

# Changes in skin blood flow during the menstrual cycle: the influence of the menstrual cycle on the peripheral circulation in healthy female volunteers

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## SUMMARY

1. It is known that females have a lower skin perfusion than males. In women there are also differences in blood flow at different reproductive stages of their lives. As an initial investigation of the possible contribution of sex hormones to these differences, we studied skin and forearm blood flow during the natural changes in hormone levels which occur during the menstrual cycle.

2. Thirty-one healthy female volunteers were studied. The effect of a standardized finger cooling test (immersion of a gloved hand in a 16°C water bath) on finger skin temperature and on laser Doppler flux in the finger, and forearm blood flow (strain gauge venous occlusion plethysmography) was assessed at four different times during one cycle: during menstruation, 1 day before ovulation, 2 days after ovulation and at the mid-luteal phase. Test days were determined by daily measurements of basal body temperature and were confirmed afterwards by determinations of serum luteinizing hormone, follicle-stimulating hormone, 17 $\beta$ -oestradiol and progesterone.

3. Peripheral skin circulation varied significantly within one menstrual cycle. The extremes were a mean finger skin temperature of 25.9  $\pm$  3.0°C in the luteal phase compared with 28.4  $\pm$  3.7°C in the pre-ovulatory phase ( $P=0.002$ ). The respective values for the mean laser Doppler flux were 18.4  $\pm$  10.9 compared with 29.2  $\pm$  16.4 arbitrary units ( $P=0.003$ ).

4. Baseline forearm muscle blood flow also varied significantly ( $P=0.04$ ) within one menstrual cycle, with low values in the menstrual phase compared with the other phases.

5. In conclusion, we have shown that peripheral skin circulation and forearm muscle blood flow exhibit significant variability during the hormonal changes in a menstrual cycle.

**Key words:** menstrual cycle, peripheral circulation, Raynaud's phenomenon, sex hormones.

**Abbreviations:** DBP, diastolic blood pressure; FBF, forearm muscle blood flow; FCT, finger cooling test; FSH, follicle stimulating hormone; FST, finger skin temperature; FVR, forearm vascular resistance; HR, heart rate; LDF, laser Doppler flux; LH, luteinizing hormone; MAP, mean arterial pressure; SBP, systolic blood pressure.

## INTRODUCTION

There are several observations indicating that sex hormones affect peripheral blood flow. Healthy women, in their fertile phase of life, show up to 50% lower peripheral skin blood flow than men, whereas there is no difference between the sexes before the menarche and after the menopause [1, 2]. The severity and frequency of cold-induced vasospastic attacks and the measured skin blood flow vary within the different phases of the menstrual cycle [3-6]. Disturbances in the peripheral circulation are not equally distributed between the sexes either. Vasospastic diseases, such as Raynaud's phenomenon, migraine and variant angina, are more common in women, whereas men more often suffer from atherosclerotic vascular diseases. In primary Raynaud's phenomenon, the female/male ratio ranges between 2 and 9 [3, 7-9]. The prevalence varies from 5% up to 20% in otherwise healthy females [7, 10-12]. The onset of vasospastic complaints shortly after the menarche [3, 8], the improvement in symptoms after the menopause [7, 10]

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and the decrease in frequency and severity of the attacks during pregnancy, all suggest the influence of female sex hormones. Moreover, it is suggested that oral contraceptives worsen the complaints [13, 14].

Unfortunately there are no studies showing a correlation between naturally occurring levels of sex hormones and changes in blood flow. We investigated the possible correlation between the level of sex hormones and peripheral blood flow by measuring vascular reactivity during the normal changes in hormone levels which occur during the menstrual cycle of healthy women.

## EXPERIMENTAL

### Subjects

We selected 31 healthy female volunteers, 15–45 years of age, with a regular menstrual cycle of between 26 and 32 days during the previous 4 months. None used medications or oral contraceptives, and no symptoms of Raynaud's phenomenon were present. They all gave informed consent to the protocol, which was approved by the hospital ethical committee.

### Methods

On four occasions within one menstrual cycle, a provocative finger cooling test (FCT) was performed in order to quantify cold-induced vasoconstriction. Previously, we have demonstrated that finger skin temperature (FST) recovery in this test has a good reproducibility if room temperature and other factors influencing the finger perfusion are standardized [15]. For this reason, all volunteers were asked to abstain from smoking for 24 h, from caffeine- and alcohol-containing beverages for 12 h and to fast for at least 2 h before the test. All FCTs were performed at the same time of the day, in a climate room (ambient temperature  $24.7 \pm 0.3^\circ\text{C}$ ; humidity  $59 \pm 2\%$ ) with the subjects in a comfortable supine position, their arms at heart level. After an acclimatization period of at least 20 min, the following measurements were performed. Systolic (SBP) and diastolic (DBP) blood pressure (in mmHg) were measured in the left arm by Arteriosonde (Roche Medical Electronics Inc., Oranjestad, NJ, U.S.A.) [16] and heart rate (HR; in beats/min) was calculated from an electrocardiogram strip. Forearm muscle blood flow (FBF; in  $\text{ml min}^{-1} 100 \text{ ml}^{-1}$ ) was measured at the left forearm by strain gauge venous occlusion plethysmography (Loosco BVP 96; Hoekloos, Amsterdam, The Netherlands), using a wrist-cuff (inflated to 40–50 mmHg) to exclude the venous return from the hand. FST (in  $^\circ\text{C}$ ) was measured on the second volar fingertip of the right hand (Thermocouple; Ellab Instruments, Copenhagen, Denmark). Laser Doppler flux (LDF; in arbitrary units) was measured on the third volar fingertip of the right hand (Periflux Pf-Id; Perimed, Stockholm, Sweden) after zero calibration. For all measurements the average of three pre-test values obtained after the acclimatization period was used as the baseline value. After this, the gloved right hand was immersed in a water bath at  $16^\circ\text{C}$  for 5 min, during which FST and LDF were

recorded every minute. During a recovery period of 20 min FST and LDF were measured every 2 min, whereas FBF, HR, SBP and DBP were measured separately every 10 min.

The first FCT took place on the second or third day of the cycle, during menstruation (menstrual phase). The second FCT was performed 1 day before the expected day of ovulation (pre-ovulatory phase). The third test was carried out 2 days after ovulation (post-ovulatory phase) and the last FCT took place 8 days after ovulation, in the mid-luteal phase. The menstrual phase exhibits low values of all the sex hormones measured. In the pre-ovulatory phase  $17\beta$ -oestradiol levels are at their peak, progesterone levels are slightly rising and levels of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are increasing. After ovulation, in the post-ovulatory phase,  $17\beta$ -oestradiol levels are falling, progesterone levels are still rising and levels of both LH and FSH are low. In the mid-luteal phase  $17\beta$ -oestradiol levels are again at their peak, progesterone levels are at their peak and levels of both LH and FSH are still low (see Table 1). The duration of previous menstrual cycles was used to determine the days on which an FCT should be performed. It was assumed that the luteal phase had a duration of 14 days and that ovulation took place 14 days before the first day of the next menstruation. All women recorded their basal body temperature during the investigation cycle starting on the first day of the menstrual period. Rectal temperature (during at least 3 min) was measured each morning at the same time, before leaving the bed. The last low point in the temperature curve before the rise towards a hyperthermic plateau started, was considered the day of ovulation. In the case of a difference between the expected day of ovulation and the recorded basal body temperature, extra FCTs were carried out.

On the days on which an FCT was performed, blood was sampled by venepuncture of the antecubital vein for determination of the serum levels of FSH, LH,  $17\beta$ -oestradiol and progesterone. The samples were frozen ( $-20^\circ\text{C}$ ) and the hormone levels were determined later by radioimmunoassay [17, 18]. After the investigation cycle, the available hormonal data and basal body temperature charts were submitted to an independent, experienced observer, who verified the phase of the cycle.

To control for a number of other influences on skin blood flow that might explain the differences in peripheral blood flow between the sexes, we also determined the body weight (in kg), the height (in m) and calculated the amount of subcutaneous fat (in %) by measuring the skin-fold thickness at four standardized places of the body [19]. The hand volume was determined (in ml) by immersing the right hand up to the wrist in a fixed amount of water.

The mean arterial pressure (MAP; in mmHg) was calculated from the formula  $\text{MAP} = (\text{SBP} + 2\text{DBP})/3$ , and the forearm vascular resistance (FVR; in arbitrary units) was obtained by dividing MAP by FBF. The Quetelet index was calculated by dividing weight by  $(\text{height})^2$  and was expressed in  $\text{kg/m}^2$ .

### Data analysis and statistics

The data from those tests which were retrospectively confirmed to be correctly timed were used. In 19 of the original 31 subjects, we obtained results in all four desired phases of the cycle. In the remaining 12 subjects, tests, mostly in the post-ovulatory phase, appeared not to have been performed at the appropriate time. Results are given for, and statistical analysis was performed on, all the data from correctly timed tests (menstrual phase  $n=31$ ; pre-ovulatory phase,  $n=30$ ; post-ovulatory phase,  $n=23$ ; mid-luteal phase,  $n=26$ ).

To obtain a measure of the overall level of FST and LDF during the FCT, the area under the curve during the test was calculated and divided by the total length (min) of the test period. The resulting quotient is a weighted average of the periods for which each value is representative. It is called 'mean (immersion and recovery)'. We used the same procedure for the cooling period (5 min), the 'mean (immersion only)' and for the level of FST and LDF during the recovery period (20 min), the 'mean (recovery only)' [20]. The absolute changes in FST and LDF from baseline values were calculated as well.

We also made an approximation of the course of FST over time (during recovery) by using an exponential model [21]. For each test the change in FST after recovery with respect to the baseline value was approximated by a simple exponential model: baseline - FST( $t$  'after recovery') =  $a \exp(-bt)$ , where  $t$  is the time in min from the start of recovery,  $a$  is the temperature drop during immersion, and  $b$  is the rate of recovery. The estimated values for  $a$  and  $b$  were used as parameters for the relation between FST and the four phases. Statistical comparisons between the test results in the different phases were performed by an analysis of variance, mixed model, with subject as the random factor and phase as the fixed factor, and allowing some empty cells. Pairwise comparison between the phases was performed by using the method of Scheffé.

For each subject we calculated the Kendall correlation coefficient between the hormone levels and mean (recovery only) FST, and to investigate the consistency of these relations in all women together, we used a signed rank test. Correlations between hormone levels and FST values for all women were calculated by Pearson correlation coefficients. A  $P$  value of less than 0.05 (two-tailed)

was considered to be significant. All results are given as means  $\pm$  SD, unless indicated otherwise.

### RESULTS

We investigated 31 females with an age of  $25.0 \pm 4.1$  years, a cycle length of  $29.3 \pm 3.5$  days, a body weight of  $62.0 \pm 9.6$  kg, a height of  $1.67 \pm 0.07$  m, a Quetelet index of  $21.5 \pm 2.5$  kg/m<sup>2</sup>, an amount of subcutaneous fat of  $26\% \pm 4\%$  of total body mass, and a hand volume of  $312 \pm 51$  ml.

Table 1 gives the means and the range of levels of the sex hormones in the different phases of the menstrual cycle. In Figs. 1 and 2, the mean of all measurements of FST and LDF during the whole FCT is shown in all four phases of the cycle. Table 2 shows the FST in the different phases. There was no significant difference in the baseline FST between the four phases. However, on further analysis significant variations were found during the menstrual cycle (all  $P < 0.005$ ) for the mean (immersion and recovery) FST, the mean (immersion only) FST and the mean (recovery only) FST. Significant Scheffé contrasts were observed between the mid-luteal phase and the pre-ovulatory phase. Also, in the exponential model, significant differences for the temperature drop  $a$  ( $P=0.03$ ) and the recovery rate  $b$  ( $P=0.005$ ) were calculated. Again, the significant contrast was between the mid-luteal phase and the pre-ovulatory phase for  $b$ , and between the menstrual phase and the post-ovulatory phase for  $a$ . Table 2 also shows that the LDF values varied significantly during one menstrual cycle. The baseline LDF showed a significant difference ( $P=0.04$ ), as did the mean (immersion and recovery) LDF, the mean (immersion only) LDF and the mean (recovery only) LDF (all  $P < 0.005$ ). Again the significant contrasts for all the parameters were found between the mid-luteal phase and the pre-ovulatory phase. Since the baseline values were already different, the changes were also expressed as absolute changes from baseline and then all the mean LDF values during the different phases were no longer significant. Table 2 gives only the most representative, the mean (recovery only) LDF value.

Table 3 gives further baseline haemodynamic data. The baseline measurements at the four phases of the menstrual cycle were similar for blood pressure and HR,

**Table 1. Hormone levels during the four phases of the menstrual cycle**

Results are shown as mean (range).

Phase...	Menstrual ( $n=31$ )	Pre-ovulatory ( $n=30$ )	Post-ovulatory ( $n=23$ )	Mid-luteal ( $n=26$ )
17 $\beta$ -Oestradiol (pmol/l)	207 (75-330)	1081 (190-2000)	550 (260-900)	733 (380-1000)
Progesterone (nmol/l)	2.4 (1.3-5.4)	4.6 (1.3-13.0)	24.6 (4.7-64.0)	68.0 (23.0-120.0)
LH (i.u./l)	9.4 (5.2-16.0)	26.8 (8.2-72.0)	15.5 (4.0-38.0)	10.1 (4.5-20.0)
FSH (i.u./l)	5.1 (1.1-9.2)	3.1 (2.7-16.0)	4.9 (1.5-9.2)	2.9 (1.1-6.1)

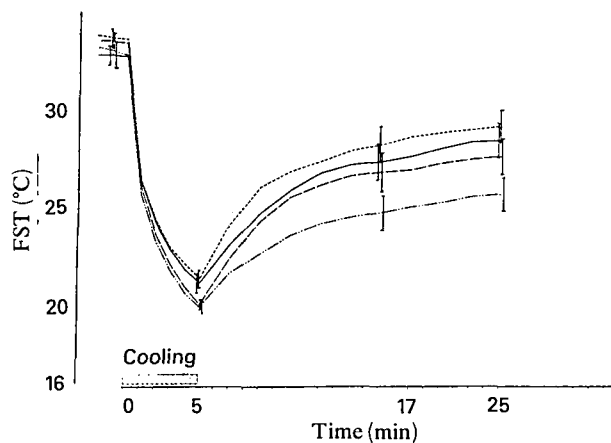
**Table 2. FST and LDF during the four phases of the menstrual cycle**

Results are means  $\pm$  SD for  $n$  tests. Abbreviation: NS, not significant.  $P$  values refer to the analysis of variance for differences between phases. Comparisons refer to significant ( $P < 0.05$ ) Scheffé comparisons.

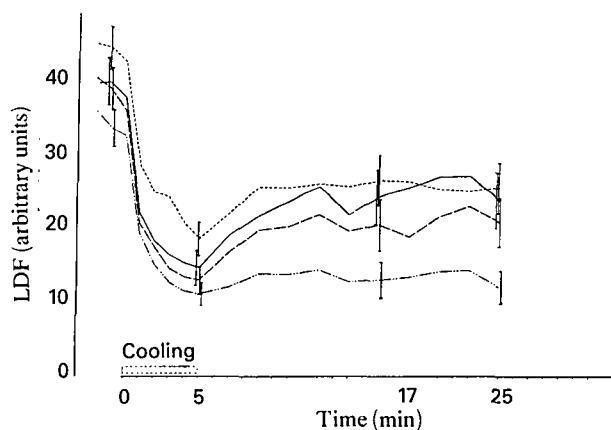
	Menstrual (M) ( $n = 31$ )	Pre-ovulatory (PrO) ( $n = 30$ )	Post-ovulatory (PO) ( $n = 23$ )	Mid-luteal (L) ( $n = 26$ )	$P$ -value	Comparisons
<b>FST (<math>^{\circ}\text{C}</math>)</b>						
Baseline value	32.9 $\pm$ 2.8	33.8 $\pm$ 2.4	33.6 $\pm$ 2.0	33.1 $\pm$ 2.6	NS	
Mean (immersion and recovery)	27.7 $\pm$ 3.6	28.4 $\pm$ 3.7	27.2 $\pm$ 3.2	25.9 $\pm$ 3.0	0.002	PrO vs L
Mean (immersion only)	24.5 $\pm$ 2.1	24.8 $\pm$ 1.8	24.0 $\pm$ 1.4	23.6 $\pm$ 1.5	0.004	PrO vs L
Mean (recovery only)	26.7 $\pm$ 4.6	27.4 $\pm$ 4.9	25.9 $\pm$ 4.3	24.1 $\pm$ 3.9	<0.001	PrO and M vs L
Baseline minus mean (recovery only)	6.3 $\pm$ 3.5	6.5 $\pm$ 4.0	7.8 $\pm$ 3.5	9.0 $\pm$ 3.6	<0.001	M vs L
Temperature drop, $a$ ( $^{\circ}\text{C}$ )	11.7 $\pm$ 3.0	12.3 $\pm$ 2.6	13.5 $\pm$ 1.9	12.9 $\pm$ 2.3	0.03	M vs PO
Recovery rate, $b$ ( $\text{min}^{-1}$ )	0.12 $\pm$ 0.13	0.18 $\pm$ 0.21	0.10 $\pm$ 0.14	0.07 $\pm$ 0.12	0.005	PrO vs L
<b>LDF (arbitrary units)</b>						
Baseline value	39.0 $\pm$ 17.9	43.9 $\pm$ 15.8	38.3 $\pm$ 13.4	33.9 $\pm$ 12.8	0.04	PrO vs L
Mean (immersion and recovery)	26.1 $\pm$ 16.7	29.2 $\pm$ 16.4	22.5 $\pm$ 12.3	18.4 $\pm$ 10.9	0.003	PrO vs L
Mean (immersion only)	19.5 $\pm$ 12.5	25.7 $\pm$ 14.4	17.8 $\pm$ 9.9	16.1 $\pm$ 9.0	0.001	PrO and PO vs L
Mean (recovery only)	23.4 $\pm$ 19.1	25.0 $\pm$ 17.7	18.6 $\pm$ 14.5	13.7 $\pm$ 12.5	0.002	PrO and M vs L
Baseline minus mean (recovery only)	15.6 $\pm$ 14.2	18.9 $\pm$ 12.2	19.1 $\pm$ 13.6	20.3 $\pm$ 13.4	NS	

but different for FBF ( $P < 0.05$ ) and calculated FVR ( $P = 0.002$ ). The most striking finding here was the lower FBF in the menstrual phase compared with more or less similar values in the other three phases. As expected, the baseline FVR was increased in the menstrual phase in comparison with the three other phases. During the FCT no changes in blood pressure, HR and forearm parameters were detected in any of the four phases (data not shown).

We looked for correlations between the results of the FCT [expressed as mean (recovery only) FST] and the levels of  $17\beta$ -oestradiol, LH, FSH, progesterone and the  $17\beta$ -oestradiol/progesterone ratio. We did not see any correlation between these hormones (for all subjects and all phases) and the test results. We further calculated correlations between the sex hormones and the test results in the different phases. We found a negative correlation ( $r = -0.48$ ,  $n = 26$ ,  $P = 0.012$ ) between the level of  $17\beta$ -oestradiol and the mean (recovery only) FST in the mid-luteal phase but not in the other three phases.



**Fig. 1.** FST before, during and after cooling, in the four different phases of the menstrual cycle. —, Menstrual phase; ---, pre-ovulatory phase; — — —, post-ovulatory phase; - · - · -, mid-luteal phase. Results are means  $\pm$  SD.



**Fig. 2.** LDF before, during and after cooling, in the four different phases of the menstrual cycle. —, Menstrual phase; ---, pre-ovulatory phase; — — —, post-ovulatory phase; - · - · -, mid-luteal phase. Results are means  $\pm$  SD.

Within subjects we found a positive correlation between the  $17\beta$ -oestradiol/progesterone ratio and the mean (recovery only) FST, by calculating the Kendall correlation coefficients between hormone level and FST for each woman. The mean of these correlation coefficients was 0.38, with  $P < 0.05$  in the signed rank test. No significant correlations were found between the results of the FCT and the Quetelet index, the amount of subcutaneous fat or hand volume.

## DISCUSSION

This study shows an effect of menstrual cycle phase on the peripheral skin perfusion and FBF. In the luteal phase, finger skin perfusion shows the greatest cold-induced vasoconstriction and the slowest recovery afterwards as compared with the other phases of the cycle. The adaptation to changes in environmental temperatures and emotions results in large physiological fluctuations in peripheral skin blood flow, which causes difficulties in measuring this parameter. Consequently, both methods, FST and LDF, have a high sd.

Lafferty *et al.* [4] and Terregino & Seibold [5] also found cyclic differences in finger skin blood flow, but in a different pattern from that which we found. In contrast with these studies, we studied a much larger group of subjects, our FCT was strictly standardized [15] and we used the combined determination of serum  $17\beta$ -oestradiol, progesterone, LH and FSH and a basal body temperature chart to determine the correct timing of ovulation.

We have also shown a cyclic change in FBF, consisting mainly of muscle flow. This appeared to be low in the menstrual phase, consistent with other reports of a lower blood flow in the calf during menstruation [22].

The particular role played by sex hormones in the regulation of the peripheral circulation is not yet known. There might be a direct influence on the blood vessel wall or through other peripheral regulation mechanisms, or an indirect systemic hormonal action causing a cyclic pattern in females. Besides, non-hormonal factors might be responsible for differences in women compared with men. The influence of natural and synthetic oestrogens on different tissues and arteries and veins has been the object of studies both *in vitro* or *in vivo*, mostly in animals. Observations are largely dependent on the species and the tissue used and cannot be automatically extrapolated to

other species or tissues. Natural and synthetic oestrogens may sometimes exhibit a completely opposite effect on the same target [23–26].

The regulation of cutaneous blood flow and the response to cold challenge is mainly under sympathetic nervous system control. The vasomotor tone within skin vessels, particularly the arteriovenous anastomoses, is reflexly influenced by both local circulating mediators and the sympathetic nervous system. Nutritional capillary skin flow is more constant and is influenced by local factors rather than directly by sympathetic nerves. The sympathetic nervous system releases catecholamines into the synaptic cleft that may stimulate vasoconstrictive  $\alpha_2$ -adrenoceptors and vasodilating  $\beta_2$ -adrenoceptors. Some investigators have found cyclical changes in platelet  $\alpha_2$ -adrenoceptors [27], others found cyclical changes only in  $\beta_2$ -receptors [28], and some no change at all [29]. Studies point to an influence of oestrogens on the sympathetic nervous system and there is evidence that oestrogens induce an up-regulation of (vasoconstrictive)  $\alpha_2$ -adrenoceptors [30–32]. Experiments in arteries and veins of rats revealed a dose-dependent reactivity of oestrogens, with vasoconstriction after low and vasodilatation after high dosage [33, 34]. Although much less investigated, the same seems true for progesterone, leading to a vasoconstrictive as well as a vasodilatory effect, the latter possibly by influencing the vasodilating  $\beta$ -adrenoceptors [26, 34]. The most satisfying hypothesis is that the cyclical  $17\beta$ -oestradiol/progesterone ratio and its continuous fluctuation is of major importance in the vascular responsiveness in women. Indeed, we did find a correlation between this ratio and the blood flow, although it was a weak.

The sex hormonal influenced premenstrual fluid and electrolyte retention or the progesterone-induced post-ovulatory elevated core temperature may play an indirect role in the regulation of the peripheral circulation. Eventually, non-hormonal factors may have an influence on the skin circulation. For example, a low Quetelet index or a low amount of subcutaneous fat may lead to less isolation against low temperature. Also, a low hand volume may imply that a relatively greater surface is in contact with the environmental temperature [35]. Yet we could not establish that any of these factors influenced the FCT.

In conclusion, this study has demonstrated that during the menstrual cycle with its natural changes in sex hormone levels, the peripheral circulation and its

**Table 3. Baseline values of blood pressure, MAP, HR, FBF and FVR in the four phases of the menstrual cycle**

Results are means  $\pm$  SD for  $n$  tests. Abbreviation: NS, not significant.  $P$  values refer to the analysis of variance for differences between phases.

Phase...	Menstrual ( $n = 31$ )	Pre-ovulatory ( $n = 30$ )	Post-ovulatory ( $n = 23$ )	Mid-luteal ( $n = 26$ )	$P$ -value
SBP (mmHg)	103.7 $\pm$ 6.5	103.8 $\pm$ 6.7	102.7 $\pm$ 5.4	103.1 $\pm$ 6.1	NS
DBP (mmHg)	69.8 $\pm$ 6.5	69.4 $\pm$ 6.0	68.1 $\pm$ 5.7	68.3 $\pm$ 5.6	NS
MAP (mmHg)	81.1 $\pm$ 6.0	80.9 $\pm$ 5.7	79.6 $\pm$ 5.1	79.9 $\pm$ 5.3	NS
HR (beats/min)	63.5 $\pm$ 8.6	64.8 $\pm$ 9.5	63.8 $\pm$ 10.0	64.7 $\pm$ 9.0	NS
FBF (ml min <sup>-1</sup> 100 ml <sup>-1</sup> )	2.90 $\pm$ 1.5	3.79 $\pm$ 2.0	3.93 $\pm$ 1.7	3.88 $\pm$ 1.8	0.041
FVR (arbitrary units)	37.6 $\pm$ 27.3	26.3 $\pm$ 10.9	24.1 $\pm$ 9.4	24.8 $\pm$ 10.2	0.002

response to cold changes. These differences are worth considering when the peripheral circulation of females is investigated without knowledge of the phase of the menstrual cycle.

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