# Acute effect of oral vitamin C on coronary circulation in young healthy smokers

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**Background** Recent studies suggest that smokers' coronary endothelial function is impaired because of increased oxidative stress, and their coronary flow velocity reserve (CFVR) is reduced. It is uncertain whether oral antioxidant vitamin C restores impaired CFVR in smokers. Recent technological advances in transthoracic Doppler echocardiography (TTDE) have resulted in the successful measurement of coronary flow velocity and noninvasive CFVR assessment.

**Methods** We studied 13 healthy young male smokers and 12 nonsmokers. Coronary flow velocities in the left anterior descending coronary artery (LAD) were recorded with TTDE at rest and during hyperemia induced with intravenous infusion of adenosine triphosphate (ATP). CFVR was calculated as the ratio of hyperemic to basal mean diastolic flow velocity. CFVR and plasma concentrations of vitamin C were assessed at baseline and 2 and 4 hours after oral intake (2 g).

**Results** Heart rate and blood pressure responses to ATP infusion were not affected by oral vitamin C, but plasma concentrations of vitamin C increased to physiological levels in both groups. CFVR was significantly higher in nonsmokers than in smokers at baseline (4.3 ± 0.4 vs 3.8 ± 0.8, $P < .05$). After oral vitamin C, it was increased significantly in smokers (3.8 ± 0.8 to 4.5 ± 0.7, $P < .005$, 4.5 ± 0.8, $P < .005$, respectively), but not in nonsmokers (4.3 ± 0.4 to 4.3 ± 0.3, 4.4 ± 0.7).

**Conclusions** This study demonstrated that oral vitamin C restores coronary microcirculatory function and impaired CFVR against oxidative stress in smokers. (Am Heart J 2004;148:300–5.)

Vitamin C is the most effective aqueous-phase antioxidant in human blood plasma, and it is a physiological antioxidant of major importance for protection against diseases and degenerative processes caused by oxidant stress.[1–3] Cigarette smokers are known to be exposed to a large number of oxidants,[4,5] and oxidative stress on the vascular endothelium has been implicated in the development of atherosclerosis associated with coronary and peripheral vascular disease.[6,7] Indeed, endothelial dysfunction in coronary[8] and brachial arteries[9] has been demonstrated in smokers. Furthermore, it has been demonstrated that coronary flow velocity reserve (CFVR) is reduced in smokers.[10,11]

Previous reports have demonstrated that vitamin C improves endothelium-dependent vasodilatation in the forearm of smokers.[12,13] Kaufmann et al[10] have shown that vitamin C restores coronary flow reserve, one index of coronary microcirculation, using positron emission tomography (PET). However, because 3 g of vitamin C was given intravenously over ten minutes, the plasma concentrations of vitamin C were probably much higher than by oral intake.[14,15] To our knowledge, the effect of oral vitamin C on coronary circulation remains unknown at physiological plasma concentrations.

Recent technological advances in transthoracic Doppler echocardiography (TTDE) have resulted in the successful measurement of coronary flow velocity and noninvasive CFVR assessment.[16–21] The purpose of this study was to assess the acute effect of oral vitamin C at physiological plasma concentration levels on coronary circulation in smokers using the measurement of CFVR with TTDE.

## Methods

### Subjects

We studied 12 male healthy nonsmokers with a mean age of 26 ± 4 years (range, 21–35 years) and 13 male smokers with a mean age of 29 ± 8 years (range, 20–49 years). These subjects were recruited from among hospital staffs. Nonsmokers did not have regular exposure to environmental tobacco smoke. In smokers, the daily number of cigarettes ranged from 10 to 50 (mean, 19.2 ± 10.0), and they all had a smoking history of at least 1.5 years (10.1 ± 8.8 pack-years' history of smoking). They had to refrain from smoking for at

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least 3 hours before the study to minimize any effect of acute smoking and short-term cessation of smoking compared with the effect of vitamin C.

None of the subjects had a history of hypertension, diabetes mellitus, hyperlipidemia, obesity, or other risk factors for coronary artery disease, and none were taking antioxidant vitamins or cardioactive drugs.

All subjects gave their written informed consent after thorough explanation of the study design and protocol before enrollment into this study.

### Study protocol

In all subjects, CFVR was assessed at baseline, and 2 and 4 hours after an oral administration of vitamin C (2 g). The dose of vitamin C was chosen so as to reach plasma concentrations that have been demonstrated to improve brachial endothelial function in smokers.[12,22]

Blood samples were taken immediately before ultrasound scanning examination and 2 and 4 hours after the initial 2 g dose of vitamin C intake for the determination of the plasma carboxyhemoglobin (HbCO) level, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and blood sugar levels and concentrations of vitamin C. The plasma HbCO level was determined with spectrophotometry as a parameter of smoking. The plasma concentrations of vitamin C were determined by high performance liquid chromatography (normal range, 10.8–85.2 μmol/L).

### Measurements of CFVR with TTDE

The method of measurements of CFVR with TTDE was described previously.[19–21] Coronary flow velocities in the left anterior descending coronary artery (LAD) were recorded with TTDE (Acuson Sequoia 512, Mountainview, Calif) using a frequency of 5 to 7 MHz (Doppler frequency, 3.5 MHz) with color flow mapping guidance. We tried to align the ultrasound beam direction with the LAD flow in as parallel a manner as possible, but the angle needed to be corrected for each examination because of the incident Doppler angle (mean angle, 38°; range, 10–61°).

Coronary flow velocity was measured at rest and during hyperemia induced by intravenous infusion of adenosine triphosphate (ATP) for 2 minutes (0.14 mg/kg/min). Arterial blood pressure and heart rate were recorded with automatic cuff sphygmomanometry at 1-minute intervals, and the electrocardiogram was monitored continuously throughout the procedure.

The investigators performing the measurements were blinded to the subjects' smoking status. Mean diastolic velocities were measured at rest and peak hyperemia by tracing contours of spectral Doppler signals using the software incorporated in the ultrasound system. An average of the measurements was obtained for 3 cardiac cycles. CFVR was calculated as the ratio of hyperemic to rest mean diastolic flow velocity.

### Statistical analysis

Parametric data were presented as mean plus or minus SD. Group comparisons with respect to baseline characteristics were performed with the unpaired *t* test.

**Table I.** Clinical characteristics of the 2 study groups

| | Smokers (n = 13) | Nonsmokers (n = 12) | *P* |
|---|---|---|---|
| Age (y) | 29 ± 8 | 26 ± 4 | .29 |
| BMI | 23 ± 3 | 23 ± 2 | .56 |
| HbCO (%) | 2.3 ± 1.3† | 0.7 ± 0.3 | .0004 |
| Lipids (mg/dL) | | | |
| Total cholesterol | 190 ± 46 | 187 ± 33 | .84 |
| LDL cholesterol | 107 ± 43 | 106 ± 30 | .97 |
| HDL cholesterol | 63 ± 27 | 68 ± 14 | .57 |
| Triglycerides | 82 ± 118 | 127 ± 96 | .22 |
| Blood sugar (mg/dL) | 110 ± 20* | 92 ± 17 | .026 |

Data are presented as mean ± SD. *BMI,* Body mass index; *HbCO,* carboxyhemoglobin; *LDL,* low-density lipoprotein; *HDL,* high-density lipoprotein.
*$P < .05$ vs nonsmokers.
†$P < .005$ vs nonsmokers.

Echocardiographyic and hemodynamic variables during ATP infusion in smokers and nonsmokers were evaluated by repeated measures analysis of variance (ANOVA), testing for the vitamin C effect, ATP effect, group effect and interaction. The Fisher protested least-significant difference test was used for the post hoc test. For all analyses, $P < .05$ was considered significant.

## Results

Clear envelopes of basal and hyperemic coronary flow velocity in the distal LAD were obtained in all subjects (100%) with the guidance of color Doppler flow mapping. None of the subjects experienced any symptoms or showed any electrocardiogram changes during ATP administration.

### Subject characteristics

The clinical characteristics of the 2 study groups are provided in Table I. There were no differences between smokers and nonsmokers in terms of age, body mass index, and lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride levels). However, plasma blood sugar and HbCO levels were significantly higher in smokers than in nonsmokers. However, the plasma blood sugar levels in smokers were still within normal range.

Lipid profiles (total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride levels) and blood sugar levels during all study conditions are summarized in Table II. There were no differences in the 2 groups during any of the study conditions. Although blood sugar levels in both groups increased significantly at 4 hours after the administration of vitamin C compared with baseline, there was no correlation with CFVR ($r = 0.13$).

### Plasma concentrations of vitamin C

At baseline, plasma concentrations of vitamin C tended to be higher in nonsmokers than in smokers

**Table II.** Lipid profile and blood sugar

| | Total cholesterol (mg/dL) | HDL cholesterol (mg/dL) | LDL cholesterol (mg/dL) | TG (mg/dL) | BS (mg/dL) |
|---|---|---|---|---|---|
| Smokers | | | | | |
| Baseline | 190 ± 46 | 63 ± 27 | 107 ± 43 | 182 ± 118 | 110 ± 20 |
| Post 2 h | 182 ± 47 | 60 ± 27 | 101 ± 40 | 153 ± 121 | 119 ± 15 |
| Post 4 h | 180 ± 50 | 58 ± 28 | 102 ± 41 | 165 ± 120 | 132 ± 22* |
| Nonsmokers | | | | | |
| Baseline | 187 ± 33 | 68 ± 14 | 106 ± 37 | 127 ± 96 | 92 ± 17 |
| Post 2 h | 179 ± 32 | 62 ± 12 | 101 ± 35 | 139 ± 110 | 109 ± 28 |
| Post 4 h | 183 ± 32 | 64 ± 14 | 103 ± 28 | 121 ± 107 | 123 ± 35† |

*Post 2 h,* Two hours after administration of vitamin C; *Post 4 h,* four hours after administration of vitamin C; *HDL,* high-density lipoprotein; *LDL,* low-density lipoprotein; *TG,* triglyceride; *BS,* blood sugar.
*$P < .05$ vs baseline of each group.
†$P < .005$ vs baseline of each group.

**Table III.** Plasma concentrations of vitamin C

| | Baseline | Post 2 h | Post 4 h |
|---|---|---|---|
| Smokers (μmol/L) | 32 ± 16 | 58 ± 21* | 73 ± 16*† |
| Nonsmokers (μmol/L) | 39 ± 12 | 79 ± 39* | 94 ± 24*‡ |

*Post 2 h,* Two hours after oral administration of vitamin C; *Post 4 h,* four hours after oral administration of vitamin C.
*$P < .005$ vs baseline.
†$P < .05$ vs post 2 h.
‡$P < .05$ vs smokers.

(39 ± 12 μmol/L vs 32 ± 16 μmol/L), but without significant difference ($P = .22$; Table III).

After oral administration of vitamin C, plasma concentrations significantly increased in both groups. They were not significantly different in nonsmokers and smokers 2 hours after oral vitamin C (79 ± 39 μmol/L vs 58 ± 21 μmol/L; $P = .09$), but the difference became significant at 4 hours (94 ± 24 μmol/L vs 73 ± 16 μmol/L; $P = .03$). In addition, in smokers, the difference between the levels at 2 and 4 hours was significant ($P < .05$).

### Hemodynamics

As shown in Table IV, systolic and diastolic blood pressures, heart rate, and rate-pressure products did not differ significantly in the 2 groups.

### CFVR

Coronary flow velocities of both groups are summarized in Table V. At baseline, resting coronary flow velocities in nonsmokers and smokers were similar. In smokers, coronary flow velocity during hyperemia was reduced significantly compared with nonsmokers ($P < .05$). This parameter was quite similar in the 2 groups at both 2 and 4 hours after oral vitamin C. Thus, CFVR in nonsmokers was significantly higher than that in smokers at baseline (4.3 ± 0.4 vs 3.8 ± 0.8, $P < .05$), but it did not differ significantly at 2 and 4 hours after oral vitamin C intake (4.3 ± 0.3 vs 4.5 ± 0.7, $P = .45$, 4.4 ± 0.7 vs 4.5 ± 0.8, $P = .55$). This means that CFVR in smokers was significantly restored by the oral administration of vitamin C (Figure 1).

### Observer variability

Interobserver and intraobserver variabilities for the measurement of Doppler velocity recordings were 5.0% and 3.9%, respectively.

## Discussion

This study is the first to demonstrate that oral administration of vitamin C at physiological plasma concentrations improves impaired CFVR in young healthy smokers to levels similar to those of nonsmokers. The effect of a single dose of oral vitamin C already appeared at 2 hours after its intake, and it continued for >2 hours.

### Oral administration and plasma concentration of vitamin C

We administered a 2-g dose of vitamin C according to the protocol of prior studies,[12,22] and the plasma concentrations attained were assumed to be mostly still within physiological range. Levine et al demonstrated vitamin C pharmacokinetics in healthy volunteers.[15] When vitamin C is given orally, it is completely absorbed until the dose exceeds 200 mg. Consumption of 200 mg of vitamin C daily results in a plasma concentration of approximately 70 μmol/L, and higher vitamin C doses (>200 mg) do not increase long-term vitamin C concentrations because of decreased absorption and increased renal excretion, which begin when plasma concentrations exceed 60 to 70 μmol/L. Kaufmann et al demonstrated with PET

**Table IV.** Hemodynamics

| | Baseline rest | Baseline hyperemia | Post 2 h rest | Post 2 h hyperemia | Post 4 h rest | Post 4 h hyperemia |
|---|---|---|---|---|---|---|
| Smokers | | | | | | |
| BPs | 109 ± 14 | 106 ± 13 | 105 ± 12 | 105 ± 14 | 106 ± 12 | 106 ± 11 |
| BPd | 57 ± 10 | 52 ± 10 | 52 ± 10 | 50 ± 11 | 54 ± 10 | 53 ± 8.6 |
| HR | 68 ± 8.9 | 73 ± 10 | 63 ± 8.6 | 70 ± 13 | 63 ± 7.5 | 61 ± 12 |
| RPP | 7448 ± 1750 | 7736 ± 1757 | 6746 ± 1605 | 7499 ± 2000 | 6711 ± 1317 | 6584 ± 1840 |
| Nonsmokers | | | | | | |
| BPs | 106 ± 11 | 105 ± 13 | 103 ± 7.7 | 103 ± 9.2 | 104 ± 9.2 | 101 ± 8.3 |
| BPd | 50 ± 8.9 | 50 ± 9.9 | 50 ± 9.5 | 47 ± 10 | 48 ± 9.4 | 50 ± 9.8 |
| HR | 63 ± 8.4 | 71 ± 10.5 | 62 ± 8.3 | 64 ± 9.4 | 62 ± 9.7 | 65 ± 11 |
| RPP | 6667 ± 1363 | 7437 ± 1757 | 6396 ± 1202 | 6663 ± 1422 | 6506 ± 1503 | 6620 ± 1592 |

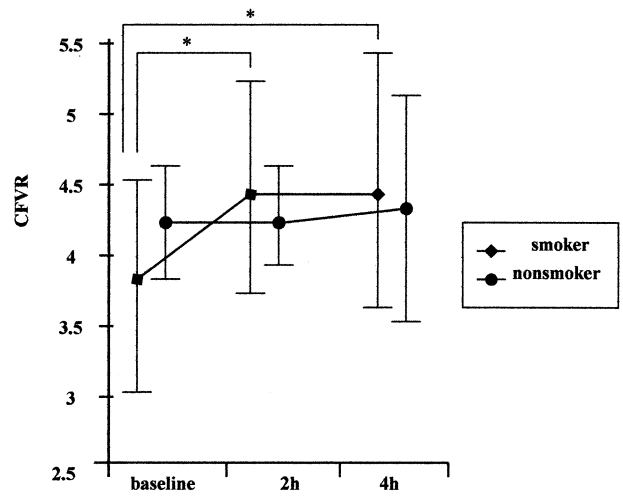
*BPs,* Systolic blood pressure (mm Hg); *BPd,* diastolic blood pressure (mm Hg); *HR,* heart rate (beats/min); *RPP,* rate-pressure product (beats/min × mm Hg); *Post 2 h,* Two hours after administration of vitamin C; *Post 4 h,* four hours after administration of vitamin C.

**Table V.** Coronary flow velocity

| | Baseline (cm/s) | Post 2 h (cm/s) | Post 4 h (cm/s) |
|---|---|---|---|
| Smokers | | | |
| Rest | 17.2 ± 4.3 | 17.3 ± 6.2 | 16.5 ± 4.1 |
| Hyperemia | 63.1 ± 14.2* | 77.1 ± 27.0 | 74.2 ± 22.2 |
| Nonsmokers | | | |
| Rest | 18.7 ± 5.6 | 16.9 ± 3.1 | 17.8 ± 3.8 |
| Hyperemia | 78.8 ± 22.9 | 74.8 ± 15.4 | 76.7 ± 15.5 |

*Post 2 h,* Two hours after administration of vitamin C; *Post 4 h,* four hours after administration of vitamin C.
*$P < .05$ vs nonsmokers.

**Figure 1**




Coronary flow velocity reserve in nonsmokers was significantly higher than in smokers at baseline, whereas it did not differ at 2 and 4 hours after administration of vitamin C. *$P < .005$.

that the intravenous administration of vitamin C restores coronary flow reserve in smokers.[10] However, Levine et al also noticed that when vitamin C is given intravenously, the limiting mechanisms are bypassed and much higher concentrations are achieved, and they estimated that 3 g of intravenous vitamin C, as was given in the study by Kaufmann et al, will result in a plasma concentration of approximately 1500 μmol/L, which is much higher than the physiological range.[14] In our study, it was demonstrated for the first time that vitamin C at physiological plasma concentrations improved CFVR in smokers.

## Smoking and CFVR

In this study, all the subjects were young and did not exhibit any coronary risk factors such as hypertension, diabetes mellitus, or hyperlipidemia, the exception being cigarette smoking, and none of the subjects had a history of coronary artery disease. Thus the clinical risk for coronary artery disease was considered to be very low,[23,24] and the subjects in our study were considered to be appropriate for estimating the effect of vitamin C on smokers' coronary circulation.

CFVR in smokers were reduced in this study, and our finding agreed with previous observations in smokers that showed impaired endothelium-dependent vasodilatation in the coronary[8] and brachial arteries.[9] It is thought that the damaging effect of smoking is at least in part explained by increased oxidative stress.[4,5] Additionally, antioxidant vitamin C restored the coronary circulation of smokers.

Because these findings strongly demonstrate that smoking is a primary risk factor of coronary vascular disease, the clear public health message is the importance of quitting smoking.

Raitakari et al[12] demonstrated that in smokers, oral vitamin C restored impaired flow-mediated dilatation of

the brachial artery, 1 index of endothelial function. Although it is not directly related to coronary circulation, peripheral flow-mediated dilatation is thought to correlate modestly with the endothelium function of coronary arteries.[25,26]

No previous studies have reported on the long-term effect of oral vitamin C on coronary circulation. These effects might be worth testing in a large-scale trial to determine whether daily oral vitamin C as a dietary supplement has preventive effects on the development of coronary artery disease in smokers.

### ATP-induced CFVR

Improvement of endothelial function with vitamin C possibly played a partial, but important, role in the improvement of ATP-induced CFVR in this study. It is known that blood flow response to adenosine is caused primarily by interaction with the $A_2$ receptor on vascular smooth muscle cell and is considered endothelial-independent. However, some studies have indicated that endothelial dysfunction should also affect the blood flow response to adenosine.[27–29] Buus et al reported that the inhibition of endogenous nitric oxide synthesis attenuates myocardial perfusion during adenosine-induced hyperemia, indicating that coronary vasodilatation by adenosine is partly endothelium-dependent.[29] Thus, impaired coronary flow reserve has been suggested as providing information about the integrated measure of both vascular endothelial function and smooth muscle relaxation of the coronary artery. Furthermore, ATP was reported to be equivalent to adenosine for assessing coronary flow reserve.[30]

### CFVR assessed with TTDE

In this study, we measured CFVR with TTDE as an index of coronary microcirculation. Although coronary flow reserve is ideal for assessing the function of coronary microcirculation, coronary flow velocity changes were measured in the epicardial coronary artery, but not coronary flow volume. Velocity changes only reflect volume flow changes in the condition that the cross-sectional area does not change. Previous reports demonstrated that angiographically smooth human coronary arteries dilated in response to an increase in coronary blood flow and to the local administration of an endothelium-dependent vasodilator.[31] Adenosine-induced increases in coronary blood flow results in endothelium-dependent dilatation of epicardial coronaries as aforementioned, so coronary volumetric flow reserve is generally greater than coronary velocity reserve. However, there was significantly strong positive correlation between coronary velocity reserve and coronary volumetric flow reserve as measured with intracoronary Doppler ultrasound scanning and quantitative angiography.[32] There was also good agreement between CFVR as assessed with Doppler guide wire and the results of perfusion scintigraphy and PET.[33,34] Moreover, assessment of CFVR with TTDE, which has been confirmed to accurately reflect the results of the invasive measurement by Doppler guide wire,[19,20] permits rapid, reproducible, and totally noninvasive assessment of coronary blood flow at a low cost. TTDE, therefore, permitted the repeated evaluation of the efficacy of oral vitamin C for coronary circulation in this study.

In conclusion, the oral administration of the antioxidant vitamin C at physiological plasma concentrations has powerful effects on the restoration of impaired CFVR in smokers.

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