, and subjects with impaired glucose tolerance [2-3]. In general population, insulin resistance is related to preclinical atherosclerosis, which is measured as intimal and medial thickness (IMT) of the carotid arterial wall [4].

In NIDDM patients, obesity [5], glycemic control [6-7] and exercise [8] have been shown to affect insulin resistance. In addition to the well known insulin resistance in the peripheral tissues, we have reported hepatic insulin resistance in subjects with genetic impairment of glucokinase [9]. Thus, the tissue specificity of insulin resistance must be further analyzed to evaluate the relationship between insulin resistance and atherosclerosis.

In this study, we evaluated the possible relation-
ship between insulin resistance and atherosclerosis in NIDDM. IMT [4, 10-12] was determined with high-resolution echo-tomography as well as the basal arterial wall thickness excluding the maximum thickness and the height of the maximum thickness, which may be the plaque lesion in diabetics with highly advanced carotid atherosclerosis [12]. It is speculated that the first event of acute coronary syndrome is the rupture of the soft plaque [13]. Insulin resistance both in the peripheral tissues and in the splanchnic tissues were quantitatively determined with a euglycemic hyperinsulinemic glucose clamp with oral glucose load (clamp OGL) [14-16]. We also evaluated the effect of habitual physical activity on insulin resistance and hence preclinical atherosclerosis.

Materials and Methods

Subjects

Fifty-three patients (36 males, 17 females) with NIDDM being treated at Osaka University Hospital participated in this study. The definition of NIDDM was based on World Health Organization criteria [17]. Every patient who fulfilled the following inclusion criteria was considered for study: 1) no episode of ketonuria, 2) insulin therapy (if any) started after at least 5 years of known disease, 3) absence of overt diabetic nephropathy or other renal tract disease, and 4) no evidence of cardiac failure. The purpose and protocol of the study were explained to each subject, and all who agreed to participate gave their informed consent. The clinical characteristics of these patients are presented in Table 1.

IMT determination

Ultrasonographic scanning of the carotid arteries was performed using an echo graphic system (EUB-450, Hitachi Medico, Tokyo, Japan) with an electrical linear transducer (midfrequency of 7.5 MHz). The axial resolution of this system was at least 0.3 mm. Scanning of the extracranial carotid arteries in the neck was performed bilaterally in two different longitudinal projections (i.e., anterior-oblique, lateral, and posterior-oblique) as well as the transverse projection. From the Polaroid photograph, the thickness was measured with calipers at three points (the thickest point (IMTp) and two ad-

Table 1. Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th>Parameters (n)</th>
<th>Males (36)</th>
<th>Females (17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>61.0 ± 9.8</td>
<td>55.9 ± 11.1</td>
</tr>
<tr>
<td>Duration (yr)</td>
<td>10.5 ± 7.6</td>
<td>13.9 ± 7.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.1 ± 6.8</td>
<td>20.0 ± 8.6</td>
</tr>
<tr>
<td>Treatment (Diet/Oral/Insulin)</td>
<td>(8/16/12)</td>
<td>(0/9/8)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.8 ± 1.7</td>
<td>10.2 ± 2.2</td>
</tr>
<tr>
<td>T-Chol (mM)</td>
<td>5.4 ± 1.1</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td>TG (mM)</td>
<td>2.0 ± 1.1</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>HDL-Chol (mM)</td>
<td>1.3 ± 0.5</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>nonHDL-Chol (mM)</td>
<td>3.7 ± 1.6</td>
<td>3.5 ± 1.5</td>
</tr>
<tr>
<td>LDL-Chol (mM)</td>
<td>2.8 ± 1.4</td>
<td>2.9 ± 1.2</td>
</tr>
<tr>
<td>sBP (mmHg)</td>
<td>124 ± 34</td>
<td>128 ± 39</td>
</tr>
<tr>
<td>dBP (mmHg)</td>
<td>75 ± 21</td>
<td>71 ± 21</td>
</tr>
<tr>
<td>Brinkman index</td>
<td>569 ± 536</td>
<td>544 ± 263</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

Abbreviations: BMI, body mass index; Oral, oral hypoglycemic agents; HbA1c, hemoglobin A1c; T-Chol, total cholesterol; TG, triglyceride; HDL-Chol, high density lipoprotein cholesterol; nonHDL-Chol, non HDL cholesterol; LDL-Chol, low density lipoprotein cholesterol; sBP, systolic blood pressure; dBP, diastolic blood pressure.

The Brinkman index was estimated as the product of years smoked and the mean number of cigarettes smoked daily.
adjacent points (IMT1, and IMT2), 1 cm away) and the average of these three thicknesses was defined as the IMT. The basal carotid arterial wall thickness was calculated as \((\text{IMT1} + \text{IMT2})/2\). The height of the maximum thickness was calculated as \(\text{IMTp} - (\text{IMT1} + \text{IMT2})/2\). Of the values from the right and left carotid the greater one was used to represent the subject, as reported in our previous studies [4, 10, 11].

*Tissue-specific insulin resistance determination*

Tissue-specific insulin resistance was determined with the euglycemic hyperinsulinemic clamp with oral glucose load (clamp OGL) described in detail elsewhere [14-16]. In brief, after an overnight fast and discontinuation of all medications on the study morning, a primed-constant infusion of short-acting insulin (Humulin R, Eli Lilly & Co., Indianapolis, in U.S.A.) at 2.6 mU/kg.min and exogenous glucose infusion were started to achieve the desired steady-state insulin concentration and to keep blood glucose levels within the euglycemic range (around 5.2 mmol/l) by using an artificial endocrine pancreas (STG-22, Nikkiso Co., Shizuoka, Japan). A steady-state of euglycemia was maintained for 90 min, and the average rate of glucose infusion (GIR, mg/kg min) during the last 15 min before the oral glucose loading (0.3 g/kg) was used as an estimate of peripheral glucose uptake. The euglycemic hyperinsulinemic clamp was continued for another 180 min after oral glucose administration. Splanchnic glucose uptake (SGU) can be calculated from the difference between the amount of ingested glucose and the cumulative decrements in GIR for 180 min after glucose loading. The SGU was expressed as the percentage of the oral glucose load (%). This indirect method of evaluating hepatic glucose uptake has been validated by comparison with a direct method using alloxan-induced diabetic dogs [18]. GIR was measured on 53 NIDDM patients. However, SGU was measured on the 45 subjects because the remaining eight subjects did not accept the clamp study after oral glucose load.

*Assessment of leisure-time physical activities*

We used a modified version of a published questionnaire to evaluate the energy expended in leisure-time physical activity (LTPA) [19]. LTPA was assessed before measurements of the carotid artery wall thickness and clamp OGL. The questionnaire lists 62 individual physical activities. Subjects indicated the number of occasions per month during the previous 12 months that they had performed each activity and its average duration in minutes. The questionnaire was self-administered under the supervision of a trained interviewer (MI). We classified the patients into three groups (i.e., high active, moderate active, and low active) according to the questionnaire replies. Examples of activities in each class are as follows: high active group, habitually engaging in a specific exercise such as tennis or jogging; moderate active group, not habitually engaging in a specific exercise, but walking to or in the workplace more than 60 min per day; and low active group, engaging in a smaller amount of exercise than the first two groups.

*Assessment of other parameters*

Lifelong exposure to smoking (Brinkman index) was estimated as the product of years smoked and the mean number of cigarettes smoked daily. Blood was withdrawn for analyses of serum total cholesterol and HDL cholesterol, serum triglycerides (TG), plasma glucose, and \(\text{HbA1c}\) levels by standard laboratory techniques. LDL cholesterol was estimated by the equation of Friedewald et al. [20]. Non HDL cholesterol was calculated as follows:

\[
\text{Non HDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol}.
\]

*Statistical analysis*

Data are given as mean±SD. The stepwise forward and backward multivariate regression analysis was performed to account for the effects of insulin resistance and other parameters on IMT. In this analysis, \(F\) values for inclusion and exclusion of variables were set at 3.0. These statistical analyses were carried out using the HALBAU statistical package (Gendai Sugaku-sha, Kyoto, Japan) on a personal computer (PC-586RA, EPSON, Tokyo, Japan). Statistical significance was determined as \(p<0.05\).
Results

GIR and IMT showed a statistically significant negative correlation ($n=53$, $r=-0.316$, $p=0.0214$) (Fig. 1). In 45 of the 53 cases we evaluated splanchnic tissue uptake of glucose by clamp OGL. SGU and IMT showed no significant correlation ($n=45$, $r=0.139$) (Fig. 2). Among those patients there was no significant correlation between GIR and SGU ($n=45$, $r=-0.24$). Age (Fig. 3A), total cholesterol (Fig. 3C), non HDL cholesterol, LDL cholesterol, and GIR (Fig. 3B) showed a significant correlation with the basal arterial wall thickness. In contrast, both age and GIR showed a significant correlation with the height of the maximum thickness (Fig. 4B).

To evaluate the role of insulin resistance as an independent risk factor in increased IMT, we performed stepwise multivariate regression analysis. For the basal arterial thickness, $F$ value of age and total cholesterol were 33.7 and 24.5 ($p<0.0001$ and $p<0.0001$), respectively, indicating that insulin resistance in the peripheral tissues might not be an individual risk factor for basal arterial wall thickness (Table 2). Both age and total cholesterol were shown to be responsible for 53.1% of the basal thickness of

![Graph](image1)

**Fig. 1.** Relationship between IMT and GIR in patients with NIDDM ($n=53$, $r=-0.316$, $p=0.0214$). IMT is the average intimal and medial thickness of the carotid artery wall. GIR is mean glucose infusion rate during euglycemic hyperinsulinemic clamp as the index of insulin resistance in the peripheral tissue.

![Graph](image2)

**Fig. 2.** Relationship between IMT and SGU in patients with NIDDM ($n=45$, $r=0.139$, $p>0.05$). IMT is the average intimal and medial thickness of the carotid artery wall. SGU is the percentage of splanchnic glucose uptake during euglycemic hyperinsulinemic clamp after oral glucose load, as the index of insulin resistance in the liver.
the carotid arterial wall. On the contrary, for the height of the maximum thickness in the carotid artery, only GIR was determined as a risk factor through the stepwise multivariant regression analysis (F = 5.4, p = 0.0245). Insulin resistance was shown to contribute to 9% of the height of maximum

![Graph A](image1)

**Fig. 3.** Relationship of the basal carotid artery wall thickness with age (panel A, r = 0.548, p < 0.0001), GIR (panel B, r = -0.280, p = 0.0423), and total cholesterol (panel C, r = 0.462, p < 0.0001). GIR is mean glucose infusion rate during euglycemic hyperinsulinemic clamp, as the index of insulin resistance in the peripheral tissue. The stepwise multivariant regression analysis showed that age and total cholesterol independently contribute to the basal carotid arterial wall thickness (see Table 2).
thickness of the carotid arterial wall (Table 2).

In the male NIDDM, GIR was 7.44 ± 1.80 mg/kg/min for the high active group (n=8), which was higher than those for the low active group (n=11, 6.19 ± 1.02 mg/kg/min) and the moderate active group (n=15, 6.38 ± 1.34 mg/kg/min) (Fig. 5). No definite tendency was observed among groups as to SGU (Fig. 5). The IMT decreased according to

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**Fig. 4.** Relationship of the height of maximum thickness with age (panel A, \( r = 0.287, p = 0.037 \)), GIR (panel B, \( r = -0.310, p = 0.024 \)), and total cholesterol (panel C, \( r = 0.209, p > 0.05 \)). GIR is mean glucose infusion rate during euglycemic hyperinsulinemic clamp, as the index of insulin resistance in the peripheral tissue. The stepwise multivariant regression analysis showed that only GIR contributes to the height of maximum thickness of the carotid arterial wall (See Table 2).
Table 2. Multivariate regression analyses of the basal thickness and the height of maximum thickness in patients with NIDDM

<table>
<thead>
<tr>
<th>Basal thickness</th>
<th>Height of maximum thickness</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>—Univariate—</td>
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<tr>
<td></td>
<td>correlation coefficient</td>
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<tr>
<td>Age</td>
<td>0.5483</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.0738</td>
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<tr>
<td>Duration</td>
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<tr>
<td>BMI</td>
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<tr>
<td>HbA1c</td>
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<tr>
<td>GIR</td>
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<tr>
<td>SGU</td>
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<tr>
<td>T-Chol</td>
<td>0.4624</td>
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<tr>
<td>TG</td>
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<td>HDL-Chol</td>
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<td>NonHDL-Chol</td>
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<tr>
<td>LDL-Chol</td>
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</tr>
<tr>
<td>sBP</td>
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</tr>
<tr>
<td>dBP</td>
<td>0.1248</td>
</tr>
<tr>
<td>Brinkman Index</td>
<td>0.1837</td>
</tr>
</tbody>
</table>

R² | 0.5307 | 0.0959 |

Stepwise multi-variant regression analyses were done on 53 NIDDM subjects.

The Brinkman index was estimated as the product of years smoked and the mean number of cigarettes smoked daily.

Abbreviations: BMI, body mass index; HbA1c, hemoglobin A1c; GIR, glucose infusion rate; SGU, splanchnic glucose uptake; T-Chol, total cholesterol; TG, triglyceride; HDL-Chol, high density lipoprotein cholesterol; nonHDL-Chol, non HDL cholesterol; LDL-Chol, low density lipoprotein cholesterol; sBP, systolic blood pressure; dBP, diastolic blood pressure.

Fig. 5. Effect of habitual exercise (L, low active; M, middle active; H, high active) on GIR, SGU, and IMT in the male NIDDM.

Values are mean ±SD. *p < 0.05. IMT is the average intimal and medial thickness of the carotid artery wall. GIR is mean glucose infusion rate during euglycemic hyperinsulinemic clamp, as the index of insulin resistance in the peripheral tissue. SGU is the percentage of splanchnic glucose uptake during euglycemic hyperinsulinemic clamp after oral glucose load, as the index of insulin resistance in the liver.
the intensity in reported exercise: 1.34±0.33 mm in the low active group, 1.20±0.31 mm in the moderate active group, and 1.12±0.29 mm in the high active group (Fig. 5).

In the female NIDDM, there was a tendency for insulin sensitivity in the peripheral tissues to be higher in the moderate active group (n=2) than in the low active group (n=15) (7.8±0.6 mg/kg·min vs. 6.1±1.6 mg/kg·min). The SGU was 23.1±15% in the low active group and 35.3±4.7% in the moderate active group. IMT was 1.31±0.25 mm in the low active group and 0.89±0.11 mm in the moderate active group.

**Discussion**

Insulin resistance may lead to atherosclerosis in subjects with insulin resistance syndrome as well as NIDDM [2]. Some reports showed an inverse relationship between insulin sensitivity and atherosclerosis in the carotid arteries in healthy subjects [21]. Insulin resistance is widely observed in subjects with hypertension [22], hyperlipidemia [23], and subjects with impaired glucose tolerance including NIDDM [24]. In subjects with NIDDM, obesity [5], hyperglycemia itself [6], or physical activity [7] have been shown to influence insulin resistance. Bonora et al. showed a relationship between the intimal-medial thickness of the carotid artery and insulin resistance measured by the intravenous insulin tolerance test in obese hypertensive NIDDM [25]. In NIDDM patients, especially ones with highly advanced atherosclerosis, the carotid arterial wall showed both the basal arterial wall thickness and the plaque lesion. Thus, what remains to be evaluated is whether insulin resistance contributes to arterial wall thickness, or plaque, or both in NIDDM. With respect to the tissue specificity of insulin resistance, we successfully showed that in patients with genetic impairment of glucokinase, according to data obtained from clamp OGL, it occurs in the liver but not in the peripheral tissues [9]. In the present study, we applied this maneuver to evaluate whether insulin resistance in the peripheral tissues or liver contributes to carotid atherosclerosis as preclinical atherosclerosis in NIDDM.

We found the average thickness of the carotid arterial wall to be inversely related with GIR as an index of insulin resistance in the peripheral tissues but not with SGU. Furthermore, multivariant regression analysis pointed to insulin resistance in the peripheral tissues as an independent risk factor for height of the maximum thickness but not for the basal arterial wall thickness. Both age and total cholesterol level were shown to contribute to more than half of the basal thickness but insulin resistance was shown to be responsible for only one tenth of the height of maximum thickness. These data would imply that insulin resistance is responsible for plaque lesion rather than for the basal thickness of the carotid artery wall. The rupture of soft plaque is observed as the first event of the acute coronary syndrome [13]. Together with this observation, it is hypothesized that insulin resistance may induce plaque formation in the coronary artery wall, which ruptures to cause coronary heart disease.

Insulin resistance is observed in subjects with varying degrees of glucose tolerance [22, 24-27]. These results suggest that in NIDDM, insulin resistance in the peripheral tissues is a primary defect rather than a secondary one due to hyperglycemia. We have shown that mildly hyperglycemic non-diabetics had advanced atherosclerosis in their carotid arteries [4]. Thus, insulin resistance in the peripheral tissue may cause progression of atherosclerosis in subjects with NIDDM far before the appearance of NIDDM, although the precise mechanisms remain to be elucidated.

Insulin does not sufficiently induce nitric oxide secretion from endothelial cells of subjects with insulin resistance [28]. This impairment of endothelial cells may be responsible for atherosclerosis. Recently, it has been shown that troglitazone, a potent insulin sensitizer, may inhibit the carotid arterial wall thickness in subjects with NIDDM [29]. This implies that improvement in insulin resistance in subjects with NIDDM could attenuate the progress of atherosclerosis.

A large-scale epidemic study showed that the mortality of physicians who engaged in habitual exercise was significantly lower than those who did not [30]. As chronic physical training was shown to increase membranous glucose transport [31] and increase insulin sensitivity [32], it was expected to prevent the progress of atherosclerosis and reduce mortality. Saito et al. reported that leisure time physical activity is correlated with the glucose/insulin ratio during
oral glucose load [33]. In this study, by using our euglycemic hyperinsulinemic glucose clamp method, we showed for the first time that male NIDDM patients engaging in a high level of physical activity have the least advanced carotid atherosclerosis and the least insulin resistance in the peripheral tissues. We also showed that the carotid arterial wall becomes thinner as the intensity of exercise increases. These data offer support for the working hypothesis that habitual exercise alleviates insulin resistance and inhibits the progress of atherosclerosis, which in turn can prevent cardiovascular incidence.

In conclusion, our current findings are compatible with the hypothesis that insulin resistance in the peripheral tissues but not in the liver independently contributes to thickening of the carotid arterial wall, especially plaque lesion, and that chronic exercise alleviates insulin resistance, thus ameliorating the progress of atherosclerosis.

References


