Arterial stiffness is not improved in long-term use of estrogen

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OBJECTIVE: The purpose of this study was to compare arterial stiffness in long-term users of estrogen who were postmenopausal, age-matched women who did not use estrogen, and women of fertile age.
STUDY DESIGN: In this clinical cross-sectional study, carotid, femoral, and aortic stiffness (estimated as elastic modulus and stiffness β index) were assessed by ultrasonic phase-locked echo-tracking in 17 women who were postmenopausal and who were treated with 17 β-estradiol implants (mean age, 68.8 years; mean duration of treatment, 20 years), 17 age-matched (±1 year) untreated women, and 20 women in the fertile age period.
RESULTS: Compared with women of fertile age, both postmenopausal groups had significantly higher stiffness elastic modulus and stiffness β index (carotid, $P < .001$; femoral, $P < .05$; aorta, $P < .001$). However, for all 3 arteries, both stiffness indices were similar in estrogen users and nonusers and did not differ significantly. These results remained after an adjustment for systolic blood pressure, body mass index, and low-density lipoprotein cholesterol levels.
CONCLUSION: These data indicate that the arterial stiffening that is related to aging/menopause is not substantially affected by long-term estrogen therapy. (Am J Obstet Gynecol 2002;186:189-94.)

Key words: Estrogen therapy, estradiol implants, arterial stiffness, cardiovascular disease, aging

A major feature of vascular aging is a change in the mechanical properties of the arterial wall.[1] Arterial stiffness, a valid indicator of these properties,[1] is closely associated with chronologic age, and deviations from the age-predicted norm may be a predictor of cardiovascular disease.[2] Arterial stiffness has been proposed as a marker of the initiation/progression of atherosclerosis and hypertension[3] and is associated with coronary heart disease[4] and a number of cardiovascular risk factors that include age,[2] male gender,[5] and a history of hypertension and diabetes.[3]

An effect of menopausal status on stiffness has been suggested from the steeper age-related increase in carotid stiffness in women than in men between the ages of 45 and 60 years.[6] Estrogen or estrogen/progestin therapy reduces carotid artery stiffness early after menopause, which indicates that estrogen therapy might modify the age/menopause-associated increase in stiffness in women in the short-term perspective.[7] The risk of cardiovascular diseases in postmenopausal women may be reduced by estrogen and estrogen/progestin therapies through other mechanisms in addition to changes in lipids and lipoproteins. Direct effects of estrogens on the arterial wall structure and function are plausible.

The aim of this study was to compare arterial wall stiffness of the common carotid artery, the common femoral artery, and the aorta in postmenopausal long-term users of estrogen and age-matched nonusers of estrogen. A group of women in the fertile age period served as a premenopausal reference group.

Subjects and methods

A cohort of postmenopausal women who had used estradiol implants for more than 5 years served as a study group. Among them, 17 volunteer residents in the Uppsala community were investigated. The mean age was 68.8 years, and the mean duration of estradiol treatment was 20.0 years (range, 6.8-35.3 years; median, 18.8 years). The administered dose of estradiol was usually 20 mg pellets implanted subdermally every 6 months (Oestradiol implant 20 mg; Organon Laboratories Ltd, Cambridge, UK). All implant users had undergone hysterectomy for bleeding problems or leiomyomata, and most of them had started with implants at the time of hysterectomy. None of them received progestogens. Indications for estrogen treatment were prevention or treatment of climacteric symptoms. In a few women, the dose was individually adjusted on the basis of climacteric symptoms. Thus, one woman was given 40 mg

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during most of the treatment periods, and 3 women (including the one who was given the higher dose) had intervals shorter than 6 months.

Seventeen women without previous or current use of estrogen therapy served as control subjects. They were selected among the consecutive women after the index subject in the population registry of the same municipality as the treated women, matched for age (±1 year) and recruited through mailed invitations. The mean age was 68.8 years. Twenty women in the fertile age without any hormonal therapy (mean age, 39.6 years) were recruited from the hospital staff and served as a premenopausal reference group (Table I). Each subject gave informed consent, and the study was approved by the local ethics committee, Uppsala University, Sweden.

Arterial stiffness assessments. Arterial stiffness was assessed noninvasively by the measurement of the pulsatile diameter changes with ultrasonography and relating the changes to the external blood pressure of the right upper arm. A B-mode real-time ultrasound scanner fitted with a 5.0-MHz linear array transducer (E2U-PL22; Hitachi, Tokyo, Japan) was used. The real-time scanner was attached to a vessel wall-moving detector equipped with an electronic echo-tracking instrument (Diamove; Teltec AB, Lund, Sweden) that automatically locked to the lumen-intima interfaces at both the near and far artery walls. A loop circuit restored the position of an electronic gate relative to the moving echo, which indicated instantaneously any vessel diameter changes. Repetition frequency of the echo-tracking loops was 870 Hz, and the time resolution was approximately 1.2 msec. The smallest detectable movement was 7.8 μm.[5]

The subjects were examined in the supine position after 15 minutes of rest. A trained technician, blinded to information on estrogen exposure, performed all examinations. The vessel was visualized in a longitudinal section on the real time image of the ultrasound scanner: 2 cm proximal to the bifurcation in the left common carotid artery and in the left common femoral artery and 3 to 4 cm proximal to the bifurcation in the aorta. Information about 3 sequential vessel diameter curves from each artery were stored on a personal computer. Each reading contained 5 to 10 cardiac cycles. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the left brachial artery by the auscultatory method, immediately after estimation of the diameters in each artery, and were used in the calculations of stiffness for the corresponding artery, as previously validated.[8] After all data had been collected, data analysis was performed blindly by 1 of the investigators (A.B.).

Two indices of arterial stiffness were used: the stress-strain elastic modulus (Ep) and the β index. The elastic modulus was calculated as the ratio of stress (arterial pulse pressure; SBP-DBP) to strain (relative diameter [D] change during the cardiac cycle)[9]:

$$Ep = K \times \frac{SBP–DBP}{(D\ systolic – D\ diastolic)/D\ diastolic} \quad (1)$$

SBP and DBP are expressed in millimeters of Mercury, and systolic and diastolic diameters (D) are expressed in millimeters. The elastic modulus (Ep) is expressed in 10^5 Newton/meter^2; K equals 133.3 and is the factor for converting millimeters of Mercury to Newton/meter^2.[8]

The β index, similar conceptually to the elastic modulus, was calculated as the natural logarithm of the systolic and diastolic pressure ratio, divided by strain. It was developed to reduce the impact of pressure on the measurements of stiffness[10]:

$$Stiffness\ \beta = \frac{\ln\ (SBP/DBP)}{(\ D\ systolic – D\ diastolic)/D\ diastolic} \quad (2)$$

The coefficient of variation, based in 10 double measurements of the carotid artery in 25 women, was 5.0% for diameter changes, 6.0% for elastic modulus, and 5.8% for stiffness index β.

Hormonal assessments. Serum samples were drawn between 8 AM and 10 AM and after an overnight fast and were frozen in –70°C until analyzed in batches. Serum levels of estradiol were measured in batches by fluoroimmune assay (AutoDelfia; Wallac OY, Turku, Finland), with an intra-assay variation of 2.7%. Sex-hormone binding globulin was measured by radioimmunassay at Medilab (Malmö, Sweden), with an intra-assay variation of 3.0%. Serum values for follicle-stimulating hormone were measured consecutively as clinical routine in the department of clinical chemistry by Delphia hFSH (Pharmacia-Wallac OY), with a total coefficient of variation of 3.6%.

Serum lipids. Serum total cholesterol and triglycerides were assayed by enzymatic techniques (Instrumentation Laboratory SpA, Milano, Italy) in a centrifugal analyzer (Monarch 2000; Instrumentation Laboratories, Lexington, Mass); the interassay coefficient of variation was <4%. Serum low-density lipoprotein (LDL) cholesterol was calculated with Friedewald's formula after separation of high-density lipoprotein (HDL) cholesterol by precipitation with magnesium chloride/phosphotungstate.

Statistical methods. Pair-wise differences were tested by paired *t* test for normally distributed variables and by Wilcoxon signed-rank test for those variables that were not normally distributed. Normality of the distributions was tested by Shapiro-Wilk *W* Test. The 2-sample *t* test or Wilcoxon rank sum test, as determined by the test for normality, was used when a postmenopausal group was compared with women in the fertile age period. The McNemar's test was used for the comparison of distributions of categoric variables. Spearman-rank correlation tests were used to test the correlation between variables. Pair-wise differences in stiffness were regressed on age to test whether the age-associated increase in stiffness behaved differently in estrogen users and nonusers.

Table I. Descriptive and clinical characteristics by study group

Variable	*Long-term estrogen users (n = 17)*	*Age-matched estrogen nonusers (n = 17)*	P *value**	*Women of fertile age (n = 20)*	P *value†*
Age (y ± SD)	68.8 ± 6.2	68.8 ± 5.9	.85	39.6 ± 4.4	.001
Height (cm ± SD)	165.5 ± 4.5	162.2 ± 5.7	.08	167.6 ± 4.4	.16
Weight (kg ± SD)	78.5 ± 15.5	65.2 ± 9.6	.07	68.6 ± 13.2	.08
BMI(kg/m^2 ± SD)	28.5 ± 4.9	24.8 ± 3.0	.02	24.3 ± 4.2	.009
Smoking (n)			.45 ‡		
Never	8 (47%)	11 (65%)		14 (70%)	
Former	6 (35%)	2 (11.5%)		2 (10%)	
Current	3 (18%)	4 (23.5%)		4 (20%)	
Antihypertensive drug use	3 (18%)	4 (23.5%)	1.00 ‡	0	
Outdoor walking (n)			.074 ‡		
Once a week	3 (17.5%)	0			
Several times a week	3 (17.5%)	1 (6%)			
Daily walking	11 (65%)	16 (94%)			
S-Estradiol (pmol/L ± SD)	§470.0 ± 349	37.2 ± 10	.00001		
S-Sex-hormone binding globulin (nmol/L ± SD)	81 ± 41	75.6 ± 38	.68		
S-Follicle-stimulating hormone (μg/L ± SD)‖	2.0 ± 1.6	14.3 ± 4.9	.00001	1.0 ± 0.5	.07
Total cholesterol (mmol/L ± SD)	6.1 ± 1.0	6.6 ± 0.9	.1	5.2 ± 0.8	.003
LDL-cholesterol (mmol/L ± SD)	3.8 ± 0.9	4.3 ± 0.8	.07	3.2 ± 0.7	.003
HDL-cholesterol (mmol/L ± SD)	1.6 ± 0.5	1.7 ± 0.5	.7	1.6 ± 0.3	.9
Triglycerides (mmol/L ± SD)	1.6 ± 0.9	1.5 ± 0.9	.7	0.9 ± 0.4	.004

*Test of pair-wise differences between postmenopausal women who used estrogen and age-matched postmenopausal women who did not use estrogen.
†Group comparisons between postmenopausal women who used estrogen and women in fertile age.
‡McNemar's test.
§Range, 150-1500 pmol/L.
‖Postmenopausal values, >5 μg/L.

From the tests of correlation, 3 covariates were selected and used in a multiple linear regression model for matched samples to test the difference between groups after adjustment. The Bonferroni correction was used in all correlation analyses to ensure an overall type I error rate of 0.05. After the Bonferroni correction, only results with a probability value of <.005 were regarded as significant, and .005 < a probability value of <.01 was regarded as marginally significant. The coefficient of variation, based on double measurements, was estimated according to the formulas: CV = 100 (SD/x) and $SD = \sqrt{\Sigma d^2/2n}$, where SD is the standard deviation, x is the mean of all measurements, d is the difference between duplicate measurement values, and n is the number of duplicate determinations. To avoid false interpretations because of low power, post-hoc power analysis was used to calculate the detectable mean difference for stiffness indices, elastic modulus and β, with an 80% power at the 0.05 level. All statistical analyses were performed with the statistical program packages JMP or SAS (SAS Institute Inc, Cary, NC).

Results

Compared with nonusers, long-term estrogen users had significantly higher values of body mass index (BMI) and nonsignificantly lower lipid levels. Estrogen users had serum estradiol and follicle-stimulating hormone levels within the normal range for women in fertile age (Table I).

Compared with women in fertile age, both postmenopausal groups had significantly higher stiffness indices of elastic modulus and β (carotid, $P < .001$; femoral, $P < .05$; aorta, $P < .001$; Fig, Table II) and significantly higher SBP and DBP levels ($P < .05$). Carotid, femoral, and aortic stiffness indices were similar in long-term estrogen users and nonusers and did not differ significantly (Table II). Further, after adjustment for SBP, BMI, and serum LDL-cholesterol and after exclusion of subjects undergoing antihypertensive therapy, the absence of significant differences for stiffness remained (data not shown). The tests of the slopes for the pair-wise differences in stiffness regressed on age were not significant for any vessel (carotid, $P = .54$; femoral, $P = .3$; aorta, $P = .88$).

Compared with women in fertile age, carotid diameters were significantly larger in both postmenopausal groups, whereas aortic diameter was larger only in estrogen users. Carotid and aortic fractional diameter changes (arterial strain) were significantly smaller in both postmenopausal groups. The 3 study groups did not differ significantly in femoral diameters (absolute values or fractional changes; Table II).

Increasing age presented no association with arterial stiffness in long-term users of estrogen but had positive, although weak, associations with carotid stiffness in postmenopausal estrogen nonusers (carotid β, $r = .56$; $P = .02$) and with aortic stiffness in premenopausal women (aortic

Table II. Arterial diameters, diameter changes, stiffness indices, and blood pressure by study group

Variable	*Long-term estrogen users (n = 17)*	*Age-matched estrogen nonusers (n = 17)*	P *value**	*Women of fertile age (n = 20)*
Left common carotid artery				
Systolic diameter (mm)	7.38 ± 0.65†	7.37 ± 0.80‡	.99	6.64 ± 0.95
Diastolic diameter (mm)	6.99 ± 0.67†	7.00 ± 0.83‡	.98	6.16 ± 0.88
Mean diameter (mm)	7.19 ± 0.66†	7.19 ± 0.81‡	.99	6.39 ± 0.91
Arterial strain	0.06 ± 0.02†	0.06 ± 0.02§	.87	0.08 ± 0.02
Elastic modulus (10^5 Newton/m^2)	1.51 ± 0.60‖	1.50 ± 0.63§	.95	0.73 ± 0.17
Stiffness β index	10.58 ± 3.66‖	10.95 ± 4.12§	.80	6.33 ± 1.32
Left common femoral artery				
Systolic diameter (mm)	8.12 ± 1.45	8.88 ± 1.21	.10	8.54 ± 0.80
Diastolic diameter (mm)	7.76 ± 1.47	8.48 ± 1.16	.10	8.11 ± 0.86
Mean diameter (mm)	7.94 ± 1.46	8.68 ± 1.18	.10	8.33 ± 0.83
Arterial strain	0.05 ± 0.02	0.05 ± 0.02	.91	0.06 ± 0.02
Elastic modulus (10^5 Newton/m^2)	2.01 ± 0.94‖	1.90 ± 0.98‡	.75	1.11 ± 0.46
Stiffness β index	14.53 ± 7.30†	13.93 ± 5.92‡	.80	9.68 ± 3.64
Aorta				
Systolic diameter (mm)	17.69 ± 3.32†	16.21 ± 2.24	.30	15.92 ± 1.37
Diastolic diameter (mm)	16.85 ± 3.08†	15.30 ± 2.33	.26	14.66 ± 1.49
Mean diameter (mm)	17.26 ± 3.19†	15.75 ± 2.28	.29	15.29 ± 1.42
Arterial strain	0.05 ± 0.02†	0.06 ± 0.03‡	.25	0.09 ± 0.03
Elastic modulus (10^5 Newton/m^2)	1.98 ± 1.21‖	1.46 ± 0.72§	.17	0.67 ± 0.25
Stiffness β index	13.99 ± 7.78‖	11.18 ± 5.31§	.26	5.79 ± 1.95
Heart rate (beats/min)	73.00 ± 9.78†	70.47 ± 9.90	.58	64.11 ± 6.55
Systolic pressure (mmHg)	138.00 ± 21.45‖	128.44 ± 13.63§	.07	107.25 ± 10.32
Diastolic pressure (mmHg)	77.33 ± 10.50‖	74.69 ± 9.91‡	.43	67.75 ± 7.86
Pulse pressure (mmHg)	60.67 ± 17.82‖	53.75 ± 10.72§	.08	39.50 ± 7.24

Data are presented as mean ± SD.
Arterial strain (fractional diameter change) = (D systolic-D diastolic)/D diastolic.
*Test of pair-wise differences between estrogen users and nonusers.
†$P < .05$, between postmenopausal estrogen users and women in fertile age.
‡$P < .05$, between postmenopausal estrogen nonusers and women in fertile age.
§$P < .001$, between postmenopausal estrogen nonusers and women in fertile age.
‖$P < .001$, between postmenopausal estrogen users and women in fertile age.

elastic modulus and β, $r = .45$; $P = .04$), which did not remain after Bonferroni correction. In the users of estrogen, no significant association was found when stiffness was regressed on estradiol levels or duration of estrogen treatment.

After Bonferroni correction, significant positive associations remained between femoral stiffness β and total cholesterol ($r = .70$; $P = .002$) and BMI ($r = .65$; $P = .004$) in estrogen users. In postmenopausal estrogen nonusers, significant positive associations remained between SBP and carotid elastic modulus ($r = .68$; $P = .003$) and both femoral stiffness elastic modulus and β ($r = .72$, $P = .001$, and $r = .65$, $P = .004$, respectively), whereas serum lipids had inverse associations with femoral stiffness β (total cholesterol, $r = -.63$, $P = .008$; LDL-cholesterol, $r = -.65$, $P = .005$). In the women of fertile age, SBP had positive associations with aortic elastic modulus ($r = .69$; $P = .0007$) and carotid elastic modulus ($r = .56$; $P = .011$).

Post-hoc power analysis revealed an 80% power at the 0.05 level to detect a mean difference of 0.3, 0.4, and 0.5 stiffness elastic modulus units and 1.8, 3.1, and 3.3 stiffness β units at the carotid, femoral artery, and aorta, respectively.

Comment

We found no significant differences between long-term estrogen users and age-matched nonusers in arterial stiffness, before and after adjustment for potential confounders or after exclusion of subjects undergoing antihypertensive drug therapy. Further, the tests of slopes for pair-wise differences in stiffness regressed on age were not significant, supporting the theory that long-term estrogen therapy has no substantial effect on the age/menopause-related changes in arterial stiffness of the central elastic and muscular arteries.

Our findings contrast with results of a recent report that indicated decreased carotid stiffness β index in postmenopausal women who underwent estrogen/progestin therapy for at least 6 months.[7] However, our results might be in accordance with investigations on regional stiffness estimated by noninvasive methods. Tanaka et al[11] found no differences in aortic and carotid stiffness that were estimated by pulse wave velocity and applanation tonometry, respectively, when comparing postmenopausal hormone replacement therapy users of approximately 60 years of age and untreated control subjects; McGrath et al[12] reported no significant difference

in carotid stiffness estimated as distensibility coefficient between women receiving hormone replacement therapy (mean age, 60 years) and untreated control subjects. Liang et al[13] found reduced carotid stiffness in estrogen users (mean age, 60 years), compared with age-matched nonusers, with the distensibility coefficient method but not with Young's modulus. The pulse wave velocity, however, estimates wall mechanics indirectly and not the local elastic properties, whereas elastic modulus and β are the indices conceptually closest to local arterial stiffness.[3] In the present study, subjects were older and had been treated with estrogen replacement therapy for a long time, compared with previous reports. The present and previous reports might indicate that any effects estrogen may have on stiffness are substantially reduced by time and aging.

In the present and previous reports,[10,14] associations between stiffness and age were less steep for the femoral artery than for the carotid artery and aorta. Differences for the femoral artery, between postmenopausal groups and women of fertile age, were smaller than for carotid artery and aorta, although still significant. Differences in structural and functional characteristics of the different vascular beds might explain why stiffness increases more in the elastic carotid arteries and aorta and less in the muscular femoral artery by age.[1,2]

Stiffness is determined principally by the elastin-to-collagen ratio in the vessel wall.[1] Both components are responsible for the nonlinear shape of the pressure/diameter curve of the vessel[1] and both change with aging. Estrogens have shown to increase the proportion of the elastic fibers over collagen, thus decreasing the stiffness of the vessel wall in rat aorta.[15] Arterial stiffness may also be influenced by more distal vascular bed characteristics or may be partly under the functional control of the endothelium,[12] as suggested by the significant relationship shown between arterial compliance and total peripheral resistance to indices of endothelial function.[16] Gangar et al[17] found a positive association between pulsatility index of the internal carotid artery and time since menopause. Reduced pulsatility index[17] and improved endothelial-dependent vasodilation[18] have also been reported after short-term estrogen therapy. Randomized controlled studies have shown a reduction of carotid pulsatility index after 6 months and 1 year of hormone replacement therapy[19, 20] or no significant changes after 6 months of hormone replacement therapy.[21] Further, no changes in arterial impedance to blood flow or endothelial vasomotor function after 1 and 3 years of hormone replacement therapy have been reported,[22,23] which suggests a transient effect of hormone replacement therapy on arterial vasodilation.

The present study is a cross-sectional comparison. Therefore, selection bias may have affected our results. However, because age is the most important determinant of arterial stiffness,[2] any bias at the time of menopause would probably have limited influence on stiffness 20 years later, compared with effects of aging and the extreme long-term estrogen therapy with a mean duration of 20 years. Further, adjustment for systolic blood pressure, BMI, and LDL-cholesterol levels, known determinants of arterial stiffness,[1,3,5] did not change the results. However, it may be inappropriate to adjust for differences in BMI and LDL-cholesterol levels, because both are most likely effects of the estrogen therapy, BMI by preserved bone mass and probably also muscle mass.[24] The cross-sectional design does not exclude a phase earlier in treatment when the treated group did exhibit lower stiffness than the untreated group, that was later outweighed by the effects of aging. However, analysis of pair-wise differences regressed on age do not support such a mechanism.

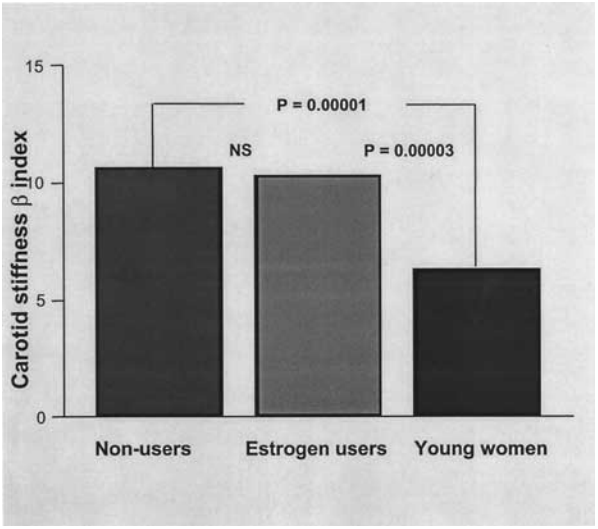

Figure. Carotid stiffness β index in the 3 study groups.

The small sample size in our study, in combination with no significant differences between estrogen users and nonusers, may raise the possibility of a type-II error. However, the mean pair-wise differences were numerically very small, and the probability values were far from statistical significance. Further, according to post hoc power calculation, mean differences of approximately one third of the SD of stiffness indices could be detected, differences that are considerably smaller than those assumed to be of clinical importance. Hirai et al[4] reported a difference of about 4 units for carotid β index and 13 units for aorta β index between patients with myocardial infarction and healthy subjects of about 60 years of age; Gatzka et al[25] reported a difference of 7 units in aorta β index between patients with newly diagnosed coronary artery disease (mean age, 63 years) and healthy age-matched subjects. Those differences were statistically significant and of apparent clinical relevance.

With regard to the validity, our estimates of stiffness indices and the SDs were numerically similar to those estimates previously reported in the literature for women of similar ages.[5,6] Moreover, we found highly significant differences (4-6 units) for stiffness β indices between premenopausal women and either of the postmenopausal groups (Table II). The coefficients of variation of about 5% in our study were also low for this type of estimates.

Our cross-sectional data might indicate that long-term estrogen therapy has no substantial effect on the age-related changes in arterial stiffness of the central elastic and muscular arteries. Accordingly, any long-term protective effect that estrogen replacement therapy has on cardiovascular disease in older women is unlikely to be mediated by an impact on arterial stiffness.

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