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# Nitroglycerin-Induced Coronary Vasodilation Is Not Enhanced in Patients With Impaired Endothelium-Dependent Dilation

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***Objectives.*** **This study was designed to determine whether enhanced sensitivity to exogenous nitrovasodilators is present in the coronary arteries of patients with impaired endothelium-dependent dilation.**

***Background.*** **Animal studies have demonstrated that the dilator response to exogenous nitrovasodilators is exaggerated in the setting of endothelial dysfunction (diminished nitric oxide activity). Whether such relative hyperresponsiveness to exogenous nitrates occurs and is important in humans is unknown.**

***Methods.*** **We assessed coronary vasomotion in 110 patients (mean [±SD] age 56 ± 10 years) by serial intracoronary infusions of acetylcholine ($10^{-8}$ to $10^{-6}$ mol/liter) to test endogenous nitric oxide and nitroglycerin (40 μg) to test responses to exogenous nitrovasodilators.**

***Results.*** **The vasomotor response to $10^{-6}$ mol/liter of acetylcholine differed between patients with (n = 95) and those without (n = 15) normal endothelial dysfunction (−21 ± 14% vs. 12 ± 8%, respectively, p < 0.001). However, neither the dilator response to nitroglycerin (21 ± 14% vs. 18 ± 13%) nor the baseline diameter differed between those with endothelial dysfunction and normal function, respectively. There was no correlation between the magnitude of the dilator response to nitroglycerin and acetylcholine. The response to nitroglycerin was decreased with increasing age (r = −0.21, p = 0.03) but was not related to any other demographic factors or to the angiographic appearance of the vessel.**

***Conclusions.*** **The coronary vasodilator response to nitroglycerin is not significantly enhanced in patients with impaired endothelium-dependent dilation but decreases with increasing age. This finding provides indirect evidence that basal coronary tone is not increased in patients with endothelial dysfunction and that supersensitivity to exogenous nitrates is not clinically important in humans.**

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The normal vascular endothelium maintains vasodilation through the release of paracrine factors such as nitric oxide (1). In response to a variety of pharmacologic stimuli, such as acetylcholine, or physiologic stimuli, such as shear stress, the endothelium releases nitric oxide, which binds to soluble guanylate cyclase, increasing the concentration of cyclic guanosine monophosphate (2). This is the same final common pathway used by nitrovasodilators to produce vasorelaxation (3). It has thus been postulated that important interactions might exist between endogenous nitric oxide and exogenous nitrovasodilators.

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In response to risk factors or overt atherosclerosis in humans, abnormal endothelium-dependent vasomotor responses have been observed as a result of a diminished nitric oxide effect (4,5). However, in these and other studies the vasodilator response to exogenous nitrates has been maintained (6,7). This finding has been interpreted as normal smooth muscle cell function, despite endothelial cell dysfunction.

Many, but not all, animal studies have shown an increased sensitivity to nitroglycerin in the setting of endothelial dysfunction, after removal of the endothelium or after blockade of nitric oxide (8–10). It has been suggested that this is similar to the supersensitivity seen after denervation, with upregulation of receptors. This may occur as a result of alterations in basal tone in the setting of endothelial dysfunction. Other suggested mechanisms include the loss of partial tolerance of endogenous nitric oxide on the effect of exogenously administered nitrates and a decrease in reactive oxygen species produced by the endothelium (11).

Previous human studies have not determined whether such a supersensitivity to exogenous nitrates exists in human coronary arteries. The purpose of the present study was to compare the coronary vasodilator response to nitroglycerin in a large



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cohort of patients with and without impaired endothelium-dependent vasomotor responses to acetylcholine.

## Methods

**Patients.** The study included 110 consecutive patients (72 men, 38 women; mean [±SD] age 56 ± 10 years, range 34 to 76) who underwent endothelial function testing in the cardiac catheterization laboratory and also received intracoronary nitroglycerin. All studies were carried out in the morning. Significant coronary artery disease (at least one stenosis >50%) was present in 78 patients (71%); the remaining 32 patients (29%) had normal coronary anatomy. Risk factors were present as follows: hypertension in 54 patients (49%), diabetes mellitus in 11 (10%), cigarette smoking in 38 (35%), family history in 47 (43%) and hypercholesterolemia in 58 (53%). Mean total, low density lipoprotein and high density lipoprotein cholesterol levels for the group were 5.2 ± 0.85, 3.4 ± 0.85 and 1.0 ± 0.26 mmol/liter, respectively.

**Study protocol: coronary endothelial function study.** Long-acting vasoactive medications, including calcium channel blocking agents, beta-adrenergic blocking agents, nitrates and angiotensin-converting enzyme inhibitors, were discontinued for at least 18 h before catheterization. Written informed consent was obtained from all patients before the catheterization procedure in accordance with the guidelines established by the Committee for the Protection of Human Subjects.

After a guiding catheter was positioned in the left main coronary artery and 10,000 IU of heparin was infused, a 2.5F infusion catheter was advanced through the guiding catheter over a 0.014-in. (0.035 mm) guide wire into the proximal portion of the left anterior descending or circumflex coronary artery. Serial intracoronary infusions were made according to an established protocol: 1) control infusion (5% dextrose in water); 2) serial 2.5-min infusions of the endothelium-dependent vasodilator acetylcholine (Miochol, Ioiab Pharmaceuticals) in doses of 0.16, 1.6 and 16 μg/min, achieving a final estimated intracoronary concentration of $10^{-8}$, $10^{-7}$ and $10^{-6}$ mol/liter, respectively; 3) 5-min recontrol infusion of 5% dextrose in water; 4) 3-min infusion of the endothelium-independent vasodilator nitroglycerin at 16 μg/min ($10^{-6}$ mol/liter) (4). During each infusion, blood pressure, heart rate and the electrocardiogram were continuously monitored.

Quantitative coronary angiographic images were taken after each intervention (4,7). Nonionic contrast medium (Omnipaque, Winthrop Laboratories) was injected into the left main coronary artery at 7 ml/s for a total of 9 ml with a power injector (Medrad) to opacify the coronary artery.

**Quantitative coronary angiography.** An automated edge detection program was used to search densities and seek inflection points, thus measuring the segment diameter of the vessel along the length of the selected segment (ImageComm, Quantum IC software). Two segments 8 to 10 mm in length were selected for analysis prospectively, on the basis of optimal regions for quantitative angiographic analysis. The vasomotor responses for the two segments in each patient were averaged to create a mean vasodilator response to acetylcholine and nitroglycerin.

For the purpose of classifying the coronary vasomotor response dichotomously, patients in whom either of the two coronary segments constricted significantly (>5% from baseline) were classified as having coronary endothelial dysfunction. All other patients were considered to have normal coronary endothelial function.

The coronary angiograms were reviewed by an observer (I.T.M.) who had no knowledge of the results of the vasodilator function studies and were coded as to the number of major epicardial vessels stenosed (>50%) and whether or not they were smooth or irregular. Only patients in whom the epicardial coronary arteries were completely smooth were classified as having no angiographic coronary disease.

**Statistical analysis.** The differences in demographics and acetylcholine and nitroglycerin responses between patients with endothelial dysfunction and normal function were compared by unpaired *t* tests. The continuous relation between patient demographics or vasomotor response to acetylcholine and that for the dilator response to nitroglycerin were compared with linear regression analysis. Statistical significance was defined as a two-tailed p value <0.05. Results are expressed as mean value ± SD. The study had a 78% power to detect a clinically significant absolute difference of 10% in the coronary response to nitroglycerin.

## Results

**Coronary artery endothelial function.** The study patients were classified as those with endothelial dysfunction (vasoconstriction >5% to acetylcholine, n = 95) and those with normal endothelial function (vasodilator or no change in diameter to acetylcholine, n = 15). The patient demographics for the two groups are shown in Table 1. There was no significant difference between the two groups with respect to risk factors, serum lipids or extent of coronary artery disease. Patients with endothelial dysfunction demonstrated constriction of 21 ± 14%, whereas patients with normal function showed vasodilation of 12 ± 8% in response to acetylcholine. There was no difference in baseline coronary diameter between the two groups (1.91 ± 0.49 vs 1.89 ± 0.36 mm, respectively).

**Vasodilator responses to nitroglycerin.** The coronary dilator response to nitroglycerin was not significantly different (21 ± 14% vs. 18 ± 13%) between patients with endothelial dysfunction and normal endothelial function, respectively (Fig. 1). By regression analysis there was no relation between the vasomotor response to acetylcholine and nitroglycerin (r = 0.06) (Fig. 2). There was also no relation between the degree of dilation to nitroglycerin and the baseline coronary diameter (r = 0.16). Neither the burden of atherosclerosis nor the presence of irregularities in the study vessel had any impact on the nitroglycerin response. The only patient demographic that correlated to dilator response was age (p = 0.029). Older patients tended to have less dilation in response to nitroglycerin (Fig. 3).

**Table 1.** Patient Demographics

| | Endothelial Dysfunction (n = 95) | Normal Endothelial Function (n = 15) |
|---|---|---|
| Age (yr) | 56 ± 10 | 59 ± 12 |
| Cholesterol (mmol/liter) | 5.2 ± 0.8 | 5.2 ± 0.7 |
| LDL cholesterol (mmol/liter) | 3.4 ± 0.8 | 3.4 ± 0.8 |
| HDL cholesterol (mmol/liter) | 1.0 ± 0.2 | 1.0 ± 0.3 |
| Triglycerides (mmol/liter) | 1.9 ± 1.0 | 2.2 ± 2.0 |
| Hypertension | 45% | 73% |
| Diabetes mellitus | 10% | 13% |
| Cigarette use | 34% | 40% |
| No. of risk factors | 1.3 ± 0.9 | 1.7 ± 1.1 |
| Coronary disease | 74% | 47% |

Data presented are mean value ± SD or percent of patients. HDL = high density lipoprotein cholesterol; LDL = low density lipoprotein cholesterol.

Because it is appreciated that the vasomotor responses along the length of the artery are heterogeneous, and averaging two segments/patient might mask a potential correlation, we also examined the relation between acetylcholine and nitroglycerin responses on a per-segment basis. A total of 220 segments (1 proximal and 1 distal/patient) were analyzed. The 163 segments with an abnormal response to acetylcholine had a mean dilation in response to nitroglycerin of 21.6 ± 16%, whereas the 57 normal segments dilated 16.7 ± 15%. This difference was not significant when the data were corrected for multiple measurements in the same subject.

## Discussion

The coronary vasodilator response to nitroglycerin is not significantly enhanced in patients with impaired endothelium-dependent dilation despite similar baseline diameters, but does decrease with increasing age. This finding is in contradistinction to many animal studies that have suggested a supersensitivity to nitrovasodilators in situations where endothelium-dependent nitric oxide activity is reduced.

**Interaction of nitric oxide and exogenous nitrates.** In response to acetylcholine, the healthy endothelium releases nitric oxide, which modulates many biologic processes, including vasodilation (12,13). Nitric oxide acts through the cyclic guanosine monophosphate second-messenger system, the same final common pathway as exogenous nitrovasodilators. Thiol groups play a role in the biotransformation of nitrovasodilators and may be important in the nitric oxide–heme interaction of soluble guanylate cyclase (14,15). There is thus a potential for interaction between nitric oxide and the effects of exogenously administered nitrates.

Moncada et al. (9) have demonstrated both in vitro and in vivo that there is an enhanced dilator response to nitroglycerin when nitric oxide is blocked or the endothelium is removed. This response appeared to occur at the level of soluble guanylate cyclase and may be analagous to the denervation supersensitivity that has been observed for >100 years. An enhanced sensitivity to nitrovasodilators in the setting of endothelial dysfunction has also been demonstrated by other investigators in a variety of animal models (8,16,17). However, this finding has not been consistent, with some models of atherosclerosis demonstrating endothelial dysfunction with no enhancement of nitroglycerin response (18). The reason for the inconsistency is not clear but may relate to the cellular characteristics of the intimal lesions created in different animal models. A recently published study (19) demonstrated a generalized enhancement of both a constrictor response to acetylcholine (endothelial dysfunction) as well as an enhanced dilator response to nitroglycerin in patients with vasospastic

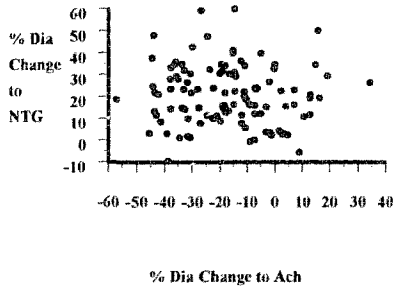



**Figure 2.** Continuous relation between dilator response to nitroglycerin (NTG, y axis) and acetylcholine (Ach, x axis). No correlation was found. Dia = diameter.

**Figure 1.** Vasomotor responses to acetylcholine and nitroglycerin for patients with and without endothelial dysfunction. No significant difference in dilator response to nitroglycerin was seen. Results shown are mean value ± SE.

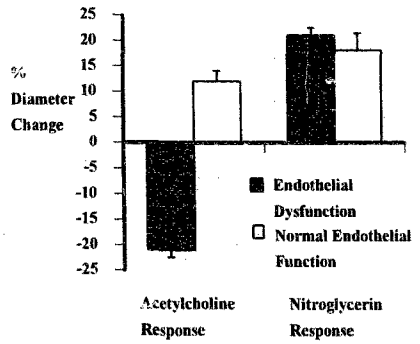
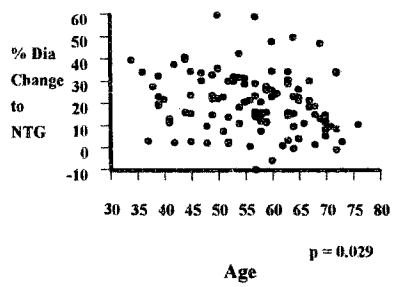



**Figure 3.** Relation between dilator response to nitroglycerin (NTG, y axis) and age. A modest inverse correlation was seen (p = 0.029). Dia = diameter.



angina. However, the study did not demonstrate an enhanced nitroglycerin response in patients without coronary vasospasm.

Rest vascular tone may be increased with endothelial dysfunction because nitric oxide has been shown to be basally released (5,13). A recent study (5) demonstrated that in the presence of risk factors that are known to impair endothelium-dependent vasodilation, basal release of nitric oxide is impaired compared with that in patients without risk factors. If the vessel starts with relative vasoconstriction, then nitrovasodilators may cause relatively greater vasodilation when the endothelium is dysfunctional. This would be analogous to the postulate that the reduced basal formation of endothelium-derived nitric oxide in veins compared with that in arteries may explain why veins are more sensitive to the effects of nitrovasodilators (20).

Another possible mechanism of enhanced sensitivity to nitrovasodilators includes the withdrawal of partial tolerance induced by nitric oxide in a healthy vessel. This withdrawal might occur as a result of alterations in the interaction between reactive thiol species, guanylate cyclase and nitric oxide.

**Current study.** Human studies have not specifically assessed the relation between responses to nitroglycerin and acetylcholine in large numbers of patients. Previous studies have demonstrated retained coronary dilator response to nitrates in the setting of atherosclerosis and endothelial dysfunction (4–7). In fact, some peripheral studies have shown a decrease in dilation in response to nitroprusside in patients with hypercholesterolemia or atherosclerosis compared with that in control subjects (21,22).

In the present study we examined this relation in 110 consecutive patients who underwent endothelial function testing with acetylcholine and intracoronary nitroglycerin. No correlation between the vasomotor response to acetylcholine and nitroglycerin was present. The vasodilator response to nitroglycerin was not enhanced in patients with endothelial dysfunction. Although basal tone is difficult to assess in vivo, the baseline diameter of the coronary arteries was not different between the two groups, indirectly suggesting that tone was not different.

It is unclear why in vitro studies have demonstrated a supersensitivity to nitrates whereas we were unable to show this response in human coronary arteries. It may be that the endothelium needs to be physically removed or the level of nitric oxide activity more completely suppressed before this effect is seen. Quyyumi et al. (5) demonstrated in human coronary arteries that nitric oxide activity is decreased in patients with coronary risk factors but that some activity is still present. Also, only one dose of nitroglycerin was given, and it may be that if dose–response curves were obtained, a difference in effect might be evident at lower concentrations of nitroglycerin. There is also a difference in the cellular characteristics of arteries from cholesterol-fed animals and atherosclerotic humans, who may have advanced fibrocalcific changes that could potentially impair an enhanced nitroglycerin effect.

The dilator response to nitroglycerin decreased with increasing age but was not dependent on coronary risk factors or the burden of atherosclerosis by angiography. This finding might represent a decreased sensitivity of the aging vascular smooth muscle cell or simply a physical abnormality in the aging vessel. Older patients tend to have more calcification in their coronary arteries, which may physically prevent dilation. Endothelium-dependent dilation of coronary resistance vessels decreases with advancing age independent of increasing burdens of atherosclerosis (23,24). The dilator response to the smooth muscle dilator papaverine was also shown to decrease with increasing age in a group of patients with normal coronary arteries (22). Thus, aging most likely has an independent detrimental effect on endothelium-dependent and endothelium-independent vasodilation in the coronary circulation.

**Study limitations.** Only one dose of nitroglycerin (40 μg over 2.5 min) was given to each patient. A dose–response curve comparison between patients with and without endothelial dysfunction may have been instructive. Perhaps there would have been a difference in dilation at lower than maximal doses. Significant dilation probably does not occur above the dose used in this study (25), suggesting that the peak dilation to nitroglycerin is not enhanced in patients with endothelial dysfunction.

The majority of the patients in the study had endothelial dysfunction, given the referral pattern to the catheterization laboratory. However, the baseline demographics were matched between the two groups. Larger numbers of patients may have allowed detection of a statistically significant but clinically unimportant difference of a few percentage points between the two groups.

No longitudinal data are present in patients to confirm that aging impairs vascular sensitivity to nitrovasodilators.

**Conclusions.** This study demonstrated that the coronary vasodilator response to nitroglycerin is not significantly enhanced in patients with impaired endothelium-dependent dilation but decreases with increasing age. This finding provides indirect evidence that basal coronary tone is not increased in patients with endothelial dysfunction and that supersensitivity to exogenous nitrates is not clinically important in humans.

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