Ascorbic acid increases cardiovagal baroreflex sensitivity in healthy older men

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Monahan, Kevin D., Iratxe Eskurza, and Douglas R. Seals. Ascorbic acid increases cardiovagal baroreflex sensitivity in healthy older men. Am J Physiol Heart Circ Physiol 286: H2113–H2117, 2004. First published February 12, 2004; 10.1152/ajpheart.01054.2003.—Cardiovascular baroreflex sensitivity (BRS) declines with advancing age in healthy men. We tested the hypothesis that oxidative stress contributes mechanistically to this age-associated reduction. Eight young (23 ± 1 yrs, means ± SE) and seven older (63 ± 3) healthy men were studied. Cardiovascular BRS was assessed using the modified Oxford technique (bolus infusion of 50–100 μg sodium nitroprusside, followed 60 s later by a 100- to 150-μg bolus of phenylephrine hydrochloride) in triplicate at baseline and during acute intravenous ascorbic acid infusion. At baseline, cardiovascular BRS (slope of the linear portion of the R-R interval–systolic blood pressure relation during pharmacological changes in arterial blood pressure) was 56% lower (P < 0.01) in older (8.3 ± 1.6 ms/mmHg) compared with young (19.0 ± 3.1 ms/mmHg) men. Ascorbic acid infusion increased plasma concentrations similarly in young (62 ± 9 vs. 1.249 ± 72 μmol/l for baseline and during ascorbic acid; P < 0.05) and older men (62 ± 4 vs. 1.022 ± 55 μmol/l; P < 0.05) without affecting baseline blood pressure, heart rate, carotid artery compliance, or the magnitude of change in systolic blood pressure in response to bolus sodium nitroprusside and phenylephrine hydrochloride infusion. Ascorbic acid (vitamin C) infusion increased cardiovagal BRS in older (ΔS8% ± 16%; P < 0.01), but not younger (Δ, −4 ± 4%) men. These data provide experimental support for the concept that oxidative stress contributes mechanistically to age-associated reductions in cardiovagal BRS in healthy men.

baroreceptors; blood pressure; antioxidants

THE SLOPE RELATING CONSECUTIVE cardiac periods (R-R intervals) and systolic blood pressures (SBP) during acute changes in arterial BP has become a widely accepted method of quantifying cardiovagal baroreflex sensitivity (BRS) in humans (2, 3, 33). Depressed cardiovagal responsiveness has been linked to impaired BP regulation with aging (32) and an increased incidence of sudden cardiac death in patients with coronary artery disease (19, 20). Thus the identification of mechanisms underlying depressed cardiovagal BRS has important clinical implications.

Many cardiovascular disease states are associated with baroreflex impairment (4, 10), with oxidative stress playing a possible mechanistic role (22, 27, 28). Aging, which is a primary risk factor for cardiovascular disease development (21), is associated with a reduction in cardiovagal BRS (9, 13, 25) and increased levels of oxidative stress (15, 17, 18, 40). However, it is unknown whether these events are mechanistically linked. Increased levels of oxidative stress with age could impair baroreflex function through a direct suppressive influence on baroreceptors (22). In addition, because nitric oxide appears to be an important modulator of baroreflex function in humans (7, 35), and its bioavailability is reduced with age (37), it is possible that oxidative stress could suppress baroreflex function indirectly by reducing nitric oxide bioavailability (29, 37).

We tested the hypothesis that oxidative stress contributes to the age-associated reduction in cardiovagal BRS. To address this hypothesis, cardiovagal BRS was determined at baseline and during acute intravenous infusion of the powerful antioxidant ascorbic acid (11) in young and older healthy men.

METHODS

Subjects

Fifteen men were studied after obtaining University of Colorado-approved written informed consent. All subjects were sedentary and between the ages of 20–35 (young) or 55–79 (older) yr. All subjects were normotensive (BP <140/90 mmHg), nonobese (BMI <27 kg/m2), nonsmokers, not taking any medications, and free of cardiovascular disease as assessed by history and physical examination, blood chemistries, and maximal exercise BP and electrocardiograms (older subjects only). No subjects took vitamin or antioxidant supplements for at least 6 wk before testing.

Measurements

Subjects were studied at least 4 h postprandial (12 h for screening blood chemistries).

Cardiovagal BRS. During these measurements, subjects were studied supine 30 min after being instrumented with an intravenous cannula for infusion of sodium nitroprusside, phenylephrine hydrochloride, and ascorbic acid and for continuous cardiac period (5-lead ECG) and beat-to-beat BP (Finapres-Ohmeda) measurements. Physiological data were digitally recorded on a personal computer.

Cardiovagal BRS was assessed using the modified Oxford technique (8, 30). Briefly, sodium nitroprusside was infused intravenously (50–100 μg) as a bolus, followed 60 s later by a bolus of phenylephrine hydrochloride (75–150 μg). Data acquisition began −10 s before sodium nitroprusside infusion and continued for 120 s after phenylephrine hydrochloride infusion. Drugs were administered at doses sufficient to elicit the desired effects on SBP (~15–25 mmHg reduction and subsequent increase in SBP from baseline levels).

Cardiovagal BRS was quantified by a blinded investigator as the slope of the R-R interval–SBP relation (binned over 2 mmHg pressure ranges) from the nadir to peak SBP response during the trial (8, 30).

Data points clearly contained within either the threshold or saturation
regions were manually removed (14). After this process, all BRS trials (≥3) with linear regression coefficients exceeding an r value of 0.70 were averaged together under each condition (e.g., baseline and ascorbic acid) and a single mean value was reported (30, 33). With the use of this approach, each individual’s cardiovagal BRS value under each condition (e.g., baseline and ascorbic acid) included the average of two (n = 9) and, in some instances, three trials (n = 6). Pilot testing established that this infusion protocol increased plasma ascorbic acid concentrations to levels previously shown to scavenge free radicals (1,000 μmol/l) (16). Fifteen minutes elapsed between each trial. Carotid artery compliance was determined before and at least 30 min after the two groups at baseline and increased to supraphysiological levels (~15-fold; >1,000 μmol/l throughout the measurement period) in both groups without affecting baseline BP, heart rate, or carotid artery compliance in either group.

Cardiovagal BRS

At baseline, cardiovagal BRS was 56% lower in older (8.3 ± 1.6 ms/mmHg) compared with young (19.0 ± 3.1 ms/mmHg; \( P < 0.05 \)) men. Ascorbic acid infusion increased cardiovagal BRS by 58 ± 16% in older men (to 13.1 ± 2.4 ms/mmHg; \( P < 0.01 \)), but had no effect in young men (18.3 ± 2.7 ms/mmHg; \( \Delta - 4 ± 4% \)) (Fig. 1 and Fig. 2, bottom). After ascorbic acid infusion, the age-associated difference in cardiovagal BRS was no longer statistically significant (\( P = 0.17 \)). Ascorbic acid-induced increases in cardiovagal BRS were consistent and effects of ascorbic acid. Specific contrasts were made using Newman-Keuls post hoc tests. Relations between variables were determined with correlation analyses. Statistical significance occurred at \( P < 0.05 \).

**RESULTS**

**Subject Characteristics**

Subject characteristics at baseline and after ascorbic acid are shown in Table 1. Ascorbic acid concentrations were similar in the two groups at baseline and increased to supraphysiological levels (~15-fold; >1,000 μmol/l throughout the measurement period) in both groups without affecting baseline BP, heart rate, or carotid artery compliance in either group.

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Young Pre (n = 8)</th>
<th>Young Post</th>
<th>Older Pre (n = 7)</th>
<th>Older Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 ± 2</td>
<td>63 ± 3†</td>
<td></td>
<td>175 ± 3</td>
<td></td>
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<tr>
<td>176 ± 3</td>
<td>70.3 ± 3.5</td>
<td></td>
<td>77.1 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.8 ± 1.1</td>
<td></td>
<td>25.2 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Body fat, %</td>
<td>16 ± 4</td>
<td>26 ± 2†</td>
<td>30 ± 2†</td>
<td></td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>117 ± 3</td>
<td>117 ± 2</td>
<td>117 ± 3</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>64 ± 2</td>
<td>71 ± 2</td>
<td>72 ± 2</td>
<td></td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>53 ± 4</td>
<td>46 ± 2</td>
<td>45 ± 2</td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>58 ± 5</td>
<td>54 ± 3</td>
<td>54 ± 2</td>
<td></td>
</tr>
<tr>
<td>Carotid artery compliance, mm²/mmHg</td>
<td>0.22 ± 0.03</td>
<td>0.23 ± 0.04</td>
<td>0.14 ± 0.02†</td>
<td>0.15 ± 0.02†</td>
</tr>
<tr>
<td>Plasma ascorbic acid, μmol/l</td>
<td>62 ± 9</td>
<td>1,249 ± 72*</td>
<td>62 ± 4</td>
<td>1,022 ± 55†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. BP, blood pressure; BMI, body mass index; \( \dot{V}O_{2max} \), maximal oxygen consumption. *\( P < 0.05 \) vs. baseline; †\( P < 0.05 \) vs. young men at same time point.

### Statistical Analysis

Differences in baseline subject characteristics were determined by t-test and repeated-measures ANOVA was used to determine the
robust among the older men (Fig. 2, top). Indeed, the increase in cardiovagal BRS with ascorbic acid in every older subject exceeded the maximal increase noted in any young subject (Fig. 2, top). Similar results were obtained when BRS was expressed as the linear portion of the SBP-heart rate relation. Specifically, BRS expressed in this manner was lower in older expressed as the linear portion of the SBP-heart rate relation.

\[ \Delta \text{Cardiovagal BRS} \] vs. young (top)

\[ \Delta \text{Cardiovagal BRS} \] vs. older (bottom)

![Figure 2](http://ajpheart.physiology.org/)

DISCUSSION

The novel finding from the present study is that acute intravenous ascorbic acid administration increases cardiovagal BRS in older men without clinical disease. This suggests that oxidative stress contributes mechanistically to the age-associated reduction in cardiovagal BRS observed in healthy men.

There is mounting experimental evidence that oxidative stress modulates baroreflex function. In rabbits with experimentally induced atherosclerosis, depressed baseline baroreceptor function was improved after exposing the carotid sinus to exogenous free radical scavenging superoxide dismutase and catalase (22). These data suggest a direct suppressive action of reactive oxygen species on carotid baroreceptors. Moreover, acute intravenous infusion of ascorbic acid increases cardiovagal BRS in patients with congestive heart failure (27, 28). Importantly, administration of antioxidants in these previous investigations, as well as in the present study, had no effect on baroreflex function in healthy young controls (22, 27), suggesting that ascorbic acid administration in the absence of oxidative stress has no influence on cardiovagal BRS. Consistent with this suggestion, exogenous administration of xanthine and xanthine oxidase, which cause oxidative stress, also impairs baroreceptor function in young rabbits (22). Collectively, the results of the present study and these previous studies support the concept that oxidative stress impairs BRS in the settings of both aging and cardiovascular disease.

It is not possible to determine the site at which ascorbic acid exerted its positive influence on baroreflex function in older men in the present study. As described above, data derived from animals strongly suggest a direct suppressive influence of reactive oxygen species on baroreceptors (i.e., a peripheral site of action) (22). However, a central effect of ascorbic acid cannot be excluded in the present study. Ascorbic acid infused systemically can cross the blood-brain barrier (1). However, this transport necessitates that ascorbic acid first be oxidized to dehydroascorbic acid (1). This process likely explains why intravenously infused ascorbic acid cannot be detected in the cerebrospinal fluid for at least 30 min after systemic infusion in rodents (34). These temporal patterns have not been established in humans. Therefore, we cannot exclude the possibility that ascorbic acid exerted a central effect in the older men and contributed to the increased levels of cardiovagal BRS observed during ascorbic acid administration.

In addition to the possibility that ascorbic acid increases cardiovagal BRS in older men due to a direct influence of reducing reactive oxygen species, it is possible that the observed effects are not direct. For example, nitric oxide has been suggested to play an important role in autonomic and baroreflex control in humans (5–7, 35). Because sedentary aging in humans is associated with a reduction in nitric oxide bioavailability (37, 38), which is caused primarily by oxidative stress, it is possible that reduced bioavailability of nitric oxide may contribute to depressed levels of cardiovagal BRS in older men secondary to increased levels of oxidative stress. Thus infusion of ascorbic acid in older adults may increase the bioavailability of nitric oxide (37), which then could exert positive influences on regions critical to the regulation of autonomic outflow such as the nucleus tractus solitarii (23) or via a direct influence on the sinoatrial node (42). We made no measure of nitric oxide bioavailability in the present study. Therefore, we can only speculate that ascorbic acid may increase cardiovagal BRS secondary to increased bioavailability of nitric oxide.

A recent study (28) reported that intravenous ascorbic acid infusion did not improve cardiovagal BRS in healthy middle-
aged adults. At least two points should be considered when interpreting these results in the context of the present findings. First, our subjects were older (mean age 63 vs. 55 yr). Because oxidative stress develops (15, 17, 18, 40) and cardiovagal BRS decreases with age (9, 13, 25), it is likely that studying individuals who were older enhanced our ability to detect an improvement in cardiac vagal BRS with ascorbic acid. Second, our dose of ascorbic acid was considerably larger than in this recent investigation, and it is well established that the antioxidant properties of ascorbic acid are dose dependent (16, 31). Moreover, because plasma concentrations of ascorbic acid were not reported in this prior study, it cannot be determined whether circulating ascorbic acid achieved levels known to scavenge reactive oxygen species in healthy adults, as was carefully documented in the present study.

The mechanism underlying the increase in cardiac vagal BRS with ascorbic acid in older men is unclear. At baseline, we demonstrated an association between the compliance of an artery in which baroreceptors are located (carotid) and cardiac vagal BRS ($r = 0.55$), which is consistent with our previous findings (24, 26). However, the lack of relation between changes in cardiac vagal BRS and carotid artery compliance from baseline in response to ascorbic acid infusion in older men suggests a compliance-independent effect of oxidative stress on cardiac vagal BRS, consistent with previous data in experimental animals (22). Collectively, these observations indicate that oxidative stress influences some other aspect of the baroreflex, such as exerting a direct effect on baroreceptors, modifying central integration of afferent barosensory stimuli, and/or altering end-organ responsiveness to alterations in cardiac vagal nerve traffic.

Our findings may have important physiological and clinical implications. A growing body of evidence in humans indicates an association between impaired cardiac vagal BRS and the incidence of sudden cardiac death (19, 20). Aging is associated with both a reduction in cardiac vagal BRS (9, 13, 25) and an increased prevalence of lethal ventricular tachyarrhythmias (12). Thus it is possible that the age-related decline in cardiac vagal BRS may predispose older adults to sudden cardiac death.

In the present study, we did not determine responses to a placebo infusion. However, we believe that our young subjects represent a valid and appropriate control group. Specifically, because oxidative stress is not present in healthy young adults, there is little experimental basis to hypothesize that ascorbic acid would exert a significant effect on baroreflex function. This suggestion is supported by the following: 1) cardiac vagal BRS did not increase in our young controls in response to raising plasma ascorbic acid concentrations to levels associated with free radical scavenging, 2) no young subject demonstrated an increase in cardiac vagal BRS that exceeded the minimal increase observed among the older subjects, and 3) intravenous ascorbic acid was shown previously not to alter cardiac vagal BRS in young healthy adults (27), suggesting that baseline oxidative stress is a prerequisite for antioxidant-mediated improvements in cardiac vagal BRS. Thus the collective body of published experimental data indicates that ascorbic acid should exert no effect in young healthy adults and supports their use as an appropriate control group. Finally, the fact that the cardiac vagal BRS analyses were performed by an investigator who was blinded to the ages of the subjects ensures the validity of the group differences observed in the responses to ascorbic acid.

It is important to reemphasize that the stated aim of the present study was to test the hypothesis that oxidative stress contributes mechanistically to age-associated reductions in cardiac vagal BRS, not to determine the efficacy of oral vitamin C supplementation as a potential intervention. However, recently a 4-wk intervention of daily oral vitamin C (4 g) showed no increase in cardiac vagal BRS in heart failure patients (27). This lack of effect likely is explained by the fact that oral vitamin C supplementation fails to raise or maintain plasma ascorbic acid concentrations at levels required to scavenge reactive oxygen species (i.e., ∼100 μmol/l compared with ≥1,000 μmol/l during acute intravenous infusion in the present study).

We have no biomarkers of oxidative stress in the present study. If we had, these would be limited to systemic plasma markers, which may not accurately reflect oxidative stress at sites critical to the baroreflex function. However, we do establish a consistent and robust increase in cardiac vagal BRS that was demonstrated during ascorbic acid infusion in older, but not young men. We are unaware of any antioxidant-independent effect of ascorbic acid that could provide an alternative explanation to our conclusions. Moreover, it is unlikely that ascorbic acid elicited an oxidative stress-independent change that would be selective to older adults. Therefore, we believe that our results can only be explained by an ascorbic acid-induced suppression of reactive oxygen species that was greater in older adults due to the presence of oxidative stress.

In conclusion, the present findings provide the first direct experimental support for the concept that oxidative stress contributes mechanistically to the age-associated reduction in cardiac vagal BRS in healthy men. As such, interventions with the potential to tonically suppress oxidative stress (e.g., habitual exercise, weight loss, and perhaps oral antioxidant therapy) may have efficacy for augmenting BRS in older adults.

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GRANTS

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