

Skin tissue water and laser Doppler blood flow during a menstrual cycle

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Summary

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Previous work offers conflicting evidence concerning whether basal skin blood flow (SBF) varies with the menstrual cycle. Our goal was to determine the extent of cycle-dependent changes in forearm SBF parameters and if they are linked to local tissue water content and arm volume changes. Both arms of 10 premenopausal women were evaluated three times during their cycle (days 4, 12 and 22) and 10 postmenopausal women were evaluated once. Each evaluation included laser Doppler blood flow parameters (perfusion, volume and velocity), skin temperature, arm volume and local tissue water determined by a new tissue dielectric constant method. Estradiol and progesterone concentrations were determined at each visit by using saliva samples and analysis showed the expected pattern of variation over the cycle. Main results showed no significant cycle-dependent variations in any SBF parameter, temperature, tissue water or arm volume. Postmenopausal women had significantly less estradiol levels, but did not differ with respect to premenopausal women in any measured parameter except for a slightly less blood velocity ($P < 0.01$). We hypothesize that the absence of changes in tissue water and arm volume in our group may account for the constancy of the measured SBF parameters. However, in other populations, hormonally induced tissue changes may occur and contribute to the cycle-dependent changes in resting blood perfusion that have been reported. This possibility suggests that it may be useful to measure and report a skin tissue water index when serial changes in blood perfusion are being investigated.

Introduction

Previous work has provided conflicting evidence concerning whether skin blood flow (SBF) in limbs of premenopausal women depends on the menstrual cycle phase in which the measurements are made (Hessemer & Bruck, 1985; Bartelink et al., 1990; Hassan et al., 1990; van Beek et al., 1996; Bungum et al., 1996; Cankar et al., 2000; Charkoudian & Johnson, 2000; Inoue et al., 2005; Kuwahara et al., 2005). As there is some evidence that there are cycle-dependent changes in skin water loss (Harvell et al., 1992) and skin thickness (Eisenbeiss et al., 1998), we considered the possibility that differences in skin tissue water and limb volume may play a role in possible cycle-dependent changes in SBF. Further, it may be that in some settings and conditions tissue water changes may be present and have an impact on the measured laser Doppler values. For example, it has been reported that the oedema can modify laser Doppler perfusion but that therapy that reduces oedema is then associated with an increase in SBF (Kalus et al., 2004). Also, reductions in arm oedema in patients with lymphedema are

associated with an increase in arm skin perfusion (Brorson & Svensson, 1997). There is also evidence that measured perfusion values are influenced by factors that effect the so called biological zero (BZ) (Abbot & Beck, 1993). For example, in patients with unilateral arm oedema secondary to therapeutic mastectomy for breast cancer, the swollen arm has a significantly higher BZ and reduced perfusion when compared with the normal arm (Stanton et al., 1996). Thus, our goal was to determine the extent of cycle-dependent changes in forearm SBF parameters and determine if they are linked to local tissue water content and arm volume changes.

Methods

Subjects

Twenty women were evaluated after signing an Institutional Review Board approved informed consent. Ten women were premenopausal (26.2 ± 3.9 years), who were not taking any hormonal form of birth control and had regular menstrual

cycles and 10 women were postmenopausal (60 ± 6.7 years), who were not taking any form of hormone replacement therapy. Entry requirements for all subjects were that they had not had any previous surgery or serious trauma to either arm, they did not have diabetes, they were not taking any vasoactive medications and they were in self-reported good health. No subject was a current smoker.

Protocol

Premenopausal women were evaluated three times during their monthly cycle at 4, 12 and 22 days after the start of their menses. These times normally correspond to low levels of oestrogen and progesterone (day 4, early follicular phase), high levels of oestrogen and low levels of progesterone (day 12, late follicular-ovulation phase) and elevated levels of both oestrogen and progesterone (day 22, mid-luteal phase). Hormonal status was confirmed retrospectively by using saliva assays taken on each evaluation day. Postmenopausal women were evaluated once. Each evaluation included the following measurements.

Skin temperature and blood flow

Subjects were comfortably seated with their arms supported on a padded surface with their palms facing upward. A standardized site located 7-cm distal to the antecubital crease was identified and marked on each arm. Skin temperature was measured and recorded by using an infrared thermometer (Model DX-501-RS; Exergen, Watertown, MA, USA) (<http://www.exergen.com>).

Laser Doppler probes (Vasamedics, model P50 right angle; St. Paul, MN, USA) were then placed on each target site and secured in place with double sided tape. Laser Doppler flow-related parameters were then recorded simultaneously for 10 min from both arms (Vasamedic Monitor BPM2) by using a time constant of 60 s and a cut-off frequency of 14.7 KHz.

The theory and applications of laser Doppler blood flow measurements is well described in the literature (Nilsson et al., 1980a; Nilsson et al., 1980b; Oberg et al., 1984; Mayrovitz, 1998). Briefly, a low intensity laser light signal is transmitted into the skin to a depth of about 1–2 mm (Jakobsson & Nilsson, 1993) and the Doppler-shifted reflected light contains information about the speed (velocity) and number density (volume) of moving red blood cells in the tissue. Velocity and volume signals are processed to yield a parameter called either red cell perfusion or red cell flux that is proportional to blood flow.

Each parameter (perfusion, volume and velocity) was recorded and averages over the 10-min sampling interval were used as indices of the prevailing skin red blood cell perfusion, volume and velocity. Each probe was calibrated in its associated channel by using a motility standard supplied by the manufacturer. In addition, at weekly intervals, outputs of each probe-channel combination were tested by using a rotating disk (two revolutions per minute) with imbedded, randomly arranged particles, to simulate moving cells. The probe-channel

system output was monitored for 4 min and its mean output averaged. This procedure was used to detect the possible long-term temporal changes in system gain and to verify correspondence between probe-channel sensitivities. During the course of the overall experimental program, changes were found to be <5% over time and between probes.

As laser Doppler does not measure blood flow directly, it is common to express the parameter outputs in relative units. For ease of possible comparison with other studies, these are here given in the exact units displayed by the instrument monitor. These units are ml/min/100 g, % rbc mm^{-3} and mm s^{-1} for the perfusion, volume and velocity signals, respectively. At the end of each experiment, the BZ, which is a measured non-zero laser Doppler signal despite an apparent no-flow condition (Colantuoni et al., 1993; Mayrovitz & Leedham, 2001), was determined by placing a blood pressure cuff around each upper arm and inflating the cuff to suprasystolic pressure for 2 min. The minimum perfusion value during this interval was taken as the BZ and during analysis subtracted from the raw averages as has been recommended (Wahlberg et al., 1992; Abbot & Beck, 1993; Kernick et al., 1999). In the present case, the BZ values obtained were always <5% of the average non-adjusted perfusion values.

Local tissue water measurement method

Local tissue water was measured with a commercial device (MoistureMeter-D; Delfin Technologies Ltd, Kuopio, Finland) (<http://www.delfintech.com>). It consists of a probe connected to a control unit that displays the tissue dielectric constant (TDC) when the probe is placed in contact with the skin. The physics and principle of operation has been well described (Nilsson et al., 1980b; Stuchly et al., 1981; Aimoto & Matsumoto, 1996; Alanen et al., 1998a; Alanen et al., 1998b; Alanen et al., 1999). In brief, a 300-MHz signal is generated within the control unit and is transmitted to the tissue via the probe that is contact with the skin. The probe itself acts as an open-ended co-axial transmission line (Stuchly et al., 1982; Aimoto & Matsumoto, 1996; Alanen et al., 1998a). The portion of the incident electromagnetic wave that is reflected depends on the dielectric constant of the tissue, which itself depends on the amount of free and bound water in the tissue volume through which the wave passes. The reflected wave information is processed within the control unit and the relative dielectric constant is displayed. Pure water has a value of about 80 and the display scale range is 1–80. The effective penetration depth depends on the probe dimensions, with larger spacing between inner and outer concentric conductors corresponding to greater penetration depths. In the present study, four different dimension probes were used with effective penetration depths of 0.5, 1.5, 2.5 and 5.0 mm. The corresponding (maximum) probe diameters were 10, 20, 23 and 55 mm with inner-outer conductor spacing of 1, 3, 5 and 17 mm, respectively. Validation studies using this device have been reported (Nuutinen et al., 1998; Nuutinen et al., 2004) and its specific

application to the measurement of forearm local tissue water has been described (Mayrovitz, 2006a).

Local tissue water measurement procedure

With the subject seated and arms positioned as with the blood flow measurements, the probes were placed in contact with the skin and held in position for 10 s using gentle pressure. For each probe, measurements were obtained in triplicate-pairs. The first pair was done by measuring one arm and then, immediately after, measuring the other arm. This procedure was repeated twice more for each probe. The order of measurement was from smallest to largest probe with a 1-min wait between changing probes. The time required to obtain a single measurement, once the probe was placed in contact with the skin, was 10 s. Preliminary work showed that repeated measurements taken at 15 s intervals for 600 s resulted in a coefficient of variation of only 2.8% indicating a good short-term repeatability of the technique (Mayrovitz, 2006b).

Arm volumes

Arm circumferences were measured at 4-cm intervals from the wrist to the axilla. Measurements were made by using a tape measure that had a calibrated spring loaded tension to insure uniform pull during circumference measurements (Gulick tape measure; Fitness Wholesale Stow, OH, USA) (<http://www.fwonline.com/gtape.htm>). Circumference values were entered into a software program that automatically calculated segmental and total arm volumes (LVP4.0; Bioscience Research Institute, Ft. Lauderdale, FL, USA) (<http://www.limbvolumes.org>). These volume calculations are based on the frustum geometric model of the arm, which has been shown to be a valid and accurate method to obtain limb volumes (Mayrovitz et al., 2000; Sander et al., 2002; Karges et al., 2003; Mayrovitz, 2003). In addition to overall arm volumes, the circumferences at the specific site at which tissue water and blood flow parameters were measured were also measured and recorded.

Hormone determinations

On the day of each visit, a saliva sample was taken and submitted to a commercial laboratory (Aeron Clinical Laboratory, San Leandro, CA, USA) (<http://www.aeron.com>) for assessment of estradiol and progesterone concentrations via radioimmunoassay. The use of saliva samples as a non-invasive index of non-bound estradiol and progesterone and the sensitivity, reliability and correlations with serum levels has previously been reported (Lu et al., 1997; Lu et al., 1999). More recently, this method has been used to characterize salivary levels of estradiol and progesterone in a large group of premenstrual women throughout the menstrual cycle (Chatterton et al., 2005). Estradiol and progesterone concentrations obtained via saliva sample analysis reflect free hormone so values measured from saliva samples are less than those measured from serum. However, estradiol values

are correlated with serum concentrations (Evans et al., 1980; Lu et al., 1999) and so are saliva progesterone values (Lu et al., 1997).

Analysis

Tests for overall changes within the menstrual cycle for arm volume, circumference, skin temperature and blood flow parameters, tissue water and hormone concentrations were done by using a general linear model (GLM) for repeated measures with visit as the repeating factor (SPSS, version 9.0; Chicago, IL, USA). Tests for differences between paired arms at each visit was done by using a paired *t*-test. Tests for differences between postmenopausal and premenopausal women were done using a *t*-test for independent samples. For these postpremenopausal comparisons, the premenopausal comparison values were those at visit 1 as hormonal concentrations during this early luteal phase corresponds most closely to postmenopausal hormone concentrations. Tests for differences among TDC vs. measurement depth were done by using a GLM for repeated measures with depth as the repeating factor. In all analyses, a minimum *P*-value to infer statistical significance was <0.05.

Results

Arm volumes and circumferences

Arm volumes and circumferences at the target sites did not significantly differ between arms at any visit nor were there any significant differences in volume nor circumference among visits for the premenopausal group (Table 1). Comparing premenopausal visit 1, arm volumes (2030 ± 459 ml) and circumferences (23.6 ± 2.4 cm) with postmenopausal volumes (2197 ± 601 ml) and circumferences (23.8 ± 2.4 cm) revealed no significant differences between groups for either parameter ($P > 0.10$).

Laser Doppler blood flow-related parameters

Neither skin temperature nor laser Doppler blood perfusion, volume nor velocity differed significantly between arms at any visit for either group nor were there any significant differences in these parameters among visits for the premenopausal group (Table 1). Comparing premenopausal visit 1, flow parameters with postmenopausal values revealed a significantly smaller blood velocity component for the postmenopausal group (0.64 ± 0.19 vs. 0.80 ± 0.16 , $P = 0.009$). Overall blood perfusion tended to be less in the postmenopausal group, but the difference was not statistically significant (0.94 ± 0.40 vs. 1.15 ± 0.40 , $P = 0.12$). Values for the volume parameter were similar for both groups (0.44 ± 0.14 vs. 0.42 ± 0.15 , $P = 0.773$).

Tissue water via tissue dielectric constant

Tissue dielectric constant at any measurement depth did not significantly differ between arms at any visit nor were there any

Table 1 Volumes, skin temperatures and laser Doppler flow parameter summary.

	Premenopausal						Postmenopausal	
	Visit 1 (day 4 of cycle)		Visit 2 (day 12 of cycle)		Visit 3 (day 22 of cycle)		Visit 1	
	Dominant arm	Other arm	Dominant arm	Other arm	Dominant arm	Other arm	Dominant arm	Other arm
Arm volume (ml)	2033 ± 481	2027 ± 463	2025 ± 502	1997 ± 476	1996 ± 495	1989 ± 466	2204 ± 642	2190 ± 631
Circumference (cm)	23.8 ± 2.5	23.5 ± 2.4	23.9 ± 2.6	23.5 ± 2.4	23.9 ± 2.7	23.6 ± 2.6	24.1 ± 2.4	23.5 ± 2.5
Skin temperature (°C)	31.9 ± 1.3	32.1 ± 1.3	31.6 ± 1.0	31.7 ± 1.0	31.9 ± 0.8	31.9 ± 0.8	31.2 ± 0.8	31.4 ± 0.8
Laser Doppler								
Perfusion (ml/min/100 g)	1.14 ± 0.41	1.16 ± 0.40	1.22 ± 0.52	1.12 ± 0.46	1.24 ± 0.61	1.14 ± 0.66	0.93 ± 0.52	0.95 ± 0.56
Volume (% rbc mm ⁻³)	0.40 ± 0.15	0.45 ± 0.17	0.48 ± 0.18	0.43 ± 0.09	0.50 ± 0.19	0.45 ± 0.11	0.45 ± 0.16	0.43 ± 0.14
Velocity (mm s ⁻¹)	0.86 ± 0.16	0.75 ± 0.15	0.76 ± 0.19	0.75 ± 0.19	0.76 ± 0.34	0.73 ± 0.32	0.61 ± 0.26*	0.67 ± 0.23*

Arm volumes, circumferences, skin temperatures and laser Doppler values did not significantly differ between arms at any visit nor were there any significant differences among visits for the premenopausal group. Except for blood velocity, postmenopausal values did not significantly differ from premenopausal values ($P>0.10$). *Significantly less ($P<0.01$) than corresponding value for visit 1 of the premenopausal group. Data values are mean ± SD.

significant differences in TDC among visits for the premenopausal group (Table 2). TDC values of the postmenopausal group did not significantly differ from the premenopausal values ($P>0.10$) for any measurement depth. For all visits, TDC values at a 5.0-mm measurement depth were significantly less than for all other depths (** $P<0.001$).

Discussion

Tissue water and arm volumes

There have been no previous studies that have investigated possible changes in local tissue water and arm volumes occurring during the normal menstrual cycle. This issue is important because of the possible impact that such changes, if present, could have on skin blood perfusion parameters and

other arm skin physiological measures. The present results show that neither arm volume nor local tissue water changes occur to any significant degree. Thus, although there are reports of cycle-dependent changes in skin thickness (Eisenbeiss et al., 1998) and skin water loss parameters (Harvell et al., 1992), these changes either did not occur in our study population or such changes if present do not significantly effect tissue water and arm volume.

The average values of the TDC, which reflect the relative tissue water content, were similar up to tissue depths of 2.5 mm although there appeared to be a trend for reduced TDC values with depth. The value obtained at 5 mm was in fact significantly less than all other values. Such depth dependence is consistent with the known variation in tissue constituents and their water content with depth below the skin surface. As effective measurement depth is determined by the depth of electro-

Table 2 Tissue dielectric constant (TDC) and hormone concentrations.

TDC	Premenopausal						Postmenopausal	
	Visit 1 (Day 4 of cycle)		Visit 2 (Day 12 of cycle)		Visit 3 (Day 22 of cycle)		Visit 1	
	Dominant arm	Other arm	Dominant arm	Other arm	Dominant arm	Other arm	Dominant arm	Other arm
0.5 mm	27.7 ± 3.6	27.8 ± 3.9	27.8 ± 2.7	27.5 ± 2.9	26.8 ± 3.4	25.8 ± 4.0	28.1 ± 4.9	27.2 ± 3.8
1.5 mm	26.6 ± 2.5	27.3 ± 2.2	26.8 ± 2.8	27.0 ± 2.4	25.7 ± 2.9	25.7 ± 2.7	26.5 ± 3.3	26.3 ± 2.5
2.5 mm	25.6 ± 3.3	26.1 ± 3.2	25.6 ± 3.0	25.8 ± 3.0	25.2 ± 2.8	25.2 ± 2.7	24.9 ± 4.0	24.9 ± 3.9
5.0 mm	20.8 ± 3.8**	21.4 ± 3.2**	21.5 ± 3.9**	21.3 ± 3.7**	21.3 ± 4.1**	21.1 ± 4.1**	20.6 ± 4.6**	20.5 ± 3.4**
Hormones	(pmol L ⁻¹)		(pmol L ⁻¹)		(pmol L ⁻¹)		(pmol L ⁻¹)	
Estradiol	5.7 ± 1.5		11.8 ± 6.8 [†]		6.8 ± 4.6		3.4 ± 1.0 [‡]	
Progesterone	105.7 ± 35.9		138.3 ± 64.4		266.8 ± 220.4 [§]		86.7 ± 21.5	

Tissue dielectric constant did not significantly differ between arms at any visit nor were there any significant differences in TDC among visits for the premenopausal group. TDC of the postmenopausal group did not differ from premenopausal ($P>0.10$). TDC values at a 5.0-mm measurement depth were less than for all other depths (** $P<0.001$). Estradiol concentration at visit 2 was greater than for visits 1 and 3 ([†] $P<0.05$). Progesterone concentrations at visit 3 were greater than for visits 1 and 2 ([§] $P<0.05$). Estradiol concentration was less for postmenopausal women ([‡] $P<0.01$). Data values are mean ± SD.

magnetic field penetration (Lahtinen *et al.*, 1997), larger diameter probes result in an increased effective measurement depth. Thus, net TDC values are increasingly influenced by deeper tissue constituents such as s.c. fat and its lower relative water content (Alanen *et al.*, 1999).

Blood flow parameters

The present findings do not show a significant change in laser Doppler SBF parameters over the monthly menstrual cycle in the young premenopausal group of women studied. This was true for overall skin blood perfusion and for the individual volume and velocity components. Further, perfusion values obtained in premenopausal women did not differ from those obtained from a control group of postmenopausal women.

These perfusion findings are consistent with some but in contrast to other reported results. Bungum *et al.* (1996) evaluated resting laser Doppler perfusion in the forearm during the follicular (days 2–7) and luteal (days 19–24) phases and reported no difference in perfusion between these phases. In contrast, van Beek *et al.* (1996) measuring during the same cycle phases reported a lower perfusion during the luteal phase. A reduced luteal phase perfusion of finger skin was also reported by Bartelink *et al.* (Bartelink *et al.*, 1990) in comparison with perfusions measured during the pre-ovulatory phase of the menstrual cycle. However, in contrast to this, Cankar *et al.* (2000) reported no difference in resting SBF of fingers between follicular and luteal phases. All of these previous studies reported only on the perfusion values and not the individual volume and velocity components of the laser Doppler measurement. Thus, the present study appears to be the first that shows that no laser Doppler flow parameter is cycle dependent. However, a small but significant reduced velocity component was found in the postmenopausal women. Although we have insufficient data to definitively explain the slightly reduced velocity component, there are several possibilities worthy of consideration. The reduced blood velocity component may be due to sampling at a slightly deeper skin depth due to naturally occurring thinning of aging skin. If so, the measured velocity component may reflect signals originating in wider venous vessels that are more abundant at a deeper level but in which the blood velocity would tend to be less than in small arterioles closer to the surface in younger women. Another possible explanation is that the slightly reduced blood velocity in the older group of women reflects an overall slightly larger diameter of the blood vessels sampled over the complete sampled volume. If this were the case, even similar blood flows would register a lesser blood velocity in the larger size vessels. As we found a tendency for overall blood perfusion to be slightly less in the older women, even a slightly overall larger diameter of sampled vessels would seem sufficient to account for the reduced velocity.

An additional relevant finding was that neither TDC nor basal SBF parameters significantly differed between dominant and non-dominant arms in either pre- or postmenopausal women. This finding complements previous work, which demonstrated

that vasoconstrictive blood perfusion responses do not differ between arms of persons with normal arm vasculatures (Mayrovitz & Groseclose, 2005). Another consequence of this finding is that use of the contralateral arm as a control in paired arm laser Doppler perfusion studies is likely justified at any time point in the menstrual cycle.

A question that still remains is what accounts for the different reported findings with respect to changes in SBF over the menstrual cycle. We cannot explain this based on the present data. In the way of speculation, we would suggest that in some women hormonally induced changes in tissue water might be present and alter the BZ and thereby directly or indirectly alter the measured perfusion. In the present case, we did not find evidence of such changes in the arm, but in other populations such changes may occur and contribute to cycle-dependent changes in resting blood perfusion. This possibility suggests that it may be useful to measure and report indices of skin tissue water when serial changes in blood perfusion are being investigated.

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