Oxidative stress contributes to chronic leg vasoconstriction in estrogen-deficient postmenopausal women

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Moreau KL, DePaulis AR, Gavin KM, Seals DR. Oxidative stress contributes to chronic leg vasoconstriction in estrogen-deficient postmenopausal women. J Appl Physiol 102: 890–895, 2007. First published November 16, 2006; doi:10.1152/japplphysiol.00877.2006.—Basal whole leg blood flow and vascular conductance are reduced in estrogen-deficient postmenopausal compared with premenopausal women. The underlying mechanisms are unknown, but oxidative stress could be involved. We studied 9 premenopausal [23 ± 1 yr (mean ± SE)] and 20 estrogen-deficient postmenopausal (55 ± 1 yr) healthy women. During baseline control, oxidized low-density lipoprotein (LDL), a marker of oxidative stress, was 50% greater in the postmenopausal women (P < 0.001). Basal whole leg blood flow (duplex ultrasound of femoral artery) was 34% lower in the postmenopausal women because of a 38% lower leg vascular conductance (P < 0.0001); mean arterial pressure was not different. Intravenous administration of a supraphysiological dose of the antioxidant ascorbic acid increased leg blood flow by 15% in the postmenopausal women as a result of an increase in leg vascular conductance (both P < 0.001), but it did not affect leg blood flow in premenopausal controls or mean arterial pressure in either group. In the pooled subjects, the changes in leg blood flow and leg vascular conductance with ascorbic acid were related to baseline plasma oxidized LDL (r = 0.46 and 0.53, P < 0.01) and waist-to-hip ratio and total body fat (r = 0.41–0.44, all P < 0.05). Our results are consistent with the hypothesis that oxidative stress contributes to chronic leg vasoconstriction and reduced basal whole leg blood flow in estrogen-deficient postmenopausal women. This oxidative stress-related suppression of leg vascular conductance and blood flow may be linked to increased total and abdominal adiposity.

Oxidative stress appears to play an important mechanistic role in some expressions of vascular aging in humans (15, 17, 40). Recently, our laboratory demonstrated that oxidative stress contributes to the reductions in large elastic artery compliance in estrogen-deficient postmenopausal women (34). In the present study, we tested the hypothesis that oxidative stress also contributes to the chronic leg vasoconstriction and reduction in blood flow in these women. To do so, we determined the effects of acute administration of the potent antioxidant ascorbic acid (i.e., vitamin C) on femoral artery blood flow and vascular conductance in groups of healthy premenopausal and estrogen-deficient postmenopausal women.

METHODS

Subjects

Twenty-nine healthy women were studied: 20 postmenopausal (48–61 yr) and 9 premenopausal (21–27 yr). Some of these women were participants in a previous study investigating the effects of oxidative stress on arterial stiffness (34). All postmenopausal women had been without menses for at least 1 yr and were not taking any estrogen preparations for at least 6 mo. All subjects were normotensive, nonsmokers, nonmedicated, and free of overt chronic diseases as assessed by medical history, physical examination, standard blood chemistries, and hematological evaluation. None of the subjects exercised or took antioxidants or other dietary supplements. Women over the age of 50 yr were further evaluated by ECG and blood pressure during incremental treadmill exercise to exhaustion. Subjects who demonstrated ankle-brachial pressure index consistent with peripheral vascular disease (<0.90) were excluded (37). All subjects gave their written informed consent to participate. All procedures were reviewed and approved by the Human Research Committee and were performed in the General Clinical Research Center.

Measurements

All measurements were performed after a ≥4-h fast (12 h for determination of metabolic parameters) and abstinence from caffeine. Premenopausal women were tested 1–6 days after onset of menstruation (i.e., 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.

Femoral artery ultrasonography. A duplex ultrasound machine (Toshiba Power Vision 6000) equipped with a high-resolution (7.5 MHz) linear-array transducer was used to measure blood velocity parameters and vessel diameter on the common femoral artery as described previously by our laboratory (9, 10). Blood flow was calculated by the equation (mean blood velocity) × (circular area) × 6 × 10⁴, with the constant 6 × 10⁴ being the conversion factor from...
Table 1. **Subject characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>Age, yr</td>
<td>23.4 ± 1</td>
<td>24.4 ± 1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.4 ± 1.1</td>
<td>24.4 ± 1.0</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>30 ± 2</td>
<td>36 ± 1*</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>72 ± 3</td>
<td>80 ± 2*</td>
</tr>
<tr>
<td>WHR</td>
<td>0.72 ± 0.02</td>
<td>0.80 ± 0.01*</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>109 ± 3</td>
<td>109 ± 2</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>63 ± 3</td>
<td>66 ± 2</td>
</tr>
<tr>
<td>Estradiol-us, pg/ml</td>
<td>70 ± 9</td>
<td>41 ± 6*</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.6 ± 0.4</td>
<td>6.0 ± 0.4*</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>2.6 ± 0.3</td>
<td>3.8 ± 0.2*</td>
</tr>
<tr>
<td>Fasting insulin, μU/l</td>
<td>4.0 ± 1.8</td>
<td>8.0 ± 1.1*</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>4.6 ± 0.2</td>
<td>5.2 ± 0.1*</td>
</tr>
<tr>
<td>HOMA</td>
<td>0.85 ± 0.42</td>
<td>1.76 ± 0.26*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. BMI, body mass index; WHR, waist-to-hip ratio; BP, resting arterial blood pressure; estradiol-us, high-sensitivity estradiol; HOMA, insulin sensitivity index. *P < 0.05 vs. premenopausal.

meters per second to liters per minute. The data were analyzed by the same investigator (K. L. Moreau), who was blinded to the group assignment and condition of the subject. Arterial blood pressure was measured over the brachial artery using the oscillometric technique as previously described (Dinamap, Critikon, FL) (9). Femoral vascular conductance (inverse of femoral vascular resistance) was calculated as femoral blood flow/mean arterial blood pressure.

**Body composition and leg tissue mass.** Total fat mass and fat-free mass were determined using dual-energy X-ray absorptiometry. Regional analysis of the tissue mass of the right leg was determined from whole body scans using dual-energy X-ray absorptiometry (DPX-IQ, Lunar, Lunar software version 3.1) as previously described (9, 10). Waist circumference and waist-to-hip ratio (WHR) were measured according to previously published guidelines (30) and were used as estimates of total abdominal fat (39).

**Plasma markers of oxidative stress and antioxidant defenses, metabolic risk factors, and vasoactive hormones.** Venous blood samples were immediately centrifuged, and plasma or serum samples were placed in aliquots and stored at −80 °C until analysis. Oxidized low-density lipoprotein (LDL), an indirect measure of systemic oxidative stress, specifically lipid oxidative damage, was determined with an ELISA plate assay (Alpco Diagnostics, Windham, NH, and R&D Systems, Minneapolis, MN). Total antioxidant status (TAS), a measure of the overall antioxidant defenses, was determined on serum samples using the Randox Laboratories enzymatic kit (Oceanside, CA). Plasma concentrations of glucose and total cholesterol (Roche Diagnostic Systems, Indianapolis, IN) and high-density lipoprotein (HDL) cholesterol (Diagnostic Chemicals, Oxford, CT) were determined by enzymatic and/or colorimetric methods, and LDL cholesterol was determined using the Friedewald equation (19). Plasma concentrations of insulin, endothelin-1, and angiotensin-converting enzyme (ACE) were measured using conventional radioimmunoassays, and epinephrine and norepinephrine by HPLC (Dionex, Sunnyvale, CA). Serum high-sensitivity estradiol was measured using the radioimmunoassay (Diagnosics Systems Laboratory, Webster, TX). The homeostasis model (fasting glucose × fasting insulin/22.5; HOMA) was used to calculate the insulin sensitivity index (21). All assays, with the exception of ACE, which was performed by the Mayo Clinic, were performed by the University of Colorado General Clinical Research Center core laboratory.

**Protocol**

To examine the contribution of oxidative stress to group differences in femoral artery blood flow and vascular conductance (resistance), measurements were obtained during saline infusion and after acute administration of a pharmacological dose of ascorbic acid (vitamin C) (American Regent Laboratories, Shirley, NY) as described recently by our laboratory (16, 17). First, a priming bolus of saline (control) was administered followed by a drip infusion of saline. Next, a priming bolus of ascorbic acid was administered (0.06 g/kg fat-free mass dissolved in 100 ml of saline infused at 5 ml/min for 20 min) followed by a drip infusion (0.02 g/kg fat-free mass dissolved in 30 ml of saline administered over 60 min at 0.5 ml/min). The total volume infused for both saline and ascorbic acid was 130 ml.

**Statistical Analysis**

An unpaired t-test was used to assess group differences in subject characteristics and baseline humoral factors and cardiovascular function. To determine the effect of acute ascorbic acid infusion on basal limb blood flow and hemodynamics, repeated-measures ANOVA was used. If significant differences were observed, post hoc analyses were performed using paired t-tests with the Bonferroni correction to identify significant differences among the mean values. Exploratory analyses were performed using Pearson product-moment correlations to test for the presence of significant linear bivariate relations between variables of interest. Data analysis was performed with SPSS software, version 12.0. Differences were considered significant at P < 0.05.

**RESULTS**

**Subject Characteristics**

Subject characteristics are presented in Table 1. There were no significant group differences in body mass index (BMI), plasma HDL cholesterol, or resting blood pressure. Body fat percent, waist circumference, WHR, plasma total and LDL cholesterol, fasting insulin and glucose, and HOMA were higher, and serum estradiol and leg fat-free mass lower, in the postmenopausal women compared with the premenopausal women (all P < 0.05).

**Humoral factors in the groups are presented in Table 2. Plasma oxidized LDL and norepinephrine were higher and TAS was lower in the postmenopausal women (all P < 0.05). There were no significant differences between the groups in circulating concentrations of ACE, endothelin-1, or epinephrine.

**Basal Leg Blood Flow**

Absolute basal femoral artery blood flow during saline control was 34% lower in the postmenopausal women compared with the premenopausal controls (252 ± 20 vs. 379 ± 30 ml/min; P < 0.001; Fig. 1A). Femoral blood flow normalized for leg fat-free mass was 24% lower in the postmenopausal than premenopausal women (38 ± 3 vs. 50 ± 4 ml·min⁻¹·kg⁻¹; P < 0.05). Com-
pared with saline control, during ascorbic acid infusion femoral artery blood flow was increased by 15 ± 3% in the postmenopausal women (to 288 ± 19 ml/min; P < 0.001), but it was unchanged in the premenopausal women (355 ± 28 ml/min; P = 0.07). Similar results were obtained when the data were expressed as femoral blood flow normalized for leg fat-free mass (postmenopausal women during ascorbic acid: 43 ± 3 ml·min⁻¹·kg⁻¹; P < 0.001 vs. saline control; premenopausal women during ascorbic acid: 47 ± 4 ml·min⁻¹·kg⁻¹; P = 0.11 vs. saline control). Mean arterial blood pressure (premenopausal: 80 ± 2 vs. 79 ± 2 mmHg; postmenopausal: 84 ± 2 vs. 84 ± 2 mmHg) and heart rate (premenopausal: 59 ± 3 vs. 57 ± 3 beats/min; postmenopausal: 63 ± 2 vs. 61 ± 2 beats/min) did not change with the ascorbic acid infusion compared with saline control.

**Leg Vascular Conductance**

The lower baseline (saline control) femoral artery blood flow in the postmenopausal compared with the premenopausal women was associated with a 38% lower femoral artery vascular conductance (P < 0.001; Fig. 1B). In the postmenopausal women, the increase in absolute femoral blood flow with the ascorbic acid infusion was associated with a 15 ± 4% increase in femoral vascular conductance compared with saline control (P < 0.002; Fig. 1B). In contrast, femoral vascular conductance was not significantly different during ascorbic acid infusion compared with saline control in the premenopausal women (P = 0.07, Fig. 1B).

**Correlates of Femoral Blood Flow and Vascular Conductance**

Baseline femoral artery blood flow and vascular conductance were positively related to plasma estradiol (both r = 0.51, P = 0.005) and TAS (both r = 0.37, P < 0.05), and inversely related to plasma norepinephrine (r = −0.40 and −0.46, P < 0.05) and oxidized LDL (r = −0.35 and −0.39, P < 0.05) concentrations. The changes in femoral artery blood flow and vascular conductance with ascorbic acid were most strongly related to baseline plasma oxidized LDL (r = 0.46 and 0.53, P < 0.01; Fig. 2), WHR (both r = 0.44, P = 0.02; Fig. 3), and total adiposity (both r = 0.41, P < 0.05). The change in femoral vascular conductance with ascorbic acid also correlated with baseline fasting insulin concentrations (r = 0.39, P < 0.05). There were no other significant relations between baseline or ascorbic acid-stimulated femoral hemodynamics and subject characteristics, including duration of menopause.

**DISCUSSION**

The present study provides novel insight into the mechanisms contributing to the reduced basal leg blood flow in healthy estrogen-deficient postmenopausal women. The key new finding is that acute administration of the potent antioxi-
The exact mechanism by which ascorbic acid increased leg blood flow in our postmenopausal women cannot be discerned from the present findings. Ascorbic acid is a potent antioxidant that scavenges reactive oxygen species. An overproduction of reactive oxygen species can influence several vasoactive fac-

LDL concentrations. Collectively, these observations suggest that oxidative stress contributes to tonic leg vasoconstriction and suppression of basal leg blood flow in healthy estrogen-deficient postmenopausal women.

We can only speculate on the mechanisms by which oxidative stress suppresses leg vascular conductance and blood flow in this setting. Oxidative stress represents an imbalance between the production and destruction of reactive oxygen species. Enzymatic and nonenzymatic antioxidant mechanisms act to maintain appropriate levels of reactive oxygen species, which are important molecules for cell signaling and other functions (12). Therefore, the development of vascular oxidative stress can occur either through an overproduction of reactive oxygen species, reduced antioxidant mechanisms, or both. We have no data regarding potential group differences in reactive oxygen species production in the present study. However, TAS, a marker of total systemic antioxidant defenses, was lower in the postmenopausal women, and it was positively related to baseline femoral blood flow in the overall group. Thus reduced antioxidant defenses may have contributed to oxidative stress-mediated leg vasoconstriction and reduced blood flow in our postmenopausal women.

The results of our laboratory’s previous finding that basal leg blood flow is reduced in estrogen-deficient postmenopausal women but not premenopausal women. The ascorbic acid-mediated increase in basal leg blood flow in the postmenopausal women was associated with an increase in leg vascular conductance. These findings suggest that oxidative stress may be a contributing mechanism to the chronic leg vasoconstriction and reduced basal blood flow observed in healthy estrogen-deficient postmenopausal women. Our findings also suggest that this oxidative stress-associated vasoconstriction may be related to the increased abdominal and total adiposity in these women.

The results of the present investigation are consistent with our laboratory’s previous finding that basal leg blood flow is reduced in estrogen-deficient postmenopausal women as a result of a decrease in vascular conductance (33). The present results extend these earlier findings by identifying oxidative stress as one mechanism involved. This conclusion is supported by the observations that 1) plasma oxidized LDL, a marker of systemic oxidative stress, was elevated in the postmenopausal compared with the premenopausal women, as our laboratory observed recently (34); 2) plasma oxidized LDL was inversely related to baseline basal leg blood flow and vascular conductance; 3) acute administration of supraphysiological concentrations of the antioxidant ascorbic acid increased femoral artery blood flow and vascular conductance only in the postmenopausal women; and 4) the changes in leg blood flow and vascular conductance with ascorbic acid administration were inversely related to baseline plasma oxidized LDL concentrations.
tors, including nitric oxide, endothelin-1, renin-angiotensin system bioactivity, and the sympathetic nervous system (1, 22, 24, 27, 38), and these factors can, in return, influence reactive oxygen species. In the present study, ascorbic acid may have increased femoral blood flow and vascular conductance in the postmenopausal women by reducing superoxide anions, thus inhibiting their reaction with nitric oxide and increasing nitric oxide bioavailability. This would, in turn, cause vascular smooth muscle relaxation either directly (27, 38) or indirectly, for example, by suppressing the release of the endothelium-derived contracting factor endothelin-1 (6) or by decreasing α-adrenergic receptor signaling by inhibiting norepinephrine release from sympathetic nerve endings and/or uncoupling norepinephrine-evoked α-adrenergic signal transduction (44).

The latter possibility is consistent with the fact that, in the present study, plasma norepinephrine levels were higher in the postmenopausal women and correlated with plasma oxidized LDL \((r = 0.44, P = 0.02)\) and baseline leg blood flow \((r = -0.40, P = 0.03)\). In addition to preventing superoxide reacting with nitric oxide, there is in vitro evidence that ascorbic acid also may influence nitric oxide production via modulation of endothelial nitric oxide synthase activity, possibly by influencing the bioavailability of a key cofactor for nitric oxide synthesis, tetrahydrobiopterin (18, 31).

The fact that leg blood flow was not restored to premenopausal levels by ascorbic acid infusion indicates that mechanisms operating independently of oxidative stress are contributing to the tonic leg vasocostriction and reduced blood flow in estrogen-deficient postmenopausal women. These may involve any of the neural, humoral, and/or local vasoactive mechanisms discussed previously. In particular, our laboratory has shown previously that α1-adrenergic receptor inhibition restores femoral blood flow and vascular conductance in older men (11). This mechanism also could be acting, at least in part, through nonoxidative stress-related processes in estrogen-deficient postmenopausal women. In addition, we cannot rule out the possibility of structural changes in the femoral artery playing a role.

In the present study, we chose to use ascorbic acid because it is one of the most potent water-soluble antioxidants in humans, and it can be acutely infused at rates that attain supraphysiological plasma concentrations known to reduce the bioavailability of superoxide anions (23). Consistent with this effect, ascorbic acid improves vascular function in groups with baseline vascular oxidative stress, including those with cardiovascular disease risk factors and patients with clinical cardiovascular disease (14, 17, 20, 32, 34–36). Importantly, although not assessed in the present study, we have established previously that the same dosing regimen of ascorbic acid as used in the present study reduces plasma concentrations of oxidized LDL and isoprostanes in older adults (3, 4), and it improves or reverses oxidative stress-mediated reductions in other physiological functions in older sedentary humans (5, 17, 32, 34). Thus ascorbic acid infusion is a well-established antioxidant with multiple beneficial effects on oxidative stress in humans.

Our findings may have important clinical implications for estrogen-deficient postmenopausal women. Reduced whole leg blood flow and vascular conductance appear to play a mechanistic role in the development of several risk factors for chronic disease and loss of physiological function. For example, reduced leg blood flow may limit peripheral glucose uptake and therefore contribute to the increased prevalence of glucose intolerance and hyperinsulinemia in middle-aged and older adults (2, 7, 28). It may also impair clearance of atherogenic lipids and contribute to chronic dyslipidemia, perhaps by affecting the amount of lipids presented to capillary lumen-bound lipoprotein lipase (29). Thus oxidative stress may represent an important therapeutic target for the prevention and treatment of vascular dysfunction in estrogen-deficient postmenopausal women.

In conclusion, the results of the present investigation provide initial insight into the role of oxidative stress as an important mechanism contributing to chronic leg vasoconstriction and reduced leg blood flow in healthy estrogen-deficient postmenopausal women. We speculate that in these women, vascular oxidative stress may develop in part as a consequence of increased abdominal and total body fat, reductions in circulating estrogens, or decreases in overall antioxidant defenses, and it may exert its vasoconstrictor effects, at least in part, via enhanced norepinephrine release and/or action.

REFERENCES


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GRANTS

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