Relationship Between Endothelial Function and Coronary Risk Factors in Patients With Stable Coronary Artery Disease

Cevat Kirma, MD; Mustafa Akcakoyun, MD; Ali Metin Esen, MD; Irfan Barutcu, MD; Osman Karakaya, MD; Mustafa Saglam, MD; Ramazan Kargin, MD; Muhsin Turkmen, MD; Bilal Boztosun, MD; Akin Izgi, MD; Kenan Sonmez, MD

**Background**
Results of experimental and clinical studies suggest that both coronary artery disease (CAD) itself and its traditional risk factors lead to endothelial dysfunction. The aim of the present study was to determine which CAD risk factors sustain their contribution to endothelial dysfunction despite the presence of established CAD.

**Methods and Results**
The study group comprised 150 patients with CAD. Using a high-resolution ultrasound, the diameter of the brachial artery at rest and during reactive hyperemia (flow-mediated dilatation, FMD%; endothelial-dependent stimulus to vasodilation), as well as after sublingual administration of nitroglycerin (NTG%; endothelium-independent vasodilatation), was measured. The relationship between FMD% and coronary risk factors [diabetes mellitus (DM), total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, triglycerides, age, family history of premature atherosclerosis, smoking, hypertension (HT), body mass index (BMI)] was investigated. In univariate analysis there was an inverse relationship between FMD% and age (r = -0.300, p < 0.001), and BMI (r = -0.230, p < 0.005) and FMD% was significantly lower in diabetic patients when compared to non-diabetic patients (p < 0.001). In stepwise multivariate regression analysis; FMD still correlated with DM and advanced age, but not with BMI (β = 0.065, p < 0.001, β = -0.001 p = 0.002, β = -0.087, p < 0.284, respectively). FMD% was found to be not associated with hypercholesterolemia, family history of premature atherosclerosis, HT and smoking.

**Conclusion**
Only aging and DM were independently associated with endothelial dysfunction in patients with established CAD. (Circ J 2007; 71: 698 – 702)

**Key Words:** Coronary artery disease; Coronary risk factors; Endothelial function

The vascular endothelium plays an integral role not only in regulation of vascular tonus, but also in prevention and formation of thrombus and inflammation.1 It is known that endothelial dysfunction is associated with coronary risk factors and atherosclerosis, and has a close pathophysiological relation with acute coronary syndromes.2-4 Endothelial dysfunction has been shown in patients with documented atherosclerosis, but it is also an early step in the pathogenesis of the atherosclerotic cascade.5-7 Among various methods to assess endothelial function, endothelium-dependent vasodilatation (EDV) is a noninvasive, highly reproducible, simple method based on high-sensitivity ultrasound waves.7,8 In this study we assessed the relationship between EDV in systemic arteries and coronary risk factors in patients with documented coronary artery disease (CAD).

**Methods**

**Patient Population**
One hundred and fifty patients with angiographically proven CAD (103 males, 47 females), age ranging between 29 and 78 years (mean: 58±10), were recruited. CAD was defined as the presence of angiographically demonstrated ≥70% stenosis in at least 1 major epicardial coronary artery. Hypertension (HT) was defined as blood pressure ≥140/90 mmHg or use of antihypertensive drugs and diabetes mellitus (DM) as fasting blood glucose level ≥126 mg/dl or use of antidiabetic agents. All study subjects underwent a complete physical examination, and biochemical, electrocardiographic and body mass index (BMI) measurements. Vascular endothelial function in the brachial artery was measured by the flow-mediated dilatation (FMD) technique. Patients with acute coronary syndromes, severe left ventricular dysfunction (ejection fraction <35%) or old myocardial infarction were excluded from the study.

**Vascular Study**
Each subject was studied in the morning, after abstaining from alcohol, caffeine and tobacco, as well as food, within 8h before the study. High-resolution echocardiography Doppler ultrasound (Technos MPX ultrasound ESOTA Inc) with an 8.0MHz transducer was used to measure the
flow velocity and diameter of the right brachial arteries. In all studies, scans were taken at rest, during reactive hyperemia (FMD%: endothelial-dependent stimulus to vasodilation), again at rest and after sublingual nitroglycerin (NTG%: endothelium-independent vasodilatation). The inter- and intra-observer variabilities for repeated measurements are 0.13±0.06 and 0.14±0.02 mm, respectively, in our laboratory.

Each study subject rested quietly for 10 min before the scan, then after being placed in the appropriate position, the skin was marked and arterial flow velocity was measured at rest using a pulsed Doppler signal at 60° in the center of the artery. Blood flow through the brachial artery was altered with an occluding cuff placed on the forearm 8 cm distal to the site of brachial artery measurement. By inflating the cuff to 250–300 mmHg, distal circulation was arrested and flow was reduced through the brachial artery measured proximal to the cuff. By deflating the cuff after 5 min of inflation, flow through the brachial artery was increased (reactive hyperemia). The brachial artery was scanned continuously 30 s before and 90 s after cuff deflation, then 10 min later, a second rest scan was recorded. NTG (400 g) was then administered sublingually, and the artery was scanned 5 min later.

All images were analyzed by 2 observers who were unaware of the clinical details. Artery diameter measurements were made at end-diastole (peak of R wave on electrocardiogram) using electronic calipers. Five cardiac cycles were analyzed, and measurements were averaged. Brachial artery diameter measurements after reactive hyperemia were taken 60 s after cuff deflation. FMD was calculated as the percent increase in arterial diameter during reactive hyperemia vs the corresponding rest value. Brachial artery diameter measurements after NTG were taken after its administration and nitroglycerin-induced dilatation was calculated as the percent increase in arterial diameter after NTG vs the corresponding rest value.

Statistical Analysis
Statistical analyses were performed with SPSS-11.5 for Windows (Chicago, IL, USA). Continuous variables are displayed as mean ± standard deviation and categorical ones as percentage. Pearson’s correlation test was performed between variables and the Mann-Whitney U-test was used to compare the means of 2 non-homogeneous groups. Multiple stepwise regression analysis was used to assess the relationship between coronary risk factors and FMD%. A value
of p<0.05 on the 2-tail test was considered statistically significant.

**Results**

**Patient Population (Table 1)**

Of the 150 patients, 68% were men and 32% were women, with respective mean ages of 57.0±11.0 years and 60.7±9.1 years; 54% had HT, 28% had DM, 36% had a family history of CAD and 45% were smokers; 70 patients had single-vessel disease, 56 had 2-vessel disease, and 24 had 3-vessel disease. In the diabetic population only 21 patients were using oral antidiabetic agents, such as metformin (n=11), gliclazide (n=7) or glimepiride (n=3). In the hypertensive population 50 patients were using an oral antihypertensive agent, including diuretics (n=21), angiotensin converting enzyme inhibitors (n=13), angiotensin II receptor blockers (n=11) or calcium antagonists (n=5). In addition, nearly 25% of the study population were using cholesterol-lowering drugs such as atorvastatin (n=14), simvastatin (n=10), pravastatin (n=6), and fenofibrate (n=5). The mean BMI of the participants was 27.4±4.5 kg/m², mean total cholesterol (TC) level was 198±50 mg/dl, low-density lipoprotein (LDL)-cholesterol level was 122±44 mg/dl, high-density lipoprotein (HDL)-cholesterol level was 45±11 mg/dl, triglyceride (TG) level was 163±89.3 mg/dl, and the TC/HDL ratio was 4.7±1.8 mg/dl.

**Vascular Study (Table 2)**

The mean basal brachial artery diameter was 4.0±0.7 mm, mean FMD% was 5.8±0.2% and mean sublingual NTG-dependent vasodilatation was 10.2±2%. status, TC, TG, LDL-cholesterol, HDL-cholesterol or the TC/HDL ratio. In the multiple stepwise regression analysis, the relationship between FMD% and age (r=–0.001, p=0.002) and the presence of DM (r=0.065, p<0.001) continued, whereas the relationship with BMI disappeared (r=–0.087, p<0.284).

**Discussion**

Many studies have investigated the relationship between coronary risk factors and endothelial function. Celermajer et al investigated the relationship and interaction between coronary risk factors and endothelial function in 500 (mean age 36±15) asymptomatic adults. In their univariate analysis they found an inverse relationship between FMD% and hyperlipidemia, smoking, blood pressure, male sex, family history and larger vascular diameter. In their multiple stepwise regression analysis the relationship between FMD% and smoking, male sex, and larger vascular diameter continued, but not with hyperlipidemia, blood pressure or family history. In that study patients with HT and DM were excluded. Herrington et al showed that age and male sex were strong parameters for determining FMD% in a population of 4,040 patients who were mostly elderly, but they did not investigate coronary risk factors.

Benjamin et al investigated the relationship between coronary risk factors and endothelial function in 2,883 patients and found a relationship between FMD% and female sex, exercise and increased heart rate, and an inverse relation with old age, HT, BMI, lipid-lowering therapy and smoking. In their multivariate analysis the effect of BMI and female sex continued for FMD%. However, in our study we established an independent relationship between FMD% and age and DM in the presence of CAD. Tanriverdi et al have suggested that FMD is significantly lower in smokers than nonsmokers among subjects with normal coronary arteries. In addition, Ishibashi et al used plethysmography to measure reactive hyperemia in healthy subjects with multiple cardiovascular risk factors and found that the number of risk factors significantly correlated with the duration of
reactive hyperemia, suggesting that endothelial dysfunction increases with the number of risk conditions clustering in a single individual. However, our study was in part different from the aforementioned studies because we intended to investigate the contribution of cardiovascular risk factors to the degree of endothelial dysfunction. Also, in contrast to the previous reports, we included patients with established CAD not normal coronary arteries. Terawa et al.13 investigated the effect of alcohol consumption on FMD and reported that it was independently associated with endothelial dysfunction in 108 men with CAD. However, in our study only 6 patients had a history of regular alcohol consumption, so we could not investigate the effect of alcohol consumption on FMD. In the study by Terawa et al only the effect of alcohol consumption, not of the other cardiovascular risk factors, on FMD was studied, whereas in the present study we investigated the relationship between multiple risk factors including aging, smoking, HDL-cholesterol, LDL-cholesterol, TC, TG, obesity, DM, HT, family history and endothelial dysfunction in a patient population with documented stable CAD.

It has been shown that endothelial function is impaired in the elderly.19,20 Although the mechanism is not clear, it has been proposed that the increase in free oxygen radicals with aging inactivates nitric oxide or has a direct toxic effect on the endothelium.15 In our study we showed that aging is independently associated with endothelial dysfunction even in the presence of CAD, which suggests an effect of aging on endothelial dysfunction. Hirai et al.16 have shown that both acute and chronic hyperglycemia and impaired glucose tolerance cause endothelial dysfunction, and in a recent study it was shown that even transient hyperglycemia induced with oral glucose in non-diabetic subjects impairs endothelial function.17 Several different mechanisms have been proposed as explanation.18,19 One of the major findings of our study is that DM still continues to be an independent factor effecting endothelial function in patients with CAD. As well, in our study the mean glucose value in patients with DM was 154±40.55 mg/dl, which is an acceptable level for glycemic control, so our results suggests that DM has a relationship with endothelial function in patients with CAD even when good glycemic control is achieved.

It is known that obesity impairs endothelial function whereas weight loss improves it.20–22 The reason is multifactorial and may in part be associated with oxidative stress and systemic inflammation. In our study, we found a weak inverse relationship between BMI and FMD%, but this lost significance in the multivariate analysis. A possible reason for this weak relation might have been that our population had a relatively low BMI because only 25% of them had a BMI >30 kg/m². There are many studies showing a connection between HT and endothelial function. Antihypertensive treatment improves endothelial function, leading to less cardiac events.24,25 Although the mechanism of the relationship between HT and endothelial dysfunction is unknown, it has been proposed that endothelial dysfunction itself causes HT.26 In our study we did not find a relationship between HT and endothelial function, which may have resulted from the patients having appropriate antihypertensive treatment.

Although endothelial dysfunction caused by smoking is multifactorial, there is sufficient evidence that free oxygen radicals have a potential role.11,27,28 Both passive and active smoking causes endothelial dysfunction.19,29,30 In contrast to previous studies we found no relationship between smoking and endothelial dysfunction; however, almost all of the present patients had quit smoking after clinical and/or angiographic diagnosis of CAD. Benjamin et al.10 found that FMD% was better in smokers who did not smoke within the 6h prior to the study hours than in those who did smoke.

It has been shown that elevated blood cholesterol impairs endothelial function11 and the lack of this association in our study may be related to the characteristics of our study population because 25% were using cholesterol-lowering drugs.

**Study Limitations**

Because the study subjects had CAD they were using medications that have some effect on endothelial function and could have partly affected the FMD results. However, all drugs were ceased at least 24h before the measurement of FMD, so we consider that drug effects on FMD were probably minimal. In addition, all patients did not undergo an oral glucose tolerance test; however, approximately 40% of population was already diabetic and none of the non-diabetic patient had symptoms or clinical finding of diabetes. Moreover, the fasting blood glucose level of these patients was less than 110 mg/dl.

**Conclusion**

The relationship between coronary risk factors and endothelial dysfunction has been shown in many studies, but in the present study using multivariate regression analysis we investigated the relationship between multiple risk factors and endothelial dysfunction in a patient population with documented stable CAD. We found that only aging and DM were independently associated with endothelial dysfunction in the presence of CAD. Obviously, these results need to be validated with further large-scale and long-term studies.

**References**


