Smooth Muscle Dysfunction Occurs Independently of Impaired Endothelium-Dependent Dilation in Adults at Risk of Atherosclerosis

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Objectives. We sought to assess smooth muscle function in adults at risk for atherosclerosis.

Background. Previous studies in subjects at risk for atherosclerosis have demonstrated arterial endothelial dysfunction, with reduced vasodilator responses after pharmacologic or physiologic stimulation of endothelial nitric oxide (NO). Most have also shown a slight but nonsignificant impairment of vasodilation in response to exogenous sources of NO, such as nitroglycerin (NTG). We hypothesized that NTG responses might be reduced in a large number of consecutively studied adults at risk for atherosclerosis, independent of any impaired endothelium-dependent responses, consistent with concomitant smooth muscle dysfunction.

Methods. Using high resolution ultrasound, the dilator response of the brachial artery to 400 μg of sublingual NTG was measured in 800 asymptomatic subjects. Subjects were also assessed for a history of vascular risk factors, blood pressure, total serum cholesterol and flow-mediated endothelium-dependent dilation (EDD).

Over the past decade, a large number of studies have demonstrated endothelial dysfunction in the coronary arteries of adults with established atherosclerosis (1–7) and even in the plaque-free peripheral arteries of asymptomatic children and young adults at high risk for atherosclerosis (8–11). These studies have documented an impaired dilator response to physiologic and pharmacologic stimuli of endothelial nitric oxide (NO) production (such as shear stress or acetylcholine infusion), in contrast to a preserved dilator response to exogenous sources of NO, such as nitroglycerin (NTG), which act as endothelium-independent, smooth muscle dependent dilators. Observers have concluded from these findings that there is impaired endothelial function early in the process of atherogenesis but that smooth muscle function remains normal (8,9). However, in most of these relatively small studies the NTG responses have actually been mildly reduced in those subjects with endothelial dysfunction, although this observation has not been statistically significant (1–3,6–12).

These in vivo observations in humans with endothelial dysfunction contrast sharply with in vitro and experimental animal data, where vasodilator responses of healthy vessels to exogenous NO are increased after endothelial denudation or acute inhibition of endothelial NO synthesis (13–16). It has been postulated (13) that this experimental effect may be due to upregulation of receptors in smooth muscle cells or to a loss of partial tolerance induced by the effect of endogenously produced NO from an intact endothelium. However, the relevance of these findings in humans is uncertain because the same pathologic processes that lead to endothelial dysfunction in adults (such as hypercholesterolemia or smoking) may also be affecting smooth muscle function.

Results. We studied 317 men and 483 women, 38 ± 17 years old (mean ± SD, range 15 to 76). The mean cholesterol level was 5.2 ± 1.3 mmol/liter, and there were 126 smokers and ex-smokers (16 ± 9 mean pack-years) and 105 diabetic subjects. On univariate analysis, a reduced vasodilator response to NTG was associated with high cholesterol, cigarette smoking, diabetes mellitus, increasing age, male gender, larger vessel size and reduced EDD (p < 0.01 for all). On multivariate analysis, diabetes, larger vessel size and reduced EDD were all independently associated with impaired NTG-related vasodilation (p ≤ 0.001 for all). In the 574 nondiabetic subjects who had never smoked cigarettes, the independent relation between EDD and NTG responses was still observed (r = 0.24, p = 0.01).

Conclusions. The vasodilator response to exogenous NO is impaired in asymptomatic subjects with reduced EDD, consistent with smooth muscle dysfunction in adults at risk for atherosclerosis.

(J Am Coll Cardiol 1998;32:123–7)
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To assess the interactions between atherosclerotic risk factors, endothelial function and smooth muscle-dependent dilation in humans, we investigated the vasodilator responses to endogenous and exogenous sources of NO. Vascular responses were examined in a large number of consecutively studied asymptomatic subjects, in a systemic artery without evidence of atherosclerotic plaque formation.

**Methods**

**Subjects.** Subjects were eligible for inclusion in the study if they were ≥15 years old, had no clinical evidence of atherosclerotic vascular disease, had not taken any vasoactive medication in the past 6 months and consented to measurement of flow-mediated and NTG-induced vasodilation of their brachial artery. Between 1993 and 1997, the same operator (J.R.) used the same ultrasound equipment and methodology (see later) to scan 800 consecutive subjects who fulfilled the above criteria; many of these subjects have contributed to previous reports of endothelial function from our groups (8,10,11,17). All were recruited from among hospital staff, friends and community volunteers, to participate in studies examining the effects of risk factors on aspects of arterial physiology. The studies were all approved by the local institutional ethics committee, and each subject gave informed consent.

**Study design.** All subjects were asked for details of their medical history, particularly for cardiovascular risk factors, and underwent a brief examination for clinical features of cardiovascular disease. In 747 subjects (93%), total serum cholesterol was also measured using either a Hitachi 747 autoanalyzer or the cholesterol C-system high performance CHOD-PAP method (Diagnostica, Boehringer Mannheim GmbH, Mannheim, Germany). All subjects had their blood pressure measured after at least 10 min of supine rest.

**Brachial artery vascular reactivity.** High resolution external ultrasound (128XP/10 mainframe with a 7.0-MHz linear array transducer, Acuson) was used to measure changes in brachial artery diameter in response to reactive hyperemia (leading to flow-mediated endothelium-dependent dilation [EDD]) and in response to 400 µg of sublingual nitroglycerin (NTG), an endothelium-independent, direct smooth-muscle dilator (8). The right brachial artery was scanned in longitudinal sections 2 to 15 cm above the elbow (control scan), after at least 10 min of rest in the supine position. Depth and gain settings were optimized to identify the vessel wall/lumen interface, and a baseline scan was recorded. Hyperemia was induced by inflation and then deflation of a pneumatic cuff placed around the forearm (below the scanned part of the artery) and inflated to ~250 mm Hg for 4.5 min. The artery was scanned before cuff inflation and for 90 s after cuff deflation. After at least 10 min rest, a further control scan was recorded. A single 400-µg metered dose of NTG (Nitrolingual spray, Fisons) was then administered sublingually by one of the investigators, in a standardized manner, and a final scan was recorded. All scans were recorded on super-VHS tape and later analyzed by two independent observers blinded to the identity of the subject, the scan sequence and the stage of the experiment. Each observer analyzed the arterial diameter for four cardiac cycles for each condition, and these measurements were averaged. Diameter measurements were taken at end-diastole, coincident with the R wave on a continuously recorded electrocardiographic trace. For calculation of EDD, the vessel diameter at 50 to 60 s after cuff deflation was divided by the average control diameter. NTG-induced dilation was calculated as the vessel diameter at 3 min after NTG administration divided by the average control diameter. For each subject, the average value of the two observers for both EDD and NTG-induced dilation is presented.

**Statistics.** Data were analyzed using SPSS for Windows 6.0. All descriptive data are expressed as mean value ± SD. Univariate and multivariate analyses of associations were assessed using standard linear regression techniques, with NTG response as the dependent variable. Of the independent variables, EDD, total cholesterol, age, cigarette smoking (pack-years) and vessel size were treated as continuous variables, and gender and diabetes mellitus were treated as categoric variables. Statistical significance was inferred at p < 0.05.

**Results**

**Subjects.** There were 800 subjects with a mean age of 38 ± 16 years (range 15 to 76). There were 317 men (40%) and 483 women (60%). There was a history of current smoking in 84 subjects (10.5%), and 40 were ex-smokers (5.0%). In those subjects with a history of current or previous smoking, the mean lifetime smoking dose was 16 ± 9 pack-years. There were 105 subjects with diabetes mellitus (13.1%) (102 with insulin-dependent diabetes mellitus, 3 with non-insulin-dependent diabetes mellitus). Mean total cholesterol in the group was 5.2 ± 1.3 mmol/liter (range 2.8 to 14.2). No subject had a history of hypertension; mean blood pressure was 122/79 ± 18/15 mm Hg. No subject had documented coronary artery disease, and none had clinical evidence of either coronary or peripheral arterial insufficiency. The ultrasound assessment of vascular reactivity was well tolerated in all subjects, none of whom had evidence of brachial artery atherosclerotic plaque on ultrasound scanning.

**Vasodilator response to NTG.** Mean NTG-induced arterial dilation in all subjects was 17.5 ± 6.0% (range 4.3% to 42%). On univariate analysis, a reduced NTG response was significantly correlated with a number of traditional risk factors for coronary artery disease, such as male gender, increasing age,
Table 1. Univariate Correlations Between Cardiovascular Risk Factors and Vasodilator Response to Nitroglycerin in All 800 Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression Coefficient</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDD</td>
<td>0.41</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>−0.15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Age</td>
<td>−0.09</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cigarette smoking (pack-yr)</td>
<td>−0.10</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>−0.15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Male gender</td>
<td>−0.26</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vessel size</td>
<td>−0.52</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

EDD = endothelium-dependent dilation.

increase total cholesterol and a history of cigarette smoking or diabetes mellitus (Table 1). In addition, there was a significant correlation between the vessel diameter and the percent dilation in response to NTG (r = −0.52, p < 0.001). When subjects with diabetes or a history of smoking were excluded, the remaining 574 subjects still demonstrated a significant relation between the response to NTG and age (r = −0.09, p = 0.009), total serum cholesterol (r = −0.15, p < 0.001), male gender (r = −0.26, p < 0.001) and vessel size (r = −0.52, p < 0.001) on univariate analysis. On multivariate analysis of the entire cohort (Table 2), the independent predictors of a reduced NTG response included larger vessel size and the presence of diabetes mellitus (consistent with our previous observations [17] in a smaller cohort of diabetic subjects).

EDD. The mean value for EDD in all subjects was 6.0 ± 3.7% (range −3.0% to 17.2%). On univariate analysis, reduced EDD was significantly associated with a history of diabetes (r = −0.08, p = 0.03), cigarette smoking (r = −0.14, p < 0.001), increasing total serum cholesterol (r = −0.21, p < 0.001), increasing age (r = −0.25, p < 0.001) and larger vessel size (r = −0.38, p < 0.001), consistent with our previous findings (8,11).

Response to NTG and endothelial function. Across the entire group of 800 subjects, EDD and NTG responses were significantly correlated (r = 0.41, p < 0.001) (Fig. 1). This correlation remained highly significant on multivariate analysis, independent of the effects of vascular risk factors or vessel size (p < 0.001) (Table 2). Similar results were obtained when male and female subjects were examined separately. This relation between NTG response and EDD was also present in a multivariate model in which only those asymptomatic subjects without diabetes or a history of smoking were considered (r = 0.24, p < 0.001).

Discussion

In this study of 800 asymptomatic subjects undergoing assessment of vascular reactivity of the brachial artery, there was a significant relation between the arterial dilator response to NTG and EDD, independent of the effects of vessel size or traditional vascular risk factors. These data demonstrate that the arterial smooth muscle response to exogenous NO is impaired in humans with risk factors for atherosclerosis. Such observations are consistent with the trend toward impaired arterial responses to NTG documented in previous smaller series in humans (e.g., 1–3,6–12), in which the impairment of endothelial function was much greater than that of the smooth muscle dilator response. Only one previous study has specifically examined the NTG dilator response in relation to endothelial dysfunction. In a smaller series of 110 patients undergoing coronary angiography for chest pain syndromes, Anderson et al. (12) found that NTG-induced vasorelaxation was slightly but not significantly lower in subjects with constrictor than in those with dilator responses to infused acetylcholine. The present study addresses this relation in a larger cohort of asymptomatic subjects and confirms a significant impairment of the NTG response in those adults with reduced EDD.

NTG and smooth muscle function. An impaired dilator response to NTG may be due to decreased bioavailability of NTG/NO to the smooth muscle cell, to mechanical forces limiting the artery’s ability to relax or to actual smooth muscle cell dysfunction. For the first possibility, it is well documented that certain vascular risk factors (e.g., hypercholesterolemia)
are associated with overproduction of oxygen-derived free radicals in the vessel wall (18–20); these may combine with endothelial derived NO, forming peroxynitrite, and thereby decreasing the bioavailability of NO to the smooth muscle cell, contributing to impaired EDD. However, this effect is unlikely to be relevant to the dilator activity of NTG because NTG activity depends on its conversion to NO inside the smooth muscle cells (21) and the subsequent activation of guanylate cyclase, the accumulation of cyclic guanosine monophosphate (GMP) and thus vasorelaxation. Previous arterial studies showing a trend toward decreased NTG responses at the site of coronary stenoses (1–3) may have been confounded by the mechanical inability of a vessel to dilate at the site of a discrete plaque. However, in the current study the NTG response was assessed in an artery without discrete plaques, minimizing the possibility that gross structural changes could explain the observed impairment of NTG responses in the adults with endothelial dysfunction.

These findings therefore suggest that, early in the process of atherosclerosis, changes in the vessel wall may not be limited to the endothelium and that the reduction in vasodilation in response to both endothelium-derived and exogenous sources of NO may be mediated (in part) by changes in vascular smooth muscle. The mechanism underlying this change in vasodilator capacity is not clear. Schächinger and Zeiher (22) have suggested that a reduced vasodilator response may be related to changes in baseline vasomotor tone in the presence of atherosclerosis; however, it is not known whether this might be the case in vessels that are structurally normal. It is also possible that microscopic structural changes, such as early fibrosis or smooth muscle atrophy, may exist in the vessel wall long before there are structural changes, such as plaques detectable by ultrasound (23). Functional changes may also exist in the vascular smooth muscle to account for the altered reactivity to NTG, such as decreased activity of intracellular guanylate cyclase, cyclic GMP or calcium-dependent relaxation.

**Endothelial function and response to NTG.** Several investigators previously examined the influence of the endothelium on NO-related vasodilation using in vitro studies and experimental models of atherosclerosis. Pohl and Busse (15) examined the vasodilator effects of sodium nitroprusside on rabbit thoracic aorta and femoral artery. They demonstrated that in the femoral artery, but not in the aorta, the absence of an intact endothelium, or blockade of endothelial nitric oxide synthase, resulted in an enhanced vasodilatory response to sodium nitroprusside. The mechanism of this effect was further investigated by Moncada et al. (13) who found that removal of the endothelium from rat aortic rings led to an increased sensitivity to exogenous nitrovasodilators, at the level of soluble guanylate cyclase. Therefore, in healthy animals in which endothelial NO production is acutely abolished, there is a supersensitivity to nitrates in smooth muscle cells, similar to that seen after denervation. In contrast, in cynomolgus monkeys with diet-induced atherosclerosis, the nitrovasodilator response is not enhanced in the presence of endothelial dysfunction (24). This response is probably much more analogous to the complex situation in humans, where endothelial dysfunction and atherosclerosis develop more slowly.

**Limitations of the study.** One limitation of the current study is the use of a single, clinically utilized dose of NTG in all subjects. We administered a pharmacologic dose of NTG (400 μg sublingually) that elicits maximal dilation and is on the plateau of the dose–response curve for human arteries (25–27). Therefore we examined the maximal dilator response achievable using this nitrovasodilator rather than a formal dose–response curve. In addition, we studied only those volunteers approached and willing to consent to studies on the effects of risk factors on arterial physiology, and some selection bias may therefore be present. Nevertheless, our subjects represented a community-based sample, studied noninvasively, with a wide range of age, cholesterol and blood pressure levels and smoking histories, which includes the average population values. Furthermore, the current results still support the previous findings by our group (8,10,11) and others (1,5,9) that endothelial dysfunction is an important early event in atherosclerosis in vivo in humans. The present large series only serves to highlight a more subtle smooth muscle dysfunction, evidenced by an impaired dilator response to NTG that is observed independently of the reduction in EDD. Given the subtle nature of the smooth muscle defect, it is unlikely that this defect would significantly influence measurements of EDD.

Although the vasodilator response to NTG may be impaired in the presence of atherosclerosis, exogenous nitrates have been used successfully for the treatment of angina pectoris for >100 years, and sublingual NTG remains the most frequently used short-acting vasodilator in the therapy of patients with symptomatic coronary artery disease (21). However, a greater understanding of the factors that modulate the vasorelaxant response to NTG may lead to improvements in achieving maximal arterial dilation at the sites of flow-limiting stenoses.

**Conclusions.** We observed that the vasodilator response to NTG is impaired in the brachial artery of asymptomatic subjects with risk factors for coronary artery disease. This finding is consistent with an abnormality in smooth muscle function in adults at risk for atherosclerosis.

**References**


