Acute effects of 17β-estradiol on ventricular and vascular hemodynamics in postmenopausal women

CHRISTOPHER S. HAYWARD,1,2 WALLY V. KALNINS,1 AND RAYMOND P. KELLY1
1Department of Cardiology, St. Vincent’s Hospital, Sydney 2010, Australia; and 2Department of Cardiac Medicine, National Heart and Lung Institute, Imperial College, London SW3 6LY, United Kingdom

Received 3 January 2000; accepted in final form 28 April 2000

Hayward, Christopher S., Wally V. Kalnins, and Raymond P. Kelly. Acute effects of 17β-estradiol on ventricular and vascular hemodynamics in postmenopausal women. Am J Physiol Heart Circ Physiol 279: H2277–H2284, 2000.—Because premenopausal women have lower cardiovascular morbidity than postmenopausal women, it has been proposed that estrogen may have a protective role. Estrogen is involved in smooth muscle relaxation both through its specific receptor as well as through calcium channel block-ade. This study examined the acute effect of estradiol on invasive cardiovascular hemodynamics in 18 postmeno- pausal women (age 62.6 ± 7.6 years, means ± SD). The effect of estradiol on left ventricular chamber performance was studied in 9 women using simultaneous left ventricular pressure-volume recordings. In a further group of 9 women, the acute effect of estradiol on arterial function was assessed using input impedance (derived from simultaneous aortic pressure and flow recordings), pressure waveform analysis, and pulse wave velocity. After 2 mg micronized 17β-estradiol was administered, serum estradiol levels increased from 50.9 ± 21.9 to 3,190 ± 2,216 pmol/l, P < 0.0001. There was no effect of estradiol on either left ventricular inotropic or lusitropic function. There was no acute effect of estradiol on arterial impedance, reflection coefficient, augmentation index, or pulse wave velocity. There was a trend to decreased heart rate and cardiac output in both groups of 9 women. Because heart rate and cardiac output were common to both hemodynamic data sets, results for these parameters were pooled. Across all 18 women, there was a small but significant decrease in heart rate (69.2 ± 10.4 vs. 67.2 ± 9.9 beats/min, P = 0.02), as well as a significant decrease in cardiac output (4.82 ± 1.77 vs. 4.17 ± 1.56 l/min, P = 0.002). Despite achieving supraphysiological serum levels, this study found no significant effect of acute 17β-estradiol on ventricular or large artery function.

Vascular smooth muscle cells have also been shown to possess the classical estrogen receptor (15) and to respond acutely through a nongenomic, receptor-specific mechanism (3). Vascular relaxation due to calcium channel antagonism has also been widely suggested as an important contributor to the effect of estrogen on the cardiovascular system (5). Previous studies have shown that acute sublingual estradiol increases time to ischemia and exercise capacity in postmenopausal women with coronary disease undergoing treadmill exercise tests (32). Whereas the mechanisms of this benefit may involve coronary vasomotor actions, alternate hemodynamic effects have not previously been studied. From the widespread distribution of calcium channels and estrogen receptors, this study was designed to determine whether acute estradiol administration had direct hemodynamic effects on ventricular or vascular function, or their interaction.

METHODS

Patients

Eighteen postmenopausal women (defined by history of amenorrhea and subsequent serum estradiol <105 pmol/l) were recruited from those undergoing elective cardiac catheterization. The Research Ethics Committee of St. Vincent’s Hospital approved the study, and all patients gave written informed consent. The acute effects of estradiol on left ventricular (LV) chamber function were examined in nine women, and its effects on aortic impedance and pulse wave velocity were examined in a further group of nine women. The mean age of the cohort was 62.6 ± 7.6 (±SD) years, and mean body mass index was 26.6 ± 5.0 kg/m². Fourteen subjects had no significant coronary artery disease, two had double vessel disease, and two had triple vessel disease. All subjects had normal global LV function (ejection fraction >50%), although one subject had minor inferior hypokinesis on ventriculography. There was no difference in effect between those with no coronary disease and the remaining few, so results are presented for the entire cohort. Two subjects were diabetic and ten had a history of treated hypertension. Patients were studied on their usual medication. Current medications included β-blockers in four subjects, calcium channel antagonists in five, angiotensin-converting enzyme inhibitors in seven, and digitalis in eight.

Address for reprint requests and other correspondence: C. Hayward, Department of Cardiology, St. Vincent’s Hospital, Victoria St., Darlinghurst, Sydney NSW 2010, Australia.
inhibitors in five, isosorbide mononitrate in three subjects, and diuretics in two subjects. Of the entire cohort, nine were taking long-acting vasodilators (angiotensin-converting enzyme inhibitors, calcium antagonists, or isosorbide mononitrate) administered on the morning of the study. Hemodynamic study was performed ~3 h postdose.

**Estradiol Administration Protocol**

All subjects had baseline hemodynamic recordings and serum estradiol assays taken before sublingual estradiol was given. Estradiol assays were taken from arterial blood from the femoral sheath. Subjects were then given crushed micronized 1 mg 17β-estradiol (Trisequens, Novo Nordisk, Denmark) sublingually with a sip of water. LV pressure volume and aortic pressure-flow recordings were taken for 10 min and then a further 1-mg micronized estradiol was given by the same route. Recordings were continued for a further 10 min, at which time the second estradiol assay was taken. This protocol was used prospectively to allow delineation of any acute (10 min) estradiol effect at the lower dose. Serum for estradiol levels was taken at the commencement of the recordings and after completion of the 20-min protocol.

**Data Acquisition**

*Pressure-volume loops* LV pressure-volume (PV) loops were obtained by simultaneous measurement of LV pressure (micromanometer) and volume (conductance method) as has been previously described (9). Briefly, an 8-Fr. conductance volume catheter (9 mm electrode spacing, Webster 7212–08, Webster Lab) was inserted into the LV cavity via a femoral arterial sheath under fluoroscopic control. The micromanometer was then inserted to the tip of the volume catheter and connected for data acquisition. Volume signals were processed via a Leycom signal conditioner (Sigma 5, Cardiodynamics, Rijnsburg, Netherlands) to a data acquisition board in a portable computer (Intel 486 processor, DX50). Data were displayed on-line at a sampling rate of 250 Hz with high-frequency, noise-filtering cut-off at 50 Hz, 20 dB/decade. End-systolic and end-diastolic volumes (ESV, EDV) were calculated off-line using single-plane contrast ventriculography, calibrated against a radioopaque sphere of known diameter. A 35-mm vena caval balloon catheter (Cordis 530000A-15565, Cordis, Miami, FL) positioned within the right atrium was used for acute preload reduction by intermittent inferior vena caval occlusion (IVCO).

*Arterial parameters.* Aortic impedance studies were performed using a Millar combined micromanometer pressure and electromagnetic velocity flowmeter (Millar Instruments, Houston, TX) connected to standard transducer box (Millar SPC-501) and Biotronix flowmeter (BLI 613). Simultaneous three-lead electrocardiogram, LV pressure, aortic pressure, aortic flow, femoral pressure and Finapres digital pressure were acquired directly to an analog-digital converter via customized software. Volume flow was calculated by multiplying integrated flow velocity by aortic root area calibrated using a radioopaque sphere. Aortic diameter measurements were taken immediately above the level of the aortic sinuses at end diastole. The mean aortic diameter for the seven subjects with adequate flow recordings was 3.39 ± 0.54 cm. Calibration for the electromagnetic flowmeter was performed using saline ejected from a pulsatile pump with fixed output and tubing of known diameter.

Femoral pressure was measured using a Millar micromanometer connected to the sidearm of an 8-F femoral sheath through which the ventricular catheter was passed. The short (20 cm) sheath sidearm was filled and flushed periodi-cally with saline to ensure the sheath remained free from thrombus. The femoral pressure was used, in combination with the aortic pressure waveform, for derivation of the aorto-femoral transfer function and for calculation of aortic pulse wave velocity. A Finapres cuff (Ohmeda) was placed around the second inter-phalangeal joint of the third finger of the left hand before cardiac catheterization to allow peripheral waveform recording.

**Data Analysis**

**Steady-state data.** Volume results were not available for two subjects due to LV catheter movement or artifact. All nine subjects had available pressure data. Steady-state data for each patient were averaged over 10–20 continuous beats after smoothing. Data in which there was a >10% change in heart rate were excluded. Preectopic, ectopic, and postectopic beats were excluded from analysis. Steady-state parameters were automatically determined from the averaged PV loop by customized software (PVAN, Pressure-Volume Analysis, Johns Hopkins University, Baltimore, MD). Steady-state variables included heart rate, cardiac output, LV systolic pressure, end-diastolic pressure (pre a wave), peak positive pressure development over time (dP/dt₉₉₅), negative dP/dt (dP/dt₉₉₅), time constant of relaxation (T, tau), duration of contraction, and end-systolic pressures and volumes. For the purposes of steady-state diastolic parameters, the point of end diastole was determined separately using pressure derivatives to calculate the pressure and volume immediately before the atrial filling wave. End systole was defined by the maximum of time-varying ventricular elastance (Eᵥ₉₉₅) as described by the equation 

\[ Eᵥ = \frac{P}{Vᵥ_0 - Vᵥ} \]

where \( Vᵥ_0 \) is the volume axis intercept as defined below. Tau was determined using a logarithmic least-squares regression commencing at peak dP/dt₉₉₅ and extending for a further 80 ms. Duration of electromechanical systole was defined from the midpoint of the electrocardiographic QR wave to dP/dt₉₉₅. This measure was used as a measure of duration of contraction (25).

**Chamber function.** The load-independent indexes end-systolic PV relations (ESPVR), preload-recruitable stroke work relations (PRSWR), and end-diastolic PV relations (EDPVR) were obtained during transient IVCO. Because linear relations were assumed for all indexes, individual IVCO runs with a linear correlation of <.80 were not included in analysis. For ESPVR, an iterative procedure was performed from the linear regression of the data using an initial estimate of \( Vᵥ_0 \) of 0 ml. Subsequent iterations were continued until errors were minimized as previously described (18). Because of poor correlations in individual runs or ventricular ectopy in response to IVCO, some studies were found to be unsuitable for load-independent analyses.

End-diastolic relationships were determined from two EDP/EDV points on each PV loop. The points chosen were those immediately before the a wave and a second point 10% of the filling volume earlier in the same loop as has been described previously (21). By examining diastole before atrial contraction, this provides an assessment of the passive diastolic properties of the left ventricle. A linear model was used to describe the EDPVR because this is simpler and has been shown to be as valid as more complex exponential regression models (16). Although the slope of the regression describes an elastance, the results are usually reported as the inverse, namely chamber compliance \( Cᵥₒₐ₃ₐ₃ \) (17). The equation for diastolic compliance is therefore 

\[ V = Cᵥₒₐ₃ₐ₃ P + Vᵥₒₐ₃ₐ₃ \]

where \( Vᵥₒₐ₃ₐ₃ \) is the volume axis intercept.
Arterial parameters. Because of problems with flow catheter positioning and flow waveform, impedance results were only available for seven of the nine subjects. Problems with the Finapres device and with the femoral pressure trace in two separate patients meant that pulse wave velocity and aorto-femoral transfer function results were available for seven of the nine patients. Data were averaged from steady-state recordings. Pressure, flow, and Finapres waveforms were ensemble averaged using electrocardiogram triggering to obtain an averaged waveform before entry into a discrete Fourier transform calculation to determine impedance and transfer functions. Individual impedance and transfer function results were derived from the ratio of the Fourier components of the aortic pressure to corresponding aortic flow and femoral pressure, respectively. Sampling rate was 250 Hz for all channels. The last one-third of the flow waveform was assumed to be zero and was used to autocalibrate for offset drift. Results for frequency-dependent parameters were reported up to the tenth harmonic, beyond which the signal-to-noise ratio is too low for meaningful analysis. Characteristic impedance ($Z_c$) was defined as the mean of impedance modulus for harmonics 4–10.

Time domain parameters such as augmentation index (19), reflection wave ratio (37), and ejection duration were derived using pressure derivatives. Whereas augmentation index is defined by the ratio of the difference between the late and early systolic shoulders to the aortic pulse pressure, the reflection wave ratio uses the ratio of the height of the late to early pressure peaks from the pressure waveform. The indexes are similar in the information derived from them. Pulse onset for pulse wave velocity calculation was determined using the peak of the second derivative. Distances were determined from the sternal notch to femoral transducer tip and from the sternal notch to Finapres cuff by surface anatomy.

Systemic vascular resistance was calculated using the ratio of the mean aortic pressure to cardiac output multiplied by 80 (to convert to units of dyn·s·cm⁻²). This assumes a right atrial pressure of zero, which may lead to a minor overestimation of systemic vascular resistance. Reflection coefficient (Γ) was calculated according to $\Gamma = (Z_e - Z_t)/(Z_e + Z_t)$, where $Z_e$ is the terminal impedance, and $Z_t$ the characteristic impedance. Absolute Finapres pressures were not found to be reliable and were not further analyzed. The Finapres waveform was used solely for timing purposes for calculation of brachial pulse wave velocity.

Statistics

Results are shown as means ± SD. The effect of estradiol on steady-state parameters was analyzed using paired $t$-tests. To determine the effect of estrogen on chamber function (ESPVR, EDPVR, PRSWR), a multiple linear regression model was used coding for the effect of estrogen on both the slope and intercept of the appropriate relationship after accounting for the baseline differences between individuals using dummy coding (34).

RESULTS

Estradiol Levels

Serum estradiol levels increased in all subjects. The smallest change in serum estradiol level in any subject was a greater than fivefold increase in concentration, from 92 to 548 pmol/l. In the entire cohort estradiol levels increased from 50.9 ± 21.9 to 3,190 ± 2,216 pmol/l, $P < 0.0001$.

Estradiol and LV Chamber Function

Steady-state recordings. Baseline steady-state parameters and results for the last 5 min of the study are shown in Table 1. Interim analysis revealed no effect of estrogen at 10 min. It is of note that, despite marked elevation of the serum estradiol level, no change in blood pressure or ejection fraction were found. There was a trend to decreased heart rate and cardiac output. There was no evidence for any lusitropic effect, as shown by either tau or $dP/dt_{\text{min}}$. There was no change in arterial elastance, suggesting no marked change in arterial or arteriolar tone. Indeed, arterial elastance tended to increase in this study ($P = 0.19$).

LV chamber function. Systolic function was assessed using ESPVR and PRSWR (Table 2). There was no inotropic effect of estradiol ($P = 0.59$ and 0.51, respectively). An example of PV loops before and at the end of the study period is shown in Fig. 1. As can be seen in this patient, there was no significant change in LV contractility due to estradiol. Passive diastolic ventric-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$n$</th>
<th>Baseline</th>
<th>SD</th>
<th>Estradiol</th>
<th>SD</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>9</td>
<td>67.6</td>
<td>12.2</td>
<td>66</td>
<td>11.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Peak systolic pressure, mmHg</td>
<td>9</td>
<td>138.8</td>
<td>17.5</td>
<td>138.4</td>
<td>13.2</td>
<td>0.87</td>
</tr>
<tr>
<td>End-systolic pressure, mmHg</td>
<td>9</td>
<td>126.5</td>
<td>14.5</td>
<td>129.7</td>
<td>11.4</td>
<td>0.28</td>
</tr>
<tr>
<td>End-diastolic pressure, mmHg</td>
<td>9</td>
<td>10.2</td>
<td>4.1</td>
<td>10.7</td>
<td>5.0</td>
<td>0.74</td>
</tr>
<tr>
<td>End-systolic volume, ml</td>
<td>7</td>
<td>39.4</td>
<td>18.5</td>
<td>41.9</td>
<td>22.2</td>
<td>0.35</td>
</tr>
<tr>
<td>End-diastolic volume, ml</td>
<td>7</td>
<td>97.4</td>
<td>27.6</td>
<td>94.2</td>
<td>32.3</td>
<td>0.52</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>7</td>
<td>60.5</td>
<td>15.3</td>
<td>55.1</td>
<td>18.7</td>
<td>0.11</td>
</tr>
<tr>
<td>Right atrial pressure, mmHg</td>
<td>8</td>
<td>3.7</td>
<td>3.0</td>
<td>2.9</td>
<td>2.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>7</td>
<td>4.094</td>
<td>0.98</td>
<td>3.62</td>
<td>1.13</td>
<td>0.06</td>
</tr>
<tr>
<td>LVEF</td>
<td>7</td>
<td>63.9</td>
<td>12.6</td>
<td>64.0</td>
<td>17.1</td>
<td>0.17</td>
</tr>
<tr>
<td>Stroke work, mmHg/ml</td>
<td>7</td>
<td>7,161</td>
<td>1,717</td>
<td>6,522</td>
<td>2,192</td>
<td>0.16</td>
</tr>
<tr>
<td>$dP/dt_{\text{max}}$, mmHg/s</td>
<td>9</td>
<td>1,535</td>
<td>333</td>
<td>1,446</td>
<td>347</td>
<td>0.12</td>
</tr>
<tr>
<td>$dP/dt_{\text{min}}$, mmHg/s</td>
<td>9</td>
<td>-1,581</td>
<td>339</td>
<td>-1,578</td>
<td>414</td>
<td>0.97</td>
</tr>
<tr>
<td>Tau (ventricular), ms</td>
<td>9</td>
<td>46.6</td>
<td>7.7</td>
<td>49.4</td>
<td>11.4</td>
<td>0.27</td>
</tr>
<tr>
<td>Arterial elastance, mmHg/ml</td>
<td>7</td>
<td>2.30</td>
<td>0.81</td>
<td>2.69</td>
<td>1.23</td>
<td>0.19</td>
</tr>
</tbody>
</table>

$P$, paired $t$-tests. Because of volume catheter movement during study period, complete results were only available for 7/9 subjects ($n$). Pressure results were available for all subjects. LVEF, left ventricular (LV) ejection factor; $dP/dt_{\text{max}}$ and $dP/dt_{\text{min}}$, maximal and minimal rate of LV pressure development, respectively.
ular function was assessed by examining EDPVR. Again, there was no effect of estradiol administration on chamber distensibility ($P = 0.68$).

Estradiol and Arterial Hemodynamics

**Baseline parameters.** There was a statistically significant, but small, decrease in heart rate (69.8 ± 9.4 vs. 66.0 ± 6.9 beats/min, $P < 0.05$) from baseline to 10 min; however, overall heart rate had not changed significantly from baseline at the end of the study period ($P = 0.12$). Whereas there was no change in any pressure-related parameter, there was a significant decrease in cardiac output and peak aortic flow velocity with time (Table 3). Calculated systemic vascular resistance increased significantly over the first 10 min of the study period, with a trend to increased vascular resistance remaining at the end of the study period ($P = 0.06$). Tau (arterial) derived from an exponential decay model of the aortic pressure waveform (windkessel model) tended to suggest a decrease in aortic compliance ($P = 0.07$).

**Aortic input impedance.** Analysis of impedance spectra showed no significant changes at any harmonic (Fig. 2). $Z_t$ (impedance at zero frequency = systemic vascular resistance) increased significantly in the interim time period but was not significantly different by the end of the study. $Z_t$ did not change significantly in response to estradiol (Table 3). Because the small changes in $Z_t$ were in a similar direction to those in $Z_r$, there was no significant change in the calculated $\Gamma$.

**Pulse waveform analysis and pulse wave velocity.** The aortic augmentation index and reflection wave ratios were used as further markers of peripheral arterial wave reflection. There was no significant change in systemic augmentation, ejection duration, or pulse wave velocity at any time during the study (Table 3).

**Aorto-femoral transfer function.** To further delineate any change in arterial function, as may be expected by smooth muscle relaxation, the aorto-femoral transfer function was derived for each time period. There was a characteristic amplification of lower frequencies with diminution of higher harmonics (Fig. 2). This is similar to that seen in brachial and radial transfer functions (14). The phase decreases in an approximately linear manner in accordance with the delay between the two pulses due to transit delay. There was no significant change in either amplification or phase delay over the first 10 min. At 20 min, there was a small but significant decrease in mean femoral arterial pressure, as shown by a decrease in amplification at zero frequency.

---

**Table 2. Effect of estradiol on left ventricular chamber function**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>SEE</th>
<th>$r^2$</th>
<th>Estradiol</th>
<th>SEE</th>
<th>$r^2$</th>
<th>$P$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESPVR ($n = 6$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{es}$, mmHg/ml</td>
<td>2.69</td>
<td>0.11</td>
<td>0.94</td>
<td>2.85</td>
<td>0.24</td>
<td>0.94</td>
<td>0.59</td>
<td>0.94</td>
</tr>
<tr>
<td>$P_o$, mmHg</td>
<td>38.5</td>
<td>4.5</td>
<td>27.0</td>
<td>8.4</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{100}$, ml</td>
<td>-14.4</td>
<td>-9.5</td>
<td>22.9</td>
<td>25.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDPVR ($n = 5$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{ed}$, mmHg/ml</td>
<td>0.15</td>
<td>0.02</td>
<td>0.93</td>
<td>0.14</td>
<td>0.01</td>
<td>0.94</td>
<td>0.68</td>
<td>0.93</td>
</tr>
<tr>
<td>$C_{Dia}$, ml/mmHg</td>
<td>6.60</td>
<td>0.65</td>
<td>7.09</td>
<td>0.70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_o$, mmHg</td>
<td>-0.6</td>
<td>1.2</td>
<td>1.9</td>
<td>0.6</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRSWR ($n = 7$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$M_{sw}$, mmHg</td>
<td>95.1</td>
<td>3.1</td>
<td>97.5</td>
<td>6.6</td>
<td>0.95</td>
<td>0.51</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>SW$_{es}$, mmHg/ml</td>
<td>-1,905</td>
<td>294</td>
<td>-2,226</td>
<td>389</td>
<td>0.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{o_{sw}}$, ml</td>
<td>20.0</td>
<td></td>
<td>22.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{7500sw}$, ml</td>
<td>98.9</td>
<td></td>
<td>99.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$n =$ number of subjects. ESPVR, end-systolic pressure-volume relation; $E_{es}$, end-systolic elastance; $P_o$, pressure at zero volume; $V_{100}$, volume at zero pressure; $V_{100}$, volume at 100 mmHg; EDPVR, end-diastolic pressure-volume relation; $E_{ed}$, end-diastolic elastance; $C_{Dia}$, diastolic compliance; PRSWR, preload recruitable stroke work (SW) relation; $M_{sw}$, slope of PRSWR; SW$_{es}$, SW at zero volume; $V_{7500sw}$, volume at 7,500 mmHg·ml; $V_{o_{sw}}$, volume at zero SW. SEE, standard error of the estimate; $r^2$, correlation coefficient.
at 20 min (Fig. 3). This may have been due to intralu-
minal thrombus deposition with time, despite regular
flushing, due to the close fit of the pressure-flow cath-
eter within the arterial sheath. There was no change in
mean aortic pressure (Table 3). There were no changes
in phase delays due to estradiol administration.

**Pooled data.** Because heart rate and cardiac output
were common to the two studies, analysis could be
performed across all 18 subjects, increasing statistical
power. Across all 18 subjects, there was a small, but
significant, decrease in heart rate across the study
period (from 69.2 ± 10.4 to 67.2 ± 9.9 beats/min, P =
0.02). Cardiac output was derived by stroke volume in
the ventricular study (n = 7) and by integrated aortic
flow velocity (n = 7) in the impedance study. Because
the results were compared using paired t-tests, this
was not considered a barrier to appropriate interpre-
tation of the results. There was a significant decrease
in cardiac output across the entire group (from 4.82 ±
1.69 to 4.17 ± 1.56 l/min, P = 0.002). From the variability
in increase in serum estradiol levels, the relation between
either change in estradiol levels or absolute estradiol
levels was also examined. There was no relationship
(P = 0.68–0.83) between either the percentage change in es-
tradiol level or absolute estradiol level and changes in
either heart rate or cardiac output.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Baseline</th>
<th>SD</th>
<th>Estradiol</th>
<th>SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline hemodynamics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>9</td>
<td>69.8</td>
<td>6.9</td>
<td>67.2</td>
<td>7.2</td>
<td>0.12</td>
</tr>
<tr>
<td>Aortic systolic pressure, mmHg</td>
<td>9</td>
<td>131.6</td>
<td>20.0</td>
<td>130.8</td>
<td>22.6</td>
<td>0.72</td>
</tr>
<tr>
<td>Aortic diastolic pressure, mmHg</td>
<td>9</td>
<td>66.3</td>
<td>7.1</td>
<td>67.1</td>
<td>7.1</td>
<td>0.59</td>
</tr>
<tr>
<td>Mean aortic pressure, mmHg</td>
<td>9</td>
<td>93.9</td>
<td>10.8</td>
<td>94.3</td>
<td>12.9</td>
<td>0.80</td>
</tr>
<tr>
<td>Peak flow velocity, cm/s</td>
<td>7</td>
<td>73.4</td>
<td>26.5</td>
<td>67.2</td>
<td>26.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>7</td>
<td>5.54</td>
<td>2.00</td>
<td>4.73</td>
<td>1.81</td>
<td>0.02</td>
</tr>
<tr>
<td>Systemic vas resistance, dyn·s·cm⁻⁵</td>
<td>7</td>
<td>1,491</td>
<td>475</td>
<td>1,812</td>
<td>732</td>
<td>0.06</td>
</tr>
<tr>
<td>Tau (arterial), s</td>
<td>9</td>
<td>1.40</td>
<td>0.30</td>
<td>1.51</td>
<td>0.39</td>
<td>0.07</td>
</tr>
<tr>
<td>Derived impedance parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zₜ, dyn·s·cm⁻⁵</td>
<td>7</td>
<td>1,491</td>
<td>475</td>
<td>1,812</td>
<td>732</td>
<td>0.06</td>
</tr>
<tr>
<td>Zᵦ, dyn·s·cm⁻⁵</td>
<td>7</td>
<td>151</td>
<td>52</td>
<td>164</td>
<td>76</td>
<td>0.72</td>
</tr>
<tr>
<td>Reflection coefficient</td>
<td>7</td>
<td>0.82</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse waveform indexes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Augmentation index, %</td>
<td>9</td>
<td>43.2</td>
<td>7.4</td>
<td>41.7</td>
<td>10.8</td>
<td>0.36</td>
</tr>
<tr>
<td>Reflection wave ratio, %</td>
<td>9</td>
<td>178.8</td>
<td>24.4</td>
<td>177.8</td>
<td>39.5</td>
<td>0.87</td>
</tr>
<tr>
<td>Ejection duration, ms</td>
<td>9</td>
<td>330.2</td>
<td>26.8</td>
<td>326.7</td>
<td>22.2</td>
<td>0.35</td>
</tr>
<tr>
<td>Aortic PWV, m/s</td>
<td>7</td>
<td>11.36</td>
<td>3.64</td>
<td>10.99</td>
<td>3.17</td>
<td>0.48</td>
</tr>
<tr>
<td>Brachial PWV, m/s</td>
<td>7</td>
<td>6.12</td>
<td>0.93</td>
<td>6.11</td>
<td>0.81</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Zₜ, terminal impedance; Zᵦ, characteristic impedance; PWV, pulse wave velocity. Because of flow catheter movement during study period, complete results were only available for 7/9 subjects (n). Pressure results were available for all subjects.

Fig. 2. Acute effect of sublingual estradiol (E2) on aortic input impedance. There was no effect of estradiol at 10 min or at completion of the study at 20 min. NS, not significant.
DISCUSSION

This study found no significant acute effects of estradiol administration on ventricular or arterial function, despite achieving markedly supraphysiological estradiol serum concentrations within the study period. There was a significant decrease in cardiac output and heart rate in the pooled data of all subjects following estrogen administration. This result is surprising because a number of studies have shown that estrogen may act directly as a smooth muscle relaxant, with consequent vasodilating properties (12, 23). At least one previous study, however, has documented a significant combined decrease in heart rate and cardiac output in association with estrogen therapy (22), similar to the current study. Whereas the decrease in heart rate may be partially responsible for the decrease in cardiac output, in the absence of changes in ventricular function, a further possible mechanism is through a decrease in preload due to venodilation. This is suggested by the tendency toward a decrease in right atrial pressure in the eight subjects in whom it was measured.

There have been no prior invasive human studies of the effect of estradiol on load-independent indexes of LV function. Two studies that have suggested a positive inotropic effect of estrogen found increases in peak aortic flow velocities in response to short-term hormone replacement therapy and attributed those changes in aortic velocities to “enhanced inotropism” (26, 28). These studies were noninvasive and did not record corresponding serum estradiol concentrations. Two further noninvasive echocardiographic studies have also found no effect on cardiac function in response to hormone replacement therapy (24, 35), in agreement with the current study.

The current study confirms what has been found in an isolated rat heart study (6). In that study, 17β-estradiol was associated with a negative chronotropic effect in the right atrium without any negative inotropic effect. Of note, however, is the fact that global ventricular function improved with estradiol only if the coronary arteries had been preconstricted with acetylcholine (6). Because the majority of subjects in the current study had normal coronary arteries, it may be expected that no significant effect on global LV function would be found. A study in the isolated rabbit heart found a significant negative inotropic effect only at very high concentrations (>1 μmol/l) (29). Consistent with a calcium blockade mechanism, a similar negative inotropic effect has been shown in an isolated guinea pig myocyte study, again at high in vitro concentrations (10–30 μmol/l) (11). Another study, in the rat heart following gonadectomy, found that estrogen (2 mg/day) tended to improve the cardiac dysfunction associated with gonadectomy (33). Serum estrogen levels were not measured in that study. A frequent problem with all of the in vivo studies in experimental animals is that significantly higher estrogen concentrations are used than are typically seen during hormone replacement therapy (400–600 pM) making direct extrapolation to effects on cardiac function in postmenopausal women difficult. The current study suggests that even at serum concentrations at around five times the standard therapeutic levels, estrogen does not significantly affect cardiac performance in the acute phase.

Preliminary reports have suggested that estrogen may acutely improve arterial compliance (36). In the study by Stefanadis et al. (36), aortic compliance was calculated from invasively measured pressure-diameter relations. Contrary to this, the current study found a tendency to decreased arterial compliance, in both pressure-volume and pressure-flow protocols. This was suggested by a trend toward an increase in effective
arterial elastance, and an increase in the aortic pressure diastolic decay constant (tau) in aortic pressure waveform analysis. There was no trend with respect to Zω, augmentation index, or pulse wave velocity. One difference with the study by Stefanadis et al. (36) was that measurements were taken for 40 min rather than 20 min in this study. This timing difference may be crucial because in the noninvasive study by Volterrani and colleagues (39), forearm blood flow changes were not apparent at 20 min but became significant at 40 min. The rationale for completing this study within 20 min was based on results that showed that both the negative inotropic effects in isolated myocytes (11) and activation of nitric oxide synthase from endothelial cells (3) occur within 5 min of estrogen exposure. Whereas longer term estrogen has been shown to improve arterial compliance (derived noninvasively) in some studies (30), this is not a universal finding (8, 10).

Possible reasons contributing to the negative results reported here may relate to the effect of radiographic contrast administered during cardiac catheterization. One effect of cardiac catheterization is to gradually deplete intravascular volume due to the diuretic effect of the contrast in fasting patients. This would be expected to increase the peripheral vascular resistance slightly, as was found in this study (with a tendency to increased arterial elastance and a tendency to increased calculated systemic vascular resistance). Any significant sympathetic response associated with volume depletion would tend to increase heart rate. This was not evident in this study, suggesting that the decrease in heart rate may be a direct effect of estrogen. This is consistent with other data demonstrating a negative chronotropic effect of estrogen (1, 22).

Because different classes of calcium antagonists have been shown to have variable effects on the periphery and on the heart (4), it is conceivable that estrogen may also exhibit selectivity of action. Because the urogenital bed may be considered the prime target for estrogen, regional increases in blood flow may have occurred that were not reflected in overall cardiac output or hemodynamic parameters (23). A number of vascular studies of the effects of estrogen have used intra-arterial injections, making vascular effects more apparent. Nonetheless in the study by Gilligan et al. (7), estradiol doses were used to achieve "premenopausal" estrogen levels. At that concentration, however, estradiol did not directly cause vasodilatation or increase blood flow but only potentiated acetylcholine responses (7). The negative chronotropic effect in the absence of significant vasodilatation remains consistent with calcium antagonism as a possible mechanism of action. Importantly, because this was an acute study, it remains possible that genomic effects of estrogen on other elements in the vascular tree or the heart may be seen with more prolonged use. This may be an important factor in the increase in arterial compliance associated with pregnancy (27).

The absence of any demonstrable benefit of acute estrogen administration in this study may relate to patients continuing their antianginal therapy at the time of catheterization. Previous (32) studies that have found an increase in exercise capacity in patients with angina due to sublingual estrogen administration have studied patients off anginal therapy, which may make any vasodilatation easier to detect. Whereas none of the women in this study were taking hormone replacement therapy, a number of the women were already taking long-acting vasodilators, including calcium antagonists, angiotensin-converting enzyme inhibitors, and long-acting nitrates. A recent study (38) has suggested that estrogen may modulate the effect of nitravasodilators. In that study, whereas higher doses of estrogen (10 μM) were associated with in vitro vasodilatation, no relaxation was seen at 1 nM. In the presence of other vasodilators, including sodium nitroprusside, however, 1 nM estradiol enhanced vasodilatation. This study may have been confounded, therefore, by concurrent anginal therapy. Nonetheless, the important conclusion can be made that acute supraphysiological levels of estradiol did not have any additive beneficial hemodynamic effect for women already receiving appropriate cardiovascular treatment. A subanalysis of subjects who were not taking vasodilators (9 of the 18 women studied) did not appreciably alter the cardiac output results. There remained a trend toward a decrease in cardiac output from 4.7 ± 0.9 to 4.2 ± 1.1 l/min (n = 6 subjects, P = 0.08). Heart rate changes following estrogen in women not taking vasoactive therapy remained significant, decreasing from 74.4 ± 9.7 to 71.4 ± 10.1 (n = 9 subjects, P = 0.03).

The kinetics of sublingual estradiol were not examined in this study. Our concern was to ensure that sufficient serum drug levels were achieved within the time frame of the study. The dose was chosen because it had previously been shown to acutely increase serum levels in a cohort of postmenopausal women (32). The levels achieved in the current study are similar (3,190 ± 2,216 pmol/l) to those from the study by Rosano et al. (32) (2,531 ± 1,192 pmol/l), despite the fact that the estradiol was given 40 min before exercise in that study. This suggests that the serum level increase quickly and remain elevated in the short term. A pharmacodynamic study in five women suggested that the peak level occurred within 2 h and had returned to baseline before 24 h (2).

In conclusion, despite increasing serum estradiol from low postmenopausal to supraphysiological levels, we found no acute alteration in either ventricular or vascular function. There was a small but significant decrease in heart rate associated with a significant decrease in cardiac output over the entire cohort without any evidence of a negative inotropic effect. Previously described improvements in arterial compliance due to estrogen may be related to structural or receptor-mediated changes associated with longer exposure.

The assistance of the staff of the St. Vincent's Hospital Cardiac Catheterisation Laboratory is gratefully acknowledged.

This work was supported by a Postgraduate Research Fellowship for C. Hayward and a Project Grant for R. P. Kelly, both from the National Health and Medical Research Council (Canberra, Australia). C. Hayward is currently supported by an Overseas Research
REFERENCES