Insulin resistance and endothelial dysfunction in smokers: effects of vitamin C

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Hirai, Nobutaka, Hiroaki Kawano, Osamu Hirashima, Takeshi Motoyama, Tomohiro Sakamoto, Kiyotaka Kugiyama, Hisao Ogawa, Kazuwa Nakao, and Hirofumi Yasue. Insulin resistance and endothelial dysfunction in smokers: effects of vitamin C. Am J Physiol Heart Circ Physiol 279: H1172–H1178, 2000.—Cigarette smoking impairs endothelial function and is one of the major risk factors for atherosclerosis and coronary heart disease. Insulin resistance is associated with major risk factors for atherosclerosis. We examined the effects of vitamin C on insulin sensitivity and endothelial function by measuring steady-state plasma glucose (SSPG) and flow-mediated dilation (FMD) of the brachial artery. We studied 16 current smokers with normal glucose tolerance, 15 nonsmokers with impaired glucose tolerance (IGT), and 17 nonsmokers with normal glucose tolerance as controls. Both SSPG and FMD were blunted in smokers and nonsmokers with IGT compared with controls. In smokers, vitamin C decreased SSPG (P < 0.01 by ANOVA) with decreasing plasma thiobarbituric acid-reactive substances (TBARS) (P < 0.05 by ANOVA) and improved FMD (P < 0.05 by ANOVA). Furthermore, vitamin C improved both SSPG (P < 0.005 by ANOVA) and FMD (P < 0.05 by ANOVA) in nonsmokers with IGT. SSPG, FMD, or TBARS in controls did not change after vitamin C infusion. There was a significant correlation between SSPG and FMD both in smokers and nonsmokers with IGT, whereas no correlation was observed in controls. In conclusion, both insulin sensitivity and endothelial function were impaired in smokers and nonsmokers with IGT, whereas no correlation was observed in controls. In conclusion, both insulin sensitivity and endothelial function were impaired in smokers and nonsmokers with IGT, whereas no correlation was observed in controls. The present study was designed to examine whether oxidative stress plays a role in the relationship between insulin resistance and endothelial function in smokers and nonsmokers with IGT.

METHODS

The study group comprised 53 subjects, including 16 current smokers with normal glucose tolerance (smoker group, age 57.2 ± 2.6 years, 13 men, 3 women), 15 age-matched nonsmokers with IGT (age 61.2 ± 3.1 years, 9 men, 6 women), and 17 age-matched nonsmokers with normal glucose tolerance (control group, age 61.7 ± 1.7 years; 9 men, 8 women). We diagnosed their glucose tolerance from a 75-g oral glucose tolerance test according to the American Diabetes Association criteria (44). The quantity and duration of smoking were 28.5 ± 2.6 cigarettes per day and 35.7 ± 2.1 years, respectively, in the smoker group. The study was performed after one night cessation of smoking. We defined nonsmokers as those who had never smoked or had stopped smoking at least 2 years prior to the study.

The present study was designed to examine whether oxidative stress plays a role in the relationship between insulin resistance and endothelial function in smokers and nonsmokers with IGT.

THE ENDOTHELIAL FUNCTION has been shown to play an important role in the regulation of the circulation. Impaired endothelial-dependent dilation has been reported in patients with various risk factors for vascular disease, such as old age, dyslipidemia, hypertension, diabetes, and smoking (5, 6, 24, 45). The endothelial dysfunction was thus assumed to be a manifestation of the genesis of the atherosclerotic process (36).

Previous studies have shown endothelial dysfunction in smokers that is caused by reactive oxygen species generated by cigarette smoking (5, 20, 24, 25, 29). Several studies have also revealed that oxygen-derived free radicals in diabetic animals (9) and humans (4, 46) induce the inactivation of endothelium-derived nitric oxide (NO) and lead to the reduction of endothelium-dependent vasodilation. In addition, vitamin C, an antioxidant, was reported to improve the endothelial dysfunction in previous studies (24, 46).

In recent years, insulin resistance was newly implicated as a common pathway of the risk factors for atherosclerosis, such as hypertension, obesity, diabetes, dyslipidemia, and impaired glucose tolerance (IGT) (15, 28, 35). However, the precise mechanism whereby insulin resistance accelerates the development of atherosclerosis remains undetermined.

The present study was designed to examine whether oxidative stress plays a role in the relationship between insulin resistance and endothelial function in smokers and nonsmokers with IGT.

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No subject had hypertension, diabetes, previous myocardial infarction, congestive heart failure, or any other diseases associated with insulin resistance. No subject was taking any drugs known to affect glucose or insulin metabolism. Written informed consent was obtained from each subject before the study was commenced. The procedures used in the study were in agreement with the protocols approved by the ethics committee at our institution.

**Measurements of insulin sensitivity.** To evaluate insulin sensitivity, we performed a steady-state plasma glucose (SSPG) method using octreotide acetate (Sandostatin, Novartis) (16). Sandostatin inhibits the endogenous secretion of insulin, glucagon, and growth hormone (3). After an overnight fast, human insulin (Novolin R-40, 7.5 mU/kg) and Sandostatin (10 μg) were injected as a bolus initially, and glucose (6 mg·kg⁻¹·min⁻¹), insulin (0.77 mU·kg⁻¹·min⁻¹), Sandostatin (150 μg/2 h), and KCl (0.5 U·kg⁻¹·min⁻¹) were infused simultaneously for 2 h using a constant infusion pump. Blood samples were obtained 2 h after the start of the infusion to determine SSPG and steady-state plasma insulin (SSPI). Plasma glucose levels were increased to increase and reach the steady state between 90 and 120 min after the start of the infusion (16). Plasma thiobarbituric acid-reactive substances (TBARS) concentration was measured after the SSPG test as a marker of lipid peroxidation (11, 24, 47).

On the following day, we performed the SSPG test again under the constant infusion of vitamin C (ascorbic acid; Takeda) with a dose of 10 mg/min for 120 min. Vitamin C concentration was also measured before and after the SSPG test.

**Measurements of FMD.** Flow-mediated dilation (FMD) of the brachial artery was measured with the ultrasound technique by two skillful examiners. The validity of this method has been confirmed in previous studies (5–7, 22, 24). Briefly, the diameter of the brachial artery was measured from B-mode ultrasound images using a 7.5-MHz linear array transducer (model SSH-160A; Toshiba). Flow velocity in the brachial artery was measured using a pulsed Doppler signal. The brachial artery was scanned in the antecubital fossa in a longitudinal fashion. Depth and gain settings were optimized at the beginning of the study and were kept constant throughout the recording period. When a satisfactory transducer position was found, the surface of the skin was marked, and the arm remained in the same position throughout the study. Each subject lay quietly for 10 min before the first scan. After baseline measurements of the diameter and the flow velocity in the brachial artery, a blood pressure cuff placed around the forearm was inflated with a pressure of 250–300 mmHg and was released after 5 min. The ultrasound images were recorded on a Super-VHS videocassette recorder (model BR-S601M, Victor). The arterial diameter was measured at a fixed distance from an anatomical marker. The measurement was made from a single point with ultrasonic calipers by two independent observers who were blinded to clinical details. The measurements were taken from the anterior to the posterior interface between the media and adventitia (m line) at the end diastole, incident with the R wave on a continuously recorded electrocardiogram (5–7, 22, 24). The diameters at four cardiac cycles were analyzed for each scan, and the measurements were averaged. The diameter measurements for the reactive hyperemia were taken 45–90 s after the cuff deflation to measure peak diameter (7). As the ultrasound display could show both the B-mode picture of the brachial artery and the Doppler flow velocity side by side, we measured the Doppler flow signal and brachial artery diameter simultaneously. Blood flow was calculated by multiplying the velocity-time integral of the Doppler flow signal by heart rate and the vessel cross-sectional area. The increase in the blood flow was calculated by dividing the maximum flow within the first 15 s after the cuff deflation by the flow at the baseline levels (7, 22, 24).

FMD was measured in a quiet and temperature-controlled (22–24°C) room in the early morning after an overnight fast. Vitamin C was then infused constantly at a dosage of 10 mg/min for 2 h, and FMD was measured again to evaluate the effects of vitamin C on endothelial dysfunction. Heart rate and blood pressure were also measured before and after vitamin C infusion. Furthermore, after confirmation that arterial diameter and flow velocity had returned to the baseline levels, brachial artery diameter was measured before and 3–4 min after sublingual nitroglycerin (0.3 mg) administration.

In the previous study, the interobserver variability for the repeated measurements of resting arterial diameter was 0.05 ± 0.02 mm. The intraobserver variability for the repeated measurements of resting arterial diameter was 0.04 ± 0.05 mm. In a preliminary study, when the procedures were performed at the same time on two separate days in 20 volunteers, the average intraindividual test-retest difference for the measurements of the arterial diameter during the reactive hyperemia was 0.05 ± 0.04 mm (24).

**Assays of blood samples.** We measured plasma glucose by an autoanalyzer (Glucose Auto and Stat GA-1160; Kyoto Daiichi Kagaku) (23). The analyzer uses the end-point assay method from glucose oxidase-immobilized enzyme membranes and hydrogen peroxide and the differentiation method.

Plasma insulin was measured manually with a radioimmunoassay kit (Eiken Kagaku, Tokyo, Japan) (26). The radioactivity was measured using a well-type scintillation counter. The coefficients of variation (CV) for interassay and intra-assay were 6.3 ± 3.1% and 6.2 ± 2.6%, respectively. The recovery rate was 100.1 ± 6.3%.

The serum total cholesterol and triglyceride concentrations in the fasting state were measured enzymatically, and the serum high-density lipoprotein (HDL) cholesterol concentration was measured by heparin-Ca²⁺/Ni²⁺ precipitation (27). Vitamin C concentration was measured by high-performance liquid chromatography (39).

The plasma lipid peroxide content was determined by measuring TBARS as a marker (11, 47). We obtained reagents of the TBARS assay from Wako Chemical, Tokyo, Japan. First, we added H₂SO₄ (4.0 ml), phosphotungstic acid (0.5 ml), and saline (0.5 ml) to the sample plasma (25 μl) and obtained sedimentation after centrifugation at 3,000 rpm for 10 min. Trichloroacetic acid-thiobarbituric acid-HCl reagent (1.0 ml) was then added to the sedimentation and well vortexed. Standards were also prepared by acid hydrolysis of 1,3,3,3-tetraethoxypropane in trichloroacetic acid-thiobarbituric acid-HCl reagent. We incubated each solution at 95–100°C for 60 min. To minimize peroxidation during the assay procedure, butylated hydroxytoluene (5.0 ml) was added to the thiobarbituric acid reagent mixture, and the cooled extraction was centrifuged at 3,000 rpm for 10 min. Finally, measurement of absorption spectra was performed with fluorophotometer (excitation 515 nm/emission 553 nm; Shimadzu). The findings were expressed as the malondialdehyde (MDA) equivalent content (nmol MDA/ml plasma).

**Statistical analysis.** The clinical characteristics and hemodynamic findings among the three groups were compared using the unpaired t-test for continuous data and the chi-square test for group data. The differences between two means among the groups in the time course of plasma con-
centration of glucose and insulin during oral glucose tolerance test were performed by Student’s unpaired t-test. We analyzed the effect of vitamin C on SSPG, SSPI, FMD, and TBARS among the three groups using repeated measures of ANOVA with a Bonferroni post hoc test. In addition, differences between two means among the groups were performed by Student’s paired or unpaired t-test, as appropriate. Correlation between SSPG and FMD was examined using a linear regression analysis. Statistical significance was defined as $P < 0.05$. All values are means ± SE.

RESULTS

Baseline characteristics. There were no significant differences in the body mass index, the serum levels of total cholesterol, LDL cholesterol, and triglyceride among the three groups (Table 1). The serum levels of HDL cholesterol were significantly lower in the smoker group and the IGT group than in the control group (Table 1).

Oral glucose tolerance test. Table 2 shows plasma glucose and insulin responses after the 75-g equivalent oral glucose loading. There were no significant differences in the plasma glucose and insulin levels among the three groups at the fasting state. After the oral glucose loading, the plasma glucose and insulin levels increased to the same extent in the smoker group and the control group. The plasma glucose levels at 1 and 2 h were higher in the IGT group than in the other two groups. The insulin levels at 1 h were comparable among the three groups, whereas those of the IGT group at 2 h were higher than those of the other two groups.

SSPG test. The baseline SSPG level was significantly higher in the smoker group and the IGT group than in the control group [179.6 ± 17.3 vs. 118.5 ± 8.2 mg/dl ($P < 0.005$), 194.3 ± 9.3 vs. 118.5 ± 8.2 mg/dl ($P < 0.001$), respectively]. There was no difference in the levels between the smoker group and the IGT group. After vitamin C infusion, the SSPG levels were significantly decreased in the smoker group (179.6 ± 17.3 vs. 133.8 ± 17.9 mg/dl, $P < 0.01$) and in the IGT group (194.3 ± 9.3 vs. 147.7 ± 11.1 mg/dl, $P < 0.001$) but not in the control group [118.5 ± 8.2 vs. 115.4 ± 8.6 mg/dl; not significant (NS)]. However, the SSPG levels were still higher in the IGT group than in the control group after vitamin C infusion (147.7 ± 11.1 vs. 115.4 ± 8.6 mg/dl, $P < 0.05$). There was a significant difference in the effect of vitamin C on SSPG levels between the smoker group and the control group ($P < 0.01$, ANOVA) and between the IGT group and the control group, ($P < 0.005$, ANOVA), as shown in Fig. 1A. There was no change of the effect of vitamin C between the smoker group and the IGT group. The SSPI levels did not change among the three groups both before and after vitamin C infusion (Fig. 1B).

FMD of the brachial artery. The baseline FMD was lower in the smoker group and the IGT group than in the control group [3.21 ± 0.63 vs. 6.71 ± 0.52% ($P < 0.001$), 3.90 ± 0.64 vs. 6.71 ± 0.52% ($P < 0.01$), respectively]. There was no difference in the baseline FMD between the smoker group and the IGT group. After vitamin C infusion, FMD did not change in the control group (6.71 ± 0.52 vs. 7.29 ± 0.69%, NS). However, it increased to the level of the control group both in the smoker group (3.21 ± 0.63 vs. 6.50 ± 0.76%, $P < 0.005$) and in the IGT group (3.90 ± 0.64 vs. 6.71 ± 0.62%, $P < 0.01$). There was a significant difference in the effect of vitamin C on FMD between the smoker group and the control group ($P < 0.05$, ANOVA) as well as between the IGT group and the control group ($P < 0.05$, ANOVA), as shown in Fig. 2A. There was no difference in the effect between the smoker group and the IGT group.

Heart rate and blood pressure at baseline, resting arterial diameter, and the increase in the blood flow during reactive hyperemia were similar among the three groups throughout the study (Table 3). Vitamin C did not change these hemodynamics in any group. Nitroglycerin-induced vasodilation after vitamin C was comparable among the three groups (control group, 21.6 ± 2.8%; smoker group, 19.4 ± 3.6%; IGT group, 19.1 ± 3.5%).

TBARS and vitamin C. The baseline TBARS levels were higher in the smoker group than in the control group ($3.83 ± 0.22$ vs. $3.03 ± 0.12$ nmol/ml, $P < 0.05$). The levels in the IGT group were comparable with those in the control group ($3.25 ± 0.09$ vs. $3.03 ± 0.12$ nmol/ml, NS) and the smoker group ($3.25 ± 0.09$ vs. $3.83 ± 0.22$ nmol/ml, NS) (Fig. 2B). Vitamin C decreased the plasma TBARS levels in the smoker group ($3.83 ± 0.22$ vs. $3.17 ± 0.17$ nmol/ml, $P < 0.05$), whereas it did not affect the plasma TBARS levels in either the control group ($3.03 ± 0.12$ vs. $2.84 ± 0.18$ nmol/ml).

### Table 1. Characteristics of study subjects

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Control ($n = 17$)</th>
<th>IGT ($n = 15$)</th>
<th>Smoker ($n = 16$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>61.7 ± 1.7</td>
<td>61.2 ± 3.1</td>
<td>57.2 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>9/8</td>
<td>9/6</td>
<td>13/3</td>
<td></td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>22.8 ± 0.5</td>
<td>23.2 ± 0.6</td>
<td>24.0 ± 1.1</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>58.2 ± 3.0</td>
<td>48.7 ± 3.1</td>
<td>47.3 ± 2.4</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>117.0 ± 7.2</td>
<td>120.8 ± 11.2</td>
<td>111.2 ± 10.4</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>199.1 ± 7.5</td>
<td>185.1 ± 5.7</td>
<td>184.3 ± 11.7</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>109.0 ± 10.5</td>
<td>112.8 ± 7.3</td>
<td>118.1 ± 11.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. *$P < 0.05$ vs. control. HDL, high-density lipoprotein; IGT, impaired glucose tolerance; LDL, low-density lipoprotein; NS, not significant.

### Table 2. 75 g oral glucose tolerance test

<table>
<thead>
<tr>
<th>Plasma glucose, mg/dl</th>
<th>Control ($n = 17$)</th>
<th>IGT ($n = 15$)</th>
<th>Smoker ($n = 16$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>90.4 ± 2.5</td>
<td>92.7 ± 4.0</td>
<td>87.8 ± 2.5</td>
</tr>
<tr>
<td>1 h</td>
<td>133.6 ± 7.6</td>
<td>171.9 ± 11.8</td>
<td>144.4 ± 8.5</td>
</tr>
<tr>
<td>2 h</td>
<td>93.8 ± 5.8</td>
<td>154.5 ± 4.8</td>
<td>109.4 ± 5.2</td>
</tr>
<tr>
<td>Plasma insulin, µU/ml</td>
<td>7.1 ± 0.4</td>
<td>7.2 ± 0.6</td>
<td>7.9 ± 0.7</td>
</tr>
<tr>
<td>Fast</td>
<td>63.2 ± 6.5</td>
<td>67.9 ± 12.0</td>
<td>75.5 ± 16.8</td>
</tr>
<tr>
<td>1 h</td>
<td>36.5 ± 6.3</td>
<td>81.1 ± 32.0</td>
<td>41.8 ± 6.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. IGT, impaired glucose tolerance. *$P < 0.05$ and †$P < 0.0001$ vs. control and smoker, ‡$P < 0.01$ vs. control.
nmol/ml, NS) or the IGT group (3.25 ± 0.09 vs. 3.01 ± 0.25 nmol/ml, NS). There was a significant difference in the effect of vitamin C on TBARS between the smoker group and the control group \( (P < 0.05, \text{ANOVA}) \), as shown in Fig. 2B. There was no difference in the effect between the smoker group and the IGT group and between the control group and the IGT group.

The fasting vitamin C levels tended to be lower in the smoker group and the IGT group compared with the control group (38.9 ± 3.0 vs. 44.8 ± 5.6 \( \mu \)mol/l and 39.7 ± 7.54 vs. 44.8 ± 5.6 \( \mu \)mol/l, respectively). However, the difference was not significant. At the end of the vitamin C infusion, the vitamin C levels had increased in all three groups. The extent of increase was comparable among the three groups (control group, 402.2 ± 29.8; smoker group, 340.1 ± 18.07; IGT group, 361.2 ± 41.6 \( \mu \)mol/l).

**Correlation between FMD and SSPG.** There was a significant correlation between FMD and SSPG \( (r = 0.52, P < 0.0001) \) in all the groups combined, as shown in Fig. 3. Furthermore, in both the smoker group and the IGT group, there was a significant correlation between FMD and SSPG \( (r = 0.57, P < 0.001; r = 0.40, P < 0.05; \text{Fig. 3, B and C, respectively}) \). However, there was no correlation between FMD and SSPG in the control group \( (r = 0.27, \text{NS}) \), as shown in Fig. 3D.

**DISCUSSION**

FMD of the brachial artery during reactive hyperemia is reported to be mainly dependent upon endothelium-derived NO (17). In the present study, FMD was lower in the smoker group than in the control group, which was improved after the treatment with vitamin C. Cigarette smoke extract is well known as an abundant source of reactive oxygen species (2, 34). We have previously shown that reactive oxygen species in cigarette smoke extract degrade the endothelium-derived NO (25, 29). Smoking impairs NO-mediated regulation of coronary vasomotor tone through the reduction of bioavailability of NO (20). The present findings are consistent with the findings of our previous study (24).

The insulin sensitivity as assessed by SSPG was blunted in the smoker group compared with the control group. These findings are in agreement with those of previous studies (1, 10). The present study further
showed that vitamin C treatment improved insulin sensitivity in the smoker group, which was accompanied by the reduction of the plasma TBARS levels. The vitamin C infusion improved FMD as well as SSPG, and there was a significant correlation between FMD and SSPG. Thus a close relationship is recognized between endothelial function and insulin sensitivity, and reactive oxygen species may play an important role in the pathogenesis of insulin resistance in subjects with endothelial dysfunction.

Other disorders associated with oxidative stress, including aging, diabetes mellitus, obesity, hypertension, dyslipidemia, coronary spasm, heart failure, and trauma, are all reported to be associated with insulin resistance (8, 9, 14, 30, 42, 43). IGT is known to have an association with insulin resistance (28). In the present study, both the insulin sensitivity and endothelium-dependent vasodilation were blunted in the IGT group compared with the control group, and vitamin C improved both. These findings suggest that the increased reactive oxygen species may play an important role in the pathogenesis of endothelial dysfunction in the insulin-resistant subjects.

We examined the FMD of the brachial artery as a marker of endothelial function, although the endothelial function in the arteriolar and capillary levels may be more important in relation to the insulin resistance (33). It is well known that atherosclerosis is a disease of large conduit arteries, and insulin resistance is associated with risk factors for atherosclerosis (8, 15, 35). In the present study, age and body mass index were matched among the three groups, and patients with hypertension, diabetes mellitus, obesity, or coronary heart disease were excluded. Serum HDL cholesterol levels were lower in the smoker and the IGT groups than in the control group. These patterns of dyslipidemia are known to be associated with insulin resistance (8, 15, 35). We therefore assume that the endothelium of conduit artery levels and capillary levels are both impaired by increased reactive oxygen species and are

![Fig. 3. Correlation between SSPG and FMD of the brachial artery. Plots include data before (open symbols) and after (solid symbols) vitamin C infusion. A: all groups. B: smoker group. C: IGT group. D: control group.](http://ajpheart.physiology.org/Downloaded from http://ajpheart.physiology.org/)

Table 3. Effects of vitamin C on hemodynamic variables

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 17)</th>
<th>IGT (n = 15)</th>
<th>Smoker (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>62.8 ± 2.9</td>
<td>60.0 ± 3.1</td>
<td>61.4 ± 3.2</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>121.1 ± 5.5</td>
<td>116.0 ± 3.8</td>
<td>125.0 ± 4.0</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>70.0 ± 2.8</td>
<td>68.9 ± 2.6</td>
<td>72.7 ± 2.9</td>
</tr>
<tr>
<td>Resting arterial diameter, mm</td>
<td>4.1 ± 0.2</td>
<td>4.2 ± 0.2</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>Increase in blood flow during reactive hyperemia, %</td>
<td>226.2 ± 23.3</td>
<td>230.4 ± 25.0</td>
<td>234.3 ± 17.2</td>
</tr>
</tbody>
</table>

Values are means ± SE.
associated with insulin resistance in the smoker and the IGT groups.

Insulin induces endothelium-dependent vasodilation by releasing endothelium-derived NO (38, 40). The endothelium plays an important role in the vascular tone and regulates blood flow to the insulin-sensitive tissues (32, 38). The impairment of the insulin-induced increase in skeletal muscle blood flow has been reported in the insulin-resistant state (33, 41). Thus it is assumed that increased reactive oxygen species cause endothelial dysfunction and serve to impair the blood flow to insulin-sensitive tissues such as skeletal muscles. However, other studies have reported that insulin resistance may occur in the absence of reduced blood flow to skeletal muscles (21, 31). It is possible that the reactive oxygen species cause flow-independent insulin resistance by inhibiting insulin signaling. A recent in vitro study has shown that oxidative stress impaired insulin signal transduction (37). Furthermore, antioxidants may improve impaired insulin-mediated glucose uptake and suppress high glucose-induced vascular smooth muscle cell migration and proliferation, reducing intracellular oxidative stress (48).

Keaney and co-workers (17) showed that normal extracellular concentrations of ascorbic acid (30 to 150 μmol/l) are unlikely to prevent the NO-superoxide interaction. Although the serum levels of vitamin C increased in smokers in the present study, whether these levels are high enough to scavenge superoxide is unclear. However, vitamin C may directly augment NO bioavailability through the increase in NO synthesis and in the release of NO from S-nitrosoglutathione or other nitrosothiols (13, 19).

Study limitations. We did not assess the effect of vitamin C on endothelium-independent vasodilation following sublingual nitroglycerin in smokers in the present study. However, vitamin C had no effect on endothelium-independent vasodilation in our previous study (24), in agreement with the results of Vita and coworkers (22).

We measured plasma glucose using the glucose oxidase method. Vitamin C may interfere with detection of glucose by more than 2% when its concentration is over 150 mg/dl (8,505 μmol/l) with this autoanalyzer (23). However, the plasma concentration of vitamin C reached in this study ranged from 0.6 to 8 mg/dl (34–454 μmol/l) and in these concentrations, vitamin C did not interfere with detection of glucose.

We measured plasma TBARS as a marker of plasma lipid peroxidation (11, 47). The measurement of TBARS is susceptible to artifacts caused by variations in sample lipid content and iron contamination of the reagents (11, 12). In the present study, we prevented amplification of peroxidation during the assay by adding the chain-breaking antioxidant, butylated hydroxytoluene, to the samples before adding the thiobarbituric acid (TBA) reagents (24). We did not examine whether vitamin C interferes with detection of “MDA” equivalents in plasma samples in the present study. Although the fluorescence measurements can often distinguish the products from the “real” TBA-MDA adduct compared with light absorption measurements, it is still unknown whether fluorescence measurements can distinguish the vitamin C-TBA adduct from the authentic MDA-TBA adduct (12).

Conclusions. Endothelial function and insulin sensitivity were impaired in smokers and nonsmokers with IGT, and both were improved by the administration of vitamin C. Furthermore, a significant correlation was observed between insulin sensitivity and endothelial function in both smokers and nonsmokers with IGT. These findings indicate that the increased reactive oxygen species plays an important role in the pathogenesis of both endothelial dysfunction and insulin resistance, and endothelial dysfunction is closely related to insulin resistance.

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