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Influence of Smoking Status on Angiotensin-Converting Enzyme Inhibition–Related Improvement in Coronary Endothelial Function

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Summary. **Our study evaluated the influence of smoking status on coronary endothelial function in normotensive patients with coronary artery disease who received placebo or the angiotensin-converting enzyme inhibitor quinapril in the TREND study (Trial on Reversing Endothelial Dysfunction). In this retrospective analysis of data from the previously published study, patients were classified as either smokers (n = 23) or nonsmokers (n = 82). Patients underwent coronary angiography at baseline and again after 6-month follow-up. The primary response variable was the net change in acetylcholine-induced diameter of the target coronary artery segments (n = 105) between the baseline and 6-month follow-up angiograms. The secondary response variables were based on analysis of all segments (n = 300) and the mean diameter responses of target and all segments at 6 months. At baseline, coronary artery vasomotor responses were similar in smokers and nonsmokers in the placebo and quinapril groups. There was a significant improvement in the primary response variable for both smokers ($P = 0.008$) and nonsmokers ($P = 0.047$) randomized to quinapril compared with placebo. At 6-month follow-up, nonsmokers in the placebo group showed no significant change in the mean vasoconstrictor responses (8.3% vs. 8.0% at acetylcholine 10^{-4} mol/L), whereas nonsmokers in the quinapril-treated group showed significantly less vasoconstriction (2.7% vs. 13.2%; $P = 0.003$). Among smokers in the placebo group, vasoconstriction increased nonsignificantly (21.7% vs. 17.2% at baseline) but decreased significantly in the quinapril group (0.5% vs. 17.9%; $P = 0.002$). These results indicate that ACE inhibition improves the coronary vasomotor response in both smokers and nonsmokers, but that smokers apparently derive greater benefit.**

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Key Words. **acetylcholine, angiotensin-converting enzyme inhibition, coronary disease, endothelium, smoking, vasoconstriction**

Epidemiologic studies document smoking as an independent risk factor for the development of coronary artery disease [1,2]. Cigarette smoke is also associated with the progression of established atherosclerosis and an increase in coronary morbidity and mortality [3,4]. The mechanisms responsible for the adverse vascular effects of smoking are not clear. One possible mechanism is through acute and chronic effects on coronary endothelium. Both active and passive smoking cause abnormal endothelial-mediated vasomotor reactivity and can increase platelet aggregation, leukocyte activation, and fibrinogen levels, all of which may contribute to the development of atherosclerosis and plaque instability [5–10]. Cessation of cigarette smoking is associated with restoration of endothelium-dependent vasomotion [5]. This may explain why smokers who give up cigarettes after myocardial infarction have a lower risk of recurrent infarction than those who do not [11]. Improvement of endothelial function may therefore be associated with a reduction in the progression of coronary atherosclerosis and vasoconstriction, leading to reduced morbidity and mortality.

Studies have suggested that angiotensin-converting enzyme (ACE) inhibitors can improve endothelial function in atherosclerotic vessels [12]. Proposed mechanisms include inhibiting local production of angiotensin II, limiting the oxidation of NADP/NADPH, and inhibiting degradation of bradykinin, all of which results in an increase in nitric oxide (NO) and prostacyclin [12]. In the recently reported Trial on Reversing Endothelial Dysfunction (TREND), the ACE inhibitor quinapril significantly improved endothelium-dependent

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vasomotor reactivity in patients with coronary artery disease [13]. Quinapril is a potent ACE inhibitor with high affinity for both plasma and vascular tissue ACE. The patients in TREND were normotensive or controlled hypertensive, and free of severe left ventricular dysfunction, hypercholesterolemia, or insulin-dependent diabetes. The purpose of this report is to evaluate the effects of smoking status on endothelial function in patients who received placebo or the ACE inhibitor quinapril.

Methods

Patient population

The TREND study has been reported in detail previously [13]. In brief, eligible patients had single or double vessel coronary artery disease (>50% diameter stenosis) requiring a percutaneous revascularization procedure and one adjacent main coronary artery with <40% diameter stenosis that had never been revascularized. This adjacent artery was designated the target artery when it exhibited endothelial dysfunction, defined as no dilation (<5% increase in mean lumen diameter) in response to intracoronary acetylcholine (Ach).

Patients were excluded if a dominant right coronary artery was the only target artery, age was >75 years, low-density lipoprotein (LDL) cholesterol was >4.3 mmol/L (165 mg/dL), systolic blood pressure was >160 mmHg or diastolic blood pressure >90 mmHg, they had a history of coronary artery bypass grafting or coronary spasm, myocardial infarction within 7 days, a percutaneous revascularization procedure within 3 months, a left ventricular ejection fraction ≤40%, type I diabetes mellitus, clinically significant hepatic or renal dysfunction, valvular heart disease, second-or third-degree heart block, or treatment with lipid-lowering agents within the previous 6 months. Patients with a history of hypertension were enrolled only if hypertension was controlled, with systolic and diastolic blood pressures ≤160 mmHg and ≤90 mmHg, respectively.

Study design

The study was a double-blind, placebo-controlled, parallel design carried out in multiple centers. The protocol was approved by the ethics committees of each institution, and written informed consent was obtained from all patients. Patients discontinued all vasoactive medications except beta-blockers and sublingual nitrates at least 12 hours before the study.

During catheterization, a 5F bipolar pacing catheter was positioned in the right ventricular apex and set in the demand mode at 10 beats/min less than the baseline heart rate. A baseline angiogram was taken and the target artery was identified. This was followed by two stepwise intracoronary infusions of Ach (10^{-6} mol/L and 10^{-4} mol/L) delivered at 0.8 mL/min for 2 minutes through the coronary catheter by a constant infusion pump. Careful attention was paid to the calculation of catheter dead space to ensure accurate delivery of Ach to the coronary ostium. Angiography was repeated immediately after each infusion. A nitroglycerin bolus (mean, 206 μg) was then administered and was followed by an angiogram identical to the one performed at baseline. The intended goal was to totally reverse any lingering effects of Ach by ensuring maximal epicardial dilation with nitroglycerin. All details of the catheterization and radiography views used were recorded to ensure duplication at the 6-month follow-up.

Patients were randomized to receive placebo or quinapril. After 6 months, study medication was discontinued and after 3 days (76 ± 2.4 hours), the patients returned for repeat angiography and Ach. The patients again discontinued all vasoactive medications except beta-blockers and sublingual nitrate 12 hours before the challenge, as was done at baseline. In the event that a patient underwent a clinically necessary coronary angiogram <3 months after randomization, the angiogram with Ach was repeated at 6 months.

Quantitative coronary angiography

All films were analyzed at the angiographic core laboratory by use of digital angiographic techniques described previously to compare luminal diameter and coronary endothelial reactivity [14]. Core lab investigators were masked to treatment assignment and clinical findings. At least one boundary (proximal or distal) for each segment was referenced to a precise anatomic landmark, usually a branch origin, to aid in precise replication of segmental analyses at baseline and follow-up. The mean diameter of these segments was recorded from angiograms before Ach infusions, after each infusion, and after nitroglycerin administration. Segment responses were calculated as the percent change in the mean diameter before and after each Ach infusion and after nitroglycerin administration. The core laboratory identified the target artery segment from the baseline angiogram by determining the segment that showed the worst endothelial dysfunction as defined earlier. Angiograms performed at 6 months were analyzed in the same way using views that were identical to the baseline study. The core angiography laboratory also reviewed procedure sheets and logs for the baseline and follow-up studies to ensure protocol adherence and replication of the radiographic conditions.

Statistical analysis

The data were analyzed by an analysis of covariance (ANCOVA) model as described in the original TREND study paper [13]. Within this model, appropriate contrasts were constructed to compare smokers and nonsmokers, both within and between treatment groups. Analyses were performed using the MIXED proce-

dure of the Statistical Analysis System (SAS), version 6 [15]. Student's *t*-tests and Fisher's Exact tests, using procedures TTEST and FREQ of SAS, version 6 [15], were used to compare the groups' characteristics at baseline. All comparisons were done using a significance level of 5%. Results are presented as mean ± SE.

Results

Baseline patient characteristics

A total of 105 patients (51 randomized to quinapril and 54 to placebo) were eligible for repeat catheterization and Ach challenge at 6 months. Of these, 15 patients receiving quinapril and 8 patients receiving placebo were classified as current smokers (defined as occasional or regular daily smokers); the remaining 82 patients were classified as nonsmokers (defined as never or past smokers) based on history. All pertinent baseline and follow-up clinical characteristics were similar between the placebo-treatment and quinapril-treatment groups as well as between smokers and nonsmokers (Table 1). There was no significant difference in the blood pressure or lipid profile between groups. However, smokers had a trend toward increased frequency of previous myocardial infarction ($P = 0.099$) and a decreased frequency of non–insulin-dependent diabetes mellitus ($P = 0.054$) compared with nonsmokers. Mean segment diameters and mean percent diameter stenosis were not different between the placebo and quinapril groups (2.2 ± 0.1 mm vs. 2.1 ± 0.1 mm and 22.9 ± 0.8% vs. 24.8 ± 0.8%, respectively). Baseline and follow-up mean diameters of the segments also were not different (1.9–2.1 mm).

Vasomotor response to Ach

At baseline (prior to randomization), the coronary artery response to Ach in the target segment was similar in smokers and nonsmokers in the placebo-and quinapril-assigned groups. However, smokers tended to have a greater constrictive response in the target segment compared with nonsmokers. At the 10^{-4} M concentration of Ach, the mean constrictor response was −17.2% for smokers and −8.0% for nonsmokers ($P = 0.111$). After 6 months of treatment, nonsmokers in the placebo group showed no significant change in the mean vasoconstrictor responses (8.3% at 6 months vs. 8.0% at baseline; 10^{-4} mol/L Ach), whereas those in the quinapril group showed a significantly less constrictor response at 6 months (2.7% vs. 13.2% at baseline; $P = 0.003$). Among smokers, there was a nonsignificant increase in the constrictor response in the placebo group at 6 months (21.7% vs. 17.2% at baseline), whereas there was a significant decrease in the

Table 1. *Patient characteristics at baseline*

	Placebo (n = 54)		Quinapril (n = 51)	
	Nonsmokers[a] (n = 46)	Smokers[b] (n = 8)	Nonsmokers (n = 36)	Smokers (n = 15)
Age (yr)	61.1 (1.4)	54.1 (2.1)	57.7 (2.0)	54.9 (2.6)
Systolic BP (mmHg)				
Baseline	127 (2.6)	122 (4.7)	120 (2.1)	118 (5.1)
Follow-up	131 (3.1)	129 (4.3)	134 (2.9)	131 (4.2)
Diastolic BP (mmHg)				
Baseline	73 (1.6)	70 (3.2)	74 (1.5)	71 (3.3)
Follow-up	77 (1.3)	82 (1.8)	78 (1.5)	81 (3.1)
LDL cholesterol (mmol/L/mg/dL)				
Baseline	3.3 (0.1)	3.6 (0.1)	3.2 (0.1)	3.2 (0.2)
	127.6	139.2	123.7	123.7
Follow-up	3.5 (0.2)	3.5 (0.2)	3.3 (0.1)	3.3 (0.3)
	135.3	135.3	127.6	127.6
HDL cholesterol (mmol/L,mg/dL)				
Baseline	1.0 (0.04)	0.9 (0.1)	1.0 (0.05)	1.1 (0.1)
	38.7	34.8	38.7	42.5
Follow-up	1.1 (0.04)	0.9 (0.1)	1.0 (0.05)	1.2 (0.1)
	42.5	34.8	38.7	46.4
Prior MI, no. (%) of patients	18 (39.1%)	5 (62.5%)	15 (41.7%)	9 (60.0%)
Prior HTN, no. (%) of patients	19 (41.3%)	2 (25.0%)	14 (38.9%)	9 (60.0%)
NIDDM, no. (%) of patients	7 (15.2%)	0	10 (27.8%)	1 (6.7%)

Values are mean ± SEM.

[a]Nonsmoker = never smoked or past smokers.

[b]Smokers = occasional and regular daily smokers.

BP = blood pressure; HDL = high-density lipoprotein; HTN = hypertension; LDL = low-density lipoprotein; MI = myocardial infarction; NIDDM = non–insulin-dependent diabetes mellitus.

constrictor response in the quinapril-treated group (0.5% vs. 17.2% at baseline; $P = 0.002$; both, Ach 10^{-4} M; Figure 1).

The primary response variable, the net change in target segment responses from baseline, is shown in Figure 2. In the nonsmoking cohort, the vasomotor response in the quinapril group improved by 2.9 ± 3.4% and 10.5 ± 3.4% at Ach doses of 10^{-6} and 10^{-4} M, respectively, whereas there was no change in the vasomoter response in the placebo group ($P > 0.047$). Among smokers, the vasomotor response in patients randomized to quinapril improved by 9.1 ± 5.0% and 16.6 ± 5.1% at Ach 10^{-6} and 10^{-4} M, respectively, whereas the vasomotor response in the placebo group worsened (− 3.9 ± 6.5% and − 3.8 ± 6.6% at Ach 10^{-6} and 10^{-4} M, respectively; $P = 0.008$).

An analysis of all segments increases the number of segments analyzed to 202 and 178 in the placebo- and quinapril-treated groups, respectively. The results of the all segment analysis parallel those in the target segment analysis (Figure 3). At 6 months, smokers in the quinapril group demonstrated significantly less vasoconstriction than those in the placebo group, whereas for nonsmokers the change in all-segment analysis is not significant. Likewise, the net percent change in the response between baseline and follow-up demonstrates a significant improvement in the quinapril group compared with placebo in the smoking cohort ($P = 0.001$). A trend toward improvement in vasomotor response with quinapril compared with placebo was observed in nonsmokers ($P = 0.09$; Figure 4).

Vasomotor response to nitroglycerin

At baseline (prior to randomization) there was no difference in the vasodilator response to nitroglycerin in target segments between smokers and nonsmokers randomized to either treatment group. Furthermore, there was no difference in vasodilator responses between smokers or nonsmokers, and between the placebo and quinapril groups based on either 6-month assessment of percent change or on the net change during follow-up (Table 2).

Discussion

Smoking has been causally related to coronary heart disease and is a major independent risk factor [1,2]. The incidence of myocardial infarction and sudden death is increased in cigarette smokers compared with nonsmokers [16,17]. Smokers also have significantly more myocardial ischemia during daily life compared with patients who do not smoke [18]. Smoking accelerates the progression of coronary atherosclerosis and new lesion formation [3,4]. The effect of smoking on mortality and morbidity may not be solely related to the atherosclerotic process itself because there is no correlation between smoking and the extent of disease

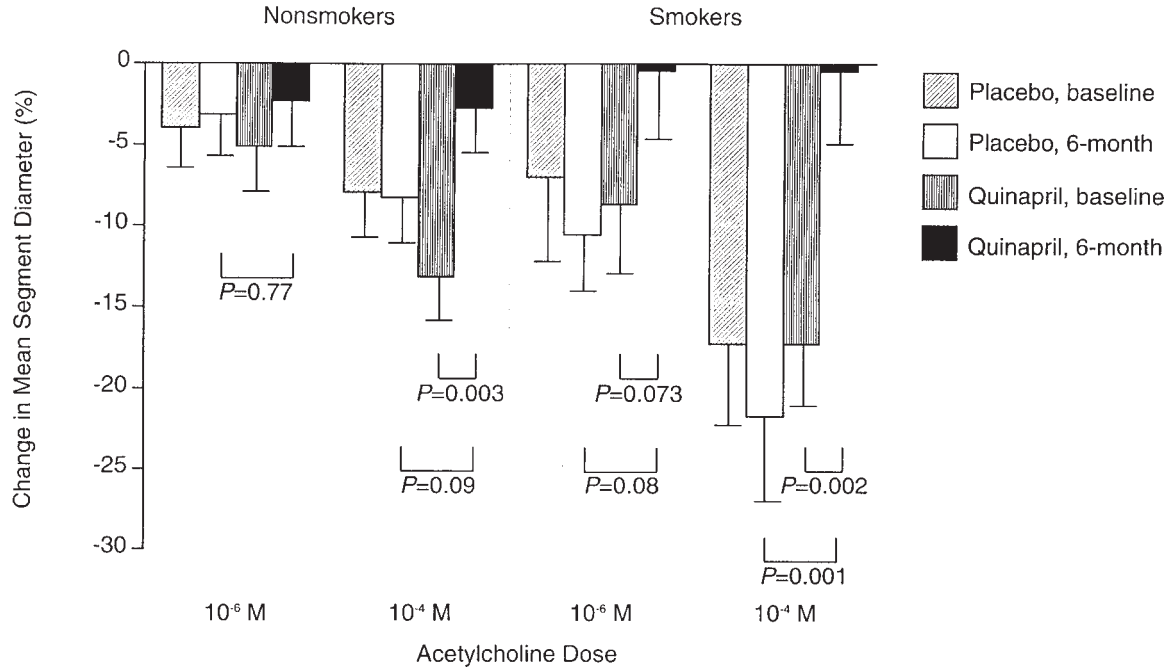

Fig. 1. *Percent changes in mean segment diameter in the target segment (expressed as percent ± SE, plotted on y axis) in each group at baseline and follow-up. Data are grouped on the basis of the acetylcholine concentration and smoking status. At the 10^{-4} mol/L dose, the quinapril-treated group showed significant improvement after 6 months ($P < 0.003$) in both smokers and nonsmokers, whereas the placebo group showed increased vasoconstriction.*

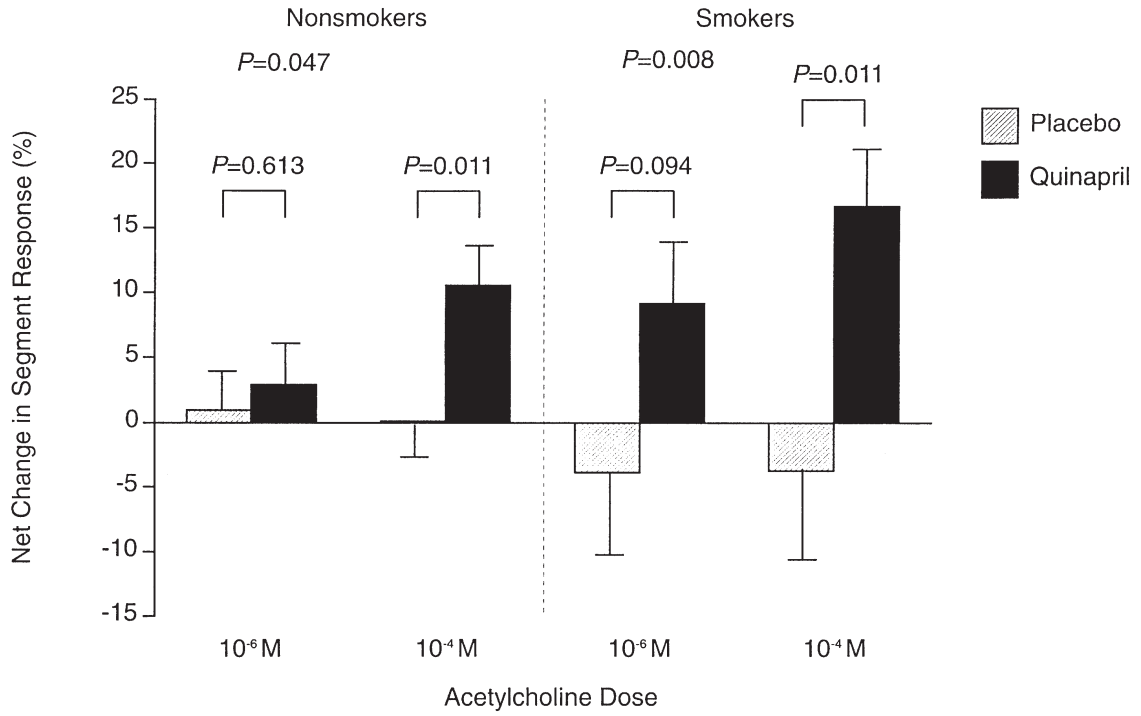

Fig. 2. *Primary efficacy parameter (net change in segment response after 6 months) in the target segment. Overall differences in response between the placebo and quinapril groups were significant for smokers (*P *= 0.008) and nonsmokers (*P *= 0.047). At the* 10^{-4} *mol/L dose, the difference between the placebo and quinapril groups was significant for smokers and nonsmokers (both,* P *= 0.011).*

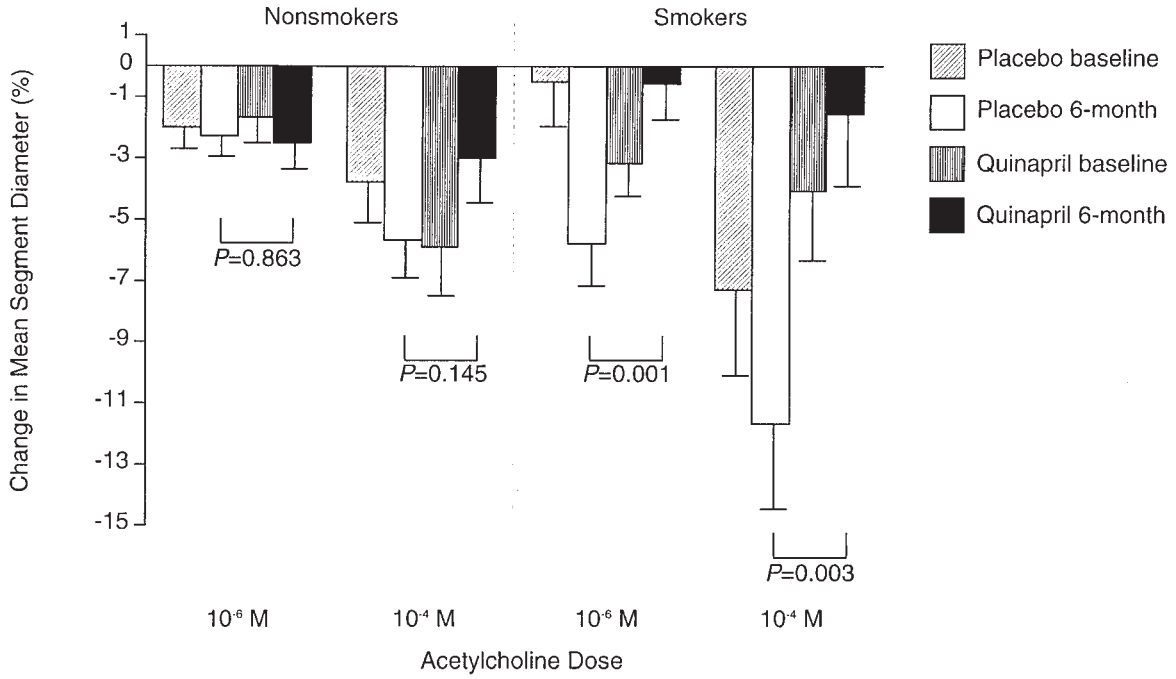

Fig. 3. *Percent changes in mean segment diameter in all segments (same format as Fig. 1). In smokers, there was significantly less constriction after 6 months in the quinapril group than in the placebo group (*P *< 0.003), even at the lower concentration of acetylcholine.*

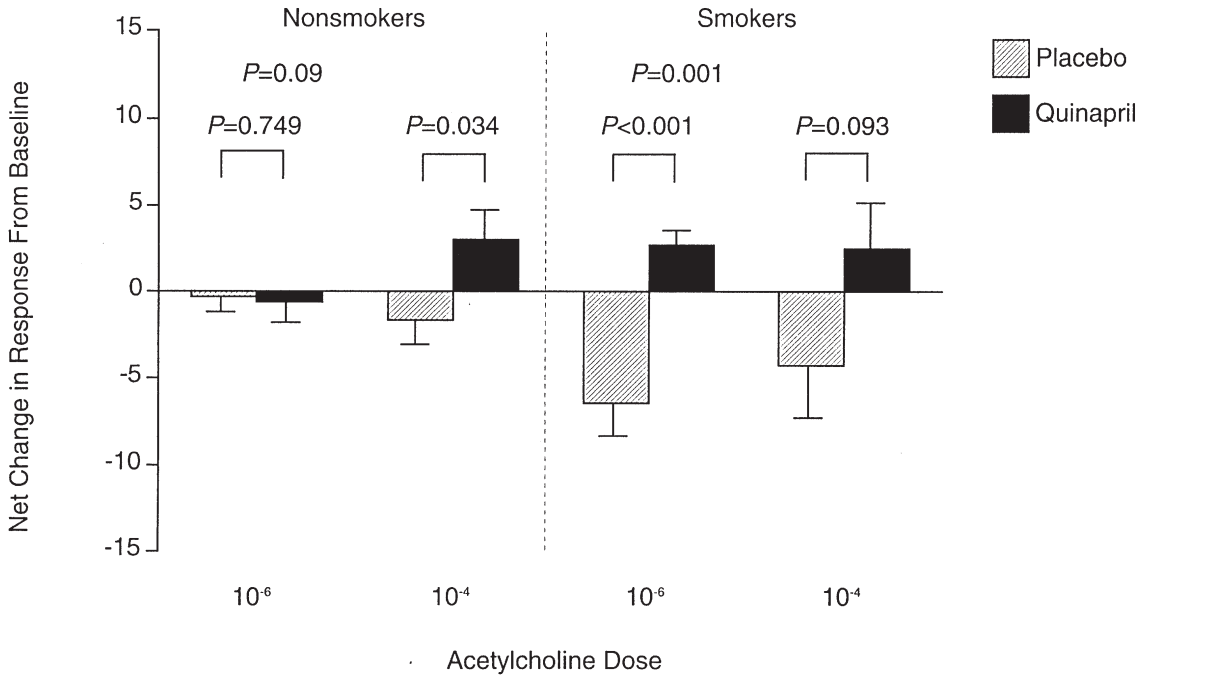

Fig. 4. *Net change in segment response after 6 months in all segments. The results are concordant with the analysis illustrated in Figure 2.*

by angiography [19]. However, disease progression and new lesion formation are important response variables shown to correlate with clinical coronary events such as myocardial infarction and cardiac death [20–23]. Although new lesions are rarely severe enough to cause obstructive coronary flow, they can be clinically relevant because they may be prone to plaque rupture and thrombosis, leading to acute ischemic syndromes.

The exact pathophysiologic mechanism by which cigarette smoking predisposes to coronary heart dis-

Table 2. *Coronary diameter responses to nitroglycerin*

	Placebo (n = 54)	Quinapril (n = 51)	*P* value
Target segments	(%Λ)	(%Λ)	
Baseline			
Nonsmokers	10.0 ± 2.2	9.1 ± 2.4	0.739
Smokers	9.9 ± 4.5	11.2 ± 3.5	0.811
6-month follow-up			
Nonsmokers	9.4 ± 2.1	10.7 ± 2.3	0.603
Smokers	6.5 ± 4.4	11.8 ± 3.5	0.293
Net change from baseline			
Nonsmokers	− 0.7 ± 2.5	1.6 ± 2.7	0.444
Smokers	− 3.4 ± 5.1	0.8 ± 4.0	0.468
All segments	(n = 202)	(n = 178)	
Baseline			
Nonsmokers	9.4 ± 1.1	11.3 ± 1.2	0.148
Smokers	13.4 ± 2.1	10.2 ± 1.7	0.174
6-month follow-up			
Nonsmokers	8.6 ± 1.1	10.6 ± 1.2	0.123
Smokers	9.2 ± 2.0	10.5 ± 1.7	0.581
Net change from baseline			
Nonsmokers	− 0.7 ± 1.3	− 0.7 ± 1.4	0.991
Smokers	− 4.2 ± 2.4	0.4 ± 2.0	0.093

ease is not completely understood. As early as the mid-1970s, endothelial damage and platelet aggregation were among the proposed mechanisms. Asmussen and associates [24] reported electron microscopic observations on umbilical cord arteries obtained from smoking and nonsmoking mothers. They observed pronounced degenerative intimal changes, such as endothelial swelling, bleeding, contraction, and subsequent opening of the interendothelial junctions with formation of subendothelial edema, in the arteries from smoking mothers. In other studies, both active and passive tobacco exposure resulted in increased platelet aggregation and an increased circulating endothelial cell count, presumably secondary to endothelial desquamation [7,8]. In human subjects and laboratory animals, acute and chronic exposure to cigarette smoke exerts peroxidative damage to endothelial cells, leading to reduced generation of prostacyclin and increased platelet adhesion [9]. Recent studies also have shown that cigarette smoking increases monocyte adhesion to endothelial cells in animal and human models [25].

Most investigators believe that the loss of endothelial function, rather than actual endothelial denudation, is key to atherogenesis and plaque instability. Celermajer and colleagues [5] demonstrated that cigarette smoking is associated with a dose-related impairment of forearm endothelium-dependent arterial dilation in otherwise healthy young adults. In their study, there was a stronger association between impaired flow-mediated dilation and pack-years smoked than with nicotine levels, suggesting that the effect of smoking on endothelial function is due to chronic rather than acute exposure to cigarettes. Impaired endothelium-dependent arterial dilation was also observed when healthy subjects had prolonged exposure to passive environmental tobacco smoke [6]. Although Vita et al. [26] found no significant association between smoking and endothelial dysfunction of epicardial coronary arteries, others have reported a significant association between long-term cigarette smoking and impaired endothelium-dependent coronary vasodilation, regardless of the presence or absence of coronary atherosclerotic lesions [27,28]. Our results agree with these findings. At baseline, smokers with coronary artery disease tended to demonstrate more coronary constriction in response to Ach than did nonsmokers, whereas there was no difference in endothelium-independent vasodilation using nitroglycerin between the two subgroups.

Recent data from Kiowski et al. [29] show that administration of L-NMMA, an inhibitor of NO synthesis, results in a reduced vasoconstrictor response in long-term smokers. This finding suggests that cigarette smoking impairs endothelial function by inhibiting NO production. In addition, the normal vasodilator response to low-dose infusions of endothelin-1 is absent in smokers, which may represent impaired endothelial release of either NO or prostacyclin in smokers. Smoking also may cause endothelial dysfunction through formation of superoxide anions, as shown in experimental animals [30]. Superoxide anion directly impairs the function of NO [31]. Free radicals generated by cigarette smoking may increase the lipid peroxidation and the formation of oxidized LDL cholesterol, which is also known to inhibit endothelium-dependent vasodilation [32]. Hietzer and associates [33] reported increased levels of autoantibody titers to oxidized LDL in both smokers and hypercholesterolemic patients; these titers were closely related to the Ach-induced forearm blood flow response.

In the recent TREND study, we demonstrated that endothelial dysfunction can be attenuated in patients with coronary artery disease using an ACE inhibitor. These improvements occurred independently of lipid or blood pressure changes [13]. Our results in this substudy show that the beneficial effects of quinapril on endothelial dysfunction are present in both smokers and nonsmokers but that smokers apparently derive a greater benefit from ACE inhibition than do nonsmokers.

The renin-angiotensin-aldosterone system has been implicated in the development of coronary artery disease and its clinical implications in a number of epidemiologic studies. Vascular ACE is located on the surface of the endothelium, where it mediates the conversion of circulating angiotensin I to angiotensin II. Angiotensin II not only causes vasoconstriction of vascular smooth muscle, but also induces activation of endothelin-1, a potent vasoconstrictor in the absence of competing NO. Angiotensin II increases monocyte adhesion to endothelial cells [34] and decreases local fibrinolytic activity in vitro by increasing endothelial production of plasminogen activator inhibitor-1 [35]. Importantly, angiotensin II also has been shown to directly stimulate the NADH/NADPH oxidases of macrophages (foam cells) and smooth muscle cells, leading to increased generation of superoxide anion, which can degrade NO and oxidize LDL [36]. Furthermore, ACE on the endothelial surface can degrade bradykinin. Bradykinin is a potent stimulator of both NO and prostaglandin synthesis and release from endothelium, which are impaired in smokers. Both NO and prostaglandin protect the vasculature from vasoconstriction and inhibit smooth muscle cell proliferation and migration, platelet aggregability, and leukocyte adhesion. ACE inhibitors may exert their beneficial action on the endothelium in smokers through one or all of these mechanisms.

In conclusion, our results indicate that smokers tended to have a greater degree of endothelial dysfunction than nonsmokers. Treatment with quinapril, an ACE inhibitor with potent binding affinity for tissue ACE, results in improved endothelial vasodilator function in normotensive patients with coronary atherosclerosis regardless of their smoking status. However, smokers appear to derive a greater benefit than nonsmokers from ACE inhibition. Endothelial dysfunction may account for the increased frequency of myocardial infarction and coronary death observed in

smokers compared with nonsmokers. However, this substudy was too small and too brief in duration to detect differences in clinical outcomes between smokers and nonsmokers, or between treatment assignments. Furthermore, because this study was not initially designed to evaluate the differences between smokers and nonsmokers, there may be unappreciated biases confounding the results. Additional investigation into this intriguing finding is warranted.

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