Neutrophil Superoxide Anion-Generating Capacity, Endothelial Function and Oxidative Stress in Chronic Heart Failure: Effects of Short- and Long-Term Vitamin C Therapy

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OBJECTIVES
First, we sought to study the effects of short- and long-term vitamin C therapy on oxidative stress and endothelial dysfunction in chronic heart failure (CHF), and second, we sought to investigate the role of neutrophils as a cause of oxidative stress in CHF.

BACKGROUND
Oxidative stress may contribute to endothelial dysfunction in CHF. Vitamin C ameliorates endothelial dysfunction in CHF, presumably by reducing oxidative stress, but this is unproven.

METHODS
We studied 55 patients with CHF (ischemic and nonischemic etiologies) and 15 control subjects. Flow-mediated dilation (FMD) in the brachial artery was measured by ultrasound wall-tracking, neutrophil superoxide anion (O$_2^-$) generation by lucigenin-enhanced chemiluminescence and oxidative stress by measurement of free radicals (FRs) in venous blood using electron paramagnetic resonance (EPR) spectroscopy and plasma thiobarbituric acid reactive substances (TBARS). Measurements were performed at baseline in all subjects. The effects of short-term (intravenous) and long-term (oral) vitamin C therapy versus placebo were tested in patients with nonischemic CHF.

RESULTS
At baseline, FRs were higher in patients with CHF than in control subjects ($p < 0.01$), TBARS were greater ($p < 0.005$), neutrophil O$_2^-$-generating capacity was enhanced ($p < 0.005$) and FMD was lower ($p < 0.0001$). Compared with placebo, short-term vitamin C therapy reduced FR levels ($p < 0.05$), tended to reduce TBARS and increased FMD ($p < 0.05$), but did not affect neutrophil O$_2^-$-generating capacity. Long-term vitamin C therapy reduced FR levels ($p < 0.05$), reduced TBARS ($p < 0.05$) and improved FMD ($p < 0.05$), but also reduced neutrophil O$_2^-$-generating capacity ($p < 0.05$). Endothelial dysfunction was not related to oxidative stress, and improvements in FMD with vitamin C therapy did not relate to reductions in oxidative stress.

CONCLUSIONS
Oxidative stress is increased in ischemic and nonischemic CHF, and neutrophils may be an important cause. Vitamin C reduces oxidative stress, increases FMD and, when given long term, decreases neutrophil O$_2^-$ generation, but the lack of a correlation between changes in endothelial function and oxidative stress with vitamin C implies possible additional non-antioxidant benefits of vitamin C. (J Am Coll Cardiol 2000;36:1474–82) © 2000 by the American College of Cardiology.

The prevalence of chronic heart failure (CHF) is increasing alarmingly against the trend in coronary artery disease (CAD) prevention, and this phenomenon is not entirely explained by demographic changes (1,2). Furthermore, despite the impressive gains resulting from advances in medical therapy over the past few years, CHF remains a disease with a high mortality rate and severe impairment of quality of life (1,2). Therefore, we need to better understand the pathophysiology, with a view to further improvements in therapy.

It has been postulated that oxidative stress plays a key role in the genesis and progression of CHF (3–8) and that, as a consequence, vitamin C may have an important potential therapeutic role (8). Although there is considerable evidence that oxidative stress is increased in CHF, previous studies have generally been based on patients with underlying CAD, which is a cause of increased oxidative stress, or have used indirect, nonspecific markers (3–7). The underlying origin and species of oxygen free radicals (FRs) in CHF have also not been defined, and the mechanism(s) of action of vitamin C on endothelial dysfunction in CHF remain(s) unclear.

The aims of this study were: 1) to assess the role of oxidative stress as a mechanism of endothelial dysfunction in CHF, using a novel, direct technique involving electron paramagnetic resonance (EPR) spectroscopy and a standard marker of lipid peroxidation; 2) to assess the effects of vitamin C therapy on these variables and on endothelial
function; and 3) to investigate neutrophil superoxide anion–generating capacity as a potentially important source of free radicals (FRs) in CHF and to test their response to vitamin C therapy.

METHODS

Subjects. We studied 55 patients with New York Heart Association (NYHA) functional class II-IV symptoms of heart failure who were recruited from a cardiology clinic, as well as 15 healthy control subjects (Table 1). Patients were clinically stable, with no signs of pulmonary or peripheral congestion, and were stable (unchanged for three months) on medical therapy. Those patients with evidence of diabetes or significant concurrent medical illness were excluded. Patients had objective left ventricular systolic impairment (ejection fraction <35%), and all had recently undergone exercise testing and coronary angiography. The diagnosis of ischemic CHF was based on evidence of reversible ischemia on stress testing or a history of myocardial infarction and significant coronary disease on the angiogram, or both. The subjects gave written, informed consent, and approval was obtained from the local Research Ethics Committee.

Study protocol. Baseline EPR spectroscopy and analysis of plasma thiobarbituric acid reactive substances (TBARS) and assessment of neutrophil NADPH oxidase activity and endothelial function were performed in all patients and control subjects at 9 AM after an overnight fast. Blood samples were obtained by venipuncture. Neutrophils were separated from whole blood for measurement of neutrophil superoxide anion–generating capacity. Plasma was also stored frozen for subsequent measurement of TBARS.

Measurement of the effects of short-term (intravenous) and long-term (oral) vitamin C therapy were assessed separately in two groups of patients with CHF of nonischemic etiology, because our aim was to study the effects on CHF, per se, rather than on CAD. Twenty of these patients were given 2 g of vitamin C (Evans Medical Ltd., Surrey, United Kingdom) intravenously over 5 min. Over a week later, 10 of the patients (matched for age and functional status) were given intravenous saline. Baseline measures were repeated 30 min later. We had previously established that plasma vitamin C levels increased by over 500% at 30 min when given intravenously.

In a separate arm of the study, we also tested the effects of long-term (oral) vitamin C therapy in patients with CHF of nonischemic etiology. Ten patients were given oral vitamin C, 2 g twice daily, and 10 different patients matched for NYHA status received placebo. Repeat measurements of endothelial function, oxidative stress and neutrophil superoxide anion (O$_2^-$)-generating capacity were performed after one month of therapy in each group and again after a six-week washout period in the active treatment group.

Table 1. Clinical Characteristics of Patients With Chronic Heart Failure and Control Subjects

<table>
<thead>
<tr>
<th>Patients With CHF</th>
<th>Control Subjects</th>
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<tbody>
<tr>
<td></td>
<td>DCM</td>
</tr>
<tr>
<td>n (females)</td>
<td>40 (7)</td>
</tr>
<tr>
<td>Mean ± SEM age (yrs)</td>
<td>53 ± 2</td>
</tr>
<tr>
<td>Passive heavy smoking (n)</td>
<td>5</td>
</tr>
<tr>
<td>Mean ± SEM total cholesterol (mmol/liter)</td>
<td>6.0 ± 2.4</td>
</tr>
<tr>
<td>Mean ± SEM triglyceride (mmol/liter)</td>
<td>1.7 ± 1.0</td>
</tr>
<tr>
<td>Mean NYHA class</td>
<td>2.8</td>
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CAD = coronary artery disease; DCM = dilated cardiomyopathy; NYHA = New York Heart Association.
THIOBARBITURIC ACID REACTIVE SUBSTANCES. The plasma level of TBARS (an indicator of lipid peroxides) was determined as previously described using a colorimetric assay available as a standard commercially available kit (Oxis International Inc., Oxford, United Kingdom) (11). Briefly, the assay is based on the reaction of a chromogenic reagent with malonaldehyde and 4-hydroxynonenals. One molecule of either malonaldehyde or 4-hydroxynonenals reacts with two molecules of this reagent to yield a stable chromophore with maximal absorbance at 568 nm. The results are expressed as μmol/liter, with the values for malonaldehyde and 4-hydroxynonenals combined.

SUPEROXIDE ANION GENERATION BY POLYMORPHONUCLEAR CELLS. We employed a refined lucigenin-based chemiluminescent assay of NADPH oxidase activity to measure basal and post-activation levels of O$_2^-$ generation by neutrophils (12). Neutrophils were separated from venous blood using a Ficoll-Hypaque density gradient, and activation of the neutrophil respiratory burst was achieved using 1 μmol/liter of N-formyl-methionyl-leucyl-phenylalanine (Sigma Co.). The peak level of O$_2^-$ generated after activation was recorded in mV/5 × 10$^5$ neutrophils.

Measurement of flow-related endothelial function. Changes in brachial artery diameter in response to reactive hyperemia were measured noninvasively using a high resolution ultrasonic wall-tracking system (Vadirec Wall-Track System, resolution ± 3 μm). This technique was previously described and validated by our group (13). The brachial artery was imaged using a 7.5-MHz transducer, and reactive hyperemia was produced by releasing a sphygmomanometer cuff inflated to suprasystolic pressure (systolic pressure + 50 mm Hg) for 5 min. Internal brachial artery diameter was measured at baseline and 60 s after cuff release. The flow-mediated dilation (FMD) data are presented as the percent change in brachial artery diameter from baseline.

Plasma vitamin C (ascorbic acid). Ascorbate was measured by reverse phase high performance liquid chromatography with the use of an ion pairing agent and electrochemical detection (14). This assay measures ascorbate in the reduced form. Levels were checked in a subset of five patients to assess their response to therapy at baseline and after intravenous (30 min after dose) and oral (1 h after dose) vitamin C therapy, as well as after a six-week washout period.

Statistical analysis. For our statistical analysis, we used SPSS 9.0. All data are presented as the mean value ± SEM, unless otherwise stated. At baseline, measures of oxidative stress, neutrophil O$_2^-$-generating capacity and FMD were compared in patients with CHF (ischemic vs. nonischemic) and control subjects, using one-way analysis of variance (ANOVA) followed by the post hoc Bonferroni multiple comparisons test. The relations between NYHA status and these variables at baseline were also assessed using ANOVA. We used multi-way ANOVA and applied Tukey’s multiple comparisons test to compare the effects of short-term vitamin C therapy versus placebo on oxidative stress, neutrophil O$_2^-$-generating capacity and FMD. The effects of long-term vitamin C therapy versus placebo were assessed using analysis of covariance. Relations between changes in FMD and measures of oxidative stress and neutrophil O$_2^-$-generating capacity were assessed individually using linear regression analysis. Correlation coefficients were calculated using Pearson’s formula. Statistical significance was accepted at the 95% confidence level (p < 0.05).

RESULTS

The clinical characteristics of the study group are shown in Table 1. All patients were on stable medical therapy, including maximally tolerated angiotensin-converting enzyme inhibitor therapy and a diuretic agent.

Baseline

Oxidative stress. Analysis of the EPR spectra and comparison with published values indicated trapping of lipid alkoxyl and carbon-centered FRs derived from lipid peroxidation (15). These lipid-derived FRs were significantly higher in patients with CHF than in control subjects (0.41 ± 0.03 vs. 0.27 ± 0.04 U, p < 0.01). Plasma TBARS were also higher in patients with CHF than in control subjects (7.0 ± 0.6 vs. 3.8 ± 0.6 μmol/liter, p < 0.005). Post hoc analysis showed that lipid-derived FR levels were higher in patients with ischemic versus nonischemic CHF (0.53 ± 0.09 vs. 0.36 ± 0.06 U, p < 0.05), but not in levels of plasma TBARS (8.2 ± 1.2 vs. 6.5 ± 0.5 μmol/liter). In the patients with nonischemic CHF, severity (NYHA status) did not correlate with lipid-derived FR levels (p = NS), nor with TBARS (p = NS).

Neutrophil superoxide-generating capacity. Neutrophil O$_2^-$-generating capacity was significantly increased in patients with CHF versus control subjects (44.6 ± 4.3 vs. 17.7 ± 2.1 mV/5 × 10$^5$ neutrophils, p < 0.005), but was similar in patients with both nonischemic and ischemic etiologies (44.4 ± 3.8 vs. 45.1 ± 8.1 mV/5 × 10$^5$ neutrophils, respectively, p = NS). The NYHA status was related in the patients with nonischemic CHF to neutrophil O$_2^-$-generating capacity (p < 0.0001) (Fig. 1).

Endothelial function. Flow-mediated dilation was significantly impaired in patients with CHF versus control subjects (2.5 ± 0.4% vs. 6.5 ± 0.9%, p < 0.0001), but did not differ between patients with ischemic and those with nonischemic etiologies of CHF (1.3 ± 0.4% vs. 2.9 ± 0.4%, p = NS). In the patients with nonischemic CHF, FMD did not significantly relate to levels of oxidative stress (p = NS), neutrophil O$_2^-$-generating capacity (p = NS) or NYHA status (p = NS).

Effects of Vitamin C

In the subsets of patients studied, plasma levels of vitamin C rose significantly after 1 month of oral administration
(measured at 1 month) and after intravenous administration (measured at 30 min) (from 28.6 ± 1.4 to 74.8 ± 1.6 μmol/liter and from 20.6 ± 0.9 to 134.5 ± 31.6 μmol/liter, respectively). In the group given long-term vitamin C therapy, levels then fell after a six-week washout period, back to 41.4 ± 0.9 μmol/liter.

Short-term vitamin C therapy, as compared with placebo, reduced lipid-derived FRs (from 0.35 ± 0.03 to 0.23 ± 0.03 U, p < 0.05) (Fig. 2a), but only tended to reduce TBARS (from 6.4 ± 0.7 to 5.2 ± 0.7 μmol/liter) (Fig. 2b). Neutrophil O$_2^-$-generating capacity was not affected by short-term vitamin C therapy (from 38.3 ± 7.6 to 39.0 ± 8.0 mV/5 × 10$^5$ neutrophils, p = NS) (Fig. 2b). Flow-mediated dilation increased after intravenous vitamin C administration (from 3.1 ± 0.6% to 6.7 ± 1.0%, p < 0.05). Improvements in FMD with short-term vitamin C therapy did not correlate with reductions in oxidative stress (FRs: r = 0.05; TBARS: r = 0.04) (Fig. 3), nor with reductions in neutrophil O$_2^-$-generating capacity (r = 0.04).

Long-term oral treatment with vitamin C, as compared with placebo, reduced lipid-derived FRs (from 0.38 ± 0.03 to 0.25 ± 0.02 U, p < 0.05) (Fig. 4a) and reduced plasma TBARS (from 7.1 ± 0.8 to 5.2 ± 0.6 μmol/liter, p < 0.05) (Fig. 4b). Neutrophil O$_2^-$-generating capacity was also reduced by long-term vitamin C therapy (from 43.4 ± 6.5 to 23.7 ± 2.9 mV/5 × 10$^5$ neutrophils, p < 0.05) (Fig. 4c). Flow-mediated dilation increased after oral vitamin C administration (from 3.0 ± 0.6% to 8.2 ± 1.1%, p < 0.05). Improvements in FMD after long-term vitamin C therapy did not correlate with reductions in oxidative stress (FRs: r = −0.04; TBARS: r = −0.2), nor with reductions in neutrophil O$_2^-$-generating capacity (r = 0.2). After a six-week washout period in the active treatment group, lipid-derived FRs tended to increase toward baseline levels (from 0.23 ± 0.02 to 0.36 ± 0.04 U, p = 0.09), but plasma TBARS increased (from 5.2 ± 0.7 to 7.1 ± 1.0 μmol/liter, p < 0.05), and neutrophil O$_2^-$-generating capacity returned toward baseline levels after withdrawal of vitamin C therapy (from 23.7 ± 2.9 to 40.4 ± 7.9 mV/5 × 10$^5$ neutrophils, p < 0.05). There was no change in NYHA status during long-term vitamin C therapy as compared with placebo (2.7 ± 0.2 to 3 ± 0.2 vs. 2.6 ± 0.2 to 2.8 ± 0.2, respectively, p = NS). The study was not powered to assess this, however.

DISCUSSION

This study makes the following important new observations. Firstly, lipid-derived FR are increased in patients with CHF even in the absence of underlying CAD, though the magnitude of oxidative stress was greater in those patients with an ischemic etiology. Secondly, these lipid-derived FR are reduced significantly by vitamin C, both acutely when given intravenously and chronically with oral therapy. Thirdly, neutrophils from patients with CHF have an enhanced capacity to generate O$_2^-$ via the NADPH oxidase enzyme system and this relates to NYHA status. Fourthly, vitamin C has no effect on the ability of neutrophils to generate superoxide anions acutely, but during long-term therapy there was a marked suppression. We confirm the earlier observation of impaired conduit artery endothelium-dependent FMD in patients with CHF (8,13) and the recent observations that this may be improved by treatment with vitamin C, both acutely and chronically (8). Finally, the absence of a relationship between the impairment in FMD and the magnitude of elevation of FR at baseline, or between change in endothelial function and the change in FR with short-term (Fig. 3) and long-term vitamin C argues against oxidative stress as the sole mechanism of endothelial dysfunction in CHF and
suggests the possibility that the beneficial effects of vitamin C may not relate solely to its FR-scavenging actions.

Oxidative stress in heart failure. Oxidative stress is known to be increased in atherosclerotic vascular disease (16). In this study, we demonstrate that oxidative stress, as assessed using both the novel and relatively direct marker of plasma lipid-derived FRs and plasma TBARS, is increased in both ischemic and nonischemic CHF, although it tends to be more elevated in the former. Increased oxidative stress in CHF has previously been implied from very indirect measures (3–7), which generally lack both specificity and sensitivity. The alkoxyl and carbon-centered FRs, as measured using EPR spectroscopy in this study, provide a much more direct and physiologically relevant measure of oxidative

Figure 2. (a) Mean plasma lipid-derived FR levels in patients with nonischemic CHF before and after short-term vitamin C (VC) therapy versus those subjects receiving placebo. *p < 0.05. (b) Mean plasma TBARS in patients with nonischemic CHF before and after short-term VC therapy versus those subjects receiving placebo. (c) Mean O$_2^-$ generation by activated neutrophils in patients with nonischemic CHF before and after short-term VC therapy versus those subjects receiving placebo. Data are shown as the mean value ± SEM. Solid bar = baseline; open bar = after treatment.
stress than those previously reported in heart failure, as alkoxy radicals are capable of reacting directly with nitric oxide (NO) (17). Short-term vitamin C therapy reduced EPR-measured FRs significantly, although there was only a trend toward a reduction in plasma TBARS. This would also suggest that our ex vivo EPR technique provided a more direct measure of oxidative stress.

Oxygen FRs may contribute importantly to the pathophysiology of CHF by initiating myocyte apoptosis through the nuclear factor κB-like transcription factors (18) and by exerting direct negatively inotropic effects through depressed calcium uptake and reduced calcium-stimulated magnesium-dependent adenosine triphosphatase activity of the cardiac sarcoplasmic reticulum (19,20). Oxidative stress may also contribute to endothelial dysfunction in CHF through direct toxic effects on endothelial cells, by reducing NO synthase activity and by reacting with NO to form peroxynitrite (3,21–23). Endothelial dysfunction may limit cardiac and skeletal muscle blood flow (particularly on exercise), impair pulmonary blood flow regulation (resulting in ventilation-perfusion mismatching) and may lead to impaired diastolic cardiac performance (24). Despite these likely effects of oxidative stress on endothelial function, we found no relation between the impairment of FMD and the magnitude of oxidative stress. This may support the involvement of additional factors in the genesis of endothelial dysfunction, including the effects of asymmetric dimethylarginine, which competes with arginine for endothelial nitric oxide synthase (eNOS) binding and is elevated in CHF (25).

The NADPH/NADH oxidase system in endothelial cells, smooth muscle cells and phagocytes has been suggested as an important potential source of FRs in heart failure (26). Angiotensin II, acting through type I receptors, may modulate the activity of this enzyme (27). Our data demonstrate that, despite the fact that all patients were being treated with angiotensin-converting enzyme inhibitors, their neutrophils exhibited an enhanced capacity to generate $O_2^-$ through the NADPH oxidase system. However, recent data suggest that tissue angiotensin II levels may be increased in CHF, despite angiotensin-converting enzyme inhibitor therapy, owing to alternative angiotensin II generating pathways (28) or to increased angiotensin II generation through the incompletely blocked classic pathway when angiotensin I levels become markedly elevated (29). Tumor necrosis factor-α, which is elevated in CHF (30), also appears to modulate the activity of the NADPH/ NADH oxidase system (31).

Effects of vitamin C. In this study, vitamin C reduced lipid-derived FR levels during both the short-term and long-term treatment studies and increased brachial artery FMD to levels similar to those of healthy control subjects. After a washout period of six weeks, FR levels rose again but failed to return to pretreatment levels. Interestingly, plasma vitamin C levels remained higher than baseline levels after the washout period (either as a result of continued vitamin supplementation, although this was denied, or because of improvements in dietary vitamin C intake), implying an enhanced antioxidant state.

Although vitamin C is recognized as a potent watersoluble free radical scavenger (32), the rate constant of its interaction with $O_2^-$ is approximately five orders of magnitude lower than those for the interaction of $O_2^-$ with the endogenous antioxidants superoxide dismutase and NO, and vitamin E exhibits similar differential kinetics (33). Therefore, it seems unlikely that the antioxidant effects of these vitamins rely solely on their extracellular scavenging effects. In a recent study in the pig coronary angioplasty model, it was suggested that these vitamins may decrease $O_2^-$ generation by inhibiting NADPH oxidase activity (34). Our data confirm this, in that long-term therapy with vitamin C reduced the ability of neutrophils to generate $O_2^-$. However, intravenous vitamin C administration did not have this effect. Nevertheless, it reduced lipid-derived FRs and normalized brachial artery endothelial function. Vitamin C is actively transported into cells through an insulin-dependent transport system and can achieve intra-
cellular concentrations in the 1 to 10 mmol/liter range, at which it is an effective scavenger of $O_2^-$ (35). Thus, its scavenging effects may be largely intracellular. Aside from its scavenging actions, vitamin C spares intracellular glutathione from oxidative degeneration (36). Depletion of intracellular-reduced thiols impairs NO synthase activity in cultured endothelial cells, hence vitamin C may increase endothelial NO production (37). Reduced thiols stabilize NO (and increase its bioavailability) by reacting to form 5-nitrosothiol species (38). Vitamin C also functions synergistically with vitamin E, promoting its regeneration and leading to enhanced intracellular antioxidant capability (39).

Two recent in vitro studies suggest that vitamin C may have additional important actions. One study reported that vitamin C increased eNOS gene expression in cultured endothelial cells (40), and in another study, vitamin C increased NO generation from eNOS by increasing tetrahydrobiopterin availability or increasing the affinity of tet-

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**Figure 4.** (a) Mean plasma lipid-derived FR levels in patients with nonischemic CHF before and after long-term vitamin C (VC) therapy versus those subjects receiving placebo. *p < 0.05. (b) Mean plasma TBARS in patients with nonischemic CHF before and after long-term VC therapy versus those subjects receiving placebo. (c) Mean $O_2^-$ generation by activated neutrophils in patients with nonischemic CHF before and after long-term VC therapy versus those subjects receiving placebo. Data are shown as the mean value ± SEM. *p < 0.05. **Solid bar** = baseline; **open bar** = after treatment.
rahydrobiopterin binding (41). The relevance of these observations to the in vivo situation is unknown; however, our observation that the improvement in FMD did not correlate with the fall in FRs supports the additional effects of vitamin C. A recent study of vitamin C in patients with CAD demonstrated a marked improvement in endothelial function with no detectable change in oxidative stress (42). The discrepant response of the neutrophil NADPH oxidase system to long-term oral versus short-term intravenous vitamin C therapy is also unexplained. There are a number of potential stages at which vitamin C may act, including the priming process and NO feedback of NADPH oxidase activity. These processes may be time dependent, and understanding them may be important in the development of vitamin C as a therapeutic tool in the prevention and treatment of CHF.

Study limitations. Endothelium-independent dilation was not assessed in this study. All patients were maintained on stable, maximal medical therapy for the duration of the study, including, in the majority of cases, long-acting nitrate preparations. Therefore, testing endothelium–independent dilation using a NO donor would have been unreliable. Other investigators have shown that the endothelium–independent response to a supramaximal dose of nitroglycerin is preserved in patients with CHF (8,13), and that vitamin C, given both long term and short term, has no effect on this variable (8).

Although a direct measure of \( O_2^- \) in vivo is, at present, technically impossible in human subjects, the ex vivo spin-trapping technique provides good evidence for the production of lipid radicals in vivo. Furthermore, although it is likely that a reduction in oxidative stress coupled with improvements in endothelial function will lead to clinical benefits, this study was not powered to assess changes in important clinical variables, such as exercise capacity and left ventricular function. A study of the longer term effects of vitamin C therapy on such clinical variables, with a larger patient group, ideally those with nonischemic CHF, would seem to be clearly warranted.

Conclusions. Oxidative stress is increased in CHF, even when the etiology is nonischemic. Neutrophils may be an important source of FRs. Vitamin C reduces oxidative stress, both short and long term. In the latter case, reduced neutrophil–generating capacity may contribute to this effect. However, the beneficial effects of vitamin C therapy on endothelial function do not seem to be explicable solely on the basis of reduced oxidative stress.

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