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J. Am. Coll. Cardiol. 2003;42;814-822
doi:10.1016/S0735-1097(03)00851-9

This information is current as of February 10, 2012

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

<http://content.onlinejacc.org/cgi/content/full/42/5/814>

JACC

JOURNAL of the AMERICAN COLLEGE of CARDIOLOGY



Coronary Vasoregulation in Patients With Various Risk Factors in Response to Cold Pressor Testing

Contrasting Myocardial Blood Flow Responses to Short- and Long-Term Vitamin C Administration

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- OBJECTIVES** We sought to determine whether abnormal myocardial blood flow (MBF) responses to the cold pressor test (CPT) in patients with various risk factors may involve different mechanisms that could lead to varying responses of short- and long-term administration of antioxidants.
- BACKGROUND** There is a growing body of evidence that increased vascular production of reactive oxygen species markedly reduces the bioavailability of endothelium-derived nitric oxide, leading to impaired vasodilator function. It is unknown whether increased oxidative stress is the prevalent mechanism underlying endothelial dysfunction in patients with different coronary risk factors.
- METHODS** Fifty patients with normal coronary angiograms were studied. The MBF responses to CPT was determined by means of positron emission tomography before and after intravenous infusion of 3 g vitamin C or saline (placebo), as well as after 3 months and 2 years of 2 g vitamin C or placebo supplementation daily.
- RESULTS** In hypertensive patients, the change in MBF (Δ MBF) was not modified significantly by short-term vitamin C administration challenges (0.20 ± 0.20 ml/g/min; $p = \text{NS}$) but was significantly increased after three months and two years of treatment with vitamin C versus baseline (0.58 ± 0.27 and 0.63 ± 0.17 vs. 0.14 ± 0.18 ml/g/min; both $p \leq 0.001$). In smokers, Δ MBF in response to CPT was significantly increased after short-term vitamin C infusion and long-term vitamin C treatment (0.52 ± 0.10 , 0.54 ± 0.13 , 0.50 ± 0.07 vs. -0.08 ± 0.10 ml/g/min; all $p \leq 0.001$). In hypercholesterolemic patients, no improvement in Δ MBF during CPT was observed after short- and long-term vitamin C treatment (0.05 ± 0.14 , 0.08 ± 0.18 , 0.02 ± 0.19 vs. 0.08 ± 0.16 ml/g/min; $p = \text{NS}$). The CPT-induced Δ MBF in hypertensive patients and smokers after follow-up was significant as compared with placebo and control subjects ($p \leq 0.001$).
- CONCLUSIONS** The present study revealed marked heterogeneous responses in MBF changes to short- and long-term vitamin C treatment in patients with various risk factors, which highlights the quite complex nature underlying abnormal coronary vasomotion. (J Am Coll Cardiol 2003; 42:814–22) © 2003 by the American College of Cardiology Foundation

The vascular endothelium plays a crucial role in the regulation of vasomotor tone and function by synthesizing and releasing a variety of substances (1). These include vasoconstricting and vasodilating factors, whereby endothelium-derived relaxing factors, such as prostacycline, hyperpolarizing relaxing factor, and, most importantly, nitric oxide (NO) (1) have been identified. Although the mechanism underlying abnormal endothelial-dependent vascular tone is

likely to be multifactorial, there is a growing body of evidence that increased vascular production of reactive oxygen species (ROS) derived from enzymes, such as nicotinamide-adenine-dinucleotide phosphate oxidase, xanthine oxidase, and uncoupled endothelial nitric oxide synthase (eNOS), markedly reduces the bioavailability of endothelium-derived NO, leading to impaired vasodilator function (1–3). Until recently, the evaluation of endothelial-dependent coronary vasomotion in humans focused primarily on the intracoronary administration of acetylcholine, bradykinin, or substance P (4). Importantly, recent studies have demonstrated that abnormal endothelial-dependent vasoreactivity of conduit and resistance vessels in response to acetylcholine reveals a significant disparity (5,6). Whether this may also apply to a physiologically more relevant endothelial function test, such as flow-dependent dilation and sympathetic activation by cold pressor testing (CPT), remains to be determined (1,6).

Accordingly, the aims of this study were to: 1) assess the

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Manuscript received November 23, 2002; revised manuscript received May 8, 2003, accepted May 13, 2003.

Abbreviations and Acronyms

ANOVA	= analysis of variance
CPT	= cold pressor test(ing)
CVR	= coronary vascular resistance
eNOS	= endothelial nitric oxide synthase
LAD	= left anterior descending coronary artery
LCx	= left circumflex artery
LDL	= low-density lipoprotein
HDL	= high-density lipoprotein
MBF	= myocardial blood flow
NO	= nitric oxide
PET	= positron emission tomography
ROS	= reactive oxygen species
RPP	= rate-pressure product

relationship between endothelial function of epicardial conductance and resistance vessels in response to flow-dependent dilation and CPT, respectively; and 2) examine whether CPT-induced changes in myocardial blood flow (MBF) may respond differently to short- and long-term treatment with antioxidants.

METHODS

Study population and design. Fifty patients (29 men and 21 women; mean age 59 ± 3 years) without angiographic evidence of coronary artery disease were studied. We included 20 hypertensive patients (systolic blood pressure ≥ 145 mm Hg), 10 chronic smokers (32 ± 9 pack-years), and 10 hypercholesterolemic patients (total serum cholesterol $\geq 263 \pm 10$ mg/dl, low-density lipoprotein [LDL] cholesterol $\geq 168 \pm 8$ mg/dl, and high-density lipoprotein [HDL] cholesterol $\geq 46 \pm 5$ mg/dl). Ten patients without coronary risk factors served as the control group.

Quantitative coronary angiographic evaluation at baseline and during CPT to establish flow-mediated vasoreactivity of epicardial coronary artery was performed as described (7) (Fig. 1). Follow-up with respect to MBF changes in response to CPT were assessed noninvasively by dynamic positron emission tomography (PET) after three months and two years of follow-up. Hypertensive patients were randomized into a placebo ($n = 8$) and vitamin C group ($n = 12$), whereas chronic smokers, hypercholesterolemic patients, and control subjects were assigned to an open-label

treatment with 2 g vitamin C daily during 2-year follow-up (Fig. 1).

Exclusion criteria included a history of unstable angina pectoris, previous myocardial infarction, malignant hypertension, diabetes mellitus, evidence of glucose intolerance, cardiac autonomic neuropathy, valvular heart disease, peripheral vascular disease, and significant endocrine, hepatic, renal, and inflammatory disease, as well as a regular dietary intake of antioxidants. Only patients not on vasoactive medication, such as angiotensin-converting enzyme inhibitors, calcium channel blockers, or statins at baseline and/or throughout the study period of two years, were recruited. In hypertensive patients, a regular intake of diuretics and beta-blockers was allowed for blood pressure control. Each hypertensive patient treated with beta-blockers discontinued the medication seven days before each study. In addition, during this period, patients were asked to use a short-term calcium antagonist to control high blood pressure. In these patients, study sessions were only performed if patients did not use a short-acting calcium antagonist for at least 24 h before the investigation. Hypercholesterolemic patients were included in the study if they were not receiving statin therapy at baseline and throughout the follow-up period. During the study period, however, patients were free to begin statin medication, and, if so, they were not considered for the follow-up evaluation. Laboratory analysis included total cholesterol, HDL and LDL cholesterol, very-LDL cholesterol, triglycerides, glucose (8), and plasma vitamin C levels (9). The dose of vitamin C was chosen in view of previous studies (10). All patients underwent echocardiographic studies to evaluate the left ventricular mass index and left ventricular ejection fraction evaluation at baseline and after two-year follow-up. The study was approved by the local ethics committee of the University of Freiburg, and written, informed consent was obtained from all patients.

Quantitative coronary angiography. Coronary angiography was performed using a biplane, isocentric, multidirectional digital angiographic system (Siemens BICOR-HICOR, Erlangen, Germany). End-diastolic images of coronary arteries were evaluated quantitatively with automatic contour detection, as described previously (7). In all 50 patients with normal coronary angiograms, as indicated

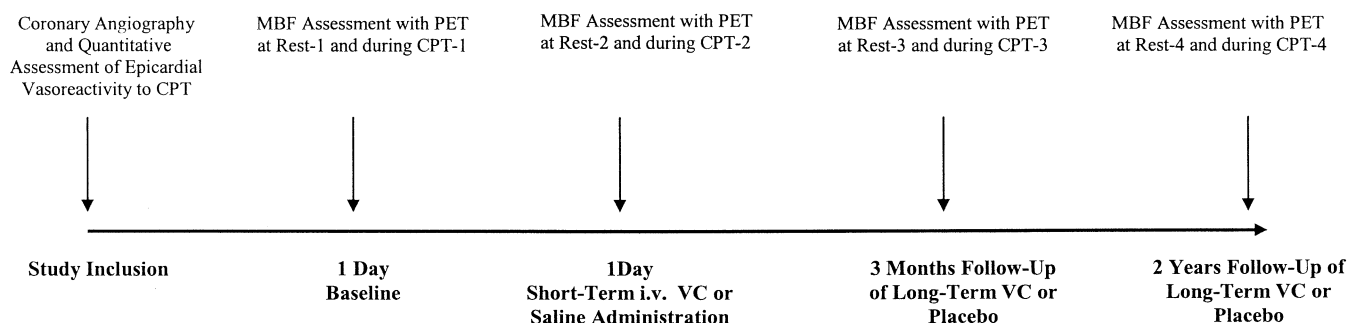


Figure 1. Study protocol. CPT = cold pressor test; MBF = myocardial blood flow; PET = positron emission tomography; VC = vitamin C.

by a smooth coronary vessel appearance without evidence of lumen wall irregularities or diffuse-caliber reduction and stenosis, quantitative measurements were performed biplane in a selected, distinct 4- to 8-mm-long, relatively straight proximal left anterior descending coronary artery (LAD) segment ($n = 21$) or left circumflex coronary artery (LCx) segment ($n = 29$).

Noninvasive quantification of MBF. Myocardial blood flow was measured noninvasively by using intravenous nitrogen-13 (^{13}N)-labeled ammonia serial image acquisition with dynamic PET (951 ECAT-HR, Siemens) and a two-compartment tracer kinetic model, as described (11). The relative myocardial perfusion was assessed visually on re-orientated, static ^{13}N -ammonia images, and regional MBF was quantified from the regions of interest positioned on three mid-ventricular slices of the left ventricle corresponding to the vascular territories of the LAD, LCx, and right coronary artery (11). The regional MBF values were averaged to calculate mean MBF (11).

Study protocol. The PET study protocol consisted of three study sessions. First, MBF was measured at rest. After immersion of the left hand into ice water for 60 s, ^{13}N -ammonia was re-injected and CPT was continued for 60 s. The same procedure was performed after intravenous infusion of 3 g vitamin C or 100 ml 0.9% saline in the placebo group. Beginning with intravenous ^{13}N -ammonia administration (15 to 20 mCi), serial transaxial emission images were acquired (12 images per frame of 10 s each, 2 frames of 30 s each, and 1 frame of 900 s), and time-activity curves from the first 12 dynamic frames (12 for 10 s) were used to calculate mean MBF. The image acquisition sequence used for the baseline study was repeated while the CPT was maintained for 2 min to permit trapping of ^{13}N -ammonia in the myocardium (11). In all patients studied, visual inspection and polar map analysis of the ^{13}N -ammonia distribution at rest and during CPT revealed homogeneous tracer uptake. No perfusion defects were identified.

Subsequent MBF assessments on the second session after three months and on the third session after two years, administration of ^{13}N -labeled ammonia, image acquisition, and quantification of regional MBF were performed at rest and during CPT with previously defined regions of interest that were identical to those in the first session. In contrast, the short-term effect of vitamin C or saline on MBF responses to CPT was not determined again. Regional MBF was averaged to calculate mean MBF by two independent investigators unaware of the patients' assignment to vitamin C or placebo. In each study session, heart rate, blood pressure, and a 12-lead electrocardiogram were recorded continuously. From the average of heart rate and blood pressure during the first 2 min of each image acquisition, the rate-pressure product (RPP) was determined as an index of cardiac work. An index of coronary vascular resistance (CVR) was calculated from the ratio of mean arterial blood pressure (mm Hg) to MBF (ml/g/min). Finally, the averaged mean MBF of the left ventricle was

given and was not specifically derived for the coronary territory of the coronary artery segment for which measurements of the lumen area and its changes during CPT were obtained.

Statistical analysis. For descriptive purposes, all data are presented as the mean value \pm SD and relative frequencies, as indicated. Absolute changes of CPT-induced vascular responses before and after vitamin C infusion or placebo within each group were analyzed using the Wilcoxon sign-rank test. A comparison of these changes between the different study groups was done by two-way analysis of variance (ANOVA) followed by the Scheffé F test. Correlations between selected variables were estimated by Spearman correlation coefficients. All test procedures were two-sided with a p value <0.05 , indicating statistical significance.

RESULTS

Clinical characteristics. The clinical characteristics of the study groups are given in Table 1. In hypercholesterolemic patients, total and LDL cholesterol levels were significantly higher than in control subjects ($p \leq 0.05$ by ANOVA). After the follow-up periods of three months and two years, the study group laboratory results were not significantly different from baseline ($p = \text{NS}$ by the Wilcoxon test). At the end of two-year follow-up, a questionnaire study revealed that the patients did not change their habits with respect to diet or exercise, and none of the smokers stopped or refrained from smoking. Regarding study co-medication, apart from diuretics in the group of hypertensive patients and placebo, three patients received combined therapy with beta-blockers. In the hypercholesterolemic group, two patients decided to start with statin medication and therefore were not considered for the follow-up evaluation.

Reproducibility of hemodynamic response to CPT. At the baseline investigation, on both study days of coronary angiography and PET, CPT induced comparable percent changes in RPP ($31 \pm 15\%$ vs. $28 \pm 12\%$, $p = 0.12$ by the Wilcoxon test), implying a comparable hemodynamic effect. Table 2 summarizes the hemodynamic measurements obtained during MBF assessment with PET at baseline and after two-year follow-up. At baseline, systolic blood pressure in hypertensive patients (hypertension and placebo groups) and RPP were significantly higher than in chronic smokers, hypercholesterolemic patients, and control subjects ($p \leq 0.05$ by ANOVA) (Table 2). In all protocols, CPT induced a significant increase in heart rate, systolic and diastolic blood pressure, and RPP, owing to sympathetic activation ($p \leq 0.05$ by the Wilcoxon test). However, the overall mean RPPs at rest and during CPT at baseline and two-year follow-up were not significantly different ($p = 0.17$ and 0.98 , respectively). In addition, there was a close correlation between the resting RPP and RPP during CPT obtained at baseline and the two-year time point ($r = 0.93$ and 0.90 , respectively; $p < 0.0001$), indicating that the

Table 1. Baseline Characteristics of Study Population

	Hypertension		Placebo		Smokers		Hypercholesterolemia		Controls	
	Day 1	2 Years	Day 1	2 Years	Day 1	2 Years	Day 1	2 Years	Day 1	2 Years
No.	12	9	8	7	10	8	10	6	10	8
Age (yrs)	63 ± 2	—	64 ± 4	—	53 ± 2	—	56 ± 4	—	57 ± 3	—
Gender (male/female)	6/6	—	6/2	—	6/4	—	5/5	—	6/4	—
Mean blood pressure (mm Hg)	101 ± 2	100 ± 3	101 ± 2	102 ± 4	87 ± 4	86 ± 2	88 ± 3	91 ± 4	95 ± 5	95 ± 5
Heart rate (beats/min)	63 ± 2	64 ± 2	60 ± 3	60 ± 3	59 ± 2	60 ± 3	63 ± 2	64 ± 2	56 ± 4	57 ± 3
Total cholesterol (mg/dl)	199 ± 8	203 ± 7	192 ± 7	194 ± 8	206 ± 10	206 ± 11	263 ± 10	261 ± 8	160 ± 12	167 ± 10
LDL (mg/dl)	133 ± 5	143 ± 4	112 ± 8	116 ± 8	140 ± 6	137 ± 7	168 ± 8	160 ± 5	93 ± 8	94 ± 8
VLDL (mg/dl)	26 ± 3	26 ± 4	20 ± 2	20 ± 2	25 ± 3	26 ± 3	42 ± 12	45 ± 12	18 ± 3	19 ± 3
HDL (mg/dl)	49 ± 3	49 ± 3	50 ± 3	55 ± 5	49 ± 4	48 ± 3	46 ± 5	47 ± 4	60 ± 3	56 ± 3
HDL/LDL ratio	0.37 ± 0.6	0.34 ± 0.8	0.45 ± 0.4	0.47 ± 0.6	0.35 ± 0.7	0.35 ± 0.4	0.27 ± 0.6	0.29 ± 0.8	0.65 ± 0.4	0.60 ± 0.4
Triglycerides (mg/dl)	134 ± 15	156 ± 18	100 ± 12	102 ± 12	164 ± 20	161 ± 20	156 ± 39	138 ± 6	88 ± 11	93 ± 14
Fibrinogen (mg/dl)	296 ± 24	294 ± 25	310 ± 23	311 ± 23	287 ± 17	274 ± 16	290 ± 11	285 ± 10	258 ± 22	253 ± 22
Glucose (mg/dl)	90 ± 4	91 ± 3	93 ± 6	93 ± 5	89 ± 5	89 ± 6	93 ± 5	94 ± 3	88 ± 4	88 ± 4
Vitamin C (mg/100 ml)	0.96 ± 0.1	1.4 ± 0.1*	0.96 ± 0.1	0.94 ± 0.1	0.55 ± 0.1	1.2 ± 0.1*	0.81 ± 0.03	1.1 ± 0.04*	1.0 ± 0.12	1.4 ± 0.08*
BMI (kg/m ²)	24.7 ± 2.7	24.8 ± 2.6	24.3 ± 2.1	24.3 ± 1.8	22.8 ± 1.8	23.0 ± 2.2	23.4 ± 1.9	23.3 ± 1.8	22.7 ± 1.3	22.8 ± 1.0
LVMI (g/m ²)	115 ± 5	115 ± 4	110 ± 6	111 ± 8	94 ± 14	94 ± 10	85 ± 6	85 ± 9	76 ± 6	75 ± 8

*p ≤ 0.05 versus day 1 for each study group (by the Wilcoxon sign-rank test). Data are presented as the mean value ± SD or number of subjects.
BMI = body mass index; HDL and LDL = high- and low-density lipoprotein cholesterol; respectively; LVMI = left ventricular mass index; VLDL = very-low-density lipoprotein cholesterol.

Table 2. Responses of Epicardial Lumen Area to CPT

	Baseline (mm ²)	CPT (mm ²)
Hypertensive patients	6.0 ± 0.5	5.3 ± 0.5*
Placebo	4.6 ± 0.4	4.2 ± 0.4
Chronic smokers	6.2 ± 0.8	4.2 ± 0.5*
Hypercholesterolemic patients	5.1 ± 0.4	4.3 ± 0.3*
Control subjects	4.7 ± 0.5	5.8 ± 0.6*

*p ≤ 0.02 versus corresponding baseline value in each study group (by the Wilcoxon sign-rank test).
CPT = cold pressor test.

hemodynamic changes to CPT on repeated assessments were relatively consistent and reproducible. Lastly, the CPT-induced percent change of RPP determined at baseline and during the follow-up period did not differ significantly within the study population (28 ± 12% vs. 25 ± 8%; p = 0.22), indicating comparable myocardial work load for repeated hemodynamic assessments.

Responses of epicardial arteries to CPT. Table 3 depicts the responses of the epicardial lumen area to CPT in each study group. The baseline values of the mean lumen area did not differ significantly between the different study groups (p = NS by ANOVA). However, the mean lumen area of the epicardial coronary artery was significantly reduced from 6.0 ± 0.5 to 5.3 ± 0.5 mm² (p ≤ 0.001) in hypertensive patients compared with control subjects. Furthermore, there was a nonsignificant decrease of the mean lumen area from 4.6 ± 0.4 to 4.3 ± 0.4 mm² (p = NS) in the placebo group. In smokers and hypercholesterolemic patients, CPT led to a significant decrease of the mean lumen area from 6.2 ± 0.8 to 4.2 ± 0.5 mm² and from 5.1 ± 0.4 to 4.3 ± 0.3 mm², respectively (p ≤ 0.002). In contrast, in control subjects CPT induced an increase of the mean lumen area from 4.7 ± 0.5 to 5.8 ± 0.6 mm² (p ≤ 0.002). The group comparison of a CPT-induced decrease of the mean lumen area in the groups of patients with various coronary risk factors was significant when compared with a blood flow-mediated increase of the mean lumen area in control subjects (p ≤ 0.03 by ANOVA).

Responses of MBF and CVR to CPT. Responses of MBF and CVR at baseline and two years follow-up for different groups are depicted in Table 4. In addition, the percent changes of MBF in response to CPT during repeated assessment of hypertensive and hypercholesterolemic patients and in smokers are summarized in Figure 2. At baseline, MBF measured by dynamic PET during CPT revealed a markedly reduced increase of MBF in hypertensive and hypercholesterolemic patients, as well as in chronic smokers, compared with the control group (p ≤ 0.001 by ANOVA) (Table 4, Fig. 2). Short-term administration of vitamin C did not augment CPT-induced increases in MBF in hypertensive patients (p = NS) (Table 4, Fig. 2). Three months and two years of vitamin C treatment, however, resulted in a significant improvement of MBF in response to CPT (p ≤ 0.001) (Table 4, Fig. 2). As expected, placebo did not modify CPT-induced changes in MBF compared with

Table 3. Hemodynamic Responses to CPT

Group	Baseline Studies								
	Rest				CPT				
	HR	SBP	DBP	RPP	HR	SBP	DBP	RPP*	%Δ RPP
Hypertension	63 ± 2	148 ± 2	77 ± 2	9,395 ± 411	69 ± 3	170 ± 3	86 ± 3	11,682 ± 421	23 ± 7%
Placebo	60 ± 3	152 ± 3	76 ± 2	9,138 ± 585	63 ± 4	169 ± 5	83 ± 3	10,744 ± 781	13 ± 3%
Smokers	59 ± 2	116 ± 3	72 ± 3	6,868 ± 359	63 ± 2	139 ± 2	77 ± 2	8,760 ± 442	23 ± 6%
Hypercholesterolemia	63 ± 2	120 ± 5	72 ± 3	7,557 ± 356	67 ± 2	140 ± 7	78 ± 3	9,350 ± 498	24 ± 4%
Controls	56 ± 4	136 ± 6	74 ± 3	7,689 ± 509	65 ± 5	161 ± 9	82 ± 3	10,431 ± 891	40 ± 11%

Group	Two-Year Follow-Up After Vitamin C or Placebo								
	Rest				CPT				
	HR	SBP	DBP	RPP	HR	SBP	DBP	RPP†	%Δ RPP‡
Hypertension	65 ± 6	149 ± 4	78 ± 7	9,719 ± 1,051	70 ± 7	170 ± 6	84 ± 7	11,911 ± 988	27 ± 4%
Placebo	62 ± 4	154 ± 5	79 ± 4	9,610 ± 995	65 ± 5	167 ± 7	82 ± 5	10,890 ± 1,409	17 ± 3%
Smokers	60 ± 6	117 ± 6	69 ± 5	7,030 ± 924	63 ± 6	137 ± 7	73 ± 5	8,644 ± 1,426	24 ± 2%
Hypercholesterolemia	63 ± 4	121 ± 4	71 ± 3	7,623 ± 344	67 ± 3	141 ± 8	79 ± 4	9,447 ± 523	20 ± 3%
Controls	56 ± 6	138 ± 19	73 ± 8	7,584 ± 1,034	65 ± 6	166 ± 26	82 ± 10	10,693 ± 1,937	36 ± 4%

* $p \leq 0.05$ CPT versus rest for each corresponding hemodynamic parameter in each study group. † $p = NS$ %Δ RPP at two-year follow-up versus %Δ RPP at baseline studies in each study group (Wilcoxon sign-rank test).

CPT = cold pressor test; DBP = diastolic blood pressure (mm Hg); HR = heart rate (beats/min); %Δ RPP = percent change of RPP from rest during CPT; RPP = rate-pressure product (systolic blood pressure × heart rate); SBP = systolic blood pressure (mm Hg).

baseline ($p = NS$) (Table 4). In chronic smokers, short-term vitamin C administration (intravenous) improved MBF responses to CPT ($p \leq 0.001$) (Table 4, Figs. 2 and 3). In addition, long-term oral therapy with vitamin C led to a sustained beneficial effect after three months and two years of follow-up ($p \leq 0.001$). The increase of MBF to CPT in chronic smokers after intravenous vitamin C administration was markedly stronger as compared with MBF responses in hypertensive and hypercholesterolemic patients (Table 4, Figs. 2 and 3) ($p \leq 0.001$). In contrast, short- and long-term vitamin C treatment did not result in a significant CPT-induced increase in MBF in hypercholesterolemic patients ($p = NS$) (Table 4, Fig. 2). Lastly, high-dose vitamin C supplementation of control subjects had virtually no effect on MBF during CPT ($p = NS$) (Table 4).

The group comparison of a CPT-induced increase of MBF in hypertensive patients and chronic smokers after three months and two years of follow-up with vitamin C supplementation was significantly different from the placebo and control groups ($p \leq 0.001$ by ANOVA). Because changes of CVR mirrored those of MBF for each study

group (Table 4), differences in hemodynamic responses are unlikely to account for the observed changes in MBF during CPT.

Correlation between responses to CPT of epicardial coronary artery and MBF. In the overall study population at baseline, there was a significant correlation between percent changes in the epicardial lumen area of the proximal artery segment and MBF during CPT ($r = 0.72$, $p = 0.0001$). However, the regression analysis between the percent changes of the lumen area of the epicardial vessel and MBF during CPT at baseline for patients with coronary risk factors did not show a significant correlation ($r = 0.34$, $p = 0.06$; $n = 40$). In addition, no correlation was observed between the percent changes of the lumen area of the epicardial artery and MBF during CPT in hypertensive patients ($r = 0.07$, $p = 0.83$; $n = 12$), placebo combined with hypertensive group ($r = 0.32$, $p = 0.17$; $n = 20$), chronic smokers ($r = -0.27$, $p = 0.45$; $n = 10$), hypercholesterolemic patients ($r = 0.42$, $p = 0.23$; $n = 10$), and control subjects ($r = 0.51$, $p = 0.13$; $n = 10$), except for just the placebo group ($r = 0.81$, $p = 0.015$; $n = 8$).

Table 4. Mean MBF Responses and Coronary Vascular Resistance to CPT for Each Study Group

Group	Baseline Studies				Two-Year Follow-Up			
	Rest		CPT		Rest		CPT	
	MBF	CVR	MBF	CVR	MBF	CVR	MBF	CVR
Hypertension	0.80 ± 0.26	143 ± 48	0.94 ± 0.26*	131 ± 33†	0.67 ± 0.08	154 ± 15	1.30 ± 0.20*	89 ± 12†
Placebo	0.71 ± 0.22	150 ± 27	0.89 ± 0.25	134 ± 30	0.66 ± 0.11	161 ± 20	0.80 ± 0.14	145 ± 21
Smokers	0.79 ± 0.09	111 ± 11	0.70 ± 0.09	141 ± 18	0.68 ± 0.06	126 ± 12	1.18 ± 0.10	82 ± 4
Hypercholesterolemia	0.70 ± 0.22	136 ± 31	0.77 ± 0.28	138 ± 40	0.75 ± 0.18	126 ± 26	0.77 ± 0.23	142 ± 33
Controls	0.75 ± 0.17	134 ± 33	1.41 ± 0.34	78 ± 18	0.69 ± 0.14	140 ± 23	1.24 ± 0.21	90 ± 16

* $p \leq 0.05$ versus corresponding MBF and CVR at rest in repeated assessment in each study group by the Wilcoxon sign-rank test. Mean (± SD) MBF is expressed as ml/g per min, and mean CVR as mm Hg/ml/g per min.

CPT = cold pressor testing; CVR = coronary vascular resistance; MBF = myocardial blood flow.

Contrasting MBF Responses to Vitamin C

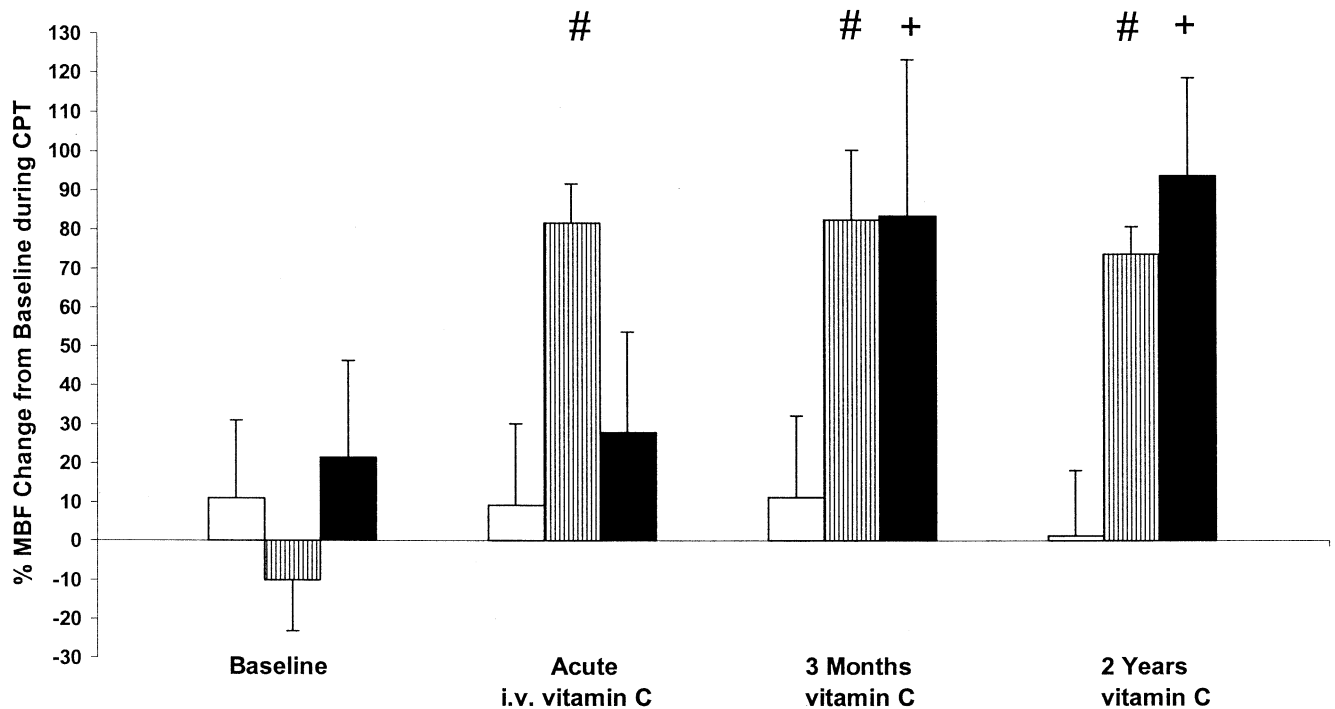


Figure 2. Percent change of myocardial blood flow (MBF) during cold pressor testing (CPT) in hypercholesterolemic patients (open bars), hypertensive patients (solid bars), and smokers (lined bars). #+ $p \leq 0.03$ compared with baseline (CPT-1) on repeated assessments for each study group.

Blood chemistry. In all patients, vitamin C plasma levels were measured before and after vitamin treatment periods of three months and two years, respectively (Table 1). In two patients, vitamin C plasma levels after the two years of follow-up could not be evaluated due to blood sampling errors. The baseline levels of vitamin C in chronic smokers were below the normal range (0.6 to 2.0 mg/dl) and significantly lower than in the other study groups ($p \leq 0.05$). Regular intake of 2 g vitamin C daily over the follow-up periods resulted in a significant increase of vitamin C concentrations in all study groups ($p \leq 0.05$), apart from the placebo group ($p = \text{NS}$).

Correlation between change of vitamin C levels and CBF during CPT after two-year follow-up. Regression analysis between the increase of vitamin C levels after the follow-up period of two years and changes of MBF during CPT did not reveal statistically significant correlations between the study groups (smokers: $r = 0.19$, $p = 0.65$; hypertensive patients: $r = 0.43$, $p = 0.29$; hypercholesterolemic patients: $r = -0.57$, $p = 0.14$; and control subjects: $r = 0.31$, $p = 0.54$).

DISCUSSION

The results of the present study provide several new findings. At first, sympathetic activation by CPT elicits markedly different vascular responses of coronary conductance and resistance vessels, depending on cardiovascular risk factors such as hypertension, hypercholesterolemia, and chronic smoking. Secondly, differences in the effect of short- and long-term vitamin C administration on abnor-

mal MBF responses in smokers and hypertensive and hypercholesterolemic patients suggest that the mechanisms underlying endothelial dysfunction may vary considerably. In addition, the good reproducibility of MBF measurements with PET may indicate that this kind of method may be used as a tool to study the effects of pharmacologic interventions on coronary microcirculatory function.

Coronary endothelial-mediated vasoreactivity and CPT.

In the absence of significant coronary artery disease, the endothelium-dependent responses of both epicardial conductance and resistance vessels to CPT in patients with various risk factors were significantly impaired compared with control subjects. When looking at the study population as a whole, there was a significant relationship between changes in the lumen area of the large conduit vessels and the MBF responses to CPT, which suggests that flow-dependent changes of the epicardial lumen area correlate well with increases in blood flow at the coronary resistance vessel level. As reported previously (4), evaluation of coronary vasomotion by CPT has demonstrated a significant correlation between relative increases in blood flow indexes and lumen area changes in epicardial arteries, indicating that changes in shear stress contribute to flow-mediated vasodilation in the coronary circulation. Thus, it is conceivable to conclude that metabolic vasodilation of the coronary resistance vessels due to sympathetic activation resulted in an increase in flow throughout the vascular bed, subsequently leading to a corresponding flow-mediated increase in epicardial conductance vessel diameter (12). The different groups at risk of coronary artery disease, such as hyperten-

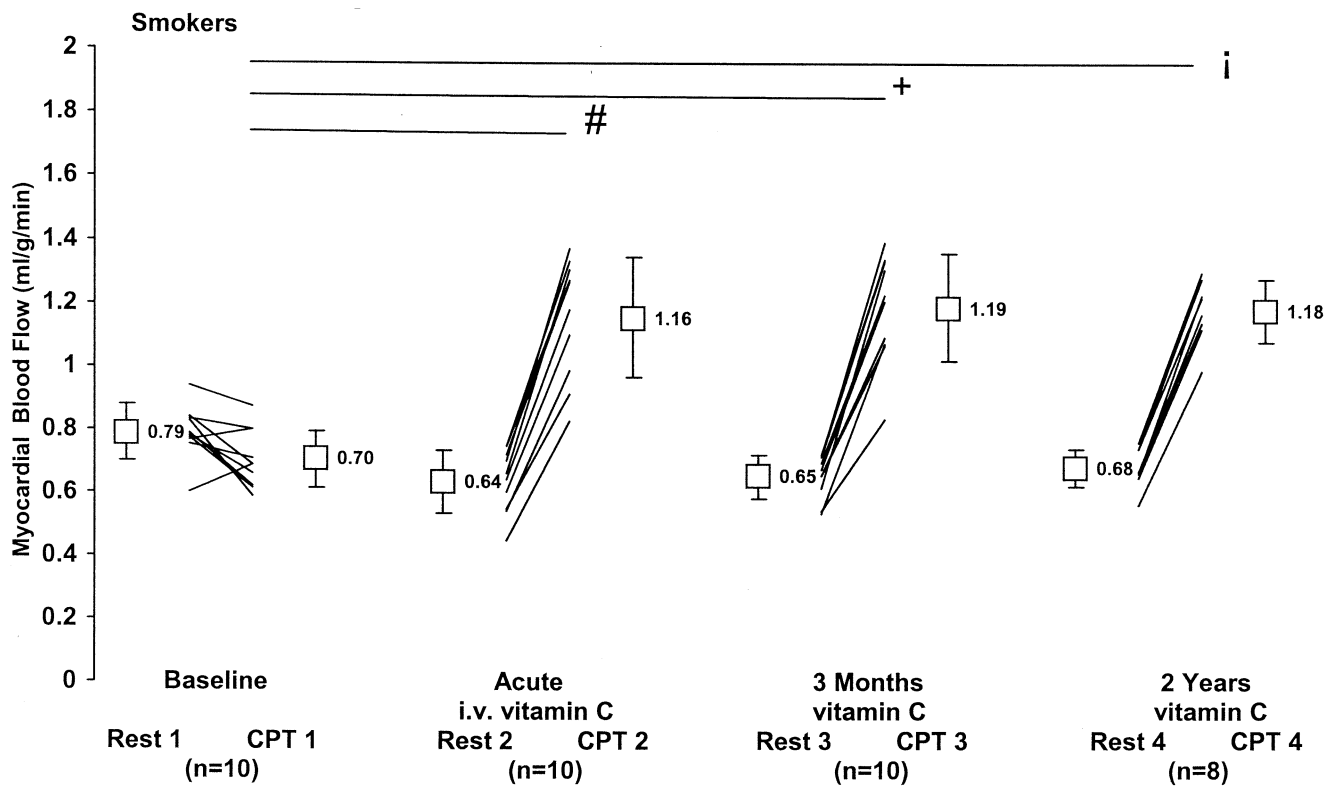


Figure 3. Changes of myocardial blood flow (MBF) during cold pressor testing (CPT) at baseline (CPT-1), after intravenous (i.v.) infusion of 3 g vitamin C (VC) (CPT-2), and after a period of three months (CPT-3) and two years (CPT-4) of 2 g VC tablets daily in smokers. #+*p* ≤ 0.001 compared with baseline (CPT-1) on repeated assessments of changes in MBF during CPT in smokers.

sion, smoking, and hypercholesterolemia, did not demonstrate a significant correlation between abnormal vasoreactivity to CPT in conduit and arteriolar vessels, supporting the contention that abnormal flow-mediated vasoreactivity of epicardial coronary arteries is a different form of manifestation from that of the arteriolar vascular bed. These observations are in keeping with the results of other investigators (5,6) who have shown a disparity between abnormal endothelial-dependent epicardial and arteriolar vascular responses to acetylcholine.

MBF responses to vitamin C in patients with various risk factors. Several investigators have previously shown that vascular and/or endothelial dysfunction of coronary and peripheral arterioles of chronic smokers respond well to short-term administration of the antioxidant vitamin C (13–15). For example, intravenous vitamin C administration improved coronary flow responses to adenosine (13). Furthermore, Heitzer et al. (14) established a marked enhancement of acetylcholine-induced increases in forearm blood flow by vitamin C in chronic smokers with a history of more than 20 pack-years. Improvement of coronary flow responses to adenosine, however, comprises endothelium-dependent and -independent mechanisms. Thus, the observed improvement of vasodilatory dysfunction by intravenous vitamin C infusion may extend previous findings to vasomotion induced by CPT, which has been previously shown to be largely endothelium-dependent (12,16). More

exciting, it also indicates that vasoconstriction induced by sympathetic stimulation in chronic smokers is largely dependent on the release of ROS. The precise sources of ROS, however, remain to be determined. Several groups have provided evidence for dysfunctional, uncoupled eNOS due to deficiency of the NOS co-factor tetrahydrobiopterin (BH₄), since BH₄ itself or its precursor sepiapterin improved endothelial dysfunction in this patient population (17). Interestingly, aggregating platelets can release large amounts of superoxide, which may be partly due to uncoupled eNOS (18). As we and others have previously shown that vitamin C has an inhibitory effect on platelet aggregation associated with an improvement of arterial vasomotion (19,20), it is tempting to speculate that the findings observed in the present study may also include beneficial effects of vitamin C on uncoupled, dysfunctional eNOS in the platelets of chronic smokers.

The observed beneficial effects of long-term vitamin C in the present study are in contrast to previous findings where regular supplementation of 1 g vitamin C daily failed to produce a sustained beneficial effect on endothelial-dependent vasomotion of arm vessels in smokers (21). The reason for this discordant observation is not clear and may be related to differences in patient characteristics, differences in the antioxidant dose, and/or different factors determining endothelial (dys)function in the forearm and coronary circulation (22,23).

Short-term administration of vitamin C improves endothelial-dependent vasodilation in the coronary conduit (10) and forearm vessels (24) of hypertensive patients, suggesting that increased vascular ROS formation contributes to endothelial dysfunction in this particular patient population. In the present study, however, we failed to demonstrate a beneficial effect of vitamin C on abnormal MBF responses, indicating that other mechanisms may contribute considerably to impaired flow-induced coronary vasodilatory capacity in the arteriolar vascular bed. Although we could not improve endothelial dysfunction when short-term vitamin C was given, long-term vitamin C supplementation for three-month and two-year follow-up periods improved abnormal MBF responses in hypertensive patients. Therefore, we speculate that the delayed onset of the beneficial effects of vitamin C on endothelial dysfunction may be secondary to an improvement of the redox equilibrium, leading to increased eNOS expression (25,26) and/or prevention of eNOS uncoupling via enhanced bioavailability of BH₄ (27). Nevertheless, it is the first study to provide evidence that oral treatment with vitamin C is able to improve endothelial dysfunction of the coronary microcirculation in hypertensive patients.

Next, we studied endothelium-dependent responses to short- and long-term vitamin C challenges in patients with hypercholesterolemia. Several possible mechanism have been proposed to account for endothelial dysfunction in this patient group, such as selective targeting of G-protein-dependent signal transduction by oxidized LDL, leading to a decrease in receptor-mediated stimulation of endothelial NO production (28). In the setting of hypercholesterolemia, an alteration in the signal transduction cascade downstream of NO may also be present, such as diminished sensitivity of guanylyl cyclase (29,30), reduced Ca²⁺ uptake via sarco/endoplasmic reticulum Ca²⁺ in smooth muscle cells (31). The most attractive concept for endothelial dysfunction in hypercholesterolemia, however, refers to increased production of ROS (32). However, in none of our interventions (acute short- and long-term vitamin C treatment) was a beneficial effect of this antioxidant on CPT induced changes in MBF observed. These negative effects are in contrast to previous observations where single-dose vitamin C challenges (2 g), as well as long-term therapy with vitamin C (500 mg/day) (33), improved flow-dependent dilation of the brachial artery in patients with coronary artery disease and may be related to different doses of vitamin C chosen for long-term therapy and/or to the different vessel region studied.

Conclusions. In the present study, we have established marked differences in MBF responses to short- and long-term vitamin C in patients with various risk factors. These results clearly emphasize the complex nature of mechanisms underlying abnormal coronary vasomotion. Although some situations might implicate a predominantly abnormal redox equilibrium between NO and ROS as a final pathway mediating endothelial-dependent vasodilatory dysfunction,

other situations point to a defect that might locate (e.g., in altered membrane receptor coupling) mechanisms affecting the release of endothelial-derived NO or may involve disturbances of the cyclic guanosine monophosphate kinase signal transduction cascade located within smooth muscle cells.

Acknowledgments

The authors thank Ursula Sahm, PhD, Kenneth Stalmo, MSc, and Bernd Morasch, MSc, for producing the radioisotope and radiopharmaceutical. We are further indebted to Claudia Santini-Böttcher, CNMT, Aasa Stalmo, CNMT, and Claudia Scheurer, CNMT, for their technical assistance. Finally, we thank the nurses of the Cardiac Catheterization Unit for their support, and Gertrud Zürcher, MD, and Gisela Baumann, LT, for the laboratory measurements of vitamin C and lipid levels.

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Coronary vasoregulation in patients with various risk factors in response to cold pressor testing: Contrasting myocardial blood flow responses to short- and long-term vitamin C administration

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J. Am. Coll. Cardiol. 2003;42:814-822
doi:10.1016/S0735-1097(03)00851-9

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