REACTIVE OXYGEN SPECIES (ROS) are generated ubiquitously in the vasculature of air-breathing organisms, and this process may accelerate as a consequence of ischemia, reperfusion, arterial balloon injury, angiotensin II binding to receptors on vascular endothelial and smooth muscle cells, and leukocyte-mediated inflammation of atherosclerotic plaques (14). ROS-mediated changes in coronary resistance and blood flow are hypothesized to be a common mechanism by which these diverse stimuli lead to adverse clinical events in patients with ischemic heart disease (IHD) (41).

The ROS hypothesis is indirectly supported by observations in the peripheral arterial circulation. Thus administration of supplemental oxygen to healthy subjects accelerates systemic ROS formation (20) and increases both systemic vascular resistance (7, 29, 36) and resistance to blood flow across limb perfusion beds (6, 25). Several lines of evidence suggest that this effect may be mediated via oxidative quenching of endothelium-derived nitric oxide (NO) by ROS generated within the arterial lumen under hyperoxic conditions (7, 19, 25, 37). Consistent with this premise, the antioxidant vitamin C prevented hyperoxia-mediated vasoconstriction, and improved the response to NO-mediated vasodilators, in the forearm circulation of subjects breathing supplemental oxygen (25).

Despite this inferential evidence from studies in the peripheral circulation, few studies have examined the effect of oxidative stress on vascular resistance and blood flow in the coronary circulation of patients with IHD in a clinical setting. The need for such an examination is illustrated by the fact that recent clinical trials testing the logical corollary that antioxidant administration should prevent adverse clinical events in patients with IHD have been disappointingly negative (15, 45). Nevertheless, millions of patients with IHD continue to ingest antioxidant substances in hopes of obtaining a therapeutic benefit. Defining the degree to which changes in ambient levels of oxidants and antioxidants actually influence coronary resistance and blood flow in the clinical setting in patients with IHD is a necessary step toward developing a rational strategy for therapeutic use of antioxidants in this disease. In this study, we used selective coronary arterial catheterization and intra-arterial Doppler sonography to test the hypothesis that acute oxidative stress substantially and acutely increases coronary resistance in patients with IHD by a mechanism sensitive to the antioxidant vitamin C.

METHODS

Subjects. Twelve adult subjects were studied during elective coronary angiography performed to evaluate stable angina pectoris or abnormal exercise tests. Subjects were selected for study if their coronary angiogram demonstrated a moderately severe stenosis in the proximal left anterior descending (LAD) coronary artery and if the performing physician planned to evaluate this stenosis for clinical reasons using intracoronary Doppler sonography. All subjects were taking a β-blocker, a statin, and aspirin, but none was taking an angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, or long-acting nitrate. No subject had experienced chest pain, nor used nitrates in any form, for at least 24 h before study. No subject had a history of diabetes mellitus, although one had an elevated fasting blood glucose (6.1 mmol/l) on the day of study. Five subjects...
were overweight (body mass index >25 kg/m²), but none was obese. The five female subjects were all postmenopausal, and none was taking hormone replacement therapy. The study was approved by the Institutional Review Board of the Penn State College of Medicine, and all subjects gave written informed consent. Subject characteristics are summarized in Table 1.

**Study protocol.** Subjects were mildly sedated with midazolam, and selective coronary angiography was performed in standard fashion. A guiding catheter was then placed into the left coronary artery and used to introduce a 0.014-in., 15-MHz Doppler flow wire (FloMap system, Jomed, Rancho Cordova, CA) into the LAD coronary artery, with the Doppler element of the wire placed distal to the moderately severe coronary stenosis. Before the beginning of the research study, LAD coronary flow velocity (CFV; cm/s) was recorded before and after a 30-µg intracoronary bolus injection of the endothelium-independent dilator adenosine. In 8 of the 12 subjects, LAD CFV increased by ≥100% in response to adenosine, indicating that the LAD stenosis was not flow limiting. In the remaining four subjects, CFV increased by <100% in response to adenosine, and a metal stent was placed to correct the flow-limiting coronary stenosis. All research observations were thus made in LAD coronary arteries that exhibited relatively advanced atherosclerosis but were free of flow-limiting stenoses and had intact vasodilator responses to adenosine.

After 10 min were allowed for the adenosine effect to dissipate, CFV was recorded during the following sequence: subjects breathed room air for 10 min, then breathed 100% oxygen via a plastic face mask for 10 min, then breathed room air again during a 10-min recovery period, then received an intravenous infusion of 3.0 g of vitamin C over 15 min, and then were rechallenged with 100% oxygen breathing for another 10-min period beginning 10 min into the vitamin C infusion. This sequence is depicted in Fig. 1A.

**Experimental controls.** To control for possible nonspecific changes in CFV over time, in three control subjects LAD CFV was monitored with the Doppler wire during 20 min of room air breathing, without exposure to 100% oxygen. To test whether changes observed during vitamin C infusion were specific to this intervention, three other control subjects breathed 100% oxygen for 10 min, and LAD CFV was subsequently recorded during a 25-min recovery period, without vitamin C infusion.

**Physiological recordings.** LAD CFV was recorded continuously throughout each experiment. Analog CFV signals recorded by the Doppler wire were converted to digital form and stored on an Apple PowerMac laptop computer using PowerLab software (ADI). During the last minute of each of the five experimental periods, heart rate and mean arterial pressure were recorded by an electronic pressure transducer connected to the femoral arterial sheath, and LAD coronary angiography was performed to measure the diameter of the LAD segment holding the Doppler wire. The Doppler wire’s position within the LAD coronary artery was recorded by digital cineangiography at the beginning of each study and checked periodically by fluoroscopy to confirm its stable position.

**Chemical analyses.** PO₂, PCO₂, and pH were measured in femoral arterial blood using a commercial blood gas analyzer (Bayer).

<table>
<thead>
<tr>
<th>Table 1. Subject characteristics</th>
<th>Means ± SD (Range)</th>
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<tbody>
<tr>
<td>n</td>
<td>12</td>
</tr>
<tr>
<td>Age, yr</td>
<td>61 ± 3 (47–78)</td>
</tr>
<tr>
<td>Female/male</td>
<td>5/7</td>
</tr>
<tr>
<td>No. of diseased coronaries</td>
<td>2 ± 1</td>
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<tr>
<td>Left ventricular ejection fraction, %</td>
<td>59 ± 5 (45–76)</td>
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<tr>
<td>Serum glucose, mmol/l</td>
<td>5.6 ± 0.7 (4.6–6.8)</td>
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<tr>
<td>Serum creatinine, mg/l</td>
<td>11 ± 4 (7–18)</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>27 ± 2 (23–29)</td>
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</table>

n, No. of subjects.

**CFV analysis.** Doppler coronary flow velocity was continuously recorded throughout each cardiac cycle. By convention, only the peak (i.e., diastolic) flow velocity during each cardiac cycle is reported. The continuous recording of diastolic CFV was sampled at 15-s intervals throughout each experiment to generate ~200 CFV values (40–50 per experimental period) for each subject. All CFV values falling within each of the five defined experimental periods were then averaged to yield one average CFV per experimental period for each subject. These average CFVs were compared across subjects using repeated-measured analysis of variance to examine the effect of each experi-
mental condition on CFV. Differences between experimental periods were considered significant if $P < 0.05$.

**LAD coronary diameter analysis.** LAD coronary diameter measurements were made by an experienced angiogram reader blinded to the treatment protocol. During each study, the segment of each subject’s LAD coronary artery holding the Doppler wire was imaged by cineangiography in an optimal angulation, using a Siemens Coroskop C-arm coronary angiography system. Identical projection angles and imaging techniques were used to acquire angiogram loops at baseline, during the final minute of the initial (untreated) 100% oxygen breathing period, and during the final minute of the second (vitamin C treated) 100% oxygen breathing period. Digitally archived cineangiogram loops were reviewed by the blinded reader at a computer workstation, and a single representative image from each loop was chosen for analysis. On this image, the diameter of the LAD coronary artery at the level of the Doppler wire was measured by electronic calipers with a Heartlab (Westerly, RI) analysis program, using the known diameter of the coronary guiding catheter as an internal reference. Baseline, hyperoxic and hyperoxic + vitamin C coronary diameters were compared using paired t-tests with a correction for one repeat measurement.

**Calculations.** Coronary blood flow (CBF) (cm$^3$/min) during the final minute of each experimental period was calculated by multiplying average CFV (cm/s) by the coronary artery cross-sectional area ($cm^2 = \pi \times$ arterial radius$^2$) and the factor 0.5 (1). Coronary vascular resistance (CVR; mmHg·cm$^{-3}$·min) was calculated by dividing mean arterial blood pressure by CBF.

**RESULTS**

**Systemic hemodynamics and oxygenation.** Administration of 100% oxygen substantially increased arterial PO$_2$, without affecting arterial blood pH, PCO$_2$, heart rate, or mean arterial blood pressure. Vitamin C infusion was without effect on heart rate, systemic arterial blood pressure, or PO$_2$. These data are shown in Table 2.

**Coronary diameter, CBF, and CVR.** Administration of 100% oxygen was associated with a prompt ~20% fall in CFV and CBF [from 26.0 ± 3.0 to 21.2 ± 3.0 cm/s ($P < 0.01$) and from 91 ± 31 to 73 ± 28 cm$^3$/min ($P < 0.01$), respectively] in every subject, corresponding to a ~23% increase in coronary resistance (from 0.9 ± 0.4 to 1.1 ± 0.6 mmHg·cm$^{-3}$·min). These changes were not accompanied by any appreciable change in the diameter of the conduit segment of LAD coronary artery holding the Doppler wire (baseline = 3.1 ± 0.3 vs. hyperoxia = 3.0 ± 0.3 cm; $P =$ not significant), suggesting they reflected a vasoconstrictor action operating primarily at the level of the coronary microcirculation. CFV remained below basal (22.0 ± 4.0 cm/s; $P < 0.05$) throughout the subsequent room air breathing recovery period, despite a prompt fall in arterial PO$_2$ to the basal level, but it rebounded to a slightly superbasal level (27.0 ± 4.0 cm/s; $P < 0.01$ vs. basal) during subsequent vitamin C infusion.

Following vitamin C infusion, repeat challenge with 100% oxygen no longer caused a reduction in CFV (27.5 ± 3.1 cm/s) nor any change in coronary diameter (hyperoxia = 3.0 ± 0.3 cm vs. hyperoxia + vitamin C = 3.1 ± 0.3 cm; $P =$ not significant). These data are displayed in Fig. 1. Control subjects in whom LAD CFV was simply monitored during 20 min of room air breathing demonstrated <10% variation in CFV over this time. In control subjects who breathed 100% oxygen for 10 min and were not treated with vitamin C, CFV remained below the basal level throughout a subsequent 25-min period of room air breathing.

There was a trend toward lower basal CFV (23.8 ± 2.4 vs. 27.4 ± 2.4 cm/s; $P = 0.013$) and CBF (84 ± 23 vs. 97 ± 28 cm$^3$/min; $P = 0.014$) in five female subjects relative to the seven male subjects, presumably due to the smaller body mass of female subjects, but no sex differences were observed in the response to oxygen breathing or vitamin C infusion (data not shown).

Real-time CFV data recorded throughout the experiment are displayed in Fig. 1A, and average CBF and CVR during the first minute of each experimental period are shown in Fig. 1, B and C.

**DISCUSSION**

This study shows that administration of supplemental oxygen to patients with stable IHD is associated with a prompt, ~20% increase in CVR and fall in CBF. This coronary constriction is both reversed and prevented by vitamin C administration, suggesting that it is mediated by substances capable of being quenched by a blood-borne antioxidant. The observation that neither 100% oxygen nor vitamin C appreciably affected the diameter of large conduit coronary arteries suggests that their effects were mediated by mechanisms operating primarily at the level of the coronary microcirculation.

These observations agree with most (2, 23, 25, 32, 43) although not all (8, 12) studies that have examined the effect of vitamin C on blood flow in the human peripheral circulation. While fewer comparison studies exist for the coronary circulation, Kauffman et al. (22) observed that intravenous infusion of 3 g of vitamin C restored normal microcirculatory flow reserve, measured by positron emission tomography, in the coronary circulation of cigarette smokers with impaired basal flow reserve, and Teramoto et al. (42) made the same observation using transthoracic Doppler ultrasonography and a 2-g oral dose of vitamin C. Acute administration of vitamin C

<table>
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<tr>
<th>Table 2. Hemodynamics and oxygenation during experimental periods</th>
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<tr>
<td><strong>Baseline</strong></td>
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<td>----------------</td>
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<tr>
<td>Heart rate, beats/min</td>
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<tr>
<td>MAP, mmHg</td>
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<tr>
<td>pH</td>
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<tr>
<td>PCO$_2$, Torr</td>
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<tr>
<td>PO$_2$, Torr</td>
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<tr>
<td>CFV, cm/s</td>
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<tr>
<td>CD, cm</td>
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Values are means ± SD for 12 subjects. MAP, mean arterial pressure; CFV coronary flow velocity; CD, coronary diameter; NA, not applicable. *$P < 0.05$ vs. baseline condition.
similarly improved endothelium-dependent coronary vasodilation in two studies of patients with chronic heart failure (17, 35). In contrast to our present findings, Solzbach et al. (39) observed that intravenous administration of vitamin C to hypertensive patients without coronary atherosclerosis improved acetylcholine-dependent dilation of conduit coronary segments, but not acetylcholine-dependent acceleration of CFV, suggesting a salutary effect on conduit arteries but not the microcirculation. A potential explanation for these observations would be that hypertension and hyperoxia exert their main vasoconstrictor influences at different anatomic sites (large vs. small vessels, respectively); however, differences in methods and subject population between our study and that of Solzbach et al. make it difficult to directly compare results.

In addition to both reversing and preventing hyperoxic coronary constriction, administration of vitamin C in this study actually increased absolute LAD CBF to a level slightly above that of the basal, untreated state. This agrees with Solzbach et al.’s (39) observations in hypertensive patients without IHD, and it suggests that, both in patients with hypertension and in those with IHD, the coronary circulation is normally in a relatively vasoconstricted state due to a predominance of oxidant in proportion to antioxidant influences on coronary tone. Vascular NO bioavailability is known to be reduced in both hypertension and atherosclerosis (1, 11, 34, 44). Because all our subjects had relatively advanced coronary atherosclerosis, the observation that vitamin C administration raised their CFV to a slightly superbasal level suggests that, at the dose used in this study, vitamin C may have increased NO bioavailability within the coronary circulation by quenching endogenous oxidants active against NO.

The heart matches coronary oxygen delivery to myocardial oxygen consumption to match energy production to energy utilization and prevent cardiomyocyte hypoxia or oxygen toxicity. This autoregulation is thought to reflect cross talk between the myocardium and coronary arteries, mediated by vasoactive and inotropic substances released by endothelial cells, cardiomyocytes, and erythrocytes (3, 11, 21, 28, 33, 46). Neither the precise signal that denotes cardiac oxygen supply nor the signal transduction pathway leading to secretion of vasoactive and inotropic substances is completely understood. ROS are recognized to function as signaling molecules in many systems (5, 18, 40) and might also plausibly regulate the balance between myocardial oxygen demand and delivery. Breathing supplemental oxygen generates a variety of ROS within the coronary lumen whose formation and subsequent metabolism vary with tissue phenotype (9, 24–26, 38). The experimental model used in this study theoretically allows manipulation of coronary oxygen supply, ROS formation, and ROS quenching within the coronary circulation of patients with cardiovascular diseases. This may facilitate study of the potential role of ROS in coordinating cardiac oxygen delivery and consumption in humans.

Because we did not directly measure NO or ROS in this study, our interpretation that vitamin C reversed and prevented hyperoxic coronary constriction by quenching ROS and thereby increasing intracoronary NO bioavailability is necessarily speculative, and other mechanisms could also be involved. Hyperoxia elicits release of the vasoconstrictor endothelin-1 (ET-1) from some types of cultured endothelial cells in vitro (16), and mice breathing high concentrations of oxygen correspondingly exhibit increased systemic levels of ET-1 (13). Vitamin C has been observed to prevent ET-1 vasoconstriction in the forearm circulation (4), although not to reverse it. These observations suggest that effects of hyperoxia and vitamin C on ET-1 release or action might have contributed to some of the changes in CFV that we observed. In addition to reducing NO bioavailability, oxidative stress may also potentially inhibit endothelium-dependent vasodilator pathways mediated by prostacyclin and endothelium-derived hyperpolarizing factor, uncouple NO synthase, and inhibit myocyte-endothelial signaling (10). Superoxide anions produced during hyperoxia may also exert a direct vasoconstrictor action, independent of NO, by inhibiting soluble guanylyl cyclase in vascular smooth muscle (10). We note that Rousseau et al. (36), in a set of experiments similar to our own, proposed that the peripheral vasoconstriction observed in subjects breathing high concentrations of oxygen may be partly attributable to reduction in blood PCO2. Because we did not observe a significant change in PCO2 in our experiments, this mechanism could not be implicated as an explanation for the effects of hyperoxia and vitamin C in our study.

Limitations. Because all subjects underwent the two hyperoxic challenges (untreated and vitamin C treated) in the same order, ordering bias is a potential limitation. It was not possible to alternate the experimental order because vitamin C remains in the circulation after its administration. The interpretation that vitamin C administration reversed hyperoxic coronary constriction assumes that CFV would not have returned to the basal level during the observation period without this treatment. Although control studies suggest this is a reasonable assumption, further studies are needed to better define the time course of hyperoxic coronary constriction in our subject population in the absence of treatment. Similarly, the interpretation that vitamin C prevented the reinduction of hyperoxic constriction presumes that this effect can be induced again when subjects are rechallenged with 100% oxygen. Although this assumption also seems reasonable, we did not formally demonstrate this because of concerns that repeatedly lowering CFV via 100% oxygen breathing might compromise subject safety. All subjects were taking statin drugs, which are known to increase vascular NO production and NO-dependent blood flow responses (30, 31). Whether our observations are relevant to subjects not taking statins will require further study.

Clinical implications. Compelling evidence suggests that CVR and CFV are important determinants of clinical outcome in IHD patients suffering from acute coronary syndromes and/or undergoing interventional coronary procedures (27). In the present study, an oxidative stressor (administration of supplemental oxygen) acutely increased CVR and reduced CFV in a group of IHD patients undergoing a coronary interventional procedure. Intravenous administration of 3 g of vitamin C both reversed and prevented this effect. Although long-term administration of antioxidants to patients with IHD has proven ineffective at preventing adverse clinical events (15, 45), the possibility that acute administration of vitamin C might improve the outcome from high-risk interventional procedures or acute coronary syndromes may deserve clinical testing.
ACKNOWLEDGMENTS

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GRANTS

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