

Vitamin C prevents hyperoxia-mediated vasoconstriction and impairment of endothelium-dependent vasodilation

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Mak, Susanna, Zoltan Egri, Gemini Tanna, Rebecca Colman, and Gary E. Newton. Vitamin C prevents hyperoxia-mediated vasoconstriction and impairment of endothelium-dependent vasodilation. *Am J Physiol Heart Circ Physiol* 282: H2414–H2421, 2002. First published January 10, 2002; 10.1152/ajpheart.00947.2001.—High arterial blood oxygen tension increases vascular resistance, possibly related to an interaction between reactive oxygen species and endothelium-derived vasoactive factors. Vitamin C is a potent antioxidant capable of reversing endothelial dysfunction due to increased oxidant stress. We tested the hypotheses that hyperoxic vasoconstriction would be prevented by vitamin C, and that acetylcholine-mediated vasodilation would be blunted by hyperoxia and restored by vitamin C. Venous occlusion strain gauge plethysmography was used to measure forearm blood flow (FBF) in 11 healthy subjects and 15 congestive heart failure (CHF) patients, a population characterized by endothelial dysfunction and oxidative stress. The effect of hyperoxia on FBF and derived forearm vascular resistance (FVR) at rest and in response to intra-arterial acetylcholine was recorded. In both healthy subjects and CHF patients, hyperoxia-mediated increases in basal FVR were prevented by the coinfusion of vitamin C. In healthy subjects, hyperoxia impaired the acetylcholine-mediated increase in FBF, an effect also prevented by vitamin C. In contrast, hyperoxia had no effect on verapamil-mediated increases in FBF. In CHF patients, hyperoxia did not affect FBF responses to acetylcholine or verapamil. The addition of vitamin C during hyperoxia augmented FBF responses to acetylcholine. These results suggest that hyperoxic vasoconstriction is mediated by oxidative stress. Moreover, hyperoxia impairs acetylcholine-mediated vasodilation in the setting of intact endothelial function. These effects of hyperoxia are prevented by vitamin C, providing evidence that hyperoxia-derived free radicals impair the activity of endothelium-derived vasoactive factors.

oxygen; acetylcholine; heart failure; endothelium

HIGH ARTERIAL BLOOD OXYGEN (O_2 tension) causes vasoconstriction in healthy humans (2, 4, 6, 7, 28). Although hyperoxic vasoconstriction was first reported at least 90 years ago (2), the mechanism(s) for this phenomenon in healthy humans is poorly understood. Several animal models of hyperoxic vasoconstriction sug-

gest that O_2 tension may influence one or more of the endothelium-derived factors that contribute to the maintenance of vascular tone, such as NO, endothelin, and vasoactive prostaglandins (5, 26, 29, 35). One potential mechanism by which hyperoxia may affect these vasoactive substances is the generation of reactive oxygen species (ROS) that occurs with increased O_2 tension. For example, it has been demonstrated in vitro that superoxide anions derived from hyperoxia react rapidly with NO (37), thereby, decreasing its bioavailability. However, in healthy humans, the hypothesis that hyperoxic vasoconstriction is related to ROS generation or that hyperoxia may impair the function of endothelium-derived vasoactive factors has not been tested.

Hyperoxia has also been associated with both increased systemic and coronary vascular resistance in patients with congestive heart failure (CHF) (11, 23). Moreover, in CHF, the rise in vascular resistance is accompanied by a fall in cardiac output and stroke volume of clinical importance, given the frequent administration of O_2 -enriched gas mixtures to patients with impaired ventricular function in the hospital setting. The potential interaction of hyperoxia-derived ROS and endothelial factors in CHF patients is of particular interest on the basis of the observation that endothelium-dependent vascular function is already impaired in these patients (19–21), possibly because of generalized oxidative stress (12, 22). As in healthy subjects, the mechanism(s) of the vascular effects of hyperoxia in CHF remain incompletely explored.

The objective of this study was to explore the possibility that hyperoxic vasoconstriction is related to an interaction between endothelium-derived factors and ROS. To investigate the effect of hyperoxia in the setting of preserved endothelial function, we studied young, healthy subjects. Vitamin C was employed as the antioxidant intervention. It is a potent aqueous phase antioxidant that has been shown to improve endothelial dysfunction due to the interaction of endothelium-derived NO and ROS (17, 40, 41). The hypotheses tested were: 1) hyperoxia vasoconstriction in the

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forearm would be attenuated by the antioxidant vitamin C; 2) hyperoxia would impair forearm vascular vasodilation to endothelial stimulation by the muscarinic agonist acetylcholine; and 3) these responses would be restored by vitamin C. To examine the effect of hyperoxia in the setting of impaired endothelial function, we tested the same hypotheses in CHF patients, a population of key interest given the clinical consequences of vasoconstriction.

METHODS

Study Population

Eleven healthy subjects participated in this study (mean age 36 ± 3 yr, 9 males, 2 females). All subjects were nonsmokers, and none were taking medications or vitamin supplements.

Fifteen male patients with CHF also participated (mean age 55 ± 3 yr, 9 patients in NYHA II, 6 patients in NYHA III). The etiology of CHF was ischemic in seven patients and idiopathic in eight patients. All patients had a left ventricular ejection fraction of $<35\%$ (mean $16 \pm 2\%$), as determined by resting radionuclide ventriculography. All patients were nonsmokers; two patients had hypertension controlled by medical therapy, three patients had noninsulin-dependent diabetes, and three patients had hypercholesterolemia controlled by medical therapy. The mean cholesterol measurement was 5.0 ± 0.3 mM. Medical therapy included angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists ($n = 15$), diuretics ($n = 13$), digoxin ($n = 7$), carvedilol ($n = 2$), other β -blockers ($n = 6$), acetylsalicylic acid ($n = 8$), and nitrates ($n = 3$).

Three different protocols were performed on separate days (see below) and individuals participated in more than one protocol. Of the healthy subjects, 9 completed the acetylcholine protocol and 8 completed the verapamil protocol. Of the CHF patients, 11 completed the acetylcholine protocol, 6 completed the verapamil protocol, and 8 completed the repeated hyperoxia protocol.

This study was approved by the Ethics Committee for Research on Human Subjects at the University of Toronto. All participants gave written informed consent.

Experimental Setup and Hemodynamic Measurements

All protocols were performed in the postabsorptive state in a climate-controlled facility. For individuals participating in more than one protocol, the studies were separated by at least 1 wk. Medications were withheld for at least 12 h before the study, except for antiplatelet medications, which were withheld for 7 days. All studies were performed in the morning and subjects were advised to consume a light, low-fat breakfast. Before the study day, all participants were asked to refrain from caffeine for at least 24 h and alcohol for at least 48 h. Subjects were also encouraged to get adequate sleep and refrain from strenuous exercise 24 h before the study. For protocols involving intra-arterial drug infusions, a 20-gauge plastic catheter (catheter set; Cook, Bloomington, IN) was inserted into the brachial artery of the nondominant arm. Heart rate was monitored continuously using a three-lead electrocardiogram, and blood pressure was measured using a noninvasive automatic blood pressure cuff (Dinamap; Critikon) placed on the dominant arm and by transducing the intra-arterial pressure via the brachial artery cannula. A rest period of at least 20 min followed catheter placement. After the rest period, measurements of forearm blood flow (FBF),

blood pressure, and heart rate were performed and then repeated to ensure stability.

FBF in the infused arm was determined by venous occlusion strain-gauge plethysmography with calibrated mercury-in-Silastic strain gauges (model EC4 plethysmograph; D. E. Hokanson, Bellevue, WA) and expressed as units representing $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}$. The arm was supported above the level of the heart and a venous occlusion pressure of 40 mmHg was used (models AG101 cuff inflator air source and E20 rapid cuff inflator; D. E. Hokanson). A wrist cuff was inflated to suprasystolic pressures for at least 1 min before each FBF measurement to exclude circulation to the hand. Each FBF measurement represents the average of five separate determinations at 10-s intervals. The plethysmograph output was aligned to an amplifier on a Gould ink recorder as well as to a digital converter and acquired on-line. Analysis of FBF was performed off-line using a customized software program (LabView version 4.1; National Instruments) that draws the line of best fit for the slope of arterial inflow for each determination. After each FBF determination, blood pressure was also measured. Forearm vascular resistance (FVR) was calculated as the ratio of mean blood pressure-to-FBF and expressed as units reflecting $\text{mmHg} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}$. Heart rate was determined after each FBF measurement using at least 15 cardiac cycles from the continuously recorded electrocardiogram signal.

Intra-arterial Drug Infusions

All brachial artery infusions were performed at a constant rate of 0.4 ml/min using a Harvard pump. Acetylcholine (Miochol-E; acetylcholine chloride 10 mg/ml; CIBA-Geigy, Mississauga, Canada) was administered to assess endothelium-dependent vascular responses. FBF was measured during infusion of acetylcholine at doses of 15, 30, and 60 $\mu\text{g}/\text{min}$ in CHF patients and at doses of 7.5, 15, and 30 $\mu\text{g}/\text{min}$ in healthy subjects. Verapamil (verapamil hydrochloride injection 5 mg/2 ml; Sabex, Boucherville, QC, Canada) was administered to assess vascular responses not dependent on endothelium-derived or exogenous NO. FBF was measured during infusion of verapamil at doses of 30, 100, and 300 $\mu\text{g}/\text{min}$ in CHF patients and at doses of 25, 50, and 100 $\mu\text{g}/\text{min}$ in healthy subjects. For both drugs, each dose was infused for 5 min with measurements performed during the last 2 min. Higher doses of acetylcholine and verapamil were used in CHF patients because blunted responses to endothelium-dependent and -independent vasodilators have been demonstrated in this population (31).

Vitamin C (ascorbic acid injection 200 mg/2 ml; Sabex) was administered via the brachial artery to assess whether this antioxidant modified the vascular responses to hyperoxia alone and to hyperoxia combined with acetylcholine. Vitamin C was infused at a dose of 24 mg/min to achieve an estimated local concentration of 1–10 mM (40, 41). This vitamin C concentration has been shown to protect human plasma from free radical-mediated lipid peroxidation (9). The vehicle solution, normal saline (0.9% sodium chloride; Astra), was infused via the brachial artery at a rate of 0.4 ml/min during periods when vitamin C was not infusing.

Acetylcholine Protocol

We examined the effect of hyperoxia on forearm vascular responses to acetylcholine, and whether vitamin C modified the vascular responses to hyperoxia with and without the administration of acetylcholine. The same tight-fitting non-rebreather mask was applied for a series of three conditions. The first two conditions were 21 and 100% O_2 (hyperoxia)

administered by the mask at the same flow rate, and the order of which was blinded and randomized. During these two conditions, the vehicle solution was infused into the brachial artery. The third condition was 100% O₂ administered by the mask with the concomitant infusion of intrarterial vitamin C (hyperoxia + vitamin C). In each of the three conditions, FBF, blood pressure, and heart rate were measured at baseline and then after 10 min of mask application. With the mask in place, the FBF response to increasing concentrations of acetylcholine was then determined. The three conditions were separated by a minimum of 12 min to reestablish the baseline state. In this protocol, hyperoxia was confirmed by measuring arterial blood PO₂ before (99 ± 3 Torr) and after (360 ± 19 Torr) the hyperoxia plus vitamin C condition. There were also no significant changes in pH (7.40 ± 0.01 to 7.41 ± 0.01) or PCO₂ (43 ± 1 to 42 ± 1 Torr), suggesting that experimental hyperoxia had no effect on ventilation.

Verapamil Protocol

We examined whether any vascular effects of hyperoxia observed in the acetylcholine protocol were specifically endothelium-dependent. In this protocol, the nonrebreather mask was applied for a series of two conditions: 21% O₂ and hyperoxia, the order of which was blinded and randomized. In each of the conditions, FBF, blood pressure, and heart rate were measured at baseline and then at 10 min of mask application. With the mask in place, the FBF response to increasing concentrations of verapamil was then determined. The two conditions were separated by at least 60 min to reestablish the baseline state.

Repeated Hyperoxia Protocol

We sought to determine that any effect of hyperoxia on FBF or FVR would be sustained over a 20-min exposure and would be reproducible on a second exposure to hyperoxia in CHF patients. In this protocol, the nonrebreather mask was applied for a series of three conditions, each lasting 20 min. The first two mask applications were for the administration of 21% O₂ and hyperoxia, the order of which was blinded and randomized. The final mask application was for the second administration of hyperoxia. During each condition, measurements of FBF, blood pressure, and heart rate were per-

formed at baseline and then at 10 and 20 min of mask application. The three conditions were separated by at least 12 min to reestablish the baseline state. This protocol was not performed in healthy volunteers, because in this population, a vasoconstrictor effect of hyperoxia persisting >1 h has been previously demonstrated in our laboratory (28).

Statistical Analysis

All data are presented as means ± SE. Analysis was performed using SigmaStat (version 1.1; Jandel Scientific). For the acetylcholine and verapamil protocol, a two-way, repeated-measures ANOVA was used to analyze the raw data for FBF, FVR, blood pressure, and heart rate. The two within-subject factors were the condition (21% O₂, hyperoxia, and hyperoxia + vitamin C) and the stage within each condition (baseline; 10-min mask breathing; acetylcholine or verapamil infusion at doses 1, 2, and 3). Comparisons of %changes from baseline in FBF and FVR were also made with a two-way, repeated-measures ANOVA. The two within-subject factors were the condition (21% O₂, hyperoxia, and hyperoxia + vitamin C) and the increasing concentrations of infused vasodilator. Comparisons of %changes from baseline in FBF and FVR after 10 min of mask breathing among the three conditions (21% O₂, hyperoxia, and hyperoxia + vitamin C) were made with a one-way, repeated-measures ANOVA. For the repeated hyperoxia protocol, changes in FVR, FBF, heart rate, and blood pressure from baseline were analyzed with a one-way, repeated-measures ANOVA. Pairwise comparisons post hoc were performed with the Student-Newman-Keuls test. Statistical significance was accepted if $P < 0.05$.

RESULTS

Healthy Subjects

Effect of hyperoxia with and without vitamin C on basal vascular tone (Table 1). In healthy subjects, the administration of high-concentration O₂ resulted in a significant 28 ± 10% increase in FVR. This increase was also significant when compared with the 21% O₂ condition ($P < 0.05$). With the concomitant arterial infusion of vitamin C during hyperoxia, no significant

Table 1. Effect of hyperoxia on endothelium-dependent vascular responses and modification by vitamin C in healthy subjects

	Baseline	Mask Breathing	ACh, 7.5 µg/min	ACh, 15 µg/min	ACh, 30 µg/min
21% O₂					
FBF, Units	2.7 ± 0.2	2.7 ± 0.2	6.1 ± 1.0	8.3 ± 1.3	10.2 ± 1.8
FVR, Units	32 ± 3	32 ± 4	17 ± 3	14 ± 4	12 ± 3
HR, beats/min	63 ± 3	61 ± 3	58 ± 3	60 ± 2	61 ± 2
MAP, mmHg	80 ± 2	81 ± 3	81 ± 2	83 ± 2	82 ± 2
100% O₂					
FBF, Units	2.9 ± 0.3	2.5 ± 0.3	5.3 ± 1.3‡	6.3 ± 0.8‡‡	9.0 ± 1.6
FVR, Units	30 ± 3	38 ± 4*	22 ± 3‡‡	16 ± 2	12 ± 2
HR, beats/min	63 ± 2	57 ± 2	56 ± 3	58 ± 2	57 ± 2
MAP, mmHg	81 ± 1	84 ± 2	86 ± 2	84 ± 2	85 ± 2
100% O₂ + vitamin C					
FBF, Units	2.7 ± 0.2	2.8 ± 0.2	8.6 ± 1.3	9.6 ± 1.6	11.0 ± 1.9
FVR, Units	33 ± 3	32 ± 3	13 ± 3	12 ± 3	10 ± 2
HR, beats/min	64 ± 3	61 ± 3	59 ± 3	59 ± 3	59 ± 2
MAP, mmHg	85 ± 2	84 ± 2	87 ± 2†	89 ± 2†	88 ± 2†

Values are means ± SE. FBF, forearm blood flow; FVR, forearm vascular resistance; HR, heart rate; MAP, mean arterial pressure; ACh, acetylcholine. * $P < 0.05$ for comparison of 10 min of mask application vs. the preceding baseline; † $P < 0.05$ vs. 21% O₂; ‡ $P < 0.05$ vs. 100% O₂ + vitamin C.

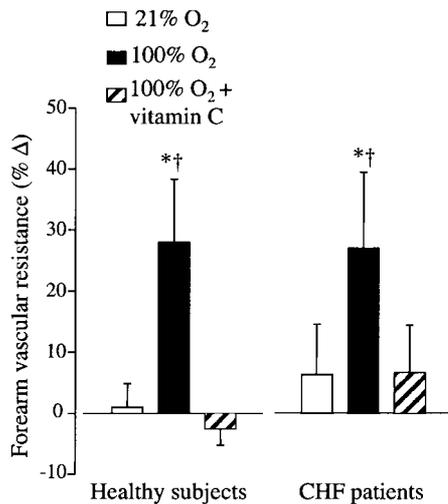


Fig. 1. Forearm vascular resistance responses (expressed as %changes from the preceding baseline) to 21% O₂ with intra-arterial saline (open bars), hyperoxia with intra-arterial saline (solid bars), and hyperoxia with intra-arterial vitamin C (hatched bars) in healthy subjects and CHF patients. * $P < 0.05$ vs. 21% O₂ with intra-arterial saline; † $P < 0.05$ vs. hyperoxia + vitamin C

change in FVR from the preceding baseline was observed ($-2 \pm 3\%$). The absence of a change was significant compared with the rise in FVR observed during hyperoxia alone ($P < 0.05$) (Fig. 1). With respect to FBF, no changes from baseline were observed during 21% O₂, hyperoxia, or hyperoxia plus vitamin C.

Effect of hyperoxia on endothelium-dependent and independent vasodilation (Tables 1 and 2). The dose-dependent, acetylcholine-mediated increase in FBF was significantly blunted during hyperoxia compared with 21% O₂ ($P < 0.05$), an effect most apparent at the two lower doses of acetylcholine. In contrast, there were no differences among the FBF responses to any dose of verapamil during hyperoxia compared with 21% O₂ (Fig. 2).

Effect of vitamin C on endothelium-dependent vasodilation during hyperoxia (Table 1). When vitamin C was infused before the administration of hyperoxia, the acetylcholine-mediated increase in FBF was no longer blunted compared with 21% O₂, and was significantly greater compared with hyperoxia alone ($P < 0.05$; Table 1, Fig. 2). These results are consistent with the hypothesis that hyperoxia impairs endothelium-depen-

dent vasodilation by a free radical mechanism in healthy humans.

Effect of hyperoxia on heart rate and mean arterial pressure (Tables 1 and 2). Heart rate tended to decrease with exposure to 100% O₂ regardless of the presence of vitamin C. However, no significant differences were observed between conditions. Mean arterial pressure (MAP) tended to increase with exposure to 100% O₂ regardless of the presence of vitamin C. The increase in MAP reached statistical significance at the 100% O₂ + vitamin C condition compared with the 21% O₂ condition ($P < 0.05$). Neither acetylcholine nor verapamil had any systematic effect on heart rate or MAP.

CHF Patients

Effect of hyperoxia with and without vitamin C on basal vascular tone (Table 3). During hyperoxia in CHF patients, FVR increased by $27 \pm 12\%$, a significant change compared with the preceding baseline and to the 21% O₂ condition ($P < 0.05$ for both). With the concomitant arterial infusion of vitamin C during hyperoxia, no significant change in FVR from the preceding baseline was observed ($7 \pm 8\%$). Absence of a change was significant compared with the rise in FVR measured in response to hyperoxia alone ($P < 0.05$), demonstrating that hyperoxic vasoconstriction was attenuated by the administration of vitamin C in CHF patients (Fig. 1). With respect to basal FBF, no changes from baseline were observed during 21% O₂, hyperoxia, or hyperoxia + vitamin C.

Effect of hyperoxia on endothelium-dependent and -independent vasodilation (Tables 3 and 4). In contrast to healthy subjects, there were no differences between hyperoxia and 21% O₂ with respect to the acetylcholine-mediated increases in FBF. There were also no differences between hyperoxia and 21% O₂ with respect to the FBF responses to any dose of verapamil (Fig. 2).

Effect of vitamin C on endothelium-dependent vasodilation during hyperoxia (Table 3). The infusion of vitamin C during hyperoxia augmented the acetylcholine-mediated increase in FBF compared with both hyperoxia alone and 21% O₂ ($P < 0.05$ for both; Fig. 2).

Table 2. Effect of hyperoxia on endothelium-independent vascular responses in healthy subjects

	Baseline	Mask Breathing	VER, 25 μg/min	VER, 50 μg/min	VER, 100 μg/min
21% O ₂					
FBF, Units	3.2 ± 0.4	3.4 ± 0.5	8.4 ± 1.0	10.4 ± 1.5	12.0 ± 2.1
FVR, Units	28 ± 3	27 ± 3	11 ± 1	9 ± 1	8 ± 1
HR, beats/min	63 ± 2	62 ± 3	62 ± 3	62 ± 3	64 ± 4
MAP, mmHg	80 ± 1	81 ± 2	80 ± 2	82 ± 2	81 ± 2
100% O ₂					
FBF, Units	3.4 ± 0.4	3.3 ± 0.4	8.5 ± 1.1	10.0 ± 1.0	12.8 ± 1.6
FVR, Units	26 ± 3	27 ± 3	11 ± 2	9 ± 1	7 ± 1
HR, beats/min	65 ± 2	60 ± 2	62 ± 3	60 ± 2	63 ± 3
MAP, mmHg	81 ± 3	81 ± 3	82 ± 3	82 ± 3	81 ± 3

Values are means ± SE. VER, verapamil.

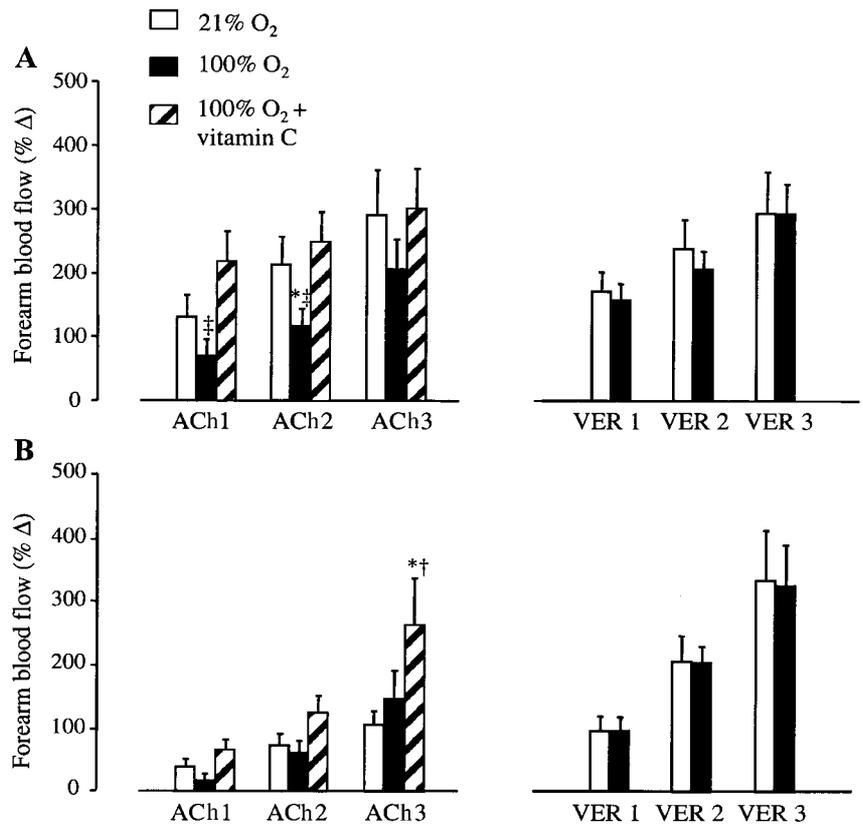


Fig. 2. Forearm blood flow responses (expressed as %changes from the preceding baseline) to increasing concentrations of intra-arterial acetylcholine (ACh 1, 2, and 3) and verapamil (VER 1, 2, and 3) in healthy subjects (A) and congestive heart failure patients (B). The experimental conditions are 21% O₂ (open bars), hyperoxia (solid bars), and hyperoxia + vitamin C (hatched bars). **P* < 0.05 vs. 21% O₂; †*P* < 0.05 vs. hyperoxia; ‡*P* < 0.05 vs. hyperoxia + vitamin C.

Effect of hyperoxia on heart rate and mean arterial pressure (Tables 3 and 4). Throughout both the acetylcholine and verapamil protocols, there was no discernible change in heart rate or MAP.

Effect of repeated exposures to hyperoxia. We observed vasoconstriction in response to hyperoxia, a response apparently abolished with the addition of vitamin C during a subsequent exposure to hyperoxia. To provide evidence that this was an effect of vitamin C and not fatigue of the vasoconstrictor response to the repeated-administration hyperoxia,

we examined vascular responses during repeated administrations of hyperoxia. During the first exposure to hyperoxia, FVR increased from 51 ± 6 to 61 ± 8 units at 10 min (*P* < 0.05 vs. baseline) and 65 ± 6 units at 20 min (*P* < 0.05 vs. baseline). During the second exposure to hyperoxia, FVR again increased from 56 ± 6 to 68 ± 8 units at 10 min (*P* < 0.05 vs. baseline) and 67 ± 6 units at 20 min (*P* < 0.05 vs. baseline). In contrast, there were no significant changes in FVR from baseline after 10 and 20 min of 21% O₂ (56 ± 6 to 55 ± 6 and 57 ± 7 units).

Table 3. Effect of hyperoxia on endothelium-dependent vascular responses and modification by vitamin C in congestive heart failure patients

	Baseline	Mask Breathing	ACh, 15 µg/min	ACh, 30 µg/min	ACh, 60 µg/min
21% O₂					
FBF, Units	2.4 ± 0.3	2.2 ± 0.3	3.3 ± 0.4	4.3 ± 0.8	5.2 ± 0.9
FVR, Units	39 ± 4	42 ± 6	28 ± 2	23 ± 2	20 ± 2
HR, beats/min	75 ± 7	75 ± 7	75 ± 7	76 ± 7	74 ± 7
MAP, mmHg	87 ± 5	85 ± 4	85 ± 5	84 ± 4	85 ± 4
100% O₂					
FBF, Units	2.4 ± 0.3	2.0 ± 0.3	2.7 ± 0.4	3.7 ± 0.5	5.4 ± 0.8
FVR, Units	40 ± 4	50 ± 6*†‡	37 ± 4†‡	28 ± 3	20 ± 2
HR, beats/min	74 ± 7	73 ± 7	74 ± 7	75 ± 7	75 ± 7
MAP, mmHg	85 ± 4	88 ± 5	87 ± 5	89 ± 5	90 ± 5
100% O₂ + vitamin C					
FBF, Units	2.4 ± 0.3	2.4 ± 0.2	3.8 ± 0.4	5.2 ± 0.8	8.1 ± 1.3†§
FVR, Units	41 ± 5	41 ± 4	25 ± 3	21 ± 3	15 ± 2
HR, beats/min	75 ± 7	74 ± 6	74 ± 6	75 ± 6	76 ± 7
MAP, mmHg	87 ± 5	90 ± 4	87 ± 4	90 ± 5	91 ± 5

Values are means ± SE. **P* < 0.05 for comparison of 10 min of mask application vs. the preceding baseline; †*P* < 0.05 vs. 21% O₂; ‡*P* < 0.05 vs. 100% O₂ + vitamin C; §*P* < 0.05 vs. 100% O₂.

Table 4. *Effect of hyperoxia on endothelium-independent vascular responses in congestive heart failure patients*

	Baseline	Mask Breathing	VER, 30 $\mu\text{g}/\text{min}$	VER, 100 $\mu\text{g}/\text{min}$	VER, 300 $\mu\text{g}/\text{min}$
21% O₂					
FBF, Units	2.3 \pm 0.5	2.5 \pm 0.6	4.4 \pm 0.8	6.4 \pm 0.9	8.6 \pm 1.0
FVR, Units	39 \pm 8	36 \pm 8	20 \pm 4	13 \pm 2	9 \pm 1
HR, beats/min	66 \pm 6	66 \pm 6	66 \pm 6	62 \pm 5	67 \pm 6
MAP, mmHg	73 \pm 4	72 \pm 4	73 \pm 3	75 \pm 4	76 \pm 4
100% O₂					
FBF, Units	2.4 \pm 0.5	2.0 \pm 0.3	4.4 \pm 0.6	6.8 \pm 0.9	9.2 \pm 1.1
FVR, Units	37 \pm 6	45 \pm 6*	20 \pm 3	12 \pm 1	9 \pm 1
HR, beats/min	67 \pm 5	64 \pm 5	64 \pm 5	66 \pm 5	65 \pm 6
MAP, mmHg	77 \pm 6	79 \pm 4	79 \pm 4	78 \pm 5	80 \pm 5

Values are means \pm SE. * P < 0.05 for comparison of 10 min of mask application vs. the preceding baseline.

DISCUSSION

In the present study, we demonstrated that hyperoxia-mediated increases in FVR in both healthy subjects and CHF patients were abolished by the administration of vitamin C. We also showed that hyperoxia was associated with the impairment of acetylcholine-dependent vasodilation, which was similarly reversed by vitamin C. This latter observation was limited to healthy subjects with presumably normal endothelial responsiveness. These data confirm that increased blood O₂ tension rapidly affects vascular tone and also suggest that hyperoxia modifies endothelial function, in part, through a ROS-related mechanism.

Hyperoxic Vasoconstriction

The observation that hyperoxia increased vascular resistance in both groups is consistent with the findings of many investigators (2, 4, 6, 7, 11, 23, 28). We have made the novel observation that hyperoxic vasoconstriction is prevented by vitamin C, suggesting that hyperoxia-associated ROS are mediators of this vascular response. High blood O₂ tension has been associated with increased production of superoxide anions as well as other ROS (14, 32), although the mechanisms by which hyperoxia may generate ROS at or near the vascular wall are unclear. Possibilities include increased mitochondrial respiration and proportional leakage of ROS (8), increased substrate availability for enzymes such as NADPH oxidase (43) and the oxidation of circulating electron-transferring components, heme-containing proteins, ferredoxins, thiols, and catecholamines (14).

Hyperoxia-generated ROS may alter vascular tone by means of an interaction with endothelium-derived NO. This is supported by *in vitro* studies that demonstrate the degradation of endothelium-derived relaxing factor after exposure to high O₂ tension (37). Superoxide dismutase attenuated the degradation of NO, providing strong evidence for the role of hyperoxia-derived superoxide anions. Depending on the vascular bed studied, some but not all, animal studies (29, 35, 38) have also suggested that hyperoxia may decrease the bioavailability of NO, whereas both animal and human studies (3, 33, 34) suggest that hypoxia increases NO. *In vivo*, hyperoxia may interact with NO by more than

one mechanism. First, hyperoxia-derived superoxide anions may react with NO, thereby, decreasing its bioavailability. This well-described reaction yields peroxynitrite and nitrogen dioxide (36). Second, ROS may potentially affect the redox state of tetrahydrobiopterin (BH₄), impairing its function as an essential cofactor for endothelial NO synthase (25). Finally, hyperoxia may interact with nitrosylated hemoglobin and/or thiol compounds that serve as vehicles for circulatory NO transport. Release of NO from these compounds is likely inversely related to blood O₂ tension (15). Vitamin C may thus prevent hyperoxic vasoconstriction by several mechanisms involving NO bioavailability, including direct quenching of superoxide anions, stabilization of BH₄, and possibly increasing the release of NO from peroxynitrite and nitrosylated compounds (24).

The possible interaction of hyperoxia-derived ROS, NO, and vitamin C provides an appealing explanation for hyperoxic-vasoconstriction. However, other mechanisms of hyperoxic-vasoconstriction for which there is some evidence, must be considered. For example, hyperoxia may inhibit the production of vasodilating prostaglandins (26). Moreover, it has been demonstrated that prolonged vitamin C supplementation may enhance the production of these vascular mediators (1). It has been demonstrated that both hyperoxia and oxidative stress may stimulate increased production of the endothelium-derived vasoconstrictor endothelin (5, 16). Finally, hyperoxia may vasoconstrict by an effect on potassium channel conductance and the resting membrane potential of vascular smooth muscle cells (29).

Hyperoxia and Endothelium-Dependent Vasodilation

To explore the existence of a specific interaction between hyperoxia and the endothelium, we examined the effects of hyperoxia on responses to acetylcholine, an endothelium-dependent vasodilator. Indeed, we demonstrated that hyperoxia blunted the FBF responses to acetylcholine in healthy subjects, whereas hyperoxia did not influence responses to verapamil, an endothelium-independent vasodilator. Acetylcholine has been used in numerous investigations as a means of examining vasodilation mediated by endothelium-derived NO. Thus our data provide support for the hypothesis that hyperoxia specifically diminishes NO

bioavailability. The observation that vitamin C restored the hyperoxia-associated impairment of acetylcholine-mediated vasodilation again suggests a ROS-related mechanism, as discussed earlier.

Our observations are consistent with the results of previous human studies that evaluated the effect of hyperoxia on limb vascular responses to ischemia and exercise. It has been demonstrated that hyperoxia reduced peak FBF and increased FVR after forearm ischemia in normal human volunteers (4); similarly, it has been shown that hyperoxia diminished the leg blood flow response to pedaling exercise (42). However, the effects of hyperoxia in these studies could not be clearly attributed to an endothelium-dependent mechanism. Although NO release contributes to reactive hyperemia, the response to ischemia is complex and may relate to the production of other metabolites and vasoactive factors (39). The vasodilator response to exercise is partly endothelium-dependent and likely related to changes in endothelial shear stress (27) but may also involve other vasoactive mediators and endothelium-independent mechanisms (18).

Hyperoxic Vasoconstriction in the CHF Setting

In CHF patients hyperoxia increased FVR, a response attenuated by vitamin C again suggesting that ROS play a role. However, in this group, hyperoxia did not discernibly impair the FBF responses to acetylcholine. This may relate to the preexisting impairment of endothelium-dependent vasodilation well described in this patient population. Of note, to our knowledge there are no reports of other experimental interventions that further depress acetylcholine-dependent vasodilation in humans with preexisting endothelial dysfunction. The possibility remains that the mechanism of hyperoxic vasoconstriction in CHF does not involve endothelium-derived NO. Regulation of vascular tone is more complex in the setting of CHF on the basis of abnormal activation of vasoconstrictor and vasodilator systems, including the sympathetic nervous system, the renin angiotensin system, endothelin, and natriuretic peptides (31). Hyperoxia may interact with one or more of these systems to produce vasoconstriction in CHF patients.

Vitamin C responses in the CHF group merit discussion. Vitamin C augmented the FBF response to acetylcholine during hyperoxia compared with both 21% O₂ and hyperoxia alone. Hornig et al. (12) have demonstrated that vitamin C reverses impaired flow-mediated dilatation of forearm conduit vessels in CHF patients. Thus in the present study, the effect of vitamin C in the CHF group may not be specific to hyperoxia. More likely, vitamin C partially restored abnormal acetylcholine-mediated vasodilation in the CHF group independently of hyperoxia. Our findings are partially consistent with those of Murchie et al. (30). These investigators also demonstrated that in CHF patients, hyperoxia had no effect on the FBF response to a single dose of acetylcholine. In their experiment, vitamin C had no effect on the response to acetylcholine whether

hyperoxia was present or not. However, these investigators did not observe the increase in basal FVR during hyperoxia described in this and other studies (11, 23), nor did they study healthy subjects.

Methodologic Considerations

The measurement of acute free radical generation is difficult in the intact human (10) and was not attempted in the present study. The dose of vitamin C used yields an estimated forearm concentration of 1–10 mM, which is sufficient to compete effectively for superoxide anions and reduce the interaction of superoxide and NO (9, 13). The observation that vitamin C prevented the functional consequences of hyperoxia provides indirect evidence of hyperoxic ROS generation. Our confidence in this observation was supported by the demonstration that repeated exposures to hyperoxia without vitamin C resulted in reproducible increases in FVR. This provided evidence that attenuation of vasoconstriction during hyperoxia with the infusion of vitamin C could not be attributed to fatigue of the vascular response to hyperoxia. Finally, it is very unlikely that vitamin C had independent vasodilator properties as an explanation for the reversal of hyperoxic vasoconstriction. The concentration of vitamin C infused in this study was similar to that used in other experiments in healthy volunteers and patients with various cardiovascular conditions, including CHF (12, 40, 41). These studies have uniformly demonstrated that brachial artery infusion of vitamin C alone does not alter FBF or FVR.

In conclusion, this investigation demonstrates that hyperoxic vasoconstriction is reversed by vitamin C in both healthy humans and CHF patients, consistent with the hypothesis that hyperoxia-derived free radicals mediate this response. In healthy humans, vitamin C also reverses the acute impairment of endothelium-dependent vasodilation by hyperoxia, suggesting that hyperoxia-derived free radicals diminish the bioavailability of NO. In CHF, hyperoxia apparently did not affect endothelium-dependent vasodilation, whereas vitamin C augmented endothelium-dependent vasodilation despite the presence of hyperoxia, suggesting that control of vascular tone is more complex in this population. Overall, these results highlight the role of acute redox modulation in control of blood vessel tone and endothelial function. Even relatively brief exposure to hyperoxia is associated with measurable physiological derangement apparently due to increased free radical generation. Although the use of O₂-enriched gas mixtures is fundamental for the treatment and prevention of hypoxia, the physiological consequences of inadvertent hyperoxia should be considered, especially for the patient with CHF.

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