Diurnal variation in uterine artery blood flow in post-menopausal women on oestrogen hormone replacement therapy

D.Jurkovic1, D.Ross, N.Aslam and M.Whitehead

Department of Obstetrics and Gynaecology, King’s College Hospital, Denmark Hill, London SE5 8RX, UK

1To whom correspondence should be addressed

Doppler ultrasound was used to investigate circadian variations in uterine artery blood flow in 20 post-menopausal women in the oestrogen-only phase of combined oestrogen hormone replacement therapy with cyclical oral norethisterone or dydrogesterone. All women were examined between 0800 and 0830 h and then again between 1800 and 1830 h on the same day. Mean arterial blood pressure, heart rate and a blood sample for measurement of serum oestradiol were taken at each visit. Indices of uterine artery blood flow included the pulsatility index, resistance index, peak systolic velocity and time-averaged maximum velocity. No significant differences in the mean arterial blood pressure, pulse rate and oestradiol concentrations were detected between morning and evening visits. Significant fluctuation was observed in the pulsatility index ($P < 0.001$), resistance index ($P < 0.001$) and time-averaged maximum velocity ($P < 0.01$). The assessment of uterine artery blood flow in post-menopausal women should take into account the presence of circadian variations to ensure accuracy and reproducibility of Doppler investigations.

Key words: blood flow/circadian rhythm/Doppler ultrasound/uterine artery

Introduction

Transvaginal Doppler ultrasound enables in-vivo studies of uterine artery blood flow (Taylor et al., 1985; Maulik, 1995). Using this technique significant changes have been described in uterine artery flow during the normal menstrual cycle (Steer et al., 1990). Blood flow differed between the proliferative and luteal phase of the cycle, but the changes observed were not directly related to the variations in ovarian steroid concentrations (Scholtes et al., 1989; Sladkevicius et al., 1993). Recently, the presence of a circadian variation in the uterine circulation has been demonstrated during the menstrual cycle with a significant reduction in blood flow in the late afternoon and early evening (Zaidi et al., 1995a,b). This finding may be responsible for the apparent inconsistencies between the results from different physiological blood flow studies.

A number of observational studies have shown cardioprotective effects of exogenous oestrogens in post-menopausal women (Bush et al., 1987; Paganini-Hill et al., 1988; Stampfer et al., 1991; Walsh et al., 1991). The reduction in cardiovascular risk can only partly be explained by changes in blood lipids and lipoproteins. Doppler studies have shown that vasodilatation in the uterine artery occurs soon after starting treatment with parenteral oestrogens (Bourne et al., 1990; de Ziegler et al., 1991; Cacciatore et al., 1998). Other studies have shown that changes in serum lipid concentrations following oestrogen therapy take ~3 weeks (Bush et al., 1987; Whitcroft et al., 1994; Kim et al., 1996) and therefore it is unlikely that the rapid response of vasodilatation of the uterine artery following administration of oestrogens is mediated by changes in blood lipid concentrations. However, it is not known to what extent uterine blood flow in post-menopausal women may be influenced by mechanisms other than hormone replacement therapy (HRT). In particular the presence of circadian rhythm may affect Doppler measurements and lead to erroneous conclusions about the effects of different ovarian steroids on uterus and systemic circulations.

In this study we investigated whether circadian changes in uterine artery blood flow are also present in post-menopausal women during the oestrogen-only phase of sequential HRT.

Materials and methods

Healthy non-smoking post-menopausal women who had not undergone a hysterectomy were prospectively recruited into the study. Women with uterine or ovarian abnormalities detected on ultrasound scan, as well as those on medication with possible effects on vascular tone, were excluded. The study was approved by the Research Ethics Committee of King’s College Hospital.

All women were prescribed sequential HRT as continuous transdermal oestradiol 50 mg/24 h (Evorel; Janssen-Cilag, Saunderton, UK) and cyclical oral norethisterone 1 mg/24 h or dydrogesterone 10 mg/24 h for 12 days each month. Patches were changed twice weekly after 3.5 days in accordance with the manufacturer’s instructions. Examinations were performed during days 2 and 4 of the second week of the oestrogen-only phase of the cycle to avoid the effects of progestogen on uterine blood flow (Marsh et al., 1994). All scans were performed 24 h after the patches were changed. Women were first seen between 0800 and 0830 h and then again between 1800 and 1830 h on the same day. Resting blood pressure and heart rate were taken once at each visit after a 30 min rest. A peripheral venous blood sample was then obtained and serum stored at −70°C for measurement of oestradiol concentrations. The hormone concentrations were measured using a commercially available direct radioimmunoassay kit (Technicon Immuno 1; Bayer Diagnostics, Newbury, Berkshire, UK). The within-batch coefficients of variation, calculated from previous assays performed in the Department of Clinical Biochemistry at King’s College Hospital, was 1.8% for oestradiol.

All ultrasound examinations were performed by a single operator (D.J.) using a 5 MHz transvaginal probe with colour and pulsed
Doppler facilities (Aloka SSD-2000; Aloka Ltd, Tokyo, Japan). Colour Doppler signal was used to identify the main uterine artery lateral to the cervix at the level of the internal os. A pulsed Doppler range gate was then placed across the vessel, aiming for an angle close to 0° between the Doppler beam and the vessel. The pulse repetition frequency ranged from 1 to 12 kHz and the filter used was 50 Hz. Flow velocity waveforms were obtained from both uterine arteries. The pulsatility index (PI), the resistance index (RI), the peak systolic velocity (PSV) and the time-averaged maximum velocity (TAMXV) were calculated electronically from regular curves fitted to good-quality, high-amplitude waveforms. PI was used as a measure of impedance to blood flow distal to the point of sampling and was calculated according to the formula:

$$\text{PI} = \frac{(S - D)}{\text{mean}}$$

where S is the peak, D is the minimum and mean is the mean maximum Doppler-shifted frequency over the cardiac cycle. RI was calculated using the formula:

$$\text{RI} = \frac{(S - D)}{S}.$$

For each individual, the mean of the left and right uterine artery PI values was calculated at each time point. Intra-observer variation in uterine artery Doppler measurements has been previously reported by our group using one-way analysis of variance of replicate data which gave coefficients of variation of 9.1% and 9.7% for the left and right uterine arteries respectively (Zaidi et al., 1995a). Statistical analysis was performed using the Wilcoxon matched-pairs signed-rank test. A non-parametric approach was chosen due to the limited sample size and non-normal distribution of the data. The morning and afternoon values of the following parameters were compared: mean arterial pressure, heart rate, PI, RI, PSV, TAMXV and oestradiol concentrations. The mean arterial pressure was calculated using the formula: pulse pressure + diastolic blood pressure, where pulse pressure = systolic blood pressure – diastolic blood pressure.

Results

Twenty-three patients consented to participate in the study. Three women were subsequently excluded at the baseline visit because of abnormal ultrasound findings: ovarian cysts were found in two women and large fibroids in the third woman. In the remaining 20 women comparisons between the visits showed no significant differences in the mean arterial blood pressure and pulse rate (Table I). Both uterine arteries were visualized in all women and good quality flow velocity waveforms were obtained. The uterine artery PI and RI were significantly higher and TAMXV was significantly lower in the afternoon compared with the morning visits ($P < 0.01$). There were no significant changes in the uterine artery PSV. Analyses of the plasma hormone concentrations showed that plasma oestradiol was not significantly different between visits (Table I).

Discussion

This study has demonstrated a circadian change in uterine artery blood flow in post-menopausal women receiving a constant dose of exogenous oestradiol. The mean PI increased by 21% and TAMXV decreased by 19% in the afternoon as compared to the morning values. The magnitude of the observed circadian blood flow changes was similar to those previously reported in healthy pre-menopausal women (Zaidi et al., 1995a). In that study there was no correlation between lutinizing hormone (LH) and oestradiol concentrations and changes in the PI and TAMXV. The findings of both studies suggest that other factors independent of the sex steroids play an influential role in the regulation of the uterine vascular tone.

Circadian rhythms are known to exist and play an important role in the regulation of physiological functions in the human body. For example, they are known to influence the secretion of many hormones, such as prolactin, adrenocorticotrophin hormone and cortisol (Weitzman, 1976). The influence of circadian rhythms on vascular reactivity and tone has been reported in previous studies (Millar-Craig et al., 1978; Panza et al., 1991; Feng and Tofler, 1995). In these studies increased vasoconstrictor activity, higher blood pressure and vascular tone have been reported during the morning periods as compared to the evening times. A circadian variation in alpha-adrenergic activity has also been demonstrated and it has been postulated that increased vasoconstriction and vascular tone is mediated by high basal alpha-adrenergic activity in the morning (Panza et al., 1991). This group demonstrated that basal forearm vascular resistance was significantly higher and the blood flow significantly lower in the morning than in the afternoon and evening. These findings contrast with the results of our study, where uterine artery blood flow impedance is higher in the evening compared with morning. Circadian variations have also been described for platelet aggregation (Petralito et al., 1982), blood viscosity (Ehrly and Jung, 1973), activated partial thromboplastin and thrombin time (Decousus et al., 1985; Tofler et al., 1987). These studies show increased tendency to thrombosis in the morning as compared with the afternoon. Melatonin has also been shown to be secreted in a circadian pattern, with nearly all being secreted at night and secretion being absent during the day (Cagnacci et al., 1998). The effects of melatonin on the circulation are mainly those of vasodilatation, which may partly explain the variations observed in uterine artery blood flow.

The majority of Doppler studies in post-menopausal women

### Table I. Comparison of various parameters at the morning and afternoon visits in 20 women on continuous oestrogen replacement therapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Morning</th>
<th>Afternoon</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>93</td>
<td>95</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(77-110)</td>
<td>(83-113)</td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>76</td>
<td>77</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(56-78)</td>
<td>(60-85)</td>
<td></td>
</tr>
<tr>
<td>Pulsatility index</td>
<td>1.73</td>
<td>2.57</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(1.05-2.89)</td>
<td>(1.18-3.82)</td>
<td></td>
</tr>
<tr>
<td>Resistance index</td>
<td>0.79</td>
<td>0.87</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(0.65-0.95)</td>
<td>(0.66-1.00)</td>
<td></td>
</tr>
<tr>
<td>Peak systolic velocity (cm/s)</td>
<td>41.4</td>
<td>41.4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(28-63)</td>
<td>(17-68)</td>
<td></td>
</tr>
<tr>
<td>Time-averaged maximum velocity (cm/s)</td>
<td>20.8</td>
<td>17.0</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>(11-35)</td>
<td>(4-27)</td>
<td></td>
</tr>
<tr>
<td>Oestradiol (pmol/l)</td>
<td>257</td>
<td>353</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(106-987)</td>
<td>(97-998)</td>
<td></td>
</tr>
</tbody>
</table>

Data are medians (range). *Twenty valid observations were made for each parameter. NS = not significant; bpm = beats per minute.
have shown a significant decrease in uterine artery impedance to flow following administration of exogenous oestrogens (Hillard et al., 1992; Marsh et al., 1994; Dören et al., 1997; Järvelä et al., 1997; Cacciatore et al., 1998). This effect appears to be counteracted to an extent by the addition of progestogens. A decrease in central retinal artery resistance was reported (Belfort et al., 1995) and consequently the cerebral microcirculation following oestrogen replacement therapy. However, not all of these studies allowed for diurnal variations in blood flow. For example a recent report demonstrated a rise in the PI value in women receiving exogenous oestradiol treatment after the administration of progestogens (Hillard et al., 1992). The average increase in the impedance to flow was 13% which is much less than circadian fluctuations observed in our study.

In another study (Järvelä et al., 1997), 13 post-menopausal women were treated with unopposed transdermal oestradiol patches alone for 1 month. This was then followed by insertion of the levonorgestrel-releasing intrauterine device. This group demonstrated an initial decrease in mean uterine artery PI after 1 month of transdermal oestradiol treatment; this was followed by an 11% increase in the mean uterine artery PI 1 month following the insertion of the progestogen-releasing intrauterine system. However, from the data it is clear that this result was due to a significant increase in resistance to flow in a minority of patients. It is, therefore, possible that underlying diurnal blood flow changes could have significantly influenced the final result. In contrast, in another randomized trial (Cacciatore et al., 1998) of women on oral and transdermal HRT, the examination time was standardized between 0800 and 1000 h. No opposing effects of progestogens on oestrogen-induced decrease in uterine artery PI were demonstrated in either treatment group. Similar results have been reported by others (Lau et al., 1998; Exacoustos et al., 1999). It remains to be seen to what extent these contradictory results can be explained by the effects of physiological blood flow variations. In addition, a different study design, for example randomized with a control group, may give more information regarding the magnitude of the effect.

Blood flow measurements in the uterine and other pelvic arteries play an increasingly important role in diagnosing gynaecological pathology (Järvelä et al., 1998) and in defining the best time for embryo transfer in IVF (Battaglia et al., 1998), amongst other applications. The accuracy of blood flow measurements may be affected by factors such as the quality of the ultrasound equipment, accessibility of the vessel of interest and the experience of the operator. In addition it is important to be aware of the influence of circadian rhythms on uterine blood flow and it is imperative that all future studies control for this to enable meaningful comparisons of the results.

References


Downloaded from https://academic.oup.com/humrep/article-abstract/14/11/2716/859598 by guest on 14 February 2019


Received on May 12, 1999; accepted on August 4, 1999