Ascorbic Acid Selectively Improves Large Elastic Artery Compliance in Postmenopausal Women

Kerrie L. Moreau, Kathleen M. Gavin, Angela E. Plum, Douglas R. Seals

Abstract—The compliance of large elastic arteries in the cardiothoracic region decreases with advancing age/menopause and plays an important role in the increased prevalence of cardiovascular diseases in postmenopausal women. We determined whether oxidative stress contributes to the reduced large elastic artery compliance of postmenopausal women. Carotid artery compliance was measured during acute intravenous infusions of saline (baseline control) and supraphysiological doses of the potent antioxidant ascorbic acid in premenopausal (n=10; 23±1; mean±SE) and estrogen-deficient postmenopausal (n=21; 55±1 years) healthy sedentary women. Carotid artery compliance was 56% lower in postmenopausal compared with premenopausal women during baseline control (P<0.0001). Ascorbic acid infusion increased carotid artery compliance by 26% in postmenopausal women (1.11±0.07 to 1.38±0.8 mm²/mm Hg×10⁻¹; P<0.001) but had no effect in premenopausal women (2.50±0.25 versus 2.43±0.20 mm²/mm Hg×10⁻¹). Carotid artery diameter, blood pressure, and heart rate were unaffected by ascorbic acid. In the pooled population, the change in arterial compliance with ascorbic acid correlated with baseline waist-to-hip ratio (r=0.56; P=0.001), plasma norepinephrine (r=0.58; P=0.001), and LDL cholesterol (r=0.54; P=0.001). These results suggest that oxidative stress may be an important mechanism contributing to the reduced large elastic artery compliance of sedentary, estrogen-deficient postmenopausal women. Increased abdominal fat storage, sympathetic nervous system activity, and LDL cholesterol may be mechanistically involved in oxidative stress–associated suppression of arterial compliance in postmenopausal women. (Hypertension. 2005;45:1107-1112.)

Key Words: aging ■ cholesterol ■ oxidative stress

Cardiovascular disease (CVD) is the leading cause of death in women in the United States. “Vascular aging” has been emphasized recently as the major risk factor for development of CVD. One clinically important change that occurs with vascular aging is a reduction in the compliance of large elastic arteries within the cardiothoracic region. In turn, this contributes to a number of adverse age-associated changes, including increased aortic impedance, left ventricular hypertrophy, and reduced cardiovagal baroreflex sensitivity. As such, identifying the mechanisms that contribute to reduced large elastic artery compliance with age is an important goal.

Cardiovascular aging in women is unique in that it appears to be delayed or occurs at a slower rate than in men during the premenopausal years, thereafter “catching up” with men during the postmenopausal period, particularly in estrogen-deficient women. The mechanisms underlying the accelerated cardiovascular aging of estrogen-deficient postmenopausal women are unclear but could be related in part to the development of oxidative stress. Markers of oxidative stress are higher and endogenous antioxidant defenses lower in some estrogen-deficient postmenopausal women compared with premenopausal controls.

Because oxidative stress modulates vascular smooth muscle cell (VSMC) tone, a key determinant of large artery compliance, we hypothesized that oxidative stress may contribute to the reduced large artery compliance of estrogen-deficient postmenopausal women. Moreover, because abdominal adiposity and LDL cholesterol levels are elevated in postmenopausal women and associated with oxidative stress, we determined whether these factors were related to oxidative stress–linked suppression of large artery compliance.

Methods

Subjects

Thirty-one healthy women were studied: postmenopausal women (50 to 63 years of age) who were estrogen deficient (n=21) and 10 premenopausal controls (20 to 27 years of age). All subjects were sedentary (no aerobic exercise >2 days per week), nonsmokers, normotensive, had a body mass index (BMI) <33 kg/m², were free of overt chronic diseases as assessed by medical history, physical examination, standard blood chemistries, and hematologic evaluation. Women >50 years of age were further evaluated by ECG and blood pressure responses during incremental treadmill exercise to exhaustion. All postmenopausal women were ≥1 year without menses (menopause duration 8±2 years) and had not taken hormone replacement therapy for ≥6 months. Four
postmenopausal women were initially taking either vitamin E or C and stopped ≥4 weeks before the main experimental protocol (methods on dietary analysis are located in the data supplement). All subjects gave their written informed consent to participate. All procedures were reviewed and approved by the University of Colorado at Boulder human research committee, and all subjects gave informed consent. All procedures were performed in the General Clinical Research Center (GCRC) and in accordance with University institutional guidelines.

Measurements

All measurements were performed after a ≥4-hour fast (12 hours for determination of metabolic parameters) and abstinence from caffeine. Premenopausal women were tested 1 to 6 days after onset of menstruation (ie, early follicular phase). During the main experimental sessions, the women were instrumented with an intravenous catheter in the arm for infusion of saline and ascorbic acid and acquisition of blood.

Large Elastic Artery Compliance

Carotid artery compliance and beta stiffness index, a less blood pressure–dependent expression of arterial compliance, were determined using high-resolution ultrasound imaging (Toshiba Power Vision 6000) along with applanation tonometry-obtained arterial pressure waveforms (SPT-301; Millar Instruments) from the contralateral artery as described previously. All images were coded by number and were blinded to group assignment.

Brachial Artery Blood Pressure

Peripheral arterial blood pressure was measured with a semiautomated device (Dinamap; Johnson & Johnson) over the brachial artery as described previously.

Metabolic Risk Factors and Oxidative Stress Markers

Fasting plasma concentrations of cholesterol, glucose, and insulin, and endothelin-1 (competitive radioimmunoassay), and catecholamines were measured by the Core Laboratory of the University of Colorado GRCC as described previously. Total antioxidant status (TAS), a measure of the overall antioxidant defense system, was determined on serum samples as described by Miller et al (methods are detailed in the online data supplement, available at http://www.hypertensionahaj.org). Oxidized LDL, an indirect measure of oxidative stress, was measured with an ELISA plate assay (Alpco Diagnostics).

Body Composition

Total fat mass and fat-free mass and abdominal-to-thigh fat distribution were determined using dual-energy x-ray absorptiometry (DXA; DPX-iQ; Lunar Corp) as described in detail previously. Minimal waist and hip circumferences were measured according to previously published guidelines, and waist-to-hip ratio (WHR) was calculated.

Protocol

To examine the contribution of oxidative stress to group differences in large elastic artery compliance, measurements were obtained during intravenous infusions of saline (baseline control) and a pharmacological dose of ascorbic acid (vitamin C) (American Regent Laboratories Inc) as described recently by our laboratory and in detail in the online data supplement.

Statistical Analysis

Unpaired t tests were used to assess group differences in subject characteristics and oxidative stress markers. To determine the effect of acute ascorbic acid infusion on large elastic artery compliance, repeated-measures ANOVA was used. In case of a significant F value, a post hoc test with the Newman–Keuls method was used to identify significant differences among the mean values. Univariate and partial correlation analyses were used to determine the relationships between variables of interest.

Results

Subject Characteristics

The characteristics of the subject groups are presented in Table 1. There were no significant group differences in body mass, BMI, arterial blood pressure, endothelin-1, HDL cholesterol, plasma epinephrine, or fasting insulin concentrations. Body fat, waist circumference, WHR, abdominal-to-peripheral body fat distribution, total and LDL cholesterol, fasting glucose, plasma norepinephrine, and oxidized LDL were higher and TAS was lower in the postmenopausal compared with the premenopausal women (all P<0.05). There was a trend for total caloric (1787±100 versus 2158±163 kcal per day) and carbohydrate (51±2% versus 58±3%) intake to be lower in the postmenopausal women (both P=0.06). There were no differences between the premenopausal and postmenopausal women in any other macronutrients, vitamins, or alcohol intake (data not shown).

Large Elastic Artery Compliance

Baseline carotid artery compliance was 56% lower (P<0.001) in the postmenopausal versus premenopausal women (Figure 1). Similar results were obtained when the data were expressed as beta stiffness index (Figure 1).

Ascorbic acid infusion increased large elastic artery compliance by 26±4% in the postmenopausal women (1.11±0.07 to 1.38±0.08 mm²/mm Hg×10⁻¹; P<0.001) but had no effect in premenopausal women (2.50±0.25 versus 2.43±0.25 mm²/mm Hg×10⁻¹; Δ=−5±6%; Figures 1 and 2). Similar results were observed when the data were expressed as beta stiffness index. Arterial blood pressure (brachial and carotid), heart rate, and
carotid artery diameter remained unchanged with ascorbic acid infusion (all \(P<0.05\); Table 2).

**Correlates of Large Elastic Artery Compliance at Baseline and With Ascorbic Acid**

In the pooled subjects, baseline carotid artery compliance was inversely related to oxidized LDL \((r=-0.46)\), fasting glucose \((r=-0.43)\), WHR \((r=-0.63)\), abdominal-to-thigh body fat distribution \((r=-0.41)\), plasma norepinephrine \((r=-0.50)\), LDL cholesterol \((r=-0.51)\) and total cholesterol \((r=-0.52\); all \(P<0.02\) and positively related to TAS \((r=0.45\); \(P<0.02\). In the pooled subjects, the changes in carotid artery compliance from baseline in response to ascorbic acid administration were most strongly related to plasma norepinephrine \((r=0.58\); \(P=0.001\); Figure 3), WHR \((r=0.56\); \(P=0.001\); Figure 3), and LDL cholesterol \((r=0.54\); \(P=0.001\); Figure 3), total cholesterol \((r=0.45\); \(P=0.007\)), fasting glucose \((r=0.48\); \(P=0.005\)), and abdominal-to-thigh body fat distribution \((r=0.32\); \(P=0.05\). When the effects of age were partialed out, only LDL cholesterol correlated with the change in arterial compliance in response to ascorbic acid. Within the postmenopausal women, LDL cholesterol \((r=0.40\); \(P<0.05\)), were related to the change in arterial compliance with ascorbic acid [plasma norepinephrine \((r=0.40)\), and WHR \((r=0.34\); both \(P=0.06)\]. There were no significant correlations within the premenopausal women. Use of beta stiffness index instead of arterial compliance resulted in the same general relations.

**Figure 1.** Carotid artery compliance and beta stiffness index during acute saline (baseline control, black bars) and ascorbic acid administration (striped bars). *\(P<0.001\) vs premenopausal; †\(P<0.001\) vs saline of the same group.

**Figure 2.** Group and individual changes in carotid artery compliance and beta stiffness index from baseline control levels in response to ascorbic acid infusion. *\(P<0.001\) vs premenopausal; †\(P<0.001\) vs saline.

**Discussion**

The novel finding of the present study is that oxidative stress appears to contribute mechanistically to the reduced large elastic artery compliance of estrogen-deficient postmenopausal women. Moreover, our results suggest that the modulatory influence of oxidative stress on large artery compliance is most closely related to baseline abdominal fat storage, sympathetic nervous system activity (plasma norepinephrine concentrations), and circulating LDL cholesterol.

**Oxidative Stress and Carotid Artery Compliance in Premenopausal and Postmenopausal Women**

Estrogen-deficient postmenopausal women appear to develop oxidative stress as a result of increased production of reactive oxygen species or reduced endogenous antioxidants. The higher oxidized LDL and lower TAS plasma concentrations in the postmenopausal compared with the premenopausal women in the present study are consistent with this idea.

The new key finding of the present study is that acute administration of the potent antioxidant ascorbic acid (vitamin C) increased carotid artery compliance in estrogen-deficient postmenopausal women but not in premenopausal controls. These results suggest that oxidative stress tonically suppresses large elastic artery compliance in healthy estrogen-deficient postmenopausal women. Administration of antioxidants, including vitamins C and E,
have been reported previously to modulate systemic arterial compliance, large artery stiffness, or pulse wave augmentation in adult humans, including patients with type 2 diabetes and CVD.\textsuperscript{27–30} The present findings extend these observations by demonstrating a tonic inhibitory effect of oxidative stress on carotid artery compliance in healthy estrogen-deficient postmenopausal women that is not observed in premenopausal females.

Consistent with the present results in premenopausal females, our laboratory demonstrated recently that acute and chronic ascorbic acid administration have no effect on carotid artery compliance in healthy young males.\textsuperscript{25} However, in apparent contrast to the present findings, no improvement in carotid artery compliance was observed in older men in response to ascorbic acid.\textsuperscript{25} It is unclear why arterial compliance increased with the ascorbic acid infusion in our postmenopausal women but not in the older men studied previously by our laboratory. Intrinsic differences may exist between women and men with aging that differentially affect arterial sensitivity to antioxidants. For example, estrogen or its metabolites have physiologically significant antioxidant properties that increase NO bioavailability and, thus, relax VSMCs.\textsuperscript{31–33} This, in turn, presumably would act to increase arterial compliance. Perhaps the loss of circulating estrogen with menopause results in an antioxidant deficit, leading to an increase in VSMC responsiveness to ascorbic acid administration.

Mechanisms of Oxidative Stress–Associated Modulation of Carotid Artery Compliance
We can only speculate on the mechanisms by which oxidative stress may contribute to impaired large artery compliance in postmenopausal estrogen-deficient women. Menopause is associated with metabolic changes, including increased visceral adiposity and plasma cholesterol,\textsuperscript{12,13} and obesity and dyslipidemia are associated with oxidative stress, independent of age and menopause.\textsuperscript{14,15} In addition, abdominal obesity is associated with elevated sympathetic nervous system activity and norepinephrine release,\textsuperscript{34} which, in itself, can contribute to in vivo oxidative stress.\textsuperscript{35} In the present study, whole-body and abdominal fat (estimated using waist

### TABLE 2. Hemodynamics During Saline and Ascorbic Acid Infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachial systolic BP, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>107±3</td>
<td>114±2</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>109±3</td>
<td>115±2</td>
</tr>
<tr>
<td>Brachial diastolic BP, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>63±2</td>
<td>68±2</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>63±2</td>
<td>68±2</td>
</tr>
<tr>
<td>Brachial MAP, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>78±2</td>
<td>86±2</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>78±2</td>
<td>86±1</td>
</tr>
<tr>
<td>Brachial PP, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>44±2</td>
<td>46±2</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>47±2</td>
<td>46±2</td>
</tr>
<tr>
<td>Carotid systolic BP, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>90±4</td>
<td>99±2</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>91±4</td>
<td>100±2</td>
</tr>
<tr>
<td>Carotid PP, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>28±3</td>
<td>32±2</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>29±3</td>
<td>32±2</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>59±3</td>
<td>63±2</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>60±3</td>
<td>64±2</td>
</tr>
<tr>
<td>Carotid diastolic diameter, mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>6.32±0.06</td>
<td>6.67±0.08</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>6.29±0.08</td>
<td>6.68±0.09</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; MAP, mean arterial pressure; PP, pulse pressure.
circumference, WHR, and abdominal-to Peripheral body fat distribution), plasma norepinephrine, and LDL cholesterol were higher in the postmenopausal compared with the premenopausal women. Moreover, measures of abdominal adiposity ($r=0.46$ to 50; $P<0.005$), norepinephrine ($r=0.35$; $P<0.05$), and LDL cholesterol ($r=0.35$; $P<0.05$) correlated with oxidized LDL and were the strongest correlates of the change in arterial compliance from baseline in response to ascorbic acid infusion. Thus, increased abdominal adiposity, sympathetic nervous system activity/norepinephrine release, and circulating cholesterol may contribute to the development of oxidative stress and its inhibition of large elastic artery compliance in postmenopausal females.

The exact mechanism by which ascorbic acid selectively improved large artery compliance in our postmenopausal women is unclear. Ascorbic acid is a potent water-soluble antioxidant that destroys reactive oxygen species. Structural changes within the elastic—collagen composition of the arterial wall are thought to occur over a period of years, making it unlikely that an acute intravenous infusion of ascorbic acid could improve large elastic artery compliance via such mechanisms.36,37 Rather, the ability of short-term administration of ascorbic acid to augment arterial compliance is more likely mediated by suppressing the effects of oxidative stress on vascular smooth muscle tone, perhaps in part through actions on the vascular endothelium.26,38 Specifically, ascorbic acid may increase NO bioavailability by protecting NO from inactivation by reactive oxygen species, which would increase the tonic state of VSMC relaxation.39 Increased NO bioavailability in the setting of reduced oxidative stress also may decrease tonic $\alpha$-adrenergic receptor–mediated contraction of VSMCs by suppressing norepinephrine release from sympathetic nerve endings or by uncoupling norepinephrine and $\alpha$-adrenergic signal transduction.40,41

Considerations and Experimental Limitations

The fact that carotid artery compliance was not restored to premenopausal levels with ascorbic acid infusion in either group of postmenopausal women indicates that other mechanisms besides oxidative stress are contributing to the reduced large elastic artery compliance in the postmenopausal state. These other mechanisms may include extracellular matrix–linked structural alterations within the arterial wall, including the formation of advanced glycation end-products.42 Additionally, it is possible that there was residual oxidative stress that was not influenced by ascorbic acid, and thus, other antioxidants (eg, vitamin E and glutathione) may have produced further improvements in or restored arterial compliance. Clearly, the most effective approach to acutely reducing oxidative stress would be to inhibit all sources of production of reactive oxygen species; however, this is not possible at the present time.

There are several limitations associated with the present study. First, we used indirect systemic plasma biomarkers of oxidative stress and antioxidant capacity, which lack sensitivity and may not accurately reflect the amount of oxidative stress in the vasculature. However, despite this, we were able to show the expected group differences in oxidized LDL and TAS, as well as significant relationships between these markers and baseline measures of large artery compliance. Second, we did not measure ascorbic acid concentrations or oxidative stress markers during the infusions. However, we have shown previously that the same infusion dose of ascorbic acid increases plasma ascorbic acid to supraphysiological levels (â~15-fold higher than baseline levels)25,26,43–45 and decreases plasma oxidized LDL and isoprostane concentrations.43,44 Moreover, in the present study, we were able to demonstrate a significant increase in large elastic artery compliance with ascorbic acid in the postmenopausal women, the group postulated to have baseline oxidative stress.

It is important to emphasize that the aim of the present study was to test the hypothesis that oxidative stress contributes mechanistically to reduced large elastic artery compliance in postmenopausal women, not to determine the efficacy of oral vitamin C supplementation as a potential intervention. Recent clinical trial data demonstrate no effect of vitamin C or E on CVD outcomes.46,47 This lack of effect is likely explained in part by the fact that oral vitamin C supplementation cannot maintain plasma ascorbic acid concentrations at levels required to scavenge reactive oxygen species.26,48 Indeed, improvements in intermediary cardiovascular risk factors in response to acute administration of supraphysiological levels of ascorbic acid are not necessarily observed with longer-term oral supplementation.26

Perspectives

Our findings may have important clinical implications. CVD is now acknowledged to be a major public health concern for women.49 In this regard, vascular aging featuring, in part, a decrease in large elastic artery compliance has been emphasized recently as the major risk factor involved in the etiology of CVD.2 As such, a better understanding of the mechanisms mediating reductions in large elastic artery compliance with aging in women is needed. The results of the present study provide new insight into the potential pathophysiological role of oxidative stress in the reduced large elastic artery compliance observed in postmenopausal women, a group at increased risk of CVD.

Conclusions

In conclusion, the results of the present study support the idea that oxidative stress plays an important mechanistic role in the reduction in large elastic artery compliance in estrogen-deficient postmenopausal sedentary women. Increased abdominal fat storage, sympathetic nervous system activity, and circulating cholesterol may be factors involved in the oxidative stress–associated reduction in large elastic artery compliance in this group.

Acknowledgments

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References


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