Ascorbic Acid Selectively Improves Large Elastic Artery Compliance in Postmenopausal Women
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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±0.08 mm²/mm Hg×10⁻⁵; P<0.001) but had no effect in premenopausal women (2.50±0.25 versus 2.43±0.20 mm²/mm Hg×10⁻⁵). 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 cardiovascual 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), normotensive, had a body mass index (BMI) <33 kg/m², were nonsmokers, nonmedicated, and 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 applation tonometric-obtained arterial pressure waveforms (SPT-301; Millar Instruments) from the contralateral artery as described previously.16,17 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.16

**Metabolic Risk Factors and Oxidative Stress Markers**
Fasting plasma concentrations of cholesterol, glucose, and insulin, and endothelin-1 (competitive radioimmunoassay),18 and catecholamines19 were measured by the Core Laboratory of the University of Colorado GCRC as described previously.20 Total antioxidant status (TAS), a measure of the overall antioxidant defense system, was determined on serum samples as described by Miller et al21 (methods are detailed in the online data supplement, available at http://www.hypertensionaha.org). Oxidized LDL, an indirect measure of oxidative stress, was measured with an ELISA plate assay (Alpco Diagnostics).22

**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.23 Minimal waist and hip circumferences were measured according to previously published guidelines, and waist-to-hip ratio (WHR) was calculated.24

**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,25,26 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.

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**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 (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.

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**TABLE 1. Subject Characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>23±1</td>
<td>55±1*</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>63.3±1.5</td>
<td>65.5±2.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.4±1.1</td>
<td>25.0±0.8</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>30±2</td>
<td>37±2*</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>72±2</td>
<td>81±2*</td>
</tr>
<tr>
<td>WHR</td>
<td>0.73±0.01</td>
<td>0.81±0.01*</td>
</tr>
<tr>
<td>Abdominal/thigh fat</td>
<td>0.6±0.1</td>
<td>0.9±0.1*</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>108±3</td>
<td>112±2</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>62±2</td>
<td>66±2</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>60±4</td>
<td>64±2</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.4±0.4</td>
<td>5.6±0.2*</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.4±0.3</td>
<td>3.5±0.2*</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.4±0.1</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>Fasting insulin, μU/mL</td>
<td>4.5±1.7</td>
<td>7.9±1.1</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>4.6±0.1</td>
<td>5.2±0.1*</td>
</tr>
<tr>
<td>Endothelin-1, pg/mL†</td>
<td>4.8±0.3</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>Oxidized LDL, U/L</td>
<td>36.9±4.5</td>
<td>50.8±3.4*</td>
</tr>
<tr>
<td>Epinephrine, pg/mL†</td>
<td>20±1.6</td>
<td>21.5±1.2</td>
</tr>
<tr>
<td>Norepinephrine, pg/mL†</td>
<td>149±37</td>
<td>295±27*</td>
</tr>
<tr>
<td>TAS, mmol/L</td>
<td>1.4±0.1</td>
<td>1.1±0.1*</td>
</tr>
</tbody>
</table>

*P*<0.05 vs premenopausal; †n=9 premenopausal and 16 postmenopausal women.

BP indicates blood pressure; abdominal/thigh fat, abdominal-to-peripheral body fat distribution.
carotid artery diameter remained unchanged with ascorbic acid infusion (all P > 0.50; 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 partitioned 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.

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.8,9 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.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.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.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.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,12,13 and obesity and dyslipidemia are associated with oxidative stress, independent of age and menopause.14,15 In addition, abdominal obesity is associated with elevated sympathetic nervous system activity and norepinephrine release,34 which, in itself, can contribute to in vivo oxidative stress.35 In the present study, whole-body and abdominal fat (estimated using waist circumferences) were highly correlated with arterial compliance in premenopausal but not in postmenopausal women (Figure 3).

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.
and sympathetic nerve endings or by uncoupling norepinephrine 
tonation of VSMCs by suppressing norepinephrine release 
and decreases plasma oxidized LDL and isoprostane concen-
trations.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 contrib-
utes mechanistically to reduced large elastic artery compli-
ance 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 supplemen-
tion 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 supraphysio-
logical 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 empha-
sized recently as the major risk factor involved in the etiolo-
y 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 compli-
ance 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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Clinical Research Center (RR-00051).

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