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17- β estradiol and progesterone independently augment cutaneous thermal hyperemia but not reactive hyperemia

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Abstract

OBJECTIVE—We examined the impact of estradiol and progesterone on skin local heating and reactive hyperemia in 25 healthy women.

METHODS—Subjects were studied 3 times over 10–12 days. Endogenous sex hormones were suppressed with agonadotropin-releasing hormone antagonist. Subjects were studied on day 4 of suppression (*study-day 1*), 3–4 days later following treatment with either 17 β -estradiol or progesterone (*study-day 2*), and another 3–4 days later, following treatment with both estradiol and progesterone (*study-day 3*). Subjects underwent identical local heating and reactive hyperemia protocols on all study days. Local heating is characterized by an initial peak in blood flow, followed by a prolonged plateau. A brief nadir is seen between the phases.

RESULTS—Blood flow values are expressed as percent maximum cutaneous vascular conductance (CVC). Estradiol alone increased initial peak CVC from 71 $\pm$ 2 to 79 $\pm$ 2% ($p=0.001$). Progesterone alone increased initial peak CVC from 72 $\pm$ 2 to 78 $\pm$ 2% ($p=0.046$). Neither estradiol nor progesterone increased plateau CVC. No significant changes were seen between study days 2 and 3 for either group. No differences were observed in reactive hyperemia.

CONCLUSION—Both estradiol and progesterone increased initial peak CVC during local heating, without altering plateau CVC. There was no additive effect of estradiol and progesterone.

Keywords

hormones; estrogen; skin temperature; vasodilation; laser-Doppler flowmetry

INTRODUCTION

The female sex hormones, estrogen and progesterone, fluctuate across the normal menstrual cycle and play significant roles in cardiovascular health. At the onset of menopause, when endogenous concentrations of these hormones are drastically diminished, cardiovascular risk is increased multi-fold (21,26). Currently, our understanding of how these hormones act on the cardiovascular system is limited. Furthermore, very little work has been done concerning the effects of the hormones on the human microvasculature.

In many disease states, dysfunction of the microvasculature occurs before that of the conduit vessels (1). Cutaneous microvascular function is also impaired in these disease states (4,20,41), and may reflect globalized microvasculature dysfunction (20,41). Thus, the skin provides a means of studying the microvasculature in a relatively non-invasive manner.

We therefore sought to test the individual and combined effects of estradiol and progesterone on the microvasculature of the skin using two tests: local heating and reactive hyperemia. Local heating (23,30,41) and reactive hyperemia (9,45) are two of the most commonly used tests to evaluate microvascular function and cardiovascular risk in both experimental and clinical research studies (8,28). Locally heating the skin causes vasodilation that is characterized by two components: an initial rapid rise in blood flow which is predominantly alocalaxon-mediated reflex, and a more gradual increase to a plateau which is predominantly mediated by nitric oxide (NO) (22,29). A brief nadir occurs between these two components. Cutaneous reactive hyperemia following vascular occlusion is characterized by an acute maximal peak followed by a prolonged total hyperemic period. Reactive hyperemia is much less dependent on NO than local heating (48,49). Recent studies indicate a role of local sensory nerves (24,25) and endothelial-derived hyperpolarizing factors (EDHFs) via $BKCa^{2+}$ channels (25).

Skin blood flow in response to local heating has been shown to be increased during the mid-luteal phase of the menstrual cycle, when serum concentrations of estrogen and progesterone are elevated, as compared to during the early follicular phase (3,6). Estrogen is thought to upregulate nitric oxide production within the endothelial cells of the vasculature (16,18), thus increasing skin blood flow. Prior studies have also shown estrogen to increase skin blood flow during reactive hyperemia (15,42). The effects of progesterone on the skin microcirculation are less understood and, to date, have not been independently investigated. During passive whole-body heating, Houghton et al. (19) found progestins to have an effect on the NO portion of active vasodilation to whole body heat stress, but not on total skin blood flow, when given with ethinyl estradiol in the form of contraceptives. Our goal in the present study was to determine the effects of estrogen and progesterone on skin blood flow during the local heating (LH) and reactive hyperemia (RH) tests. We hypothesized that estradiol would increase the response to both LH and RH, and that progesterone would not independently affect skin blood flow during either test. We also expected there to be no difference in skin blood flow between estrogen alone and both hormones in combination during either test.

MATERIALS AND METHODS

Twenty five young healthy (age 22 ± 1 years, BMI 23 ± 1 kg/m²) female subjects participated in the study. All subjects were non-smokers and were not taking any medications, with the exception of oral or vaginal combined hormonal contraceptives, which were discontinued at least 48 hours prior to participation in the study. Subjects were screened by a physician specializing in gynecology and obstetrics and excluded if they had any of the following: cardiovascular disease, hypertension, hypercholesterolemia, metabolic disease, menstrual disorders, or a personal or family history of blood clots. Approval of this protocol was granted by the Institutional Review Board of the University of Oregon. Subjects gave oral and written consent before participation in this study.

A hormone suppression, add-back model was used to study the individual and combined effects of estrogen and progesterone, as depicted in Figure 1. Endogenous sex hormones were suppressed in all subjects throughout the duration of the study (10–12 days) with a daily 250 µg/0.5 ml gonadotropin-releasing hormone antagonist (GnRHa) subcutaneous injection (Ganirelix acetate; Organon International, Roseland, NJ), beginning at the onset of

menses. Following four days of GnRHa treatment, subjects underwent study day 1. Subjects were then randomly divided into two groups. Subjects in group 1 (n=12) were supplemented orally with 200 mg per day progesterone (P₄; Prometrium; Solvay Pharmaceuticals, Marietta, GA). Following 3–4 days of P₄ add-back, subjects underwent study day 2 (GnRHa + P₄). Subjects in group 2 (n=13) were supplemented with 0.1–0.2mg 17β-estradiol (E₂; Estradiol; Mylan Pharmaceuticals, Inc., Morgantown, WV) via transdermal patch. Following 3–4 days of E₂ add-back, subjects underwent study day 2 (GnRHa + E₂). Following study day 2, subjects in both groups continued hormone supplementation and were additionally supplemented with the other hormone such that all subjects were now receiving both P₄ and E₂. After 3–4 days of receiving both hormones, subjects underwent study day 3. The initial subjects in both groups were supplemented with 0.1 mg E₂. However, after analyzing the blood values for E₂ in those subjects, we observed that the E₂ values were not as high as predicted. Thus, we doubled the E₂ dose in the remaining subjects.

Study Days

Subjects in both groups underwent identical study protocols on all three study days. Subjects reported to the lab having abstained from physical exercise, vitamins, alcohol, and over-the-counter medications for at least 24 hours, and from food and caffeine for at least 12 hours. Subjects were required to demonstrate negative results on a pregnancy test before the administration of study drugs and at the start of each study day.

Subjects were studied at the same time of day on all three study days. Subjects were instrumented with five-lead electrocardiogram (ECG) to continuously monitor heart rate (CardioCap; Datex-Ohmeda, Louisville, CO), a blood pressure cuff on the left upper arm to monitor blood pressure by automatic brachial auscultation (CardioCap; Datex-Ohmeda, Louisville, CO), and an occlusion cuff on the right forearm just below the antecubital fossa. Single-point laser-Doppler probes (DRT-4, Moor Instruments, Devon, UK) and local skin heaters (Skin Heater/Temperature Monitor SH02, Moor Instruments, Devon, UK) were placed on non-glabrous skin on the ventral surface of the right forearm just below the occlusion cuff. The local skin heaters cover approximately 700 mm² of skin and are used to control local skin temperature during the local heating protocol (below). The laser-Doppler probes were placed in the center of the local skin heaters to measure red blood cell flux as an index of skin blood flow.

Subjects rested supine for at least 40 min before the study protocols were conducted.

Reactive Hyperemia—Following 2 min of baseline recording, the occlusion cuff was rapidly inflated (E20 Rapid Cuff Inflator, D.E. Hokanson, Bellevue, WA) and held constant at 300 mmHg for 5 min. After cuff release, skin blood flow continued to be recorded for 5 min, or until flux measurements had returned back to baseline values. Subjects then rested supine for 20 min before a second trial was performed.

Local Heating protocol—Subjects rested supine for 20 min following completion of the two reactive hyperemia trials before the local heating protocol was begun. Baseline skin blood flow was recorded for 10 min with the local heaters set to 33°C. Following baseline measurements, the local heaters were raised 0.1°C per second until the temperature was raised to 42°C. The temperature then remained constant at 42°C until a plateau was seen in flux for at least 5 min, usually taking 30–40 minutes. At the end of the protocol, the local heaters were raised to 44°C (at 0.1°C per second) to attain maximal skin blood flow. Heating to greater than 42°C has previously been shown to elicit maximal vasodilation (29,43).

Venous blood samples were collected from an antecubital vein on each study day for analysis of estrogen and progesterone serum concentrations. Samples were collected (BD Vacutainer, Franklin, NJ) and centrifuged at 1300 g relative centrifugal force for 15 minutes at 4°C. They were then separated and stored frozen at -70°C, followed by transfer to Oregon Clinical and Translational Research Institute (OCTRI, Portland, OR) for analyses.

Data analysis

Skin blood flow values were expressed as cutaneous vascular conductance (CVC), calculated as the quotient of red blood cell (RBC) flux and mean arterial pressure (MAP), as follows: $CVC = RBC \text{ flux} / MAP$. All CVC values are presented as a percentage of maximal CVC. Maximal flux (100%) was determined by heating to 44°C. Minimum flux (0%) was determined as flux during the 5 minute arterial occlusion.

The local heating response was characterized by an initial peak in CVC, a nadir, and a secondary plateau, as shown in Figure 2. The reactive hyperemia response was characterized by an initial peak and the area under the curve minus baseline of the total hyperemic period, before recovery back to baseline, as shown in Figure 3. Time until initial peak, following release of the occlusion, and time of the total hyperemic response were also measured. All data were averaged across the two reactive hyperemia trials.

Statistical analysis

Subject demographic data was analyzed between groups using one-way repeated measures ANOVA. The effects of the hormone treatments on the local heating and reactive hyperemia responses were also compared with one-way repeated measures ANOVA with Tukey's post hoc test. Statistical significance was defined as $p < 0.05$. Data are presented as mean $\pm$ S.E.M.

RESULTS

Subject demographic and baseline data were similar between groups. Neither age, height, weight, BMI, baseline heart rate, nor baseline blood pressure (systolic, diastolic, and mean arterial) were significantly different between subject groups. Baseline heart rate and blood pressure refers to those taken on study day 1 when the subjects were in a suppressed state (GnRHa only). Heart rate and blood pressure did not vary across study days, except for a slight significant decrease in both diastolic and mean arterial pressure in group 2, between study days 1 and 3 ($p < 0.05$).

In group 1, estradiol serum concentration was significantly higher during study day 3 (GnRHa + P₄ + E₂) compared to day 1 (GnRHa) and day 2 (GnRHa + P₄). Progesterone concentration was significantly higher during day 2 compared to day 1, and remained elevated during day 3, with no significant difference in P₄ concentration between days 2 and 3. In group 2, estradiol concentration was significantly higher during study day 2 (GnRHa + E₂) compared to day 1 (GnRHa), and remained elevated during day 3, although dropped significantly compared to day 2. Progesterone concentration on day 3 (GnRHa + E₂ + P₄) was significantly higher than both day 1 and day 2. Estradiol concentration was also significantly higher in subjects supplemented with 0.2mg E₂ compared to subjects supplemented with 0.1mg E₂ in both groups. However, no differences were seen in skin blood flow trends between the two doses, and so data have been averaged.

In all experiments, local heating of the skin resulted in a large increase in vasodilation in the area being heated. Furthermore, all subjects on all study days demonstrated an initial peak in CVC, a brief nadir, and a more gradual secondary plateau. Figure 2 displays a representative tracing showing the baseline, initial peak, nadir, secondary plateau, and maximal local

heating responses in one subject. Across study days, estradiol (alone and in combination with progesterone) augmented the initial peak and nadir. Progesterone significantly increased only the initial peak. Progesterone also was associated with higher nadir CVC values, but this effect only achieved statistical significance in combination with estrogen, as a larger amount of variability was seen in the data. No differences were observed between the subjects in group 2 that received 0.1 versus 0.2 mg/day E₂. Figure 4 shows the increase in initial peak CVC from study day 1, when all hormones were suppressed, and the increase in nadir CVC from study day 1. No significant differences were seen in the secondary plateau, baseline, or maximal flux between study days or between groups.

Figure 3 displays a representative tracing of the reactive hyperemia response, showing the peak hyperemia followed by the total hyperemic response. There were no statistically significant differences between the first and second RH on any study day, and thus all data has been averaged between RH trials. No statistically significant changes were observed for peak hyperemia, area under the curve (AUC), time to peak, or time of total hyperemia between study days or between groups.

DISCUSSION

In this study, we explored the effects of exogenous estrogen and progesterone on the cutaneous response to local heating and reactive hyperemia. We found that both estrogen and progesterone increased the initial peak and nadir of local heating. However, estrogen and progesterone had no significant effects on either peak hyperemia or the AUC of the total hyperemic period during the RH test.

Local heating

The initial peak response to local heating is predominantly mediated by a sensory axon reflex. Minson et al (29) showed that blockade of the sensory nerves using a topical anesthetic, EMLA cream, dramatically attenuated the initial peak during local heating. However, when NO production was blocked using the L-arginine analog L-NG-nitroarginine methyl ester (L-NAME), there was also a small but significant attenuation of the initial peak. These results indicate that both the sensory nerves and NO are involved in this early stage of the local heating response, although the release of NO is not the primary mechanism.

Estrogen has well-known effects on increasing NO production (16,18,34). Estrogen may also act on the local sensory nerves, thereby augmenting the initial peak and nadir responses to local heating. In ovariectomized rats, estrogen receptors are found in peripheral sensory nerves (35,36). Furthermore, estrogen administration increases the production of neuropeptides, such as calcitonin gene-related peptide (CGRP) (13,32) and substance P (33) in sensory neurons, both of which are thought to be involved in the axon-reflex (46). Unfortunately, these studies have yet to be translated to humans.

There is mixed evidence regarding the actions of progesterone on the vasculature. Some studies in animal conduit arteries have found progesterone to enhance NO production (5,31,38); whereas others have found progesterone to possibly counteract the effects of estrogen on NO production (7,27). In the microvasculature, progesterone was shown to increase eNOS expression in the myometrial circulation in ovariectomized sheep, but only in conjunction with estradiol, and not in other vascular beds tested (38). In human conduit vessels, progesterone does not seem to have an effect on the vasodilatory effect of estrogen, as shown during flow-mediated vasodilation (FMD) (14); however, progesterone may act differently on the conduit vessels compared to the microvasculature.

From this evidence, it is hard to conclude that the increases in skin blood flow induced by progesterone administration was a result of increased NO production, although we cannot rule out the possibility. There is some evidence to suggest that progesterone can also increase the production of neuropeptides, specifically CGRP, within the sensory nerves (12,13), although these studies are limited and have only been performed in animals.

Contrary to our hypothesis, we did not see any effect of estrogen or progesterone on the secondary plateau, a predominantly nitric oxide-mediated response. Minson et al (29) and Kellogg et al (22) both demonstrated a reduction in plateau skin blood flow of at least 50% following the administration of L-NAME. During this phase, CVC was already very high (average = 91.0% of maximal CVC across all subjects and hormone treatments). There may be a ceiling effect such that the degree of vasodilation is already so high that increases in eNOS caused by the presence of estrogen and/or progesterone do not lead to further increases in CVC in young healthy women. Along these lines, we did not observe an increase in maximal RBC flux with any hormone treatment.

Reactive hyperemia

There is strong evidence for a role of the local sensory nerves (24,25), and a role of endothelium-dependent hyperpolarizing factors (25), specifically $BKCa^{2+}$ channels, in the RH response. Evidence as to whether NO plays a role is less consistent (9,11,48,49). There is also some question regarding the involvement of the cyclooxygenase (COX) pathway. For example, Larkin and Williams (24) showed COX inhibition to attenuate the RH response, whereas Dalle-Ave et al (10) showed no change in RH following COX inhibition.

In addition to the influences of estrogen and progesterone on NO and the sensory nerves already discussed, estrogen increases production of prostacyclin, through upregulation of various enzymes in the COX pathway, specifically COX-1 (39) and prostacyclin synthase (38,40). Estrogen may also increase expression of the prostacyclin receptor on vascular smooth muscle (44). Progesterone seems to act on the cyclooxygenase pathway in a similar manner to estrogen: by upregulating COX-1 (17) and prostacyclin synthase (38) in some vascular beds. However, these findings have not yet been explored in the cutaneous circulation. Limited evidence exists on the role of sex hormones on the $BKCa$ channels. Estrogen has been shown to open $BKCa$ channels in porcine coronary arteries (47), although no evidence exists as to whether progesterone does the same.

As discussed, research regarding the mechanism behind RH is not yet complete, and it indicates possible roles of multiple substances. Estrogen and progesterone also exhibit many effects and our understanding of those effects is limited. Unfortunately, the majority of the studies cited have not been performed in the skin, and many investigated the endothelium of larger vessels rather than that of the microvasculature.

Given the broad scope of effects estrogen and progesterone appear to have, we would expect to have seen some changes in the reactive hyperemia skin blood flow response as a result of the hormone treatments; however, the mean values were all similar. We also observed large variability in the hyperemic response across subjects and study days. Recently, Roustit et al (37) tested the reproducibility of forearm post-occlusive reactive hyperemia, finding it to be poorly reproducible from day to day. This may explain why we saw no significant changes or consistent differences between the hormone treatments. Estrogen and progesterone may, in fact, act on the sensory nerves, as suggested by the local heating data. However, we were unable to observe an influence of the hormones on the reactive hyperemia response.

Combined effects of estrogen and progesterone

Our results for study day 3, when estrogen and progesterone were given in combination, showed no further changes in the LH and RH responses. These findings support that estrogen and progesterone may act on the same pathways. If the hormones were acting on different pathways we might expect to see a greater increase in LH peak and nadir compared to supplementation with only one of the hormones. Another explanation is a ceiling effect, as might explain why no increase was seen in the plateau phase of LH. For instance, if production of NO is already increased maximally by estrogen, the addition of progesterone will not be able to increase production of NO further.

Limitations

We administered two different doses of estradiol. The initial subjects in group 2 (E_2 first) received 0.1 mg E_2 per day, resulting in an elevation of serum estradiol concentration at the time of study day 2. E_2 concentration remained elevated for study day 3, but was significantly lower than the concentration measured at the time of study day 2. This observation could possibly be due to the action of the transdermal patch. The majority of the women in group 2 wore the patch for the entire week, per instructions on the packaging. Those who changed it only did so if it fell off. Although the patch was designed to deliver a constant dose every day, it may instead be delivering smaller doses later on in the week. Furthermore, the addition of P_4 may be responsible for the fall in serum E_2 concentration.

To ensure the subjects were receiving high enough doses of estradiol by the third day, we doubled the dose and supplemented 5 of the women with 0.2mg E_2 . We also required these 5 women to change their patches following study day 2 in the case that the daily doses diminished over time. As shown in Table 2, the E_2 concentration remained high in these 5 subjects by study day 3. We cannot conclude whether the differences in E_2 concentration were caused by the doubling of the dose or by using the patches for fewer days at a time. However, we saw no differences in the skin blood flow responses based on the E_2 concentration, and so the data have been combined.

We were unable to randomize the order in which the reactive hyperemia and local heating tests were performed. RH had to occur before LH as the LH response is quite prolonged and often results in a die-away phenomenon (2). We had concerns that subsequent RH trials would be affected. On the other hand, multiple RH trials can be performed without a significant impact on vascular responses. Furthermore, we ensured baseline laser-Doppler flux following reactive hyperemia was not different from baseline flux before reactive hyperemia on both RH trials. Stable baseline flux was maintained for at least 20 minutes before the start of the local heating protocol.

Summary

The major new findings of this study are that estrogen and progesterone augment the initial peak and nadir of the LH response, without affecting the plateau. It seems that the hormones do not affect the RH response, although this may be due to the high level of variability in the test, in which case, we have shown the accuracy of RH in determining microvascular reactivity to be less than ideal. While we have a fairly good idea of the mechanisms behind the LH response, our understanding is not yet complete. On the other hand, RH is possibly regulated by multiple mechanisms, all of which may be influenced by estrogen and progesterone. While it is impossible for us to conclude exactly how the sex hormones act on the cutaneous microvasculature, if anything, this study opens a door to future research into the mechanisms of LH and RH and the effects of estrogen and progesterone on those mechanisms.

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ABBREVIATIONS USED

GnRHa	Gonadotropin-releasing hormone antagonist
E₂	17 β -estradiol
P₄	Progesterone
CVC	Cutaneous vascular conductance
%CVC_{max}	Percent maximal cutaneous vascular conductance
NO	Nitric oxide
EDHFs	Endothelial-derived hyperpolarizing factors
BKCa²⁺	Big potassium calcium
LH	Local heating
RH	Reactive hyperemia
ECG	Electrocardiogram
RBC	Red blood cell
MAP	Mean arterial pressure
AUC	Area under the curve
L-NAME	L-NG-nitroarginine
CGRP	Calcitonin gene-related peptide
eNOS	Endothelial nitric oxide synthase
NOS	Nitric oxide synthase
L-NMMA	L-NG-monomethyl arginine
COX	Cyclooxygenase

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Days of GnRHa Treatment										
	1	2	3	4	5	6	7	8	9	10
Group 1	GnRHa			GnRHa + P4			GnRHa + P4 + E2			
Group 2	GnRHa			GnRHa + E2			GnRHa + E2 + P4

 Study
Day 1

 Study
Day 2

 Study
Day 3

Figure 1. Schedule for treatment with study drugs. Endogenous hormones were suppressed with a gonadotropin-releasing hormone antagonist (GnRHa). Subjects were then treated with 17 β -estradiol (E₂) or progesterone (P₄), followed by treatment with both hormones.

Figure 2. Representative tracing of skin blood flow throughout the protocol. Blood flow is given as percentage of maximal cutaneous vascular conductance (%CVCmax).

Figure 3. Representative tracing of skin blood flow during reactive hyperemia. Measurements for peak hyperemia and area under the curve are indicated. Blood flow is given as percentage of maximal cutaneous vascular conductance (%CVCmax).

Figure 4.

A) Initial peak of the local heating response over all three study days given as percent maximal cutaneous vascular conductance (%CVCmax). Initial peak was increased significantly by the addition of the hormones, individually and in combination, following suppression with gonadotropin-releasing hormone antagonist (GnRH). Values are mean $\pm$ S.E.M. for n=11 for Group 1 (progesterone, P₄, first) and n=13 for Group 2 (estradiol, E₂, first). *Significantly different from study day 1 with GnRH only, p $\leq$ 0.05.

B) Nadir change in %CVCmax over all three study days. Nadir was increased significantly by the addition of estradiol individually and in combination with progesterone, but was not significantly increased by the addition of progesterone alone. Values are mean $\pm$ S.E.M. for n=11 for Group 1 (P₄ first) and n=13 for Group 2 (E₂ first). *Significantly different from study day 1 with GnRH only, p $\leq$ 0.05.

Table 1

Subject hormone concentrations and blood pressure across study days

	Group 1			Group 2		
	Day 1 (GnRHa)	Day 2 (GnRHa + P4)	Day 3 (GnRHa + P4 + E2)	Day 1 (GnRHa)	Day 2 (GnRHa + E2)	Day 3 (GnRHa + E2 + P4)
Estradiol, pg/ml						
0.1 mg	15.5 ± 2.4	21.5 ± 3.2	94.6 ± 17.0 ^{*,†}	16.0 ± 1.6	128.2 ± 21.8 [*]	68.0 ± 8.0 ^{*,†}
0.2 mg	14.0 ± 0.9	15.9 ± 1.6	193.4 ± 27.2 ^{*,†}	12.4 ± 2.0	142.9 ± 34.7 [*]	133.7 ± 23.5 [*]
Progesterone, ng/ml	2.0 ± 0.1	4.5 ± 0.4 [*]	5.7 ± 0.6 [*]	1.9 ± 0.1	1.8 ± 0.1	5.2 ± 0.5 [†]
Blood Pressure, mmHg						
Systolic	109.2 ± 2.1	109.5 ± 1.6	107.0 ± 1.9	111.8 ± 1.8	107.8 ± 1.7	108.2 ± 1.6
Diastolic	68.9 ± 1.8	68.2 ± 1.8	66.5 ± 1.5	71.2 ± 1.5	69.0 ± 1.9	65.0 ± 1.8 [*]
Mean arterial	82.3 ± 1.8	82.0 ± 1.6	80.0 ± 1.5	84.7 ± 1.6	81.9 ± 1.7	79.4 ± 1.4 [*]

Data are presented as mean ± S.E.M.

^{*}

p<0.05 compared to study day 1

[†]

p<0.05 compared to study day 2

Estradiol was administered in a dose of either 0.1 mg/day (Group 1: n=7, Group 2: n=8) or 0.2 mg/day (Group 1: n=5, Group 2: n=5).

Table 2

Local heating and reactive hyperemia responses

	Group 1			Group 2		
	Day 1 (GnRHa)	Day 2 (GnRHa + P4)	Day 3 (GnRHa + P4 + E2)	Day 1 (GnRHa)	Day 2 (GnRHa + E2)	Day 3 (GnRHa + E2 + P4)
Local Heating						
Max flux	224.8 ± 23.8	232.0 ± 11.9	241.3 ± 16.1	241.8 ± 31.7	249.5 ± 32.8	233.8 ± 17.7
Baseline, % max CVC	4.7 ± 0.4	5.1 ± 0.5	5.8 ± 0.5	5.0 ± 0.3	5.2 ± 0.6	4.9 ± 0.5
Initial peak, % max cVC	72.4 ± 1.7	78.2 ± 1.6*	78.7 ± 2.0*	71.0 ± 1.8	78.7 ± 1.7*	76.5 ± 2.5*
Nadir, % max CVC	60.2 ± 3.0	65.9 ± 2.5	66.7 ± 2.5*	57.0 ± 2.6	65.1 ± 2.0*	64.6 ± 2.2*
Plateau, % max CVC	90.2 ± 1.0	91.8 ± 1.1	90.3 ± 1.2	89.6 ± 1.6	92.4 ± 0.7	91.2 ± 1.6
Reactive Hyperemia						
Peak, % max CVC	32.4 ± 3.1	30.8 ± 2.0	34.5 ± 2.7	32.4 ± 1.9	29.0 ± 1.3	32.0 ± 1.9
AUC, %max CVC sec	1418 ± 192	1359 ± 135	1456 ± 158	1547 ± 146	1381 ± 97	1359 ± 99

Data are presented as mean ± S.E.M.

* p< 0.05 compared to study day 1±