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Author manuscript

Microcirculation. Author manuscript; available in PMC 2020 October 21.

Published in final edited form as:

Microcirculation. 2018 October ; 25(7): e12490. doi:10.1111/micc.12490.

Ovarian Hormones Modulate Endothelin-1 Receptor Responses in Young Women

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Abstract

Objective: We recently demonstrated endothelin-B receptors (ETB_R) mediate vasodilation in young but not postmenopausal women; it is unclear if this is related to age or a decline in ovarian hormones. The purpose of this study was to test the hypothesis that ETB_R responses are modulated by ovarian hormones.

Methods: We measured cutaneous vasodilatory responses in 12 young women (22±1 years, 23±1 kg/m^2) during the mid-luteal (ML; days 20-25) and early follicular (EF; days 2-5) phases of the menstrual cycle. Cutaneous microdialysis perfusions of lactated Ringer (control), ETB_R antagonist (BQ-788, 300nM), and ETA_R antagonist (BQ-123, 500nM) were performed, followed by local heating to 42°C.

Results: Serum estradiol (ML: 118±16 vs. EF: 44±9 pg/ml, $P < 0.05$) and progesterone (ML: 8.3±1.0 vs. EF: 0.7±0.2 ng/ml, $P < 0.05$) were higher during ML vs. EF phase. ETB_R blockade decreased vasodilation during ML (control: 91±2 vs. BQ-788: 83±2 %CVCmax, $P < 0.05$) but not EF (control: 89±2 vs. BQ-788: 89±1 %CVCmax). ETA_R blockade also decreased vasodilation during ML (control: 91±2 vs. BQ-123: 87±2 %CVCmax, $P < 0.05$) but not EF (control: 89±2 vs. BQ-123: 92±2 %CVCmax).

Conclusions: These data suggest that fluctuations in ovarian hormones modulate ETB_R and ETA_R responses in young women.

Keywords

cutaneous microdialysis; endothelin-B receptor; sex hormones

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Research was performed at the University of Delaware

AUTHOR CONTRIBUTIONS

KNS, AVK and MMW performed the experiments; KNS, AVK, RTP, and MMW analyzed the data; KNS, RTP, SLL, DGE and MMW interpreted the results of the experiments; AVK and MMW prepared the figures; DGE and MMW contributed to the conception and design of the research; KNS, AVK, and MMW drafted the manuscript; KNS, AVK, RTP, SLL, DGE and MMW edited and revised the manuscript; KNS, AVK, RTP, SLL, DGE and MMW approved the final version of the manuscript.

DISCLOSURES

None

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INTRODUCTION

ET-1 is a powerful vasoconstrictor that is released by the endothelium and is involved in regulating vascular tone and function [1,2]. ET-1 binds to two different receptors in the vasculature: ETA_R and ETB_R [3]. Both receptors are present on vascular smooth muscle cells and elicit vasoconstriction and cell proliferation. Conversely, ETB_R are also found on the vascular endothelium and facilitate vasodilation through the stimulation of, at least in part, release of NO [4-6]. Given the contrasting actions of these two ET-1 receptors [7], much attention has been given to the influence of these receptors on cardiovascular function.

Sex differences in cardiovascular function are well recognized, and the mechanisms contributing to sex differences in cardiovascular disease rates have been the focus of numerous investigations in recent years. Sex differences in the ET-1 system are also apparent, and may be a primary contributing factor to the divergent cardiovascular disease rates between men and women throughout the lifespan. There is accumulating evidence demonstrating ETB_R are important for vascular health in women [5,8-10]. For example, the proportion of ETB_R to ETA_R is greater in women (1:1) compared to men (1:3) [4]. In young women, ETB_R mediate vasodilation [5,8,10], but elicit vasoconstriction in young men [5]. In postmenopausal women, ET-1 mediated vasoconstriction is lower compared to men of similar age, but blockade of ETB_R ameliorates this sex difference in ET-1 constriction [9]. We recently demonstrated that ETB_R mediated vasodilation in young women is lost after menopause [10]. However, it is not clear whether this is due to age-related changes in the ET-1 system, or primarily to the fluctuations in endogenous ovarian hormones.

Fluctuations in circulating ovarian hormones influence the ET-1 system. Studies in animal and cell models indicate that ovarian hormones, specifically E_2, modulate ET-1 and its receptors. E_2 significantly lowers ET-1 concentration, mRNA expression, and secretion from HUVECs [11,12]. Administration of E_2 up-regulates the expression of ETB_R in the coronary arteries of hyperlipidemic rabbits [13], while down-regulating ETB_R expression on the smooth muscle cells of the ventricular myocardium of rats [14]. In humans, plasma ET-1 is lower in women than in men and is even lower in pregnant women [15], showing an inverse relationship with E_2 and P_4. However, the influence of hormonal fluctuations in E_2 and P_4 on ET-1 receptor responses in humans is unclear.

With this background in mind, and given the impact of circulating sex steroids on vascular function, it is important to understand if fluctuations in ovarian hormones modulate ET-1 receptor responses. To our knowledge, no studies have examined their effects in young women. Accordingly, the purpose of this study was to examine ET-1 receptor responses during two distinct phases of the menstrual cycle: the EF phase (when both E_2 and P_4 are low), and the ML phase (when both E_2 and P_4 are elevated). We hypothesized that ETB_R mediated vasodilation would be greater during ML compared to EF.

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MATERIALS AND METHODS

Subjects

Twelve young women between the ages of 18-30 years participated in this study. All subjects were non-obese (BMI < 30 kg/m^2), non-smokers, free from any known cardiovascular disease, and had regular menstrual cycles (1 cycle every approximately 28 days). Women were excluded if they were taking oral contraceptives, had irregular cycles, or if they were pregnant or breast-feeding, all of which affect endogenous sex steroid levels [16]. Women were also excluded if they had any history of cardiovascular or metabolic disease, or any other chronic disease. All experimental procedures and protocols were approved by the Institutional Review Board at the University of Delaware and conformed to the guidelines set forth by the Declaration of Helsinki. All women gave verbal and written consent prior to study participation.

Screening Visit

Women reported to the Nurse Managed Primary Care Center at the University of Delaware in the morning after an overnight fast for a standard physical exam to ensure eligibility for study participation. A medical history and menstrual history questionnaire were completed and reviewed by a Nurse Practitioner. Height, weight, resting BP (seated), and resting electrocardiogram were measured. A blood sample was taken to assess standard blood chemistry such as cholesterol, glucose, hemoglobin, and hematocrit.

Experimental Visits

Women participated in two identical experimental visits, which were conducted during two different phases of their menstrual cycle (EF and ML). Consistent with previous studies, the EF phase visit occurred during days 2-5 of the menstrual cycle and the ML phase visit occurred during days 20-25 of the menstrual cycle [17-20]. Cycle phase was confirmed by serum sex hormones; a serum P_4 level of >5ng was used to confirm ovulation [21]. Women refrained from exercise for 24 hours, alcohol and caffeine for 12 hours, and food for at least 4 hours prior to testing. Upon arrival, women provided a small urine sample for an over-the-counter pregnancy test and were weighed. After lying supine for at least 20 minutes, a blood sample was taken to assess serum E_2 and P_4, as well as plasma ET-1. Women were instrumented for measures of BP (Dinamap DASH 2000, GE Medical, Chicago, IL) and intradermal microdialysis coupled with LDF.

Microvascular Vasodilatory Function Assessment

We measured vasodilatory function in the cutaneous microcirculation using LDF coupled with cutaneous microdialysis. This approach allows for the measurement of SkBF while simultaneously administering pharmacological substances to garner insight into the specific mechanisms regulating vascular function [22,23]. Importantly, the cutaneous circulation has been used as an accessible and representative vascular bed to examine the mechanisms of peripheral microvascular vasodilatory function in humans [10,23-30]. Three microdialysis fibers (CMA 31 Linear, Harvard Apparatus, Holliston, MA) were inserted intradermally on the dorsal side of the right forearm as previously described [10,27-30]. The forearm was cleaned

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with betadine and alcohol, and ice was applied for 10 minutes to the skin surface in order to provide a short-term local anesthetic. A 23-gauge needle was then inserted through the intradermal space to serve as a guide cannula. The entry and exit points of each needle were 2 cm apart, with each site separated by at least 1 inch. After insertion of all three needles, microdialysis fibers were threaded through the lumen of the needle and the needles were removed, leaving only the semi-permeable portion of the fiber under the dermis. All fibers were taped down and connected to a syringe pump (Bioanalytical Systems, West Lafayette, IN), and perfused with lactated Ringers solution (B. Braun Medical, Bethlehem, PA) at a rate of 2 μl/min for 60-90 minutes to allow for recovery of local SkBF following fiber insertion. SkBF was measured as cutaneous red blood cell flux from 1.5 mm^2 of skin using a multi-fiber laser Doppler flow probe (Moor Instruments, Devon, UK) placed in a local heater (Moor Instruments), and secured on the skin with tape directly above each microdialysis site.

Experimental Protocol

Local skin temperature was maintained at 32°C. The microdialysis sites were randomly perfused with either the ETB_R antagonist BQ-788 (300 nM, Sigma-Aldrich, St. Louis, MO), the ETA_R antagonist BQ-123 (500 nM, Sigma-Aldrich), or lactated Ringers solution to serve as the control site [10,27,28]; all sites were perfused for 45 minutes at a rate of 5 μl/min. After the ET-1 receptor blocker perfusion, the temperature of the local heaters was increased to 42°C for approximately 30 minutes, or until a stable plateau in SkBF was reached; this plateau is largely mediated by NO [31-33]. Finally, all three syringes were perfused with SNP (28 mM, Marathon Pharmaceuticals, Northbrook, IL) at a rate of 5 μl/min for approximately 15-20 minutes with the temperature of the local heaters set to 43°C to elicit maximal vasodilation [27].

Blood Analysis

Venous blood samples were collected in separate tubes without anticoagulants for the analysis of E_2 and P_4. A separate blood sample was collected in an EDTA tube for the analysis of plasma ET-1. Tubes were centrifuged, the serum or plasma pipetted off, and frozen at −80°C until analysis. Serum E_2 and P_4 were measured using competitive ELISA (Alpco, Salem, NH, USA). The range for the E_2 assay was 20 - 3200 pg/ml with a sensitivity of 10 pg/ml. Intra-assay and inter-assay coefficients of variation for the E_2 assay were 2.71% and 1.42%, respectively. The range for the P_4 assay was 0.3 - 60 ng/ml with a sensitivity of 0.1 ng/ml. Intra-assay and inter-assay coefficients of variation for the P_4 assay were 3.52% and 1.42%, respectively. Plasma ET-1 was analyzed using an ELISA (R&D systems, Minneapolis, MN, USA). Intra- and inter-assay coefficients of variation were less than 2.5%. All samples were measured at a wavelength of 450nm on an Infinite F200 Pro microplate reader and data was analyzed with Magellan IQ software (Tecan Group Ltd., Männedorf, CH).

Data Analysis

LDF data was recorded at 1000 Hz using PowerLab (ADInstruments, Bella Vista, New South Wales, Australia). We analyzed a 5-minute segment after the local heating plateau was reached (42°C), and a 2-minute segment during SNP + local heating to 43°C (maximum

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dilation) for each site. CVC was calculated as SkBF/MAP. CVC was expressed as a percent of maximum dilation to account for site-to-site variations in SkBF and was used as an indicator of vasodilatory function [10,28].

Statistical Analyses

We compared vasodilatory responses to local heating in the ET-1 receptor-blocked sites (BQ-788 and BQ-123) and control site (lactated Ringers) during the EF and ML phases of the menstrual cycle using a 2x3 repeated measures ANOVA (cycle phase x SkBF site). Paired t-tests were used to compare serum E_2 and P_4, along with weight and MAP between EF and ML visits. Significance was set at $\alpha < 0.05$. Results are expressed as mean ± SEM.

RESULTS

Subject Characteristics

Subject characteristics from the screening visit are presented in Table 1. All women were normotensive, with standard blood chemistry levels within normal clinical limits. The average cycle length was 29±1 days. The racial demographics of our participants were as follows: 7 women were Caucasian, 2 Asian, 2 African American, and 1 woman was Hispanic. Subject characteristics from the two experimental visits (EF and ML phases of the menstrual cycle) are presented in Table 2. Blood analyses of ovarian hormones confirmed that women were in the appropriate phase (EF vs. ML) for the experimental visits. Serum E_2 and P_4 levels were significantly higher in women during the ML compared to the EF phase ($P<0.05$, Table 2). Weight and BP were similar between EF and ML phases, as well as plasma ET-1 (Table 2).

Vasodilatory Responses to ET-1 Receptor Blockade

During the ML phase, vasodilatory responses to ETB_R blockade were attenuated compared to control conditions (control: 91±2% vs BQ-788: 83±2%, $P<0.05$; Figure 1). There was no change in %CVCmax with ETB_R blockade during EF (control: 89±2% vs BQ-788: 89±1%, $P>0.05$; Figure 1). However, when comparing ETB_R blockade responses between phases, vasodilation was attenuated during ML compare to EF (ML: 83±2% vs EF: 89±1%, $P<0.05$; Figure 1). Cutaneous vasodilatory responses to ETA_R blockade were also lower during ML (control: 91±2% vs BQ-123: 87±2%, $P<0.05$; Figure 2), whereas there were no differences during EF (control: 89±2% vs BQ-123: 92±2%, $P>0.05$; Figure 2). When comparing ETA_R blockade responses between phases, vasodilation was attenuated during ML compare to EF (ML: 87±2% vs EF: 92±2%, $P<0.05$; Figure 2). Vasodilatory function at control sites were not different between ML and EF phases (91±2 vs 89±2%, $P>0.05$).

DISCUSSION

The novel finding of the current investigation is that endogenous fluctuations in ovarian hormones during the menstrual cycle modulate ET-1 receptor responses in women. Specifically, ETB_R mediate dilation during the ML phase, when endogenous E_2 and P_4 are elevated. To our knowledge, this is the first study to examine *in vivo* ET-1 receptor responses to changing endogenous ovarian hormone levels in young women. We previously examined

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ET-1 receptor responses in young and postmenopausal women, and demonstrated that ETB_R mediated dilation is lost after menopause [10]. Results from the current study extend our previous findings by suggesting that changes in sex steroid levels alter ETB_R and ETA_R responses in women. Taken together, these data are an important first step in demonstrating the interactions between ovarian hormones and ET-1 receptor responses in women.

Although the importance of ET-1 and its involvement with vascular tone and function are well known, less is understood regarding regulation of ET-1 and its receptors by ovarian hormones. Evidence in animal models suggest a direct relation between alterations in ovarian hormones and ET-1 receptor expression. For example, ovariectomy caused an increase in ETB_R mRNA and protein expression in the myocardium [14] and kidney [34] of animal models, which was reversed with administration of either E_2 [14] or E_2 and P_4 [34]. Although we did not measure changes in receptor expression in the current study, it is possible that the ETB_R-mediated dilation during the ML phase was related to an upregulation of endothelial ETB_R or downregulation of vascular smooth muscle ETB_R. However, while E_2 treatment increased ETB_R expression and elicited vessel relaxation in rabbits, the addition of P_4 negated these changes in receptor expression and abolished the E_2 vasorelaxation [13]. With this in mind, and given that both E_2 and P_4 were elevated during the ML phase in our study, changes in receptor expression may not solely explain our findings. Stimulation of the ETB_R can also result in increased NO and prostacyclin [35], which may be in part related to the results of the current study. It is well appreciated that E_2 increases NO release to elicit dilation. Furthermore, both E_2 and P_4 increase prostacyclin production in animal and cell culture models [36-38]. Thus, our findings may also be related to pathways downstream of ETB_R and/or other signaling mechanisms related to changes in ovarian hormones.

Our data also suggest that endogenous hormone production impacts ETA_R responses, as we observed a reduction in vasodilation with blockade of ETA_R during the ML phase. Since ETA_R mediate vasoconstriction, we interpret these findings to suggest that elevations in endogenous ovarian hormones attenuate the vasoconstrictor capacity of ETA_R. In this manner, these findings are consistent with data in animal models where ETA_R expression on vascular smooth muscle cells was reduced with E_2 administration [39]. However, ETA_R and ETB_R can form heterodimers and thus functionally interact [40,41], whereby stimulation of one receptor could impact the function of the other. It is possible that this receptor interaction occurred in our sample and may have impacted our results. In other words, if ETB_R expression was greater during the ML phase, then stimulation of these receptors may impact the ETA_R and partially explain the reduction in vasodilation with blockade of ETA_R during the ML phase. Alternatively, there may also be biased ligands for ETA_R such that blockade of this receptor may elicit differential signaling [41-43], contributing to the reduction in vasodilation with blockade of ETA_R. Finally, it is also possible that changes in estrogen receptors interact with ET-1 signaling and have impacted our data. Expression of the ERα receptor changes during the menstrual cycle [44], and an over-expression of ERα receptors in cell models inhibits ET-1 release [45]. Further, activation of estrogen receptors such as ERα or GPER using selective estrogen receptor modulators reduce ET-1 mediated constriction [46-48]. Thus, ET-1 signaling and receptor activity may be influenced directly by fluctuations in ovarian hormones or also through changes in gonadal steroid receptors.

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In the current study, we demonstrate that ETB_R mediate dilation during the ML phase of the menstrual cycle, and observed no impact of blocking ETB_R during the EF phase. This was in contrast to our recent publication where we demonstrated ETB_R mediate dilation in young women tested during the EF phase [10]. However, our prior publication included women using hormonal contraceptives, and were tested during the 'low hormone' or 'placebo' week of the pill. Although we were not powered to examine differences in ETB_R responses between those who were using hormonal contraceptives and those who were not, it is possible that the chronic exposure from exogenous hormones impacted ETB_R responses, and may partially explain in the current study (with no women using exogenous hormones) why we saw a minimal effect of blocking ETB_R during the EF phase. Alternatively, while often termed the 'low hormone' phase of the pill (placebo week), women may experience an endogenous surge in hormone production during the placebo week [16,49-52]; based on the finding from the current study, it is likely that endogenous hormone production impacts ETB_R responses. Our findings may also help to explain prior reports of sex differences in ET-1 receptor responses on vascular function, as we demonstrate that female reproductive hormones have a strong influence on ET-1 receptor responses. The receptor density of ETA_R to ETB_R is greater in men compared to women, and predominately mediate vasoconstriction. Based on current literature, we speculate that the greatest difference in ET-1 receptor responses would be between men and women tested during the ML phase. Further study is needed to characterize the impact of sex vs. sex hormones on the ET-1 system.

We did not observe statistical differences in the vasodilatory responses at the control site between the two phases of the menstrual cycle. We utilized a standard heating protocol that has been well established to elicit NO-mediated dilation [31-33] and mirrors the response to acetylcholine perfusions via microdialysis [53]. However, the amount of dilation achieved via local heating is proportional to the temperature used [54,55], and therefore we may have achieved maximal dilation in both phases. To this end, a recent paper by Choi and colleagues [56] demonstrated that a rapid heating protocol to 39°C may offer an improved approach to isolate NO-depend dilation in the microcirculation. Using a ramping protocol for local skin heating (a slower and graded increase), vasodilatory responses were shown to be greater during the high hormone phase of oral contraceptive use compared to the placebo / low hormone phase [57]. To our knowledge, ours is the first study to examine changes in cutaneous vasodilation during *endogenous* hormonal fluctuations. Interestingly, our findings in the cutaneous microcirculation also mirror those of conduit artery endothelial function; although flow-mediated dilation of the brachial artery increases during ovulation, it declines during the luteal phase such that there are no differences between EF and ML phases [58]. Based on findings using a controlled hormone intervention to isolate the independent effects of E_2, the increase in vasodilation is primarily related to E_2, and these effects are negated by the addition of P_4 [59]. Taken together, we speculate that chronic exposure to exogenous sex steroids alters vasodilatory function in women, and further supports that exogenous and endogenous hormones may have differentiating effects on vascular function. Indeed, there are many formulations of hormonal contraceptives which can impact tissues differently, in particular the vascular endothelium [60-62]. Further study is needed to fully understand the

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impact of endogenous versus exogenous hormones on vascular function, especially given the number of women using hormonal contraceptives.

Limitations

Several vasoactive substances are involved in regulating vascular function, such as NO, prostaglandins, angiotensin II, and norepinephrine [63-65], all of which are influenced by E_2 [66] and interact with ET-1 [67]. It is possible that the effects we observed in our study are related to the influence of E_2 on one or more of these substances; we are not able to determine which additional mechanisms may be involved from our data. Unfortunately, we did not have additional blood samples to also measure NO, angiotensin II, prostaglandins, or norepinephrine to gain insight into these other pathways. Additional study is needed to further characterize the interactions among ovarian hormones and the mechanisms regulating vascular function. Also, since E_2 has been shown to reduce circulating ET-1 [11,12], it is also possible that our findings are related to a reduced production of ET-1. However, we did not observe differences in plasma ET-1 between ML and EF. Vasoconstrictor responses to ET-1, independent of maximum vasoconstrictor capability, do not differ between men and women, despite showing sex differences in ETA_R and ETB_R responses [9], suggesting that the receptors – and not ET-1 itself – play a crucial role in determining the vascular response. Nevertheless, our data indicate that ovarian hormonal fluctuations influence both ETB_R and ETA_R *in vivo*.

Conclusions

In conclusion, we demonstrate that ETB_R blockade reduced vasodilation in young women during the ML phase of the menstrual cycle. These data suggest that ETB_R mediate vasodilation when endogenous E_2 and P_4 levels are high. This extends previous findings of ETB_R mediating vasodilation in young women [5,8,10] by demonstrating fluctuations in hormones play a primary role in modulating these responses. In addition, we saw a reduction in vasodilation during ETA_R blockade, suggesting that high levels of circulating ovarian hormones impact ETA_R responses as well. These findings are an important first step in understanding the mechanisms by which changes in endogenous hormone exposure may impact vascular function in women.

Perspectives

Our data demonstrate that fluctuations in endogenous ovarian hormones that occur during the menstrual cycle impact ETB_R and ETA_R in healthy women. Alterations in endogenous hormone production that occur with menopause [10] or reproductive disorders such as polycystic ovary syndrome [27] display a dysregulation of ETB_R responses in the vasculature. These data further support the link between sex hormones and the ET-1 system on cardiovascular function.

ACKNOWLEDGMENTS

The authors wish to thank Stephanie Mraz and Kenneth Kirschner for assistance with data collection/analysis, and the participants for their time.

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Funding Support: This research was supported in part by the DE-CTR Accel Program (U54-GM104941), the DE-INBRE Program (5 P20 GM103446-13), the University of Delaware Research Foundation, and P20 GM113125 (Center of Biomedical Research Excellence).

Funding Support: U54-GM104941, 5 P20 GM103446-13, P20 GM113125, and the University of Delaware Research Foundation

List of Abbreviations

BP	blood pressure
BMI	body mass index
CVC	cutaneous vascular conductance
EF	early follicular
E_2	estradiol
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assays
ET-1	Endothelin-1
ETA_R	endothelin A receptor
ETB_R	endothelin B receptor
HUVECs	human umbilical vein endothelial cells
LDF	laser Doppler flowmetry
MAP	mean arterial pressure
ML	mid-luteal
NO	nitric oxide
P_4	progesterone
SkBF	skin blood flow
SNP	sodium nitroprusside

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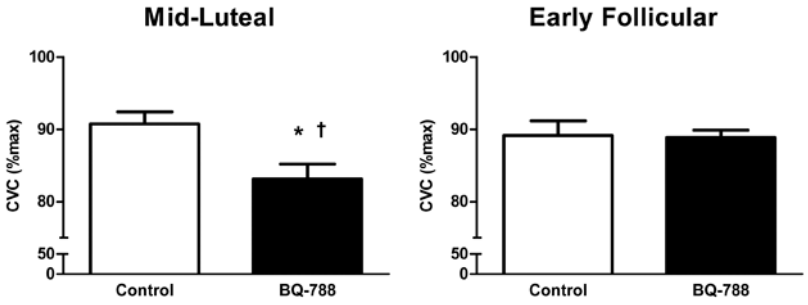

Figure 1:

Cutaneous vasodilatory responses expressed as cutaneous vascular conductance (CVC %max) during microdialysis perfusion of lactated Ringer (Control) and ETB_R blockade (BQ-788) during the Mid-Luteal Phase (left) and Early Follicular Phase (right) of the menstrual cycle. Data are presented as means ± SEM. * $P < 0.05$ vs control. † $P<0.05$ vs. Early Follicular Phase.

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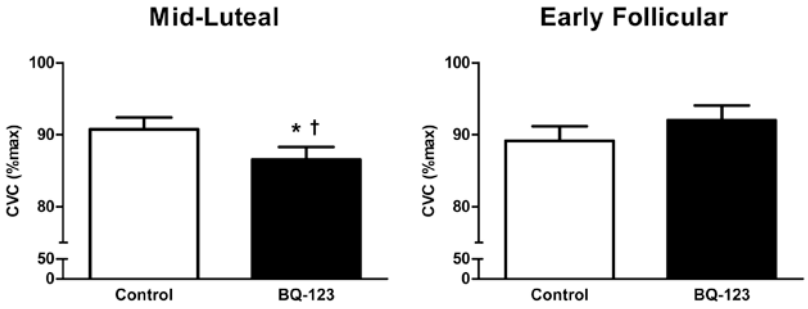

Figure 2:

Cutaneous vasodilatory responses expressed as cutaneous vascular conductance (CVC %max) during microdialysis perfusion of lactated Ringer (Control) and ETA_R blockade (BQ-123) during the Mid-Luteal Phase (left) and Early Follicular Phase (right) of the menstrual cycle. Data are presented as means ± SEM. * $P < 0.05$ vs control. † $P<0.05$ vs. Early Follicular Phase.

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Table 1:

Demographic and Screening Characteristics

Variable	***n*=12**
Demographic Information	
Age (years)	22 ± 1
Height (cm)	165 ± 2
Weight (kg)	62 ± 3
BMI (kg/m^2)	23 ± 1
Hemodynamic Measurements	
Heart rate (beats/min)	61 ± 4
Systolic BP (mmHg)	111 ± 3
Diastolic BP (mmHg)	72 ± 2
MAP (mmHg)	85 ± 2
Blood Chemistry	
Total cholesterol (mg/dl)	154 ± 6
High-density lipoprotein (mg/dl)	62 ± 4
Low-density lipoprotein (mg/dl)	76 ± 7
Triglycerides (mg/dl)	76 ± 14
Hemoglobin (mg/dl)	12.9 ± 0.2
Hematocrit (%)	39.8 ± 0.6
Glucose (mg/dl)	74 ± 2

Body mass index (BMI), blood pressure (BP), mean arterial pressure (MAP).

Data are presented as mean ± SEM.

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Table 2:

Subject Characteristics during Experimental Visits.

Variable	Early Follicular Phase	Mid-Luteal Phase
Weight (kg)	62 ± 3	62 ± 3
Systolic BP (mmHg)	105 ± 3	101 ± 2
Diastolic BP (mmHg)	65 ± 2	61 ± 1
MAP (mmHg)	78 ± 2	74 ± 2
Day of Cycle Tested	4 ± 1	23 ± 1 *
Plasma ET-1, pg/mL	1.3 ± 0. 1	1.4 ± 0.1
Estradiol, serum (pg/ml)	44 ± 9	118 ± 15 *
Progesterone, serum (ng/ml)	0.7 ± 0.2	8.3 ± 0.8 *

Blood pressure (BP), endothelin-1 (ET-1), mean arterial pressure (MAP).

Data are presented as mean ± SEM.

* $P < 0.05$ vs. Early Follicular Phase.