Reproducible assessment of vaginal and rectal mucosal and skin blood flow: laser doppler fluximetry of the pelvic microcirculation

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ABSTRACT

Pelvic venous congestion is a common cause of chronic pelvic pain in women of reproductive age. Although this condition represents a functional disturbance of the pelvic circulation which is related to the menstrual cycle, its aetiology remains unknown. Indirect techniques demonstrate that the vasoconstrictive reflex response of the microcirculation of the foot to a rise in venous pressure is attenuated throughout the menstrual cycle. We wished to develop a simple and non-invasive direct measure of pelvic blood flow to aid diagnosis of this condition. Laser doppler blood flux measurements of the skin of the big toe and of the vaginal and rectal mucosa in the follicular and luteal phases of the menstrual cycle in 12 healthy asymptomatic premenopausal women (mean age 30 years) with regular cycles and in four healthy asymptomatic postmenopausal women (mean age 59 years) were carried out both in the supine position and in response to 40° head-up tilt. The coefficient of variation of resting vaginal flux was lower for measurements in postmenopausal women (0.04) and in premenopausal women in the follicular phase (0.07) compared with those in the luteal phase (0.16). At rest, vaginal blood flow was higher than rectal and skin flux in both premenopausal and postmenopausal women. In the follicular phase a decrease in flow was observed in response to head-up tilt in the skin (−32.0%), vagina (−34.3%) and rectum (−9.4%). In the luteal phase this reflex was attenuated at these three sites (−8.6%, +6.7% and +7.4% respectively). There were no significant reflex changes in postmenopausal women. Thus laser doppler fluximetry is a reproducible method for comparing the flux of blood in the microcirculation of the skin and of the vaginal and rectal mucosa. The skin is the least sensitive site for testing vascular reactivity in response to cyclical changes. The vaginal and rectal microcirculations are the most sensitive sites for testing visceral cyclical reactivity, and have the advantage of direct anatomical relevance. The follicular phase of the menstrual cycle is associated with greatest vascular reactivity and is the most appropriate phase during which to test for abnormal vascular responses.

INTRODUCTION

Chronic pelvic pain in women is a common complaint which affects women of childbearing age [1]. A large proportion of women with this functionally disabling and chronic pain have no visible cause found at laparoscopy [2]. Pelvic veins are deficient of valves, and as long ago as 1949 Taylor [3] suggested that dilatation of these veins may be responsible for the generation of such pain. Beard et al. [4] found that 84% of women with
We wished to develop a simple and non-invasive direct measure. We also wished to determine if the test is sensitive to changes in regulatory flow, and therefore investigated the peripheral skin, vaginal and rectal mucosal flux of blood in response to a provocative test of autonomic blood flow regulation, namely head-up tilt in healthy women during both stages of the menstrual cycle.

**MATERIALS AND METHODS**

**Subjects**
A total of 12 healthy women (mean age 30 years; range 19–42 years) with a normal menstrual cycle (mean cycle length 29 days; range 25–31 days) and no gynaecological or intestinal symptoms were studied in the follicular phase (days 6–10) and the luteal phase (days 17–22) of the cycle. Four postmenopausal women (mean age 59 years; range 56–61 years), all taking Premiap C (Wyeth Laboratories; containing premarin 0.625 mg and nor-gestrel 0.15 mg), and with no intestinal or gynaecological symptoms, were also studied. None of the subjects was taking any regular medication and none gave a history of any circulatory disturbance, in particular Raynaud’s phenomenon. Informed verbal consent was obtained from all subjects. Ethical approval to perform the study was obtained from the Northwick Park and St Mark’s Hospital Trust Ethics Committee.

**Principles and technique of laser doppler fluximetry**
We used a DRT4 laser doppler fluximeter (Moor Instruments, Axminster, Devon, U.K.), which produces a low-intensity beam of predominantly monochromatic coherent 780 nm light generated by an infrared laser diode source and delivered by a fibre optic probe. Laser doppler fluximetry measures the frequency shift in light reflected from a moving object. In tissue, red blood cells account for virtually all moving structures, and the speed of their movement determines the frequency of light that is reflected. Reflected light is detected by a photocell and the signal is processed to determine the frequency shift. As the volume under observation is constant, the volume flow in ml of blood per min per 100 g of tissue (flux) can be calculated. The approximate area of measurement is 1 mm² at 1 mm from the tip of the probe. The DRT4 instrument has an inbuilt microprocessor which calculates the flux over any desired time period. The probe also monitors the temperature of the surface under study.

**Protocol for measurement of blood flux**
Patients were examined in the left lateral position with no prior bowel preparation in a room with a constant
ambient temperature of 22 °C. Rectal examination with a rigid sigmoidoscope using minimal insufflation confirmed that the rectum was empty. The probe was then placed against the mucosa 100 mm above the lower limit of the anal margin under direct visualization after air had been released. The vaginal mucosa was visualized using a speculum, and the probe was applied 100 mm above the introitus and taped to the thigh distally. Four recordings of blood flow were made at 90° circumferentially, and the mean of these was taken as the mucosal flux. The probe was also applied to the skin over the pulp of the big toe to measure peripheral blood flow, as described by Foong [7]. The pulp of the big toe was used because this vascular bed is comparable with the pelvic circulation, since both are densely populated with arteriolar-venous shunts. Stable baseline readings were always obtained after 30 s at each vascular bed, following which a recording was taken for 30 s. Following head-up tilt, flux measurement was carried out for 30 s after tilt.

Baseline measurements
All premenopausal subjects underwent skin, vaginal and rectal blood flux measurements in the mid-follicular and in mid-luteal phases of the menstrual cycle. Measurements were made on three consecutive days in each phase of the cycle, as previously described, in the fasted state with the subject in the left lateral position [17]. On the first day rectal and vaginal flux were measured, on the second day rectal and skin flux were measured, and on the third day skin and vaginal flux were measured. Premenopausal women underwent this series of tests in both the follicular and luteal phases, while postmenopausal women underwent the series of tests once only. Thus, in each phase of the cycle, and in postmenopausal women, vaginal, rectal and peripheral skin flux of blood were each measured twice, and simultaneously.

Measurements during head-up tilt
Head-up tilt brings about an increase in venous pressure and a transient drop in systolic blood pressure, and this in turn causes a reflex increase in peripheral vascular resistance with a consequent decrease in lower limb and visceral blood flow. With the subject comfortably rested on a tiltable platform, a standard 40° tilt was completed within 2 s. Simultaneous measurements of vaginal mucosal and peripheral skin blood flux were made before and after tilt on each day.

Previous studies [7] from our group have shown that 40° head-up tilt does not normally produce significant peripheral cardiovascular effects. Only one of 15 healthy control subjects experienced a change (decrease) in blood pressure, and there were no significant changes in heart rate.

Statistical analysis
Mucosal flux data were normally distributed, and the paired t-test was used to study differences between different measurement sites and between phases of the menstrual cycle. Statistical significance was declared at the $P < 0.05$ level.

RESULTS
Reproducibility of measurements (Table 1)
The reproducibility of measurements was determined from baseline values obtained for subjects at rest, in the follicular and luteal phases for those who were cycling, and in postmenopausal women, to reduce or exclude the effects of day-to-day fluctuations in steroid hormones. The coefficient of variation was determined by comparing the blood flow values on each of three consecutive days of measurement at each of three sites of measurement. In the follicular phase, the coefficient of variation obtained from subjects ranged from 7 to 10%. In the postmenopausal subjects the coefficient of variation ranged from 4 to 8%. A coefficient of variation of 10% or less is considered satisfactory [16].

Vaginal and rectal mucosal flux was significantly more reproducible in the follicular phase than in the luteal phase, with a lower coefficient of variation ($P = 0.03$ and $P = 0.04$ respectively). Skin flux was not significantly different in different phases of the cycle. Vaginal and rectal mucosal flux was significantly more reproducible in the postmenopausal women than in premenopausal women in the luteal phase ($P = 0.03$ and $P = 0.028$ respectively), but there was no significant difference in reproducibility for skin flux between postmenopausal women and premenopausal women in the follicular phase ($P = 0.36$).

There was no significant difference in the reproducibility of flux measurement between the vaginal and rectal mucosa in either phase of the menstrual cycle or in postmenopausal women.

Baseline flux measurements (Table 1)
In all 12 premenopausal women, vaginal flux in the follicular phase was significantly higher than rectal flux ($P = 0.018$). This difference failed to reach statistical significance in the luteal phase ($P = 0.087$). However, at both sites, mean blood flow was significantly higher in the luteal phase than in the follicular phase ($P = 0.05$ and $P = 0.226$ respectively). Skin flux was unaffected by the phase of the cycle, and was lower than rectal and vaginal blood flow in all patients in both the follicular ($P = 0.006$) and ($P = 0.012$) luteal phases.

Postmenopausal women had significantly lower vaginal ($P = 0.022$), rectal ($P = 0.005$) and skin ($P =
Table 1  Flux at different sites in each menstrual phase

CV, coefficient of variation. Median values are shown, with ranges in parentheses. Significance of differences: *P < 0.05 compared with vaginal blood flow in the follicular phase; †P < 0.05 compared with vaginal blood flow in postmenopausal women.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Vaginal mucosa</th>
<th>Rectal mucosa</th>
<th>Skin flux</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Flux CV</td>
<td>Flux CV</td>
<td>Flux CV</td>
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<tr>
<td>Pre menopausal</td>
<td></td>
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</tr>
<tr>
<td>Mid-follicular (n = 12)</td>
<td>225.5 (185–252)</td>
<td>0.07</td>
<td>175.8* (144–204)</td>
</tr>
<tr>
<td>Mid-luteal (n = 12)</td>
<td>248.3 (164–297)</td>
<td>0.16</td>
<td>216.6 (153–290)</td>
</tr>
<tr>
<td>Postmenopausal (n = 4)</td>
<td>155.8* (144–160)</td>
<td>0.04</td>
<td>134.3† (121–139)</td>
</tr>
</tbody>
</table>

Figure 1  Example of flux recordings from a 25-year-old subject in the follicular phase of the menstrual cycle

Shown are measurements of the vaginal (upper trace) and skin (lower trace) microcirculations at baseline and in response to head-up tilt (arrow). Each horizontal division represents 4 s, with data being dot-plotted every 0.05 s (i.e. 20 values per s). The trace shows the characteristic ‘saw-tooth’ variability of the trace observed both at baseline and after equilibration following tilt. In Figure 2 the change in vascular response has been compared between phases of the menstrual cycle for each patient in each of the three vascular beds; these changes are expressed as the percentage change in flux relative to the baseline values for each subject, obtained in the supine position. A negative sign indicates a decrease in flux and a positive sign an increase in flux. Figure 2 shows that a decrease in flux in response to head-up tilt was most pronounced in the follicular phase in premenopausal subjects, with all 12 women showing such a response in the circulation of the skin, 11 in vaginal flow and nine in rectal flow. The magnitude of this decrease was from a mean of 123.6 to 81.4 (32.0%) in the skin, from 225.5 to 144.2 (34.3%) in the vagina and from 175.8 to 157.6 (9.4%) in the rectum. The percentage decrease was not significantly different between the skin and vaginal sites (P = 0.62), and was greater in both of these sites compared with the rectal mucosal flux (P = 0.003 and P = 0.002 respectively).

Flux changes in response to head-up tilt

Figure 1 shows a typical trace for a patient at baseline and then following the induction of 40° head-up tilt. It is evident that there was an instantaneous response to tilt. This immediate decrease in flux was more marked in the vaginal microcirculation than in the skin. We feel that the acuteness of the response renders it unlikely that it is due to local or paracrine factors and rather that it is likely, in part at least, to reflect venous flow. Figure 1 shows the characteristic ‘saw-tooth’ variability of the trace observed both at baseline and after equilibration following tilt.

In contrast with the change in flux in the follicular phase, the changes in flux in the luteal phase in response to head-up tilt were more variable. While a decrease in flux in the skin was seen in nine of the 12 subjects, in the vagina in four subjects and in the rectum in one subject, in individual subjects the decrease was significantly less than that observed in the follicular phase. There was also a significant difference in the mean change in flux in the skin compared with the vaginal and rectal mucosal circulations (P = 0.041 and P = 0.047 respectively). The mean proportional change in flux in response to head-up tilt was significantly different in the skin compared with that seen in the pelvic organs, with a mean decrease of 8.6 % in the skin compared with mean increases of 6.7 % and 7.4 % in the vaginal and rectal circulations respectively (P = 0.033 and P = 0.44 for skin compared with vaginal and rectal flux respectively). None of the four postmenopausal women showed a significant change in flux compared with premenopausal women in both the follicular phase and the luteal phase (P = 0.034, P = 0.016 and P = 0.044 respectively).
blood flow from the baseline values in response to head-up tilt (skin, −3.1%; vagina, +2.2%; rectum, +2.7%).

DISCUSSION

The present study has shown that laser doppler fluximetry provides a reliable method for studying flux in the peripheral circulations of the skin, vagina and rectum, both at rest and in response to a change of posture. This means that, in the future, it should be possible to obtain reproducible recordings simultaneously from these sites and from the bladder [18]. While we acknowledge that the technique accurately measures microcirculatory flux rather than venous flow, we feel that the instantaneous response to tilt is unlikely to be a reflection of local or paracrine factors and is likely, in part at least, to reflect upon venous flow.

The study has also laid the basis for an improved understanding of the influence of human cyclical ovarian activity on pelvic and peripheral flux. In the absence of ovarian activity, as is the case in postmenopausal women, peripheral blood flow is relatively lower at rest than that found in premenopausal women. It is also much less responsive to a rise in venous pressure induced by head-up tilt. In contrast, premenopausal women have greater flow at rest in the skin and the pelvis in both phases of the cycle. The flow rate and variability between subjects increase in the luteal phase compared with the follicular phase of the cycle.

Peripheral vascular reflexes are known to be attenuated and blood flow altered by ovarian hormones, and this is best exemplified in the menstrual cycle. In the follicular phase there was a consistent tendency for a decrease in flux in response to a rise in venous pressure at all three sites. In contrast, in the luteal phase the response was much more variable.

The changes in peripheral flux that we have observed in relation to the ovarian cycle are likely, at least in part, to be due to the secretion of oestrogen and progesterone. Both of these hormones are strongly vasoactive, but their effects are often difficult to interpret, with variations depending on the site of action, duration of exposure and species in animal studies. In studies of the human menstrual cycle, the vasoconstriction in the skin that is a
constant response to leg lowering during the follicular phase of the menstrual cycle is attenuated during the luteal phase [8]. Bungum et al. [19] showed that the extent of the attenuation is correlated with the oestrogen/progesterone ratio. The mechanism by which attenuation occurs is not clear. Oestrogen is a potent dilator of smooth muscle, but this effect is likely to be mediated through vasoactive hormones. Catecholamines increase directly in relation to the phase of the cycle, being highest in the luteal phase [20]. This increase appears to be confined mainly to noradrenaline, the concentration of which is correlated with that of plasma oestriadiol [21]. Other possible mediators of changes in vascular responsiveness are acetylcholine [22], endothelins such as calcitonon-gene-related peptide [23], prostaglandins [24] and nitric oxide [25].

In the present study, simultaneous measurement of peripheral flux at rest has shown that vaginal mucosal flux is greater than that of the rectal mucosa, which in turn is greater than that of the skin. However, the stage of the cycle influences mucosal blood flow at rest and in response to head-up tilt in the vagina and rectum, but not in the skin. Differences in median baseline flux at rest between the three sites are more evident during the follicular phase than the luteal phase, and do not achieve statistical significance because of the marked variability of the flux during the luteal phase. The absence of cycle-related changes in the skin at rest suggests that, in contrast with flux in the vagina and rectum, this circulatory bed is less sensitive to the hormonal milieu and more dependent on other factors, such as surface temperature and local vasoactive substances. This view is supported by the markedly higher concentration of oestriadiol in pelvic venous blood compared with peripheral blood [26]. However, the finding of some cycle-related attenuation of peripheral vasoconstriction in the skin in response to head-up tilt suggests that, although the influence of ovarian hormones may not be so apparent at this site, it is still present.

In conclusion, laser doppler fluximetry offers a highly reproducible method of obtaining a measure of vaginal and rectal blood flow and has considerable potential for investigating a number of pathologies of the pelvic organs, such as inflammatory conditions of the bowel and pelvic congestion. The vaginal and rectal microcirculations are the most sensitive sites for testing visceral cyclical reactivity, and have the advantage of direct anatomical relevance. The study also confirms the importance of taking into account the phase of the menstrual cycle when studying any form of pelvic pathology, whether the cycle is suppressed (as occurs in women taking the contraceptive pill) or not. The results of our study also suggest that, when conducting peripheral blood flow studies, the follicular phase is the most appropriate time, as vascular reactivity is more stable and predictable than in the luteal phase.

**REFERENCES**

10. Stones, R. W., Loesch, A., Beard, R. W. and Burnstock, G. (1995) Substance P: endothelial localisation and responsiveness are acetylcholine [22], endothelins such as calcitonon-gene-related peptide [23], prostaglandins [24] and nitric oxide [25].


Received 25 March 1999/3 September 1999; accepted 25 October 1999