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Effects of oestrogen replacement on steady and pulsatile haemodynamics in ovariectomized rats

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1 The effects of ovariectomy (Ovx), menopause and oestrogen replacement on the haemodynamics remain controversial. The present study employed the technique of arterial impedance analysis to measure and calculate the steady and pulsatile haemodynamics. The purpose was to determine the haemodynamic consequence of ovariectomy and oestrogen replacement.

2 Ovariectomy was carried out under anaesthesia on female Sprague Dawley rats aged 9 weeks. Oestrogen (17 β-estradiol or E_2) replacement started 1 week after ovariectomy for 4 weeks. Ovx increased the body weight (BW), while it greatly reduced the uterus weight. Left ventricular weight (LVW) was slightly increased, but LVW/BW ratio was slightly reduced. These changes were reversed after E_2 replacement.

3 Compared to sham group, Ovx with or without E_2 replacement did not significantly affect the systolic, mean and diastolic pressure. In Ovx, pulse pressure (PP) and heart rate were significantly increased, while stroke volume and cardiac output were slightly decreased. Total peripheral resistance (TPR) was largely elevated, indicating Ovx induced systemic vasoconstriction. These changes all returned to close normal values (sham group) after E_2 replacement, except PP.

4 Ovx increased the characteristic input impedance (Zc) and pulse wave reflection, while it decreased arterial compliance. E_2 treatment reversed these changes, except Zc.

5 These results demonstrate that Ovx influences both the resistance and Windkessel functions of the artery. E_2 treatment effectively reverses most the effects of Ovx both on the steady and pulsatile haemodynamics.

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Abbreviations: AF, aortic flow; ANOVA, analysis of variance; AP, aortic pressure; BW, body weight; C, arterial compliance; CO, cardiac output; ERT, oestrogen replacement therapy; FAP, femoral arterial blood pressure; HR, heart rate; LVW, left ventricular weight; Ovx, ovariectomy; Ovx + E_2, ovariectomy with chronic 17β-estradiol replacement; *Pb*, backward wave; *Pf*, forward wave; PP, pulse pressure; SV, stroke volume; TPR, total peripheral resistance; UW, uterine weight; *W*, external power; *Zc*, characteristic input impedance

Introduction

The incidence of cardiovascular disease in women under age of 50 years is lower than in men of similar age, but it markedly increases after menopause, and it is almost the same in the two sexes between the ages of 70–90 years (Wenger *et al*., 1993). So sex hormone may affect the cardiovascular system in women. Oestrogen replacement therapy (ERT) reduces cardiovascular disease in postmenopausal women (Barrett-Connor *et al*., 1989). The possible mechanisms of the cardioprotective effect of oestrogen are not completely understood and several possibilities have been proposed, including modulation of the autonomic nervous system (Du *et al*., 1995), improvement in lipoproterin and carbohydrate metabolism (Wild, 1996), inhibition of vascular smooth muscle proliferation (Dai-Do *et al*., 1996), enhancement of nitric oxide (NO)-dependent and independent effects (White *et al*., 1997). In many studies, oestrogen has been found to have significant short- and long-term haemodynamic effects. Significant increases of mean, systolic and diastolic pressure were observed after menopause (Manson *et al*., 1990; Brosnihan *et al*., 1994). Endothelium-dependent coronary artery vasodilation is enhanced by oestrogen treatment in ovariectomized monkey (Williams *et al*., 1990; 1992). ERT improves the pulsatile vascular afterload by decreasing the augmentation of the late systolic blood pressure in postmenopause women (Hayward *et al*., 1997). Oestrogen can diminish the resistance to blood flow in various vascular beds (Magness & Rosenfeld, 1989). Acute administration of pharmacological 17 β-estradiol therapy improves aortic function and decreases wave reflection in postmenopause women (Stefanadis *et al*., 1999). However other studies of long-term ERT have shown no significant effect on blood pressure (Nabulsi *et al*., 1993; Writing Group, 1995). Hayward *et al*. (1997) reported that there was no significant change in heart rate after menopause. Inversely, Sabb *et al*. (1989) reported that there was a significant increase in heart rate after menopause, and that ERT

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reversed the effect of menopause. Acute administration of oestrogen significantly decreases the heart rate and cardiac output in postmenopausal women without structural heart disease (Sbarouni *et al*., 1997). According to these studies, the haemodynamic effects of oestrogen are controversial; the possible reason being that this study cannot completely measure the total haemodynamic changes. However, little information is available with respect to the influence of oestrogen on complete arterial haemodynamics, using the method of arterial impedance analysis.

The technique of arterial impedance analysis has been developed for the complete assessment of arterial haemodynamics and quantitation of ventricular afterload (O'Rourke & Taylor, 1967; Milnor, 1975; Nichols *et al*., 1977; O'Rourke, 1982), and utilized extensively in recent years (Zuckerman & Yin, 1989; Chang *et al*., 1990; 1994; Chen *et al*., 1996; Hu *et al*., 1994; 1997; Su *et al*., 1999). Its purpose is to analyse the instaneous relationship between aortic pressure and flow through Fourier transform (frequency analysis) of phasic pressure and flow waves. The spectral analysis derives many haemodynamic parameters, including characteristic impedance (Zc), pulse wave reflection, and ventricular work. These parameters are considered to be pulsatile and frequency-dependent and reflect the main viscoelastic properties of the aorta and large arteries or 'Windkessel' functions of the arterial system. Furthermore, the steady components of arterial haemodynamics such as arterial pressure, cardiac output (CO) and total peripheral resistance (TPR) can also be obtained from the measurement.

These conflicting studies raised the question – whether the long-term oestrogen replacement has effects on the Windkessel and resistance vessels in the ovariectomy state compared to those in normal animals or not? The present study, employed the technique of arterial impedance analysis to study the effects of ovariectomy and oestrogen replacement on arterial pressure, vascular resistance, cardiac output, arterial impedance, compliance, pulse wave reflection and ventricular work. This complete haemodynamic analysis was designed to provide more information with respect to the influences of oestrogen on the Windkessel and resistance vessels as well as on the ventricular loading.

Methods

Experimental animals

The female Spragus Dawley (SD) rats were housed in an animal room with a constant temperature of $22 \pm 1^{\circ}C$ and maintained on a 12 h light/12 h dark schedule (lights on at 0600 h, off at 1800 h), which is fully accredited by the American Association of Animal Care. Rat chow and tap water were provided *ad libitum*.

At 9 weeks of age rats were anaesthetized with sodium pentobarbital (40 mg kg^{-1}, i.p.) and underwent bilateral ovariectomy. A small incision was made on bilateral sides of the back to expose the ovaries retroperitoneally. The ovaries were clamped and removed; the uterine tubes were ligated; then the skin was sutured. The sham procedure consisted of anaesthesia, visualization of the ovaries through incisions into abdominal cavity, and closure of the wounds. One week after the operation, rats were randomly divided into three groups: (1) sham group: rats who underwent sham-operation ($n=6$); (2) Ovx group: ovariectomized rats were injected intramuscularly (i.m.) with vehicle for 4 weeks ($n=7$); and (3) Ovx + E_2 group: ovariectomized rats injected with 17β-estradiol (50 μg kg^{-1} day^{-1}, i.m.) for 4 weeks ($n=7$).

Experimental preparation

Eight hours before the experiment, food was withdrawn, but water was provided. On the day of experiment, rats were anaesthetized with pentobarbital sodium (40 mg kg^{-1}, i.p.) and placed on heat blanket (MinK™, U.S.A.) to keep the body temperature at $37 \pm 1^{\circ}C$. The trachea was cannulated to facilitate respiration, and rectal temperature was recorded by thermometer. The right femoral artery was cannulated and connected to pressure transducer (P23ID, Statham, Oxnard) for the measurement of phasic and mean arterial blood pressure and heart rate (HR). The recordings were displayed on MacLab model 4e computer (MacLab Adinstruments, U.S.A.). The right femoral vein was cannulated for the administration of drugs and fluids. Measurements of aortic flow and pressure in rats were made according to the procedures described in previous studies (Chen *et al*., 1996; Chen & Hu, 1997; Hu *et al*., 1994; 1997). A midline cervical incision was performed to expose the right common carotid artery. A Millar catheter with high-fidelity pressure sensor (Millar instruments Co. Model SPR-407, Size 2F) was inserted *via* the right common cartoid artery into the ascending aorta to continuously measure the aortic pressure (AP) by Millar transducer control unit (Millar instruments Inc. Houston, U.S.A.). To minimize baseline drift, the catheter was soaked in saline at room temperature for at least 1 h before insertion. A thoracotomy was performed to expose the aorta. An electromagnetic flow probe (Carolina Medical Electronics Inc. Model SF 408 series, internal circumference 8 mm) was placed around the ascending aorta to continuously measure the aortic flow (AF) and monitored by an electromagnetic blood flowmeter (Carolina Medical Electronics Inc. Model FM 701 D). The electrocardiogram (ECG) of lead II was monitored on MacLab bio-amplifier.

The femoral arterial blood pressure (FAP), AP (systolic, mean and diastolic), HR, AF and ECG were continuously monitored on MacLab/4e computer (MacLab Adinstruments, U.S.A.). In addition, these parameters were stored in a Macintosh computer at a recording speed of 200 sampling points s^{-1} for off-line analysis. All data were registered after the pressure and flow signals had become stable for 3 – 5 min. The ascending aorta was looped by silk thread to measure the aortic diameter after the experiment *in vivo*.

Calculations and data analysis

The pressure and flow signals were digitized at 1 ms intervals using a 12-bit analogue-to-digital converter (Microstar Laboratories Inc., Model DAP 1200/4) interfaced to a personal computer. Four consecutive beats at stable state were selected for analysis. Zero flow was assumed to be the value of flow in middle-to-late diastole. The largest modulus of this portion of the flow was considered to be the noise level. The calibration of the flow velocity signal was performed after the experiment. The descending aorta was cannulated and connected to a resistor. The aortic flow was

calibrated by an infusion of heparinized blood (collected from other rats) at a different rate through the aorta. From the digitized flow velocity signal, we determined a time-averaged flow velocity for at least 30 separate beats. This mean velocity was converted to volume flow by multiplying the cross-sectional area of the flow probe. The appropriated calibration factor for each animal was then determined by matching the cardiac output obtained from the heparinized blood with the mean outputs calculated form the digitized flow signal. The flowmeter had a frequency response that was decreased by 3 dB at approximately 100 Hz. The phase lag was almost linear with frequency (1.2 degrees Hz^{-1}). Appropriate corrections were applied at each impedance harmonic to take the phase delay into account. All haemodynamic parameters were calculated beat by beat. The average value of four beats was obtained for an individual data point.

The calculations of the haemodynamic components are essentially the same as previous reports (Chen *et al*., 1996; Chen & Hu, 1997; Hu *et al*., 1994; 1997). The aortic pressure and flow waves are subjected to Fourier transform to derive the pressure and flow harmonic:

$$P(k) = \sum_{n=0}^{N-1} p(n)\ \omega_N^{kn}$$
$$\dot{Q}(k) = \sum_{n=0}^{N-1} q(n)\ \omega_N^{kn} \qquad (1)$$

where $k = $ O, 1, 2, 3, , N-1; $p(n)$ is the sampled sequence of pressure wave; $q(n)$ the sampled sequence of flow wave; $P(k)$ the modulus of pressure at kth harmonic: $\dot{Q}(k)$ the modulus of flow at kth harmonic. $P(k)$ and $\dot{Q}(k)$ can be rewritten as:

$$P(k) = |P(k)|\ e^{j\phi(k)}$$
$$\dot{Q}(k) = |\dot{Q}(k)|\ e^{j\phi(k)} \qquad (2)$$

For each beat, the impedance modulus is the ratio of aortic pressure harmonic to flow harmonic:

$$Z(k) = \frac{|P(k)|}{|\dot{Q}(k)|} \qquad (3)$$

The flow phase is subtracted from the pressure phase at each harmonic to yield the impedance phase angle:

$$\theta(k) = \varphi(k) - \phi(k) \qquad (4)$$

Any flow harmonic with a modulus <1.5 times the noise level was not used for impedance calculation. The characteristic impedance (Zc) was the average of impedance modulus in the frequency range of 15–45 Hz with coefficients of variation $<10\%$. First zero-crossing of impedance phase angle (fo) was evaluated by linear interpolation method from the data. Systolic, diastolic, mean aortic pressure (APs, APd, APm), HR, stroke volume (SV) and total peripheral resistance (TPR) were also determined each beat. Cardiac output (CO) was the product of SV and HR. Because of a curvilinear relation between pressure and intravascular volume in the arterial tree, an acute increase in pressure was associated with reduction in arterial compliance. The arterial compliance (C) at pressure (P; systolic, diastolic, or mean) was obtained from the equation according to Liu *et al*. (1986) for an exponential pressure-volume relationship:

$$C(P) = \frac{SV}{K}\ \frac{b\ \exp^{bp}}{\exp^{bp_s^*} - \exp^{bp_d^*}} \qquad (5)$$

where SV is the stroke volume, K the ratio of total area under the aortic pressure curve to the diastolic area, b the coefficient in the pressure/volume relation (-0.0131 in the aortic arch), P_s the pressure at the time of incisura, and P_d the end-diastolic pressure.

Total, pulsatile and steady external powers (Wt, Wo, Ws) consisting of pressure and flow terms were also calculated (Milnor, 1989):

$$W_s = \overline{P} \cdot \dot{\overline{Q}}$$
$$W_o = \frac{1}{2}\sum \left|\dot{Q}(k)^2\right| |Z(k)|\ \cos\theta(k) \qquad (6)$$
$$W_t = W_s + W_o$$

where P is the mean pressure; $\dot{Q}$ the mean flow. The ratio of oscillatory to total power (Wo/Wt) was also calculated as an index for the efficiency with which the pulsatile energy was converted into forward flow. Finally, we decomposed the measured pressure and flow waves into forward and backward components (Westerhof *et al*., 1972):

$$P_m = P_f + P_b$$
$$\dot{Q}_m = \dot{Q}_f + \dot{Q}_b$$
$$P_f = Z_c \cdot \dot{Q}_f \qquad (7)$$
$$P_b = -Z_c \cdot \dot{Q}_b$$

Where P is the pressure wave, $\dot{Q}$ the flow wave, m the measured wave, f the forward wave, b the backward wave. Thus the measured pressure and flow waves are equal to the sum of a forward wave and a backward wave. Zc relates the forward pressure and flow waves. The magnitudes (pulse pressure) of Pf and Pb components, with the ratio of backward to forward magnitude (Pb/Pf) were used to characterize the wave reflection properties. Computer programmes developed previously were used to analyse all data and to derive haemodynamic parameters (Chen *et al*., 1996; Chen & Hu, 1997; Hu *et al*., 1994; 1997).

The measurements of aortic pressure and flow with the arterial impedance analysis in small animals, like rats, were essentially similar to those procedures described previously (Zuckerman & Yin, 1989; Chen *et al*., 1996; Chen & Hu, 1997; Hu *et al*., 1994; 1997). Since the Millar catheter (size 2F) used in small animals is only equipped with a pressure sensor for monitoring the aortic pressure, the measurement of aortic flow requires open-chest surgery to place an electromagnetic flow probe around the aorta. The procedures caused a fall in AP as reported in another study (Zuckerman & Yin, 1989; Hu *et al*., 1994; Chen & Hu, 1997). The extent to which surgical procedures and blood pressure reduction will affect the haemodynamic data is not certain. Thus we discarded the data in which the fall in AP was more than 15 mmHg after thoracotomy and flow-probe placement in all-experimental rats. Although the selection could minimize the effects of haemodynamic perturbation, the results only pertained to measurements in the open-chest condition in

all experimental rats. In fact, about 1–2 rats were discarded in each group.

Radioimmunoassay for 17 β-estradiol measurement

Plasma concentration of 17 β-estradiol was measured using a ^{125}I radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, U.S.A.). ^{125}I Estradiol (1 ml) was added to assay tubes containing 100 μl of plasma or standard solution. They were incubated at 37°C for 60 min. The content of all tubes was aspirated. Then, the tubes were counted for ^{125}I activity in a gamma counter (Cobra II, Packard Co, U.S.A.) for 1 min.

Drugs

All chemicals were obtained from Sigma Chemical Co. 17 β-Estradiol was dissolved in 0.2% DMSO with sesame oil. The solvent was used as vehicle. The vehicle solution did not significantly affect the haemodynamic parameters.

Statistical analysis

After experiments, rats were sacrificed with a large dose of intravascular pentobarbital. The left ventricular weight (LVW), body weight (BW), LVW to BW ratio (LVW/BW) and uterine weight (UW) were measured. All data were expressed as means ± s.e.mean and were evaluated statistically by one-way analysis of variance (ANOVA) with Newman-Keuls multiple comparisons test for the *post hoc* determination of significant differences. Differences were considered significant at $P<0.05$.

Results

Plasma estradiol level

Ovariectomy significantly reduced the plasma estradiol level. Oestrogen replacement increased the estradiol level close to that in the sham group (Figure 1).

Cardiac, uterine mass and aorta diameter

Data in Table 1 shows that ovariectomy increased the body weight and LVW significantly, and replacement of estradiol returned these parameters to normal. Although the LVW was significantly decreased after oestrogen replacement, but LVW/BW ratio was elevated. The uterine weight was greatly reduced by ovariectomy, but reversed to normal after estradiol replacement. Aortic diameter was not significantly different between sham, Ovx and Ovx + E_2.

Steady haemodynamics: AP, PP, SV, CO and TPR

Table 2 summarizes AP, PP, HR, SV, CO and TPR in sham, Ovx and Ovx + E_2 groups. The measured aortic pressures after anaesthesia and open chest surgery were not signifi-

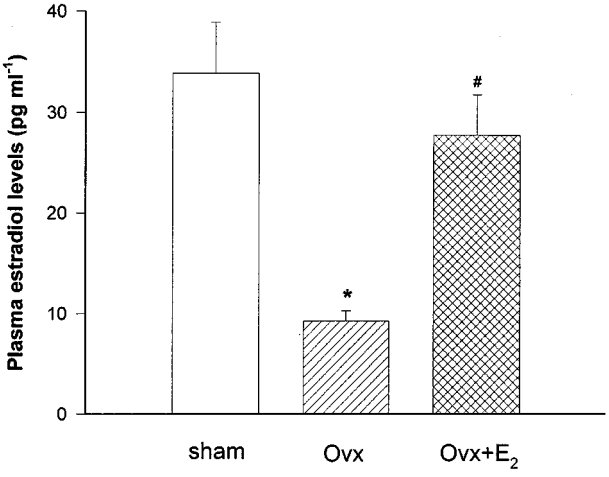

Figure 1 Effects of ovariectomy (Ovx) and ovariectomy with chronic 17 β-estradiol replacement (Ovx + E_2) on plasma estradiol level in female SD rats. Data are expressed as mean ± s.e.mean. ($n=6-7$) * $P<0.05$ Ovx versus sham group. # $P<0.05$ Ovx versus Ovx + E_2 group.

Table 1 Effects of ovariectomy (Ovx) and ovariectomy with chronic 17β-estradiol replacement (Ovx + E_2) on body weight, left ventricular (LV) weight, LVW/BW ratio, uterus weight and aortic diameter in female SD rats

	Body weight (g)	*LV weight* (g)	*LVW/BW ratio*	*Uterus weight* (g)	*Aortic diameter* (mm)
Sham ($n=6$)	284.3 ± 4.6	0.62 ± 0.02	0.22 ± 0.01	0.67 ± 0.07	1.60 ± 0.09
Ovx ($n=7$)	341.5 ± 6.6*	0.70 ± 0.02*	0.20 ± 0.01*	0.15 ± 0.01*	1.58 ± 0.11
Ovx + E_2 ($n=7$)	294.3 ± 3.8†	0.63 ± 0.01†	0.22 ± 0.00†	0.56 ± 0.03†	1.62 ± 0.18

LVW indicates left ventricular weight; LVW/BW, the ratio of left ventricular weight to body weight; Sham, rats received sham operation; Ovx, ovariectomy; Ovx + E_2, ovariectomy with chronic 17β-estradiol replacement. Data are expressed as mean s.e.mean. *$P<0.05$ versus Sham. †$P<0.05$ versus Ovx.

Table 2 The haemodynamics of steady component in Sham, Ovx and Ovx + E_2 female SD rats

	Aortic pressure (mmHg)			*PP*	*HR*	*SV*	*CO*	*TPR* ($\times 10^3$)
	AP_s	AP_m	AP_d	(mmHg)	(Beats min^{-1})	(ml)	(ml min^{-1})	(Dyne s cm^{-5})
Sham ($n=6$)	114 ± 5	96 ± 4	80 ± 4	32 ± 3	328 ± 23	0.196 ± 0.018	62.8 ± 5.3	128 ± 10
Ovx ($n=7$)	128 ± 8	107 ± 7	91 ± 7	38 ± 5*	371 ± 16*	0.144 ± 0.011*	53.3 ± 4.6*	154 ± 12*
Ovx + E_2 ($n=7$)	123 ± 7	103 ± 6	87 ± 6	37 ± 4*	354 ± 15	0.187 ± 0.015	66.1 ± 4.6†	120 ± 12†

APs, APm, APd, aortic pressure corresponding to peak systolic, mean and end diastolic pressure; PP, aortic pulse pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance. Data are expressed as mean ± s.e.mean. *$P<0.05$ versus Sham. †$P<0.05$ versus Ovx.

cantly different among these three groups. AP in Ovx had a tendency to increase, but these changes were not statistically significant. PP in Ovx and Ovx + E_2 group were higher than the sham group by 6 ± 2 and 4 ± 1 mmHg, respectively. HR in Ovx group was increased over the sham group by 43 ± 6 beats min^{-1}. SV in Ovx group was lower than the sham group by 0.052 ± 0.006 ml. CO in Ovx group decreased by 9.5 ± 0.8 and 12.8 ± 0.1 ml min^{-1} compared with sham and Ovx + E_2 groups. TPR after Ovx was significantly increased by 26 ± 2 ($\times 10^3$) dyne s cm^{-5}, and it returned to nearly normal after estradiol replacement.

Pulsatile haemodynamics: arterial impedance, compliance, ventricular work and wave reflection

Figure 2 illustrates the average impedance modulus and impedance phase in sham, Ovx and Ovx + E_2. In Ovx, the impedance modulus was elevated in comparison between sham and Ovx + E_2. In addition, the phase angle was shifted to the right. However, defect of oestrogen remarkably increased the impedance modulus at 0 Hz, which is the value corresponding to TPR. After chronic 17 β-estradiol treatments, the impedance modulus at 0 Hz recovered to normal. Table 3 summarizes the haemodynamic data of pulsatile components. *Zc* in Ovx and Ovx + E_2 were higher than sham. The values of arterial compliance (*Cd, Cs, Cm*) in Ovx were less than those in sham and Ovx + E_2 by 17–18 and 25–30%. *Ws* and *Wo*/*Wt* in Ovx were significantly lower than the other groups. *Pf*, *Pb* and *Pb*/*Pf* in Ovx were increased more than sham, but *Pb*/*Pf* was decreased significantly with chronic 17 β-estradiol treatment. The other parameters including *fo* and *Wo* were not significantly altered after ovariectomy with or without chronic 17 β-estradiol treatment. First harmonic of impedance modulus in Ovx was significantly higher than sham and Ovx + E_2 (154097 ± 12168, 127863 ± 9564, 120471 ± 11591 dyne s cm^{-5}, respectively). The comparison for impedance modulus in the frequency range which includes wave reflection (around 20 Hz) were as follows: 13063 ± 1054 in sham, 44923 ± 4760 in Ovx and 18332 ± 1702 dyne s cm^{-5} in Ovx + E_2. The impedance modulus in Ovx was elevated more than sham, while significantly reversed by ERT, but still higher than sham.

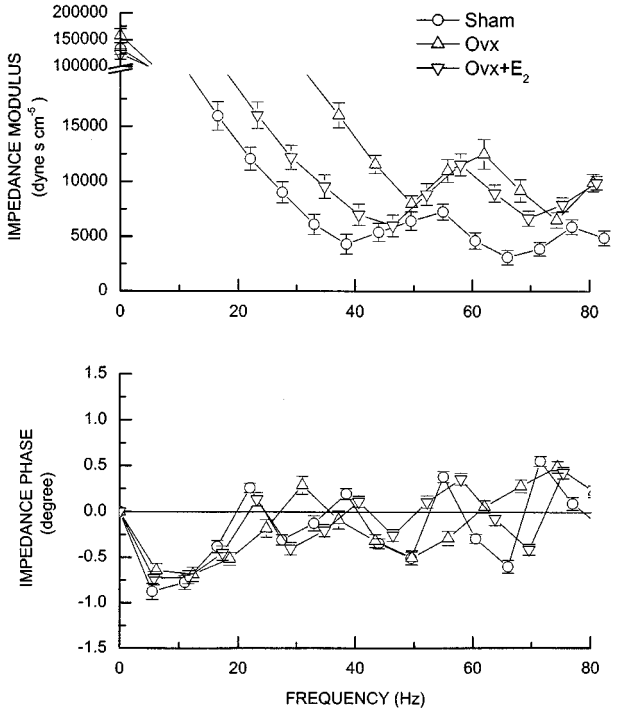

Figure 2 Comparison of average impedance modulus and impedance phase from female rats among sham, ovariectomy (Ovx) and ovariectomy with chronic 17 β-estradiol replacement (Ovx + E_2) rats.

Discussion

The technique of impedance analysis provides a complete assessment of the arterial haemodynamics, including steady and pulsatile components. In the present study, the major findings were as follows: (1) Ovariectomy caused a decrease in CO and SV, while there was an increase in PP, HR and TPR; (2) Increase in *Zc*, *Wo*/*Wt*, *Pb* and *Pf* and decrease in arterial compliance and *Ws* in ovariectomized rats indicated that oestrogen deficiency greatly affected the arterial impedance modulus, wave reflection and ventricular work; (3) After chronic oestrogen replacement, most of these haemodynamic changes were recovered to nearly normal. The results indicated that oestrogen might affect both steady and pulsatile components of arterial haemodynamics, including both resistance and Windkessel functions.

There is general agreement that cardiovascular biomechanics may be depressed after menopause, and oestrogen may normalize these changes by increasing relaxation and ventricular contractility (Samaan & Crawford, 1995; Guetta & Cannon, 1996). It has been well documented that oestrogen has direct effects on the vessel wall. Oestrogen can diminish the resistance to blood flow in various vascular beds (Magness & Rosenfeld, 1989). Normal arteries show endothelium-dependent vasodilation in response to acetylcholine infusion (Furchgott & Zawadzki, 1980). Acetylcholine-mediated coronary vasodilation was produced by infusion of

Table 3 The haemodyanamics of pulsatile components in Sham, Ovx and Ovx + E_2 female SD rats

	Zc (Dyne s cm^{-5})	*Cs*	*Cm* (μl $mmHg^{-1}$)	*Cd*	*fo* (Hz)	*Wo*	*Ws* (mW)	*Wo*/*Wt* (%)	*Pf*	*Pb* (mmHg)	*Pb*/*Pf* (%)
Sham ($n=6$)	5997 ± 707	5.32 ± 0.54	6.11 ± 0.58	6.37 ± 0.62	20 ± 4	1.13 ± 0.11	13.36 ± 0.48	8.4 ± 0.9	16.7 ± 1.1	8.8 ± 0.9	54 ± 7
Ovx ($n=7$)	7819 ± 932*	4.35 ± 0.56*	5.03 ± 0.52*	5.26 ± 0.57*	23 ± 4	1.11 ± 0.15	11.48 ± 0.56*	9.7 ± 1.1*	19.2 ± 2.1*	11.4 ± 1.1*	59 ± 9
Ovx + E_2 ($n=7$)	7581 ± 851*	5.86 ± 0.60*†	7.20 ± 0.81*†	7.53 ± 0.92*†	21 ± 4	1.10 ± 0.13	13.94 ± 0.52†	7.9 ± 0.8†	18.5 ± 1.3	9.2 ± 1.0†	50 ± 7†

Zc, characteristic input impedance; *Cm*, *Cs*, *Cd*, arterial compliance corresponding to mean, peak systolic, end diastolic pressure; *fo*, first zero crossing frequency of impedance phase angle; *Ws*, *Wo*, *Wt*, external power corresponding to steady, oscillatory and total power; *Pb* and *Pf*, backward and forward components of pressure wave. Data are expressed as mean ± s.e.mean. *$P<0.05$ versus Sham. †$P<0.05$ versus Ovx.

17 β-estradiol at physiological concentrations in 20 postmenopausal women (Gilligan *et al*., 1994). Thus, an improvement of endothelial function may be an important mechanism by oestrogen treatment. It has been proposed that one of possible mechanisms of the cardioprotective effect of oestrogen is enhancement of nitric oxide production on the vascular endothelium and improvement of haemodynamic state (White *et al*., 1997). With respect to its effects on arterial resistance, several studies determining regional blood flow or employing direct arterial perfusion have demonstrated that oestrogen deficiency causes a decrease in blood flow and an increase in resistance in various vascular beds (Charkoudian *et al*., 1999; Mercuro *et al*., 1999). The present study, found that the AP did not significantly change among sham, Ovx and Ovx + E_2 groups. However, AP was slightly increased, with a marked increase in total peripheral resistance and decrease in cardiac output in the Ovx rats. The reduction in CO of ovariectomized rats was resulting from a decrease in SV with an increase in HR. After chronic oestrogen replacement, CO was increased with an elevation of SV. In addition, elevation of HR was reduced presumably because of beta-adrenergic blocking effect and centrally mediated reflex bradycardia of oestrogen (Mohamed *et al*., 1999). Our results were similar with other studies in cardiovascular biomechanics (Evans *et al*., 1998; Taskin *et al*., 1998; Gallinelli *et al*., 1999). In our study, heart rate in Ovx was significantly higher than that of sham, but the compliance in Ovx was significantly lower than sham. After E_2 treatment, these changes return near to normal. A similar result demonstrated that a link between high resting heart rate and increased arterial stiffness (Sa Cunha *et al*., 1997), suggested that arterial stiffness *per se* was influenced by heart rate. However, Stefanadis *et al*. (1998) reported that during incremental ventricular pacing in man, the aortic stiffness reduced and aortic distensibility increased. Thus, the relationship between heart rate and stiffness of large artery is still controversial and further efforts are needed to elucidate this issue. Although AP did not significantly increase, PP significantly increased in Ovx. PP was not reversed to normal by E_2 treatment. Increased PP is an independent risk factor for cardiovascular morbidity and mortality. Both increased arterial stiffness (coming from central arteries) and alterations of wave reflections (generated at peripheral large and small arteries) contribute independently to the predominant or selective increase in PP observed with age and hypertension (van Bortel *et al*., 2001). In the present study, heart rate in Ovx was higher than sham but after E_2 treatment, this change returns to normal. Observational studies imply a link between high resting heart rate and increased arterial stiffness (Sa Cunha *et al*., 1997), suggesting that arterial stiffness *per se* may be influenced by heart rate. Stefanadis *et al*. (1998) (using ultrasound), reported reduced aortic stiffness and increased distensibility, during incremental ventricular pacing in man. Wilkinson *et al*. (2000) reported that augmentation index of central arterial waveform was inversely and linearly related to heart rate and tachycardia in Ovx influences large artery biomechanical properties.

These pulsatile haemodynamic components reflect mainly the changes in viscoelastic properties of the aorta and large arteries, and are referred to the Windkessel functions of cardiovascular system (O'Rourke, 1982; Chang *et al*., 1990, 1994; Chen *et al*., 1996; Chen & Hu, 1997; Hu *et al*., 1994, 1997; Su *et al*., 1999). In the present study, we found a marked increase in *Zc* and wave reflection with decrease of arterial compliance and ventricular work after menopause. Accordingly, after chronic oestrogen replacement therapy, the reduction of arterial compliance and ventricular work were returned to nearly normal. In a recent study, Nagai *et al*. (1999) reported that oestrogen replacement after postmenopause reduced age-associated increase in common cartoid arterial stiffness. Waddell *et al*. (1999) also reported that withdrawal of ERT for 4 weeks decreased systemic arterial compliance and pulse wave velocity in postmenopausal women. These results support our findings and might lead to the conclusion that chronic oestrogen replacement therapy could improve the large arterial compliance and ventricular intropic function. In contrast, Hayward *et al*. (2000) and Sbarouni *et al*. (1997) reported that acute administration of 17 β-estradiol caused a reduction in cardiac output, but did not significantly affect either left ventricular inotropic function or arterial impedance. This conclusion appears contrary to the results in this study, and is subject to discussion. First, supraphysiological serum estradiol levels reduced ventricular contraction, possibly through the calcium-blocking effect of oestrogen (Crews & Khalil, 1999) and reduced basal release of NO in rat aorta (Bolego *et al*., 1997). Second, acute effect of oestrogen could not affect the arterial compliance, but chronic effects of oestrogen could improve these haemodynamic properties of large arteries. The possible mechanisms of chronic oestrogen replacement therapy improved vascular elasticity through reduced collagen synthesis (Beldekas *et al*., 1981) and decreased tendency to myointimal hyperplasia (Cheng *et al*., 1991). It was surprising that our results revealed that elevation of *Zc* did not completely return to normal after chronic oestrogen replacement therapy. The possible explanation for the result was that acute or chronic effect of oestrogen did not increase aortic size (Chelsky *et al*., 1997; Stefanadis *et al*., 1999). *Zc* is relative to aortic diameter, aortic viscoelastic properties and wave reflection. As *Zc* is inversely related to the aortic lumen (Chang *et al*., 1990), an increase in aortic diameter tends to reduce the value of *Zc*. Although this study revealed that the arterial compliance was markedly elevated by chronic oestrogen replacement therapy, the aortic diameter has no significant changes among these three groups.

It is well known that haemodynamic parameters can be influenced by anaesthesia (Wenzel *et al*., 2000). It is not certain the extent of influence of anaethesia on the haemodynamics of oestrogen. This is the major limitation of arterial impedance analysis in the current study. However, we have measured tail-cuff blood pressure and heart rate with tail sphygmorgraphy (MOD59, Blood pressure Meter/Amplifier, Itic Inc, Woodland Hills, CA, U.S.A.) in conscious rats. The values of blood pressure in sham, Ovx and Ovx + E_2 were 118.0 ± 2.8, 114.9 ± 3.1 and 105.2 ± 4.0 mmHg, respectively, there is no significant difference among these groups. The heart rate of Ovx (399.2 ± 8.4 beats min^{-1}) is significant higher than those of sham (360.3 ± 11.0) and Ovx + E_2 (376.8 ± 8.3) ($P < 0.05$). These results of conscious rats are similar to those of current studies, under anaesthesia. In addition, Gallinelli *et al*. (1999) reported that postmenopausal women treated with a 6-month course of ERT have significantly improved end-diastolic index, heart contractility index, cardiac index, and SVR. These results are consistent

with those of our current studies under anaesthesia. These studies imply that anaesthesia seems not confound the haemodynamic effects of oestrogen. Further studies are needed to elucidate this point.

In summary, by using arterial impedance analysis for a complete assessment of arterial haemodynamics demonstrates that chronic oestrogen replacement can markedly improve both resistance and Windkessel functions in ovariectomized rats.

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