Vitamin C Augments the Inotropic Response to Dobutamine in Humans With Normal Left Ventricular Function

Susanna Mak, MD; Gary E. Newton, MD

**Background**—We studied the effect of an antioxidant, the intracoronary infusion of vitamin C, on basal and dobutamine-stimulated left ventricular (LV) contractility.

**Methods and Results**—Nineteen patients with normal ventricular function participated in this study. A micromanometer-tipped catheter was inserted into the LV. In the experimental group (n=10), an infusion catheter was positioned in the left main coronary artery. LV peak +dP/dt (LV +dP/dt) was measured in response to the intravenous infusion of dobutamine before (Dob) and during (Dob+vit C) the intracoronary infusion of vitamin C. The intracoronary infusion of vitamin C had no effect on basal LV +dP/dt or any other hemodynamic parameter. The infusion of vitamin C augmented the LV +dP/dt response to dobutamine by 22±4% (Dob, 1680±76 mm Hg/s; Dob+vit C, 1814±97 mm Hg/s, P<0.01). In the control group (n=9), LV +dP/dt was measured in response to sequential infusions of dobutamine (Dob, Dob-2) given at the same time intervals as in the experimental group but without the intracoronary infusion of vitamin C. In contrast to the experimental group, no difference in LV +dP/dt was observed between the 2 infusions of dobutamine (Dob, 1706±131 mm Hg/s; Dob-2, 1709±138 mm Hg/s, P=NS).

**Conclusions**—The administration of the antioxidant vitamin C augments the inotropic response to dobutamine in humans. This suggests that redox environment contributes to the adrenergic regulation of ventricular contractility. (Circulation. 2001;103:826-830.)

**Key Words:** antioxidants ■ contractility ■ receptors, adrenergic, beta

Cardiac function is regulated by a complex interplay of neural, hormonal, and mechanical controls. In addition, myocardial function can be altered by free radical generation, an example being the impairment of cardiac function that occurs in the setting of ischemia/reperfusion, a model of acute free radical excess.1 Free radicals may also play a role as tonic modulators of cardiac function in the setting of congestive heart failure (CHF).2 a model of chronic oxidative stress.3 Free radical activity may affect cardiac function by multiple possible mechanisms, including peroxidation of lipid membranes with consequent perturbation of membrane-bound enzymes and receptors.4 For example, free radical generation impairs Na⁺,K⁺-ATPase and Ca²⁺ pump activity in sarcoplasmic membranes, as well as Ca²⁺ transport in the sarcoplasmic reticulum.5–7 In addition, free radical activity may interact with membrane components that modulate contractile responses. It has been demonstrated that free radicals alter β-adrenergic receptor function and postreceptor signal transduction.8–10 These data provide evidence that the regulation of cardiac function may be altered by excess free radical generation and/or impaired antioxidant defense mechanisms.

Information regarding the effect of free radical processes on cardiac function is derived primarily from in vitro and in vivo animal experiments. The objective of our investigation was therefore to explore whether redox status contributes to the regulation of cardiac function in humans. The use of controlled free radical generating systems is not readily applicable to human studies. Therefore, we tested the hypothesis that an antioxidant can acutely enhance basal left ventricular (LV) contractile function, as well as the inotropic response to an exogenous β-receptor agonist, in patients undergoing elective cardiac catheterization for clinical indications.

**Methods**

**Study Population**

Nineteen patients referred for elective diagnostic heart catheterization participated in this study. All patients were being evaluated for a chest pain syndrome, and no patient had significant valvular disease or ventricular dysfunction detected by 2D echocardiography. The experimental group (n=10, 8 men, 2 women, age 55±4 years) included 6 patients with stable coronary artery disease and 4 patients with normal coronary arteries. The left anterior descending coronary artery was patent in all patients. Two patients had treated hypertension, 6 patients had hypercholesterolemia controlled by medical therapy, and 2 patients had non–insulin-dependent diabetes. Medical therapy in this group included β-blockers (n=5), calcium channel blockers (n=3), enteric coated aspirin (n=9), ACE inhibitors (n=5),...
and nitrates (n = 1). The control group (n = 9, 8 men, 1 woman, age 52±4 years) included 7 patients with stable coronary artery disease and 2 patients with normal coronary arteries. Four patients had treated hypertension, 4 patients had hypercholesterolemia controlled by medical therapy, and 2 patients had non-insulin-dependent diabetes. Medical therapy in this group included β-blockers (n = 8), calcium channel blockers (n = 4), enteric coated aspirin (n = 9), ACE inhibitors (n = 1), and nitrates (n = 3). All patients in this study were nonsmokers. Vitamin and/or antioxidant supplements were withheld for ≥7 days before the study.

This study was approved by the University of Toronto Ethical Review Committee for Experimentation Involving Human Subjects, and all patients gave written informed consent.

Cardiac Catheterization Procedure and Hemodynamic Measurements

Patients were studied after diagnostic left and right heart catheterization via the femoral approach. All medications were withheld on the morning of the investigation. In all patients, a 7F micromanometer-tipped catheter (Millar Instruments) was advanced via the right femoral artery into the LV for measurement of LV pressure. In the experimental group, a 6F L4 Judkins catheter (Cordis Laboratories) was advanced from the opposite femoral artery to the ostium of the left main coronary artery. When vitamin C was not infusing, the catheter was continuously flushed with the vehicle for drug infusion (0.9% saline) at a rate of 1.6 mL/min with a Harvard infusion pump. Femoral artery pressure was monitored via the 7F sidearm sheath (Terumo Medical Corp) that was used to insert the 6F Judkins catheter.

The ECG, femoral artery pressure, LV pressure, and LV peak positive dP/dt (LV +dp/dt) were recorded on a strip-chart recorder. Each description of heart rate and blood pressure represents the mean of ≥15 consecutive beats. The ECG, LV pressure, and +dp/dt were also continuously digitally recorded (300 Hz) online. LV +dp/dt was calculated offline with a customized software program (Labview version 5.0, National Instruments Corp). The mean of ≥50 consecutive beats was used for analysis. These methods are established in our laboratory.11

Study Protocol

All patients received heparin (5000 U IV) and a rest period after placement of the catheters. In the experimental group, hemodynamic and inotropic measurements were made sequentially at each of the following conditions: (1) The intracoronary infusion of the vehicle solution (0.9% saline) at 1.6 mL/min (Baseline). (2) Dobutamine (Lilly Inc) diluted in 5% dextrose in water infused via a systemic vein at a rate of 2.5, 5.0, or 7.5 μg·kg⁻¹·min⁻¹ titrated to achieve a ≥25% rise in LV +dp/dt and until LV +dp/dt remained stable (±5%) for 3 consecutive measurements, each separated by 1 minute (Dob). (3) After the dobutamine infusion was stopped for ≥10 minutes and peak LV +dp/dt was similar to baseline (within 10%) (Recontrol). (4) Vitamin C (ascorbic acid injection 500 mg/2 mL, pH adjusted with sodium hydroxide, Sabex Inc) diluted in the vehicle solution (60 mg/mL) and infused into the left main coronary artery for 10 minutes at a rate of 1.6 mL/min (96 mg/min) (Vit C). (5) During the continued intracoronary infusion of vitamin C, dobutamine was rein infused intravenously at the same rate as in the Dob group until peak LV +dp/dt remained stable (±5%) for 3 consecutive measurements, each separated by 1 minute (Dob+Vit C). (6) For completion of the same protocol, the control group was subjected to the experimental intervention (Recontrol). (7) Vitamin C (ascorbic acid injection 500 mg/2 mL, pH adjusted with sodium hydroxide, Sabex Inc) diluted in the vehicle solution (60 mg/mL) and infused into the left main coronary artery for 10 minutes at a rate of 1.6 mL/min (96 mg/min) (Vit C). (8) During the continued intracoronary infusion of vitamin C, no treatment was administered to the control group until peak LV +dp/dt remained stable (±5%) for 3 consecutive measurements, each separated by 1 minute (Vit C).

To ensure that any augmentation of the LV +dp/dt response to the second dobutamine infusion was not due to the previous dobutamine infusion, the control group underwent a similar protocol, with the following exceptions. A second recontrol (Recontrol-2) replaced the Vit C condition and was similar in duration but without the intracoronary infusion of vitamin C. The second intravenous dobutamine infusion (Dob-2) also occurred without infusion of vitamin C.

Statistical Analysis

All data are presented as mean±SEM. A statistical software package was used for the analysis (Sigma Stat 1.0, Jandel Corp). Hemody-

### TABLE 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Experimental Group (n = 10)</th>
<th>Control Group (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>65±3</td>
<td>59±4</td>
</tr>
<tr>
<td>FA systolic, mm Hg</td>
<td>125±6</td>
<td>126±4</td>
</tr>
<tr>
<td>FA diastolic, mm Hg</td>
<td>66±1</td>
<td>70±3</td>
</tr>
<tr>
<td>RAP, mm Hg</td>
<td>4±1</td>
<td>6±1</td>
</tr>
<tr>
<td>PAP, mm Hg</td>
<td>16±1</td>
<td>18±1</td>
</tr>
<tr>
<td>Cardiac output, L/min</td>
<td>5.8±0.5</td>
<td>6.1±0.5</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>12±2</td>
<td>16±2</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>120±5</td>
<td>123±4</td>
</tr>
<tr>
<td>LV +dp/dt, mm Hg/s</td>
<td>1209±34</td>
<td>1222±70</td>
</tr>
</tbody>
</table>

FA indicates femoral artery; RAP, right atrial pressure; PAP, pulmonary artery pressure; LVEDP, LV end-diastolic pressure; and LVSP, LV systolic pressure.

Results

Baseline Characteristics

There were no significant differences between the experimental and control groups with respect to any hemodynamic or inotropic parameter (Table 1).

Effect of Vitamin C on Basal and Dobutamine-Stimulated LV Contractility

The intracoronary infusion of vitamin C alone had no effect on basal contractility or on any hemodynamic parameter measured in this study (Table 2).

In the experimental group, dobutamine (mean infusion rate 3.4±0.4 μg·kg⁻¹·min⁻¹) caused a 474±60 mm Hg/s increase in LV +dp/dt, whereas the simultaneous infusion of dobutamine and vitamin C increased LV +dp/dt by 581±76 mm Hg/s (Dob+Vit C versus Dob, P<0.01) (Table 2 and Figure, panel A). This represented a 22±4% augmentation in the inotropic response to dobutamine associated with the coinfusion of vitamin C. Qualitatively, results were similar in patients with and without coronary artery disease (Figure, panel A).

In the control group, dobutamine (mean rate 2.8±0.3 μg·kg⁻¹·min⁻¹) caused an increase in LV +dp/dt (484±71 mm Hg/s) very similar to that of the experimental group. In contrast to the experimental group, there was no significant difference between the inotropic responses to Dob (484±71 mm Hg/s) and Dob-2 (448±64 mm Hg/s) (Table 3 and Figure, panel B).

Effect of Dobutamine and Vitamin C on Other Hemodynamic Parameters

In response to dobutamine, heart rate increased slightly from the preceding baseline, reaching statistical significance during the second dobutamine infusion in both groups (Tables 2 and 3). There was no difference with respect to heart rate
between Dob and Dob-2/Dob+Vit C in either group. In both groups, femoral artery systolic pressure and LV systolic pressure increased significantly in response to both dobutamine infusions (Tables 2 and 3). Again, there was no difference with respect to these parameters between Dob and Dob-2/Dob+Vit C in either group.

Discussion

In this study, the intracoronary infusion of vitamin C augmented the positive inotropic response to dobutamine in humans with normal LV systolic function. In contrast, the infusion of vitamin C alone had no effect on contractility, suggesting that basal free radical activity was not sufficient to significantly affect resting contractile state in this patient population. Although basal LV contractile state was not altered, our data indicate that β-adrenergic stimulation of ventricular contractility can be sensitive to modulation of the redox environment. To the best of our knowledge, this represents the first observation that the administration of an antioxidant can influence directly measured ventricular function in humans.

The observation that an antioxidant alters the contractile response to dobutamine in humans is consistent with previous in vivo studies in animals that have demonstrated an interaction between oxyradicals and several components of the β-adrenergic response. In the isolated rat heart, perfusion with hydrogen peroxide (H2O2) produced a dose-dependent depression of isoproterenol-stimulated LV contractility.8,12 Several abnormalities of β-receptor function and signal transduction were also identified, including decreased affinity as well as density of β-receptors and depressed activity of postreceptor components, including Gi protein and adenylyl cyclase activity.8,12 In addition, many of the downregulatory effects on the β-adrenergic pathway were prevented by antioxidant pretreatment with superoxide dismutase (SOD) and catalase. In a study of ischemia/reperfusion in isolated guinea pig hearts, the application of the antioxidant N-acetylcysteine did not affect basal contractility but did potentiate β-adrenergic responses.13

In our study, vitamin C augmented inotropic responses to dobutamine in the absence of an acute free radical stimulus. This observation suggests that β-adrenergic receptor–stimulated ventricular function may be regulated by redox status, at least in this particular patient population. Whether this response is a physiological characteristic of human myocardium or indicative of a pathological state of oxidative stress is unknown. The age range (41 to 76 years) of the patients studied is noteworthy, because there is experimental evidence of a relationship between aging and oxidative stress.14 It has been demonstrated in some mammals and insects that the generation of reactive oxygen species by mitochondria is increased with age.15 Furthermore, the susceptibility of both tissue homogenates and live animals to experimentally induced oxidative stress increases with age, suggesting a possible decline in antioxidant defenses.14 The patients in this study also exhibited a variety of conditions associated with oxidative stress, including coronary artery disease, diabetes, and hypertension. Thus, augmentation of the inotropic response to dobutamine by vitamin C may not be observed in a population of younger healthy subjects. Relevant to this concept is the response of the endothelium to acetylcholine, which can also be altered by redox environment. In patients with coronary artery disease, diabetes, and hypertension, endothelial responses are blunted and improved by vitamin C, whereas in healthy subjects, vitamin C has no effect on endothelial function.16–18

The results of this study are of potential relevance to patients with CHF, a syndrome associated with oxidative stress.3 A positive effect of an antioxidant in this setting was

| TABLE 2. Hemodynamic and Inotropic Measurements in the Experimental Group |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Heart rate, bpm | 68±2            | 69±3            | 66±3            | 65±2            | 71±3*           |
| FA systolic, mm Hg | 123±4         | 141±6*          | 125±4           | 127±5           | 144±7*          |
| FA diastolic, mm Hg | 61±2          | 62±2            | 61±2            | 62±2            | 62±2            |
| LVEDP, mm Hg   | 11±2           | 13±3            | 10±1            | 11±1            | 12±2            |
| LVSP, mm Hg    | 119±4          | 132±5*          | 118±5           | 122±5           | 137±5*          |
| LV + dP/dt, mm Hg/s | 1209±30      | 1680±76*        | 1233±29         | 1234±25         | 1814±97*†       |

Abbreviations as in Table 1. *P<0.05 vs preceding baseline or recontrol; †P<0.01 vs Dob.

A. In experimental group, measured increase in peak positive LV dP/dt (ΔLV + dP/dt) in response to infusion of dobutamine (Dob) was augmented by addition of intracoronary vitamin C (Dob+vit C). *P<0.01, Dob+vit C vs Dob. Results in patients with coronary artery disease (solid circles) were qualitatively similar to patients without coronary artery disease (open circles). B. In control group, without coinfusion of vitamin C, there was no difference in ΔLV + dP/dt between first (Dob) and second (Dob-2) infusions of dobutamine.
recently demonstrated by Ekelund et al.\textsuperscript{2} In that experiment, allopurinol increased cardiac mechanical efficiency in a dog model of heart failure, possibly as a result of inhibition of xanthine oxidase–mediated generation of superoxide anion. The short-term administration of an antioxidant may have the beneficial effect of reducing myocardial levels of peroxynitrite, a product of the reaction of superoxide anion and nitric oxide with potential negative effects on myocardial function and adrenergic responsiveness.\textsuperscript{19} The impact of vitamin C on cardiac function in patients with CHF, however, is likely to be complex. In this setting, vitamin C may have negative effects on cardiac function as a result of increased bioavailability of nitric oxide. Hare et al\textsuperscript{20} demonstrated that blockade of nitric oxide synthase resulted in augmented inotropic responses to dobutamine in patients with CHF, but not in subjects with normal LV function. This observation provided evidence that myocardial nitric oxide synthase activity attenuated \(\beta\)-adrenergic responses in humans with LV failure. Because nitric oxide is sensitive to redox environment,\textsuperscript{21} its bioavailability may be augmented by the administration of an antioxidant. Given the possible conflicting mechanisms by which vitamin C may or may not improve adrenergic responses in the setting of CHF, the results of the present study cannot be generalized to this patient population.

The administration of dobutamine by the intravenous or intracoronary route is a well-established method of assessing \(\beta\)-adrenergic responsiveness in humans.\textsuperscript{11,20,22} In this study, the vitamin C–mediated augmentation of the dobutamine response was similar in magnitude to that observed with parasympathetic blockade in humans with normal ventricular function\textsuperscript{11} as well as nitric oxide synthase blockade in patients with CHF.\textsuperscript{20} In the experimental group, it is unlikely that the inotropic response to the second dobutamine infusion was potentiated by the previous dobutamine infusion, because no significant difference in the \(dP/dt\) response was observed between successive dobutamine infusions in the control group. The vitamin C infusion rate used in this study approximated an intracoronary concentration of between 1 and 10 mmol, assuming coronary blood flow of 100 mL/min. This concentration is sufficient to inhibit free radical activity in vitro.\textsuperscript{23,24} Sherman et al\textsuperscript{25} recently demonstrated that this pharmacological concentration was necessary to alter endothelial reactivity to methacholine in humans, another physiological response that is modulated, in part, by redox environment.

The index of contractility used in the present investigation was the direct measurement of LV \(+dP/dt\). This index has been demonstrated to also be sensitive to changes in preload and afterload, although the magnitude of this sensitivity is controversial.\textsuperscript{26,27} In the present study, dobutamine was infused intravenously, so the \(dP/dt\) response may have been altered as a result of changes in loading conditions. The reason for infusing dobutamine intravenously was to ensure that its intracoronary concentration would not be affected by changes in coronary blood flow. Importantly, any load-mediated changes in \(dP/dt\) were controlled for by the use of serial identical dobutamine infusions. There were no significant changes in LV end-diastolic pressure or systemic blood pressure between the first dobutamine infusion and the second infusion with the addition of vitamin C. Vitamin C was infused by the intracoronary route in the present study both to ensure adequate cardiac concentrations and to minimize any systemic effects that might alter loading conditions. In the present study, the infusion of vitamin C caused no changes in LV filling pressures or systemic blood pressure. This is consistent with previous studies that demonstrated that vitamin C has no acute hemodynamic effect when infused either intravenously or by the intracoronary route.\textsuperscript{28,29} Contractility, as measured by LV \(+dP/dt\), may also be increased by increases in heart rate: the Treppe effect.\textsuperscript{30} In the present study, there was a small increase in heart rate in response to the second dobutamine infusion in both the experimental and control groups. Despite this change in heart rate, there was no augmentation of the inotropic response between successive infusions of dobutamine in the control group, suggesting that the Treppe effect was not relevant to our findings.

The present study had limitations that merit discussion. From our data, the component(s) of the \(\beta\)-adrenergic pathway affected by the administration of vitamin C cannot be elucidated. Both receptor and postreceptor elements may be involved. The results of this acute hemodynamic study cannot be extended to, or predict, the effect of chronic antioxidant therapy on LV function. Furthermore, the plasma concentration of vitamin C achieved by direct intra-arterial infusion is difficult to duplicate with oral supplementation.\textsuperscript{24} Indeed, large clinical trials evaluating oral antioxidant therapy on cardiovascular events in patients at high risk have yielded either negative or very modest results.\textsuperscript{31–33} It has been demonstrated that endothelium-bound extracellular (EC) SOD can be released by heparin injection, and this has been used as a method of assessing EC-SOD activity in humans.\textsuperscript{34}

| TABLE 3. Hemodynamic and Inotropic Measurements in the Control Group |
|-----------------|----------------|--------------|--------------|--------------|
| Baseline        | Dob            | Recontrol    | Recontrol-2  | Dob-2        |
| Heart rate, bpm | 63±4           | 65±4         | 62±3         | 64±3         | 67±3*        |
| FA systolic, mm Hg | 127±5         | 140±5*       | 128±5        | 130±4        | 141±5*       |
| FA diastolic, mm Hg | 67±3          | 64±3         | 66±3         | 67±3         | 67±4         |
| LVEDP, mm Hg    | 16±2           | 18±2         | 13±2         | 15±2         | 15±2         |
| LVSP, mm Hg     | 123±4          | 134±5*       | 121±4        | 124±4        | 135±5*       |
| LV +dP/dt, mm Hg/s | 1222±70       | 1706±131*    | 1261±91      | 1230±89      | 1709±138*    |

Abbreviations as in Table 1.

\(P<0.05\) vs preceding baseline or recontrol.
This raises the possibility that antioxidant status may have been altered by the systemic heparin injection required for catheterization protocols. Vitamin C may have restored antioxidant capacity rather than having a specific effect on dobutamine responses. This seems unlikely, however, because the release of EC-SOD by heparin represents a very small proportion of total EC-SOD. Finally, it is possible that bias may have been introduced in the interpretation of our results, because this investigation was not blinded. Blinding was not performed because of the invasive nature of this protocol.

This investigation demonstrates that an antioxidant acutely potentiates the inotropic response to dobutamine in humans. To the best of our knowledge, this is one of the first observations to suggest that β-adrenergic-mediated contractility in humans is modulated in part by basal free radical activity. The potential impact of redox manipulation on ventricular function in disease states characterized by greatly increased oxidative stress or abnormal β-adrenergic responsiveness requires further investigation.

Acknowledgments

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References


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