Estrogen Replacement Therapy Improves Baroreflex Regulation of Vascular Sympathetic Outflow in Postmenopausal Women

Brian E. Hunt, PhD; J. Andrew Taylor, PhD; Jason W. Hamner, BS; Margaret Gagnon, RN; Lewis A. Lipsitz, MD

Background—Menopausal estrogen loss has been associated with increased cardiovascular disease in postmenopausal women. However, the link between estrogen and cardiovascular disease remains unclear. Some data suggest estrogen mediates its effect through changes in arterial pressure and its regulation. However, the data available in older women are equivocal regarding estrogen’s ability to reduce resting arterial pressure or to improve its regulation.

Methods and Results—We studied 11 healthy, postmenopausal women before and after 6 months of estrogen administration. Arterial pressure was measured by brachial auscultation and finger photoplethysmography. Vascular sympathetic nerve activity was measured in the peroneal nerve by microneurography, and the slope of the relations between changes in heart period, sympathetic activity, and arterial pressure caused by bolus infusions of nitroprusside and phenylephrine were used as an index of baroreflex gain. Estrogen therapy did not change systolic pressure (128±2 versus 123±2 mm Hg) or cardiac-vagal baroreflex gain (6.6±0.9 versus 6.7±0.7 ms/mm Hg). However, vascular sympathetic baroreflex gain was increased (−4.6±0.6 versus −7.4±1.0 arbitrary integrated units/mm Hg; \( P = 0.02 \)).

Conclusion—These findings suggest long-term estrogen replacement therapy has effects on cardiovascular regulation that may not be reflected in resting arterial pressures. (Circulation. 2001;103:2909-2914.)

Key Words: aging ■ blood pressure ■ baroreceptors

The incidence of cardiovascular disease in women increases sharply after menopause.1 Although the causes of this postmenopausal upsurge in cardiovascular morbidity and mortality are unclear, the increase may involve changes in arterial pressure and its regulation with estrogen loss. For example, the risk for hypertension rises precipitously after menopause.2 Moreover, low arterial baroreflex gain is associated with both higher arterial pressure and severity of cardiovascular disease.3 Thus, it is feasible that the loss of estrogen with menopause results in increased arterial pressure due to decreased baroreflex function. If true, supplemental estrogen may reverse deficits in cardiovascular control and protect against the development of cardiovascular disease in postmenopausal women.

Previous data have shown that only 1 month of estrogen replacement therapy can reduce arterial pressures in postmenopausal women.4 If estrogen replacement therapy does have beneficial effects on arterial pressure, this might be achieved through the primary regulator of pressure, the arterial baroreflex. However, the sparse data available are equivocal; some data suggest baroreflex regulation is enhanced after estrogen therapy,4 whereas other data suggest baroreflex function is unaffected.5 These divergent results may be due to the different time courses of estrogen treatment (1 versus 3 months) or the use of indirect clinical assessments of baroreflex gain (Valsalva maneuver). Moreover, only the vagal arm of the baroreflex arc is examined in most studies, thus shedding no light on the important vascular sympathetic limb.

Recently, more direct measures in premenopausal women indicate the hormonal milieu can impact baroreflex function. Minson et al6 used direct pharmacological manipulation of pressure to examine both the vagal and vascular sympathetic arms of the baroreflex and compared gains during the luteal and the follicular phases of the menstrual cycle. They found vagal baroreflex gain was unchanged, whereas the sympathetic baroreflex increased during the high estrogen luteal phase of the cycle. Interestingly, a subsequent examination of changes associated with oral contraceptive use in younger women found lower baroreflex gains during the high estrogen phase.7 Thus, even in young healthy women, the effect of estrogen on arterial pressure and its regulation is unclear. Furthermore, these data do not indicate how estrogen might impact postmenopausal women, who are most at risk for
cardiovascular disease. Therefore, the purpose of this study was to determine if long-term estrogen administration improves baroreflex function and reduces resting arterial pressure in healthy, postmenopausal women. Our data show long-term estrogen replacement had no effect on cardiovagal baroreflex gain but improved vascular sympathetic baroreflex gain. However, this was not associated with a lowering of arterial pressure.

**Methods**

This study was reviewed and approved by the clinical investigations committee at the Hebrew Rehabilitation Center for the Aged. All procedures, risks, and benefits were explained, and informed written consent was obtained from each subject before testing began.

**Subjects**

Eleven healthy, postmenopausal women successfully completed the study (mean age, 60±2 years; years postmenopausal, 20±3). For recruitment, newspaper advertisements were posted, and members of the Harvard Cooperative Program on Aging Subject registry were notified. A total of 134 women, 55 to 75 years of age, responded to our solicitations. Of these, 49 declined to participate and 55 were excluded due to a history of smoking, cancer, vaginal bleeding, thrombophlebitis, migraine headaches, body mass index >30 kg/m², hypertension (systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg), use of medications with cardiovascular effects, or previous intolerance to oral estrogen.

The remaining 30 eligible subjects were given a physical examination and exercise stress test; 7 subjects were ineligible due to indications of overt cardiovascular disease (ST-segment depression >1.0 mm from baseline, chest pain, shortness of breath/wheezing, leg cramping or intermittent claudication, and/or systolic pressure >260 mm Hg or diastolic pressure >115 mm Hg). 4 and 2 women declined further participation. Of the remaining 19 healthy, postmenopausal women, 15 completed baseline measures and began continuous estrogen therapy. Treatment consisted of estrogen (0.625 mg/d) taken orally for 6 months, with 1 week of progesterone (10 mg daily) at months 3 and 6; the latter was given after measurements were completed. Three subjects withdrew due to excessive vaginal bleeding, and one subject was excluded due to an inadequate sympathetic nerve recording. This resulted in 11 women with complete data sets.

**Procedures and Protocols**

**Arterial Pressure**

Resting arterial pressures were determined during pretreatment medical examinations by auscultation and before baroreflex testing using an automated oscillometric device (Dinamap). Auscultation, when performed by trained personnel using established guidelines, is considered a valid and reliable technique. In this study, auscultatory- and Dinamap-derived systolic, diastolic, and mean pressures were not different (128±1 versus 128±2, 72±2 versus 69±1, and 90±1 versus 89±1 mm Hg, respectively) between estrogen therapy and control blood pressures. During baroreflex assessment, beat-to-beat blood pressure was monitored during each experimental protocol by photoplethysmography (Finapres), which was adjusted during supine rest to closely match mean arterial pressures derived via Dinamap. The subjects reported to the laboratory at ~8:00 AM, after at least a 12-hour fast, and were set up with Finapres and Dinamap pressure cuffs and ECG electrodes for a standard V-V interval to measure R-R interval changes during baroreceptor activation. A venous catheter was placed in an arm vein to administer depressor and pressor agents, and microneurography microelectrodes were inserted for peroneal nerve recordings. Baroreceptor engagement was accomplished through an application of the modified Oxford technique. This involves a bolus injection of 100 μg of sodium nitroprusside followed 60 s later by a bolus of 150 μg of phenylephrine hydrochloride; this generally produces an initial ~15 mm Hg drop in arterial pressure followed by an ~15 mm Hg rise in pressure above resting supine levels. Waveforms were digitized at 500 Hz and stored for subsequent off-line analysis with signal processing software (WINDAQ).

**Baroreflex Gain**

Baroreflex gains were estimated from the relations between changes in heart period or vascular sympathetic activity and arterial pressure during baroreceptor engagement via the modified Oxford technique. R-R intervals were associated with appropriate systolic blood pressures, and sympathetic activity was associated with appropriate diastolic pressures after accounting for baroreflex delays. Presures, R-R intervals, and sympathetic activities were averaged across 3 mm Hg pressure increments to account for the variability associated with ventilation and measurement error and to increase confidence in the derived relation. This procedure (binning) also allows an estimation of the linear gain of the cardiovagal and sympathetic baroreflexes. In most cases, a sigmoid nature of the relations is revealed with this pharmacological paradigm, allowing exclusion of threshold and saturation regions to derive robust linear gains (Figure 1). Correlation coefficients for gain estimates ranged from 0.83 to 0.99 (mean, 0.94±0.01).

**Resting Relations**

Previously, Wallin et al. measured muscle sympathetic nerve activity in subjects with a large variability of diastolic pressures secondary to respiratory sinus arrhythmia or atrial fibrillation during supine rest. These investigators binned the sympathetic burst activity for each level of diastolic pressure and plotted muscle sympathetic activity as a function of diastolic pressure. To examine if changes in baroreflex gain might be recapitulated in the resting beat-by-beat relations between heart period or vascular sympathetic activity and arterial pressure, we used an approach similar to that described by Wallin et al. 13 Subjects breathed at 6 breaths per minute during quiet supine rest to induce consistent arterial pressure swings. Subjects maintained a comfortable, consistent tidal volume throughout.

Further, to increase average vascular sympathetic activity and provide better resolution for determining relations, a second session of paced breathing was performed during a continuous intravenous infusion of sodium nitroprusside (0.5 mg - kg⁻¹ - min⁻¹). This low dose caused slight hypotension (~2.8±1.7 mm Hg), mild tachycardia (5±2 bpm), and sympathoexcitation (213±37%). R-R intervals were associated with preceding systolic pressures, and sympathetic bursts were associated with preceding diastolic pressures, as shown in Figure 2. Arterial pressures, R-R intervals, and sympathetic activity were averaged for each 3-mm Hg increment in pressure, and linear relations between variables were determined. Moment-to-moment changes in resting arterial pressure and auto-
nomic outflow are influenced by a number of physiological inputs, including beat-to-beat changes in stroke volume, ventilation, central respiratory drive, neuronal function, and cardiopulmonary and arterial baroreflexes. Thus, our index encompasses all inputs to autonomic activity and arterial pressure under resting conditions, providing broad insight into resting arterial pressure regulation.

Statistical Analysis

Epidemiological data suggest the loss of estrogen may be associated with an increase in arterial pressure. Thus, estrogen supplementation in postmenopausal women would be expected to result in a decline in arterial pressure. The recent data of De Meersman et al supports this hypothesis. However, many data show that estrogen therapy has little effect on arterial pressure in normotensive adults. On the basis of this large body of evidence, we hypothesized that estrogen would either have no effect (null hypothesis) or lower blood pressure. Therefore, we used a 1-tailed \( t \) test to assess the efficacy of estrogen to lower blood pressure. With regard to baroreflex gain, previous data in postmenopausal women indicate vagal baroreflex gain will not change or may increase. Thus, we used a 1-tailed \( t \) test to assess whether estrogen administration would cause an increase in baroreflex gain after estrogen therapy. Moreover, given the inherent limited statistical power due to the relatively small sample size, we felt it appropriate to offset the constrained ability to assess estrogen-related differences in autonomic function by the increase in statistical power. The \( \alpha \) level was set at 0.05. Data are reported as mean ± SE.

Results

Resting Arterial Pressure

The Table shows that heart rate, arterial pressures, and sympathetic activity were unchanged by estrogen treatment.

<table>
<thead>
<tr>
<th>Measurements Taken Before and After Estrogen Therapy</th>
<th>Before Estrogen Therapy (Baseline)</th>
<th>After Estrogen Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>60 ± 3</td>
<td>59 ± 2</td>
</tr>
<tr>
<td>Systolic pressure, mm Hg</td>
<td>128 ± 2</td>
<td>123 ± 2</td>
</tr>
<tr>
<td>Diastolic pressure, mm Hg</td>
<td>69 ± 1</td>
<td>71 ± 1</td>
</tr>
<tr>
<td>Sympathetic activity, Bursts/min</td>
<td>20 ± 1</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>Sympathetic activity, aiu/min</td>
<td>612 ± 109</td>
<td>501 ± 115</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

Heart rate and diastolic pressure were nearly identical before and after treatment. Systolic pressure and sympathetic activity were reduced somewhat, although not to a statistically significant extent. Assuming our 11 volunteers are a representative sample, power calculations indicate an additional 17 subjects would be necessary for the decline in systolic pressures to reach significance, and an additional 132 subjects would be needed for sympathetic activity after estrogen therapy to reach significance.

Baroreflex Gain

On average, cardiac-vagal baroreflex gain was unchanged after 6 months of estrogen administration compared with the pre-therapy baseline.
though estrogen administration was associated with improved vascular effects of estrogen replacement therapy. First, although sympathetic baroreflex gain was greater after estrogen compared with baseline (−7.4±1.0 versus −4.6±0.6 aiu/mm Hg; Pa1=0.02; Pa2=0.04). The increase in reflex gain ranged from 14% to 173% in 8 of the 11 subjects. The remaining 3 subjects had small decreases in gain ranging from 10% to 16%.

**Resting Relations**

Both beat-by-beat R-R interval and burst-by-burst sympathetic activity were closely related to arterial pressures during supine rest with and without low-dose nitroprusside infusion (r=0.82±0.03 and r=0.89±0.05, respectively). Changes in these relations with estrogen treatment tended to parallel those in arterial baroreflex gain (Figure 4). The relation between heart period and systolic pressure was not different after 6 months of estrogen compared with baseline, either with (2.5±0.5 versus 2.3±0.8) or without nitroprusside infusion (2.6±0.6 versus 3.0±1.7). After estrogen therapy, the relation between sympathetic activity and diastolic pressure tended to show greater sympathetic activity for a given level of diastolic pressure (5.3±1.0 versus 6.8±1.2; Pa1=0.08; Pa2=0.15). Nitroprusside infusion tended to slightly augment this relation and revealed greater vascular sympathetic activity with diastolic pressure changes after estrogen therapy (6.3±1.1 versus 9.3±1.5 aiu/mm Hg; Pa1=0.04; Pa2=0.09; Figure 4).

**Discussion**

The results of this study provide 3 insights into the cardiovascular effects of estrogen replacement therapy. First, although estrogen administration was associated with improved baroreflex function, this was limited to the sympathetic arm of the reflex arc. Second, estrogen had little effect on resting arterial pressure in healthy, normotensive, postmenopausal women. Third, relations between arterial pressure and autonomic activity during supine rest recapitulate changes in arterial baroreflex gain with estrogen treatment. These findings suggest that long-term estrogen replacement therapy does have significant effects on cardiovascular regulation that are not obviously reflected in resting arterial pressures.

**Baroreflex Function**

Our findings of unchanged vagal baroreflex function and augmented sympathetic baroreflex gain after estrogen therapy are in agreement with other human data, despite differing populations and methodologies. Virtanen and colleagues examined baroreflex function in postmenopausal women by using a common clinical tool, the Valsalva maneuver. These authors reported no change in vagal baroreflex function after 3 months of estrogen therapy. Minson et al. studied both vagal and sympathetic baroreflex regulation in young, premenopausal women using direct pharmacological manipulation of arterial pressure via the modified Oxford technique. Their data indicate that vagal baroreflex gain was unchanged, whereas sympathetic baroreflex gain was greater during the high estrogen compared with low estrogen phase of the menstrual cycle. The factor(s) mediating this increased sympathetic gain are not known.

Estrogen can have potent vasodilatory effects, perhaps through augmented release of vascular nitric oxide. This may blunt vasoconstrictor effects of vascular sympathetic outflow, necessitating greater sympathetic activity to elicit a vascular resistance response. However, sympathetic cotransmitter release is increased after estrogen administration, which should potentiate vascular responses to neural sympathetic outflow. Accordingly, Minson and colleagues found the relation of sympathetic activity to vascular resistance was unchanged during the high estrogen phase of the menstrual cycle in young women. This suggests neural-vascular sympathetic transduction is unaffected by estrogen, at least in young healthy women, and indicates that the augmented sympathetic baroreflex gain seen in our subjects should result in more sensitive blood pressure control.

**Resting Arterial Pressure and Sympathetic Activity**

Although sympathetic baroreflex gain was enhanced after estrogen therapy, resting arterial pressure was unchanged. Arterial pressure in women reportedly increases with age and menopause. However, pretreatment pressures in our subjects did show mild to moderate increases in vagal baroreflex gain. However, the remaining subjects demonstrated either no change or profound declines. Sympathetic baroreflex gain was greater after estrogen compared with baseline (6.7±0.7 versus 6.6±0.9 ms/mm Hg; Figure 3). Six of the 11 subjects did show mild to moderate increases in arterial pressure during supine rest with and without low-dose nitroprusside infusion (2.6±0.6 versus 3.0±1.7). After estrogen therapy, resting arterial pressure was unchanged. Although sympathetic baroreflex gain was enhanced after estrogen therapy, resting arterial pressure was unchanged. Their data indicate that vagal baroreflex gain was unchanged, whereas sympathetic baroreflex gain was greater during the high estrogen compared with low estrogen phase of the menstrual cycle. The factor(s) mediating this increased sympathetic gain are not known.
jects were within the optimal range for healthy, premenopausal women. Our findings are consistent with data from cross-sectional, longitudinal, and large clinical trials showing estrogen replacement therapy has little effect on arterial pressure in normotensive, postmenopausal women. Considering that baroreflex function is the key mediator of moment-to-moment arterial pressure, it might be expected that resting levels of pressure would be altered. However, long-term regulation of pressure is critically dependent on many factors, including renal input through the renin-angiotensin system. Thus, the elevated levels of angiotensin II that reportedly occur in postmenopausal women on estrogen replacement therapy may oppose reductions in arterial pressure associated with enhanced baroreflex function.

Moreover, in our subjects, the average resting heart rate and vascular sympathetic activity, which are key hemodynamic inputs to arterial pressure, remained unchanged after estrogen treatment. However, we did not measure arterial pressure throughout the 6-month intervention period, and we cannot rule out the possibility that estrogen therapy has transitory effects on arterial pressure that are manifest over days to weeks. This may explain a previous report in which arterial pressure measured in the finger declined during 1 month of estrogen therapy in a similar group of postmenopausal women.

Although average levels of arterial pressure at rest were unchanged with estrogen therapy, its underlying regulation may still have been altered. Indeed, our index of the relations between swings in resting arterial pressure and autonomic outflow directly paralleled those seen during pharmacological baroreceptor engagement, suggesting that the regulation of arterial pressure at rest may be enhanced. This may relate to previous data showing 24-hour blood pressure variability is lower after estrogen therapy, possibly due to greater sympathetic buffering of blood pressure oscillations at rest. Thus, part of the decline in cardiovascular risk suggested to occur with estrogen replacement in healthy, postmenopausal women might be related to altered beat-by-beat sympathetic regulation of arterial pressure at rest.

Our data must be interpreted within the constraints of the experimental design and methodology employed. First, the use of automated Dinamap-derived brachial pressures may be suspect. Indeed, several studies have reported Dinamap-derived brachial pressures are generally 5 to 10 mm Hg lower than direct intra-arterial measures of pressure. However, the values have been shown to be highly reproducible. Moreover, auscultatory- and Dinamap-derived systolic and mean pressures were virtually identical, whereas diastolic mean pressures tended to be slightly lower when estimated by Dinamap (≈3 mm Hg). This excellent agreement, along with the multiple measurements taken before and after treatment, give us confidence that arterial pressure during supine rest was not different after 6 months of estrogen therapy in our subjects.

Second, although the ≈5 mm Hg lower systolic pressures after estrogen treatment were not statistically significant, a decline of this magnitude might be clinically important, reducing the short-term risk of cardiovascular disease. However, the reduction in risk is greatest for those whose pretreatment systolic pressures exceed 140 mm Hg. Thus, it is unclear if the small decline in systolic pressure observed in the current study would have any protective effect for subjects who were normotensive at baseline.

Third, the effect of estrogen was largely unopposed by progesterone. Data in both animals and humans suggest progesterone may antagonize the cardiovascular effects of estrogen. If true, conventional hormone replacement therapy with both estrogen and progesterone may not augment vascular sympathetic control. In fact, young women taking oral contraceptives demonstrate blunted cardiovascual and vascular sympathetic baroreflex gain. Therefore, the effects of conventional hormone therapy on autonomic function remain unclear.

Finally, our use of a 1-tailed statistical model, although increasing power, increases the probability of a type-I error. However, even a 2-tailed model revealed sympathetic baroreflex gain was greater (P<0.04), and the relation between resting sympathetic activity and diastolic pressure tended to be greater (P<0.09) after estrogen therapy.

Despite these constraints, our data show that although estrogen did not significantly affect resting levels of arterial pressure, regulation of vascular sympathetic outflow was augmented in healthy, postmenopausal women. This seemed to be reflected in resting relations between vascular sympathetic activity and blood pressure. These findings support a possible link between postmenopausal estrogen use and a decrease in cardiovascular disease due to improvement in arterial baroreflex regulation.

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