

Coronary Heart Disease in Smokers

Vitamin C Restores Coronary Microcirculatory Function

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Background—Coronary endothelial function and vasomotion are impaired in smokers without coronary disease, and this is thought to be due to increased oxidative stress.

Methods and Results—We used positron emission tomography to measure the coronary flow reserve, an integrated measure of coronary flow, through both the large epicardial coronary arteries and the microcirculation in 11 smokers and 8 control subjects before and after administration of the antioxidant vitamin C. At baseline, coronary flow reserve was reduced by 21% in smokers compared with control subjects ($P<0.05$) but was normalized after vitamin C, whereas the drug had no effect in control subjects.

Conclusions—The present study is the first to demonstrate that the noxious prooxidant effects of smoking extend beyond the epicardial arteries to the coronary microcirculation and affect the regulation of myocardial blood flow. Vitamin C restores coronary microcirculatory responsiveness and impaired coronary flow reserve in smokers, which provides evidence that the damaging effect of smoking is at least in part accounted for by an increased oxidative stress. (*Circulation*. 2000;102:1233-1238.)

Key Words: blood flow ■ coronary arteries ■ circulation ■ tomography ■ smoking ■ vitamins

Cigarette smoking is a well established risk factor for cardiovascular disease,¹ and it affects both the coronary and the peripheral circulation.² Because cigarette smoke contains a large number of oxidants,³ it has been hypothesized that the adverse effects of smoking may result from oxidative damage to vascular endothelium. Indeed, endothelial dysfunction in brachial⁴ and coronary⁵ arteries has been demonstrated in long-term smokers and even in passive smokers.^{6,7}

Ascorbic acid, or vitamin C, is the main water-soluble antioxidant in human plasma⁸; it protects lipids against peroxidative damage by scavenging superoxide and other reactive oxygen species.⁹ In smokers, plasma¹⁰ and tissue¹¹ vitamin C levels are lower than in nonsmokers. In addition, vitamin C has been reported to improve endothelium-dependent vasodilation in the forearm of smokers.¹² In hypertensives, vitamin C improved endothelium-dependent vasomotion of epicardial coronary arteries,¹³ providing evidence that their coronary dysfunction is at least in part caused by increased oxidative stress.

We hypothesized that the noxious prooxidant effects of smoking extend beyond the epicardial arteries to the coronary microcirculation and affect the regulation of myocardial blood flow (MBF). To test this hypothesis, we measured

MBF and coronary flow reserve (CFR) with PET in asymptomatic smokers and in nonsmoking control subjects before and after the administration of vitamin C.

Methods

Study Population

We studied 8 male healthy nonsmokers (control subjects) with a mean age of 42 ± 5 years (range 36 to 50 years) and 11 asymptomatic male smokers with a mean age of 43 ± 4 years (range 38 to 50 years). Smokers were included if they had been smoking ≥ 1 pack of cigarettes for at least the past consecutive 10 years. They had to refrain from smoking for ≥ 3 hours before the study to minimize any relevant effect of acute smoking and short-term cessation of smoking compared with the effect of vitamin C. As a consequence of their smoking habit, the smokers' carboxyhemoglobin level was $3.5 \pm 0.9\%$ of the total hemoglobin versus $0.7 \pm 0.3\%$ in nonsmokers ($P<0.001$). None of the subjects had a history of cardiovascular disease or coronary risk factors (except for smoking). Entry criteria included normal heart rate, blood pressure, ECG, and 2-dimensional echocardiogram, as well as low clinical probability for coronary artery disease.¹⁴ The lipid profile was assessed in all individuals, and those with a total cholesterol level of >6.4 mmol/L (250 mg/100 mL) were excluded from the present study according to the exclusion criteria used in the West of Scotland (WOSCOP) Study.¹⁵ In addition, all subjects were carefully instructed to refrain from the intake of caffeine-containing beverages within 24 hours before the study. A screening test for caffeine was performed on a blood sample

Received January 21, 2000; revision received April 10, 2000; accepted April 13, 2000.

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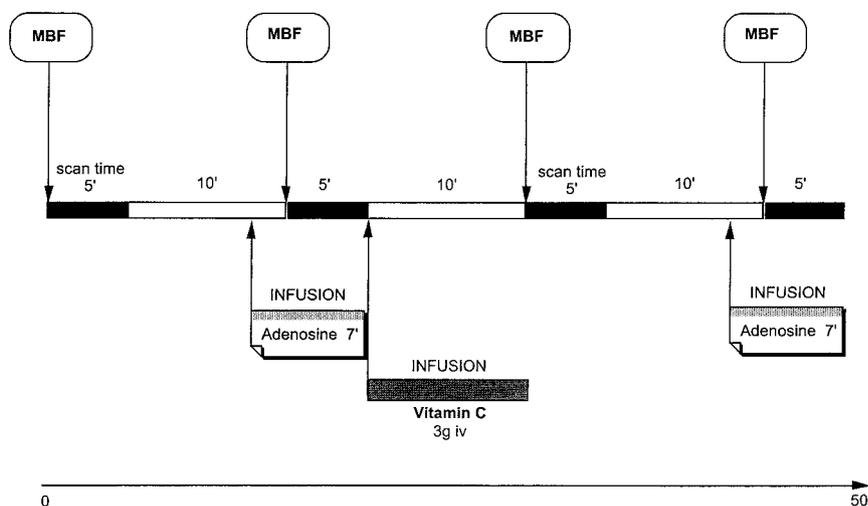


Figure 1. Study protocol.

taken immediately before the PET scan from each subject; caffeine was not detectable in any of the blood samples.

PET Scanning

Scanning was performed with an ECAT 931-08/12 15-slice tomograph that gave a 10.5-cm axial field of view (CTI/Siemens); characteristics of this tomograph have been reported previously.¹⁶ MBF was measured with ¹⁵O-labeled water (H₂¹⁵O) as reported elsewhere.¹⁷ Briefly, H₂¹⁵O (700 to 900 MBq) was injected as an IV bolus over 20 seconds at an infusion rate of 10 mL/min, and then the venous line was flushed for an additional 2 minutes with saline. The following acquisition frame times were used: 14×5 seconds, 3×10 seconds, 3×20 seconds, and 4×30 seconds.

To define regions of interest, myocardial and blood pool images were then generated directly from the dynamic H₂¹⁵O study as reported previously.¹⁸ Subsequently, regions of interest were drawn within the left atrium and ventricular myocardium on consecutive image planes. These were projected onto the dynamic H₂¹⁵O images to generate blood and tissue time activity curves. Arterial and tissue activity curves were fitted to a single tissue compartment tracer kinetic model to give values of MBF (in mL · g⁻¹ · min⁻¹) as previously described.¹⁹

CFR Calculations

MBF was measured at rest and during pharmacologically induced hyperemia with adenosine at a standardized rate²⁰ of 140 μg · kg body wt⁻¹ · min⁻¹ IV during 7 minutes. This dosage is in line with the guidelines of the American College of Cardiology and the American Heart Association²¹ for the application of adenosine in nuclear cardiac perfusion studies, and it has been shown to induce maximal myocardial hyperemia²² comparable to that achieved with intracoronary papaverine. PET flow studies have been shown to be accurate and reproducible,²³ even in patients with coronary artery disease,^{24–26} of whom many are smokers.

CFR, which is an integrated parameter of endothelial function and vascular smooth muscle relaxation, was calculated as the ratio of hyperemic to baseline MBF. In normal human subjects, myocardial oxygen consumption is linearly related to the heart rate–blood pressure product (RPP), an index of external cardiac work, and both are related to coronary blood flow.²⁷ To allow meaningful interpretation of the quantitative data, it has been proposed that resting MBF be corrected for RPP.²⁸ To account for the variability of coronary driving pressure, coronary resistance (mm Hg · min · g · mL⁻¹) was also calculated as the ratio of mean arterial pressure to MBF.

Arterial blood pressure was recorded with automatic cuff sphygmomanometry at 1-minute intervals, and the ECG was monitored continuously throughout the procedure. A 12-lead ECG was recorded at baseline and every minute during adenosine administration.

Study Protocol

A baseline CFR was assessed in all subjects. Fifteen minutes later, a repeat measurement of CFR was carried out after a 10-minute infusion of 3 g vitamin C IV (Figure 1). The dose of vitamin C was chosen to reach plasma concentrations that have been demonstrated to inhibit superoxide anion-mediated lipid peroxidation⁸ and to improve brachial²⁹ and coronary¹³ endothelial function in patients with hypertension and brachial endothelial function in smokers.¹²

Dose-Finding Substudy

Because smoking could alter the sensitivity of the coronary smooth muscles to adenosine, a dose that causes maximal dilation in nonsmoking patients may not produce the same maximal dilation in smokers. Therefore, we performed a dose-finding study to test the MBF responsiveness for 3 different doses of adenosine. In 8 additional age-matched (mean age 43±6 years) male smokers, flow was measured at rest and during the standard 140 μg · min⁻¹ · kg⁻¹ adenosine dosage. Thereafter, a second resting flow measurement was carried out followed by a second hyperemic flow measurement with a dose of adenosine that was 20% (ie, 170 μg · min⁻¹ · kg⁻¹, n=4) or 40% (ie, 200 μg · min⁻¹ · kg⁻¹, n=4) higher, with both administered as a 7-minute infusion.

TABLE 1. Hemodynamic Measurements

Measurement	Control Subjects (n=8)	Smokers (n=11)	P
Baseline			
Rest			
MAP	87±12	83±8	NS
RPP	7097±2538	7022±1165	NS
Adenosine			
MAP	84±9	84±8	NS
RPP	10 179±2667	10 537±2214	NS
Vitamin C			
Rest			
MAP	84±10	85±10	NS
RPP	7128±3006	7271±1387	NS
Adenosine			
MAP	88±11	88±9	NS
RPP	11 316±3560	11 507±2004	NS

MAP indicates mean arterial pressure (mm Hg); RPP, rate-pressure product (bpm×mm Hg).

TABLE 2. PET Measurements

Measurement	Control Subjects (n=8)	Smokers (n=11)	P
MBF			
Baseline			
Rest	0.90±0.22	0.90±0.12	NS
Adenosine	3.99±0.82	3.38±0.33	<0.05
Vitamin C			
Rest	0.94±0.18	0.99±0.18*	NS
Adenosine	4.17±0.45	4.13±0.53‡	NS
CFR			
Baseline			
Baseline	4.55±0.84	3.79±0.60	<0.05
Vitamin C	4.54±0.68	4.26±0.78*	NS

MBF indicates myocardial blood flow; CFR, coronary flow reserve.

* $P < 0.05$ vs baseline.

The study protocol was approved by the Research Ethics Committee of Hammersmith Hospital, and radiation exposure was licensed by the UK Administration of Radioactive Substances Advisory Committee. All patients gave informed and written consent before the study.

Statistical Analysis

The comparison of hemodynamic data, MBF, and CFR between baseline and drug infusion was carried out by a 1-way ANOVA for repeated measurements, with Scheffé's procedure applied when the *t* test result was statistically significant. Data are reported as mean±SD values.

Results

All procedures were well tolerated apart from the common side effects caused by adenosine, such as flushing and feeling of tightness in the chest. None of the subjects experienced any symptoms or had any ECG change during or after the infusion of vitamin C.

Hemodynamics

At the baseline study, heart rate and mean arterial blood pressure were similar in control subjects and smokers both at rest and during adenosine infusion. They remained unchanged after vitamin C infusion (Table 1). The RPP did not differ between the 2 groups during all study conditions.

MBF, CFR, and Resistance

Mean values of MBF and CFR for both groups are summarized in Table 2. At baseline, resting MBF was similar in

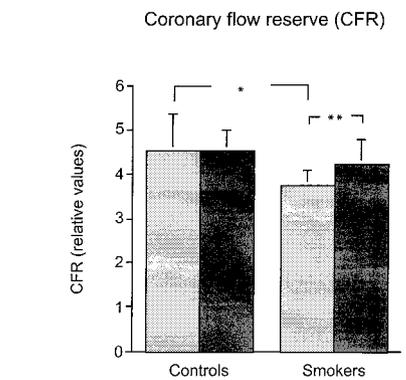
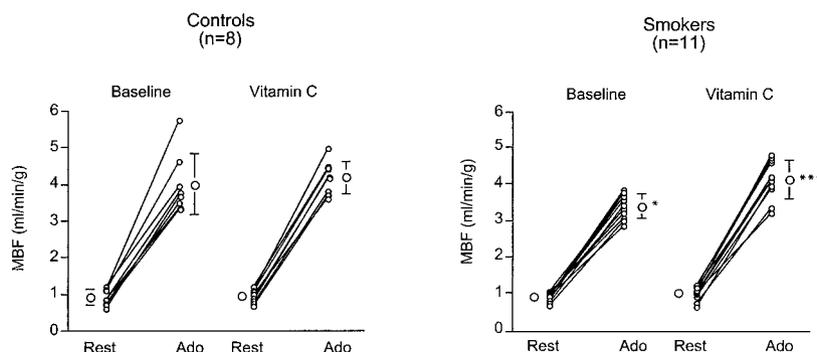


Figure 3. CFR values.

control subjects and smokers. In smokers, adenosine-induced hyperemia was reduced by 17% compared with control subjects ($P < 0.05$) (Figure 2). After vitamin C infusion, resting MBF was unchanged in control subjects but significantly increased in smokers (+11%, $P < 0.05$ versus baseline). Similarly, vitamin C did not affect hyperemic flow in control subjects but significantly increased hyperemic flow in smokers (+25%, $P < 0.001$ versus baseline), to a value comparable to that for the control subjects (Figure 2). At baseline, CFR was reduced by 21% in smokers compared with control subjects ($P < 0.05$). Coronary vasodilator reserve in smokers was normalized after vitamin C, whereas the drug had no effect in control subjects (Figure 3). Because of the similarity of RPPs between the 2 groups, correction of resting MBF and CFR for this parameter did not change the significance of these findings.

Resting coronary resistance (Table 3) was comparable in control subjects and smokers at baseline and after vitamin C infusion. Adenosine induced a greater reduction in coronary resistance in control subjects than in smokers. Vitamin C infusion significantly decreased the resistance in response to adenosine in smokers to a value comparable to that of control subjects.

Dose-Finding Substudy

In the substudy, coronary resistance fell from 102 ± 20 mm Hg \cdot min \cdot g \cdot mL⁻¹ to 26 ± 6 , 27 ± 6 , and 29 ± 6 mm Hg \cdot min \cdot g \cdot mL⁻¹ after the infusion of 140, 170, and 200 μ g \cdot min⁻¹ \cdot kg⁻¹ adenosine during 7 minutes, respectively. There was no significant difference between the minimal resistance at the 3 different adenosine doses (Figure 4).

Figure 2. Individual MBF values. Ado indicates adenosine. * $P < 0.05$ vs control subjects. *** $P < 0.001$ vs baseline.

TABLE 3. Coronary Resistance

Measurement	Control Subjects (n=8)	Smokers (n=11)	P
Baseline			
Rest	102±32	94±10	NS
Adenosine	22±4	25±3	<0.05
Vitamin C			
Rest	91±15	88±16	NS
Adenosine	21±2	21±2*	NS

Coronary resistance as an index of the microvascular function is calculated as the ratio of arterial pressure divided by myocardial blood flow and is given as mean±SD in mm Hg · min · g · mL⁻¹.

*P<0.05 vs baseline.

Discussion

The present study is the first to demonstrate that the noxious prooxidant effects of smoking extend beyond the epicardial arteries to the coronary microcirculation to affect the regulation of MBF and that a normal CFR can be restored with vitamin C. This suggests that increased production or activity of oxygen-derived free radicals contributes to vascular damage and heart disease in chronic smokers.

CFR, defined as the ratio of near-maximal to basal MBF, has been proposed as an indirect parameter to evaluate the function of the coronary circulation.³⁰ It is an integrated measure of coronary flow through both the large epicardial coronary arteries and the microcirculation. Therefore, an abnormal CFR can be due to narrowing of the epicardial arteries,¹⁹ as well as to dysfunction of the microcirculation.³¹ The latter can be caused by structural (eg, vascular remodeling with reduced lumen-to-wall ratio)³² or functional changes, which may involve neurohumoral factors or endothelial dysfunction.³³ Endothelial dysfunction has been found to be caused by coronary risk factors such as hypercholesterolemia,³⁴ essential hypertension,³⁵ diabetes mellitus,³⁶ and smoking.⁵

Endothelium-Dependent and -Independent Coronary Hyperemic Responses to Adenosine

Until recently, the vasodilator effect of adenosine was thought to be based solely on the direct stimulation of A₂ adenosine receptors on vascular smooth muscle cells, which mediate an increase in the second-messenger cAMP by

stimulating adenylate cyclase. Therefore, this agent has been used frequently in animal and human studies to evaluate endothelium-independent vasodilation.³⁷ However, in the past decade, it has been appreciated that adenosine also acts as an endothelium-dependent vasodilator,³⁸ both via flow-mediated dilation³⁹ and via direct stimulation of A₁ adenosine⁴⁰ and other purinergic⁴¹ receptors on endothelial cells. Although our results reflect coronary microcirculatory function,⁴² with the use of adenosine, no definite evidence can be provided on whether the reduction in flow reserve in smokers is due to endothelium-dependent or -independent mechanisms. However, based on experimental data, it is most likely that the endothelium is the source of the oxidative stress.⁴³

Mechanisms of Smoking-Associated Vascular Damage

Our findings are in agreement with previous observations in smokers that show blunted endothelium-dependent vasodilation in the coronary⁵ and brachial^{4,6} arteries. The findings of the present study extend these observations and demonstrate that smoking leads to a dysfunction of the coronary microcirculation.

Although the mechanisms of smoking-associated vascular damage are not yet fully established, several factors have been proposed. Nicotine has been shown to produce structural damage in aortic endothelial cells of animals.⁴⁴ Smoking is associated with a direct toxic effect on human endothelial cells.⁴⁵ The gas phase of cigarette smoke contains large amounts of free radicals and prooxidants, and the particulate phase contains high concentrations of lipophilic quinones,³ which can form the highly reactive hydroxylperoxide radical (OH[•]). In addition, the vasoactive level of nitric oxide can be reduced by superoxide anion (O^{•2-}) that directly originates from cigarette smoke and results in the formation of peroxynitrite anion (ONOO⁻), a highly reactive compound with strong cytotoxic potency.⁴⁶ In addition, these oxidants may increase the amount of oxidized LDL, which is markedly more effective than native LDL in causing endothelial⁴⁶ and microcirculatory dysfunction through the reduction in nitric oxide synthesis.⁴⁷

In the present study, we have shown that the short-term administration of the antioxidant vitamin C restores coronary microcirculatory responsiveness and impaired CFR in smokers without having an effect in nonsmoking control subjects. This supports the hypothesis⁴⁸ that the damaging effect of smoking is at least in part explained by an increased oxidative stress and is in line with the results of a recent study⁴⁹ in which reduced glutathione, another antioxidant, was shown to improve endothelial dysfunction in patients with cardiovascular risk factors but had no effect in subjects without risk factors. Similarly, vitamin C has been reported to attenuate abnormal coronary vasomotor reactivity in patients with vasospastic angina by scavenging oxygen free radicals.⁵⁰

A shift of the dose-response curve to adenosine in smokers as a cause for the reduced hyperemic response can be excluded on the basis of our dose-finding study.

Study Limitations

It cannot be entirely excluded that some of the smokers had epicardial coronary artery disease (albeit without significant

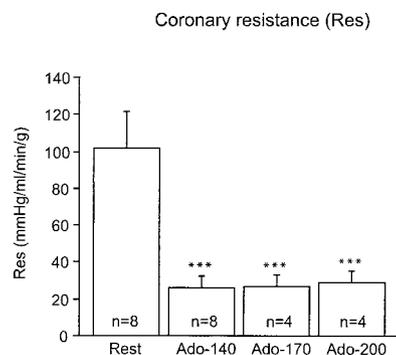


Figure 4. Dose-finding study. Ado indicates adenosine; Res, coronary resistance. ***P<0.0001 vs rest.

stenosis), which in turn would have induced endothelial dysfunction. This could have been ruled out with certainty only with coronary angiography, which seemed unjustified in these asymptomatic volunteers. With current techniques, the distinction between endothelial dysfunction due to early and nonobstructive coronary artery disease and endothelial dysfunction due to the effect of smoking cannot be made with absolute certainty. However, endothelial dysfunction due to smoking may represent an early stage in the development of coronary artery disease. None of the subjects had hypertension, diabetes, hyperlipidemia, or a history of coronary artery disease or atherosclerosis (determined by the absence of angina, intermittent claudication, and cerebrovascular disease). Thus, their clinical risk for coronary artery disease was assessed as low.¹⁴ In addition, it has been recently demonstrated that even in patients with mild coronary artery disease, CFR assessed with PET can still be used to evaluate and follow up the functional response of the coronary circulation.²⁶

Clinical Implications

Our findings provide evidence that the short-term administration of vitamin C almost completely reverses microcirculatory dysfunction in asymptomatic smokers. Because PET flow studies have been shown to be accurate,^{51–53} the method appears appropriate for the study of the effects of any intervention with each subject used as his or her own control. This was confirmed in a recent reproducibility study from our laboratory.²³

Although our study design does not allow us to comment on long-term effects of vitamin C, these effects might be worth testing in a large-scale trial of whether daily oral vitamin C as a dietary supplement has preventive effects on the development of coronary artery disease in smokers. In fact, the larger amount of vitamin C in the Mediterranean diet⁵⁴ could contribute to the fact that in northern Europe, the absolute risk of coronary artery disease is higher than that in the Mediterranean area,⁵⁵ despite the higher prevalence of smoking among the Mediterranean populations.⁵⁶ Although a recent report found prooxidant properties of vitamin C when given as a dietary supplement at a dosage of 500 mg/d in healthy volunteers,⁵⁷ this does not necessarily apply to smokers because the latter have reduced plasma¹⁰ and tissue¹¹ levels of vitamin C due to dietary differences⁵⁸ and to increased consumption as the result of a greater oxidative stress.⁵⁹

Acknowledgments

Dr Kaufmann was funded by a grant from the Swiss National Science Foundation (SCORE B, grant 3232-055002.98). Dr Schäfers was funded by a grant from the "Interdisziplinäre Medizinische Forschung" (grant Sc-1-5-II/96-20) of the University of Muenster. We are grateful to Flemming Hermansen for the use of his software and to the staff of the MRC Cyclotron Unit, especially the radiographers Andy Blyth and Hope McDevitt, for their excellent technical assistance.

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Circulation. 2000;102:1233-1238

doi: 10.1161/01.CIR.102.11.1233

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:

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