Short-term oral progesterone administration antagonizes the effect of transdermal estradiol on endothelium-dependent vasodilation in young healthy women

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Abstract

Background. Very few studies have explored the cardiovascular effects of progesterone in premenopausal women. This study aimed to examine the short-term effects of oral progesterone-only, transdermal estrogen-only, and progesterone and estrogen combined on flow-mediated dilation (FMD) in healthy reproductive-aged women.

Methods. We suppressed endogenous estrogens and progesterone in 17 premenopausal women for 10-12 days using a gonadotropin-releasing hormone antagonist (GnRHa). On day 4 (hormone suppression condition), subjects were tested (n=17) and then were supplemented with either 200 mg micronized progesterone (n=8) orally or 0.1 mg estradiol (n=9) transdermally per day. On day 7 (progesterone-first or estradiol-first condition) subjects were tested and began supplementation with both hormones (n=17), and were tested again on day 10 (combined hormone condition). FMD of the brachial artery was assessed using B-mode arterial ultrasound, combined with synchronized Doppler analysis.

Results. Significant differences in FMD were observed between hormone suppression (7.85±1.06%) and estrogen-first conditions (10.14±1.40%; p<0.05). The estradiol-induced increase was abolished when oral progesterone was also supplemented (6.27±0.96%). In contrast, we observed a trend toward a decrease in FMD with unopposed progesterone administration, but no statistically significant differences were found between the progesterone-first (6.66±1.23%), hormone suppression (7.80±1.23%), and combined hormone conditions (7.40±1.29%).

Conclusion. These data suggest that short-term oral micronized progesterone administration antagonizes the beneficial effect of transdermal estradiol on flow-mediated dilation.

Key Words

Flow-mediated vasodilation, endothelial function, sex hormones, birth control, estrogen
Introduction

With over 76% of childbearing-aged women in the United States taking exogenous hormones for contraceptive and gynecological purposes (1), the exploration of how exogenous hormones affect cardiovascular health is imperative. Although research on sex hormones has mainly focused on the effects of estrogens and manufactured progestins, there is relatively little known regarding the effect of progesterone on the vasculature. With progesterone production occurring naturally within the body, and its bio-identical exogenous form being one of the most frequently prescribed progestogens, the need to understand the influence of progesterone on cardiovascular health is great.

One of the primary methods used to investigate the effect of sex hormones on vascular health is via flow-mediated dilation (FMD). FMD, measured as the percent change in brachial artery diameter in response to an increase in shear stress, has been utilized widely as a non-invasive technique to assess endothelial function, and is known to parallel endothelial function in the coronary arteries (3). FMD can be used as a predictor of future cardiovascular events across many different ages and disease states (16, 18, 27, 29, 35, 39, 53), and adds independent prognostic value in determining cardiovascular risk in women (35).

FMD values have been shown to vary between sexes (21), and the technique has been used to investigate changes in endothelial function during the menstrual cycle (13, 51). FMD is lowest during menstruation (low-estrogen, low-progesterone phase) (13), and has been shown to vary with the rise and fall of estrogen throughout the menstrual cycle (13, 51). However, it is unclear whether the lower FMD during the mid-luteal phase, compared to the ovulation phase (high-estrogen, low-progesterone), is due to the rise in progesterone or the lowered estrogen during this phase. Administration of estradiol has also been demonstrated to increase FMD compared to hormone-suppressed values (5, 10, 11, 25, 26, 47). However, cardiovascular
responses to different types of exogenous progestogen administration vary widely (2, 7, 23, 36).

Our lab has previously shown that levonorgestrel, desogestrel, and medroxyprogesterone acetate (MPA) all antagonized ethinyl estradiol’s effects on FMD (25, 26, 47). Conversely, etonorgestrel (via a vaginal ring) and drospirenone did not (24, 48). Whereas various progestins produce different effects on the vasculature, it is unknown what effect progesterone itself might have on endothelial function.

Research on the effects of unopposed progesterone administration on the vasculature of young healthy women is non-existent. Therefore, this study aimed to evaluate the effects of progesterone, both in combination with estradiol and without, on endothelial function in healthy young reproductive-aged women. Through our design of suppressing endogenous sex hormones and then adding back hormones exogenously, we hypothesized that the beneficial effects of estradiol on endothelial function would not be antagonized by progesterone, and that progesterone administration alone would not decrease endothelial function.

### Methods

#### Subjects

Seventeen healthy, recreationally active females (exercise limited to 1-3 days per week, for less than 1 hour per day), between the ages of 18 and 29, completed the protocol. All subjects were nonsmokers, had a BMI less than 25, and were not taking any medications, with the exception of combined hormonal contraceptives (n=13). Subjects taking contraceptives discontinued use and began menstruating prior to starting GnRHa suppression. Before participating in the study, a physician specializing in gynecology screened all subjects for a history of cardiovascular disease, hypertension, hypercholesterolemia, diabetes, medical allergies, clotting disorders, endocrine and/or menstrual disorders, and recent surgical
procedures. Subjects were required to take a pregnancy test, demonstrating negative results, at
the beginning of each testing session. Subjects abstained from exercise, alcohol, vitamins, and
over-the-counter medications for 24 hours prior to each trial. Additionally, subjects abstained
from caffeine for 12 hours, and subjects were either fasted for 12 hours prior to each trial (if the
study occurred in the morning), or instructed to eat a light, low-fat meal no less than two hours
prior to the start of the protocol (for afternoon studies, in order to prevent a hypoglycemic
episode). All testing was conducted in a temperature-controlled room (21°C to 23°C), and each
testing session was conducted at the same time of day across trials for each individual subject
(time of sessions was counter-balanced across conditions). Approval for this study was granted
by the Institutional Review Board at the University of Oregon, and each subject provided written
and oral consent for the protocol prior to enrolling in the study.

**Study Design**

After a successful screening interview, subjects initiated endogenous female sex hormone
suppression via 250 μg/0.5 ml subcutaneous injection of the gonadotropin-releasing hormone
antagonist (GnRHa), ganirelix acetate (Organon International, Roseland, NJ), on the first day of
their next menstruation (day 1 of study). Subjects continued GnRHa suppression daily for the
entirety of their participation in the study (10-12 days, in total). Within 36-48 hours of beginning
GnRHa treatment, endogenous estrogens and progesterone are fully suppressed (31). On day 4 of
the study, subjects came in to the lab to participate in Trial 1 (hormone suppression condition).
Upon completion of testing on this day, subjects continued endogenous sex hormone
suppression, and were randomly assigned to one of two exogenous hormone add-back
conditions. These conditions consisted of supplementation with either 200 mg progesterone
orally each evening (n=8) or 0.1 mg estradiol transdermally (n=9) per day. Estradiol (Estradiol;
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Mylan Pharmaceuticals Inc, Morgantown, WV) was given as a weekly 31 mm² transdermal patch that contains 3.88 mg of estradiol USP, ultimately delivering 0.1 mg of estradiol per day. The progesterone administered (Prometrium; Solvay Pharmaceuticals, Marietta, GA) is a bio-identical equivalent to endogenous progesterone. On study day seven, subjects returned to the lab to participate in Trial 2 (progesterone-first or estradiol-first conditions), and then began administration of both estradiol and progesterone at the dosages stated above. Subjects returned for Trial 3 (combined hormone condition) on day 10. Figure 1 provides a schematic diagram of our study design.

After conclusion of this original study, we discovered subjects had lower serum levels of estradiol than predicted in our combined hormone condition in the estradiol group. To explore this issue further, we conducted a follow-up study in which we suppressed endogenous hormones in four additional subjects, studying them on day 4 for the hormone suppression condition. We then administered 0.2 mg of transdermal estradiol (double estradiol group), and studied them again during a combined hormone condition (double-dose of estradiol and single-dose of progesterone) on day 10. Subjects were also instructed to replace the patches every three days, to avoid modulation of the medication dose. Results and discussion for this follow-up study are included in appropriate sections below.

**Protocol**

Subjects entered the lab for each trial and were instrumented with a five-lead electrocardiogram (ECG) and a blood pressure cuff on the left brachium. Heart rate was monitored continuously throughout the protocol (CardioCap, Datex-Ohemda, Louisville, CO), and was recorded during baseline, during each subsequent 20-minute resting period, and after completion of testing. Blood pressure was measured noninvasively from the left arm via
automated brachial oscillation (CardioCap 5, Datex-Ohemda, Louisville, CO) and was recorded simultaneously with heart rate.

To measure FMD, an inflatable occlusion cuff was placed on the right forearm, approximately 2 cm distal to the antecubital fossa. Using a high-resolution Doppler ultrasound machine (Terason t3000cv, Teratech Corporation, Burlington, MA), a 10.0-MHz linear array ultrasound transducer probe was placed on the brachial artery (using an insonation angle of 60°), between 3-10 cm proximal to the antecubital fossa. Probe placement, occlusion cuff placement, and arm-trunk angle were recorded to ensure consistency between trial days. Endothelial function testing was performed as previously described by Celemajer et al. (8), followed the guidelines suggested by Corretti et al. (9) and Thijssen et al. (43), and are summarized below.

After a clear image of the brachial artery was obtained and the subject rested for a minimum of 20 minutes, measurements of brachial artery diameter and blood velocity were recorded. After one minute of baseline measurements, an occlusion cuff (Zimmer, Dover, OH) was rapidly inflated to 300 mmHg (E20 Rapid Cuff Inflater, D.E. Hokanson, Bellevue, WA). The occlusion was maintained for five continuous minutes, and recording continued for three minutes post release. Upon release, brachial artery blood flow was increased, causing a shear stress on the vessel walls. In response to this shear stress, nitric oxide (NO) is released from the endothelium, causing a reactive vasodilation, known as FMD (17). FMD was assessed as the percent change in brachial artery diameter from baseline to peak dilation. After a 20-minute supine rest, a second FMD test was conducted.

Doppler ultrasound data was collected at 20 frames/s (Camtasia Studio, TechSmith, Okemos, MI). After completion of an entire study day, data files were transferred to a computer that operates a custom-designed edge-detection and wall-tracking analysis software (DICOM, Perth, Australia), which can track the vessel walls, measuring the average of all diameters
throughout the entire cardiac cycle and the peak blood velocity tracing in real-time. These synchronous measurements allow blood flow (product of cross-sectional area and Doppler velocity) to be calculated (52). FMD is calculated as the percent change between baseline diameter and peak dilation post occlusion cuff release (FMD = \[\text{peak diameter (mm)} - \text{baseline diameter (mm)}\] \times 100). This software has demonstrated the ability to detect a 1.5-2.0% change in dilation within 8 subjects with a power of 80%, requiring fewer subjects than other methods of analyses (52).

Since peak dilation may not be achieved until 40 to 90 seconds post cuff release, we continued recording and analyzing three minutes post release (6, 43). We calculated shear rate as the product of four times the velocity, divided by the diameter. By plotting shear rate as a function of time (until peak dilation is reached), we determined the time to peak dilation shear rate area under the curve (AUC), which is the relevant individual area under the shear rate curve that is the responsible stimulus for the peak dilation (34, 43). There was no correlation between shear rate and FMD percentage within the progesterone-first group (r=0.09, p>0.05), however there was a correlation between these two measurements in the estradiol-first group (r=0.45, p=0.033). Due to a weak correlation between shear rate and FMD percentage across the two groups (r=0.25, p>0.05), we chose not to normalize our data for shear rate, as recommended by Atkinson et al. (4) and Thijssen et al. (43).

After a 20-minute rest, we assessed each subject’s endothelium-independent vasodilation (EIVD). After one minute of baseline data collection, 0.4 mg of nitroglycerine (Nitrolingual; Sciele Pharma Inc, Atlanta, GA) was administered sublingually. Data were collected and analyzed for nine minutes post administration. The administration of nitroglycerine elicits maximum dilation of the conduit arteries (25, 26, 47, 48). EIVD is calculated as the percent
change between baseline diameter and peak dilation post nitroglycerine administration (EIVD = [peak diameter (mm) – baseline diameter (mm)] / [baseline diameter (mm)] x 100).

After completion of endothelial function testing, an intravenous catheter was inserted in an antecubital vein of the non-tested arm, blood samples were taken, and subjects rested in a sitting position until blood pressure returned to baseline. Samples were collected in appropriate blood collection tubes (BD Vacutainer, Franklin, NJ), centrifuged at 1300 g relative centrifugal force for 15 minutes at 4°C, separated and stored frozen at -70°C within 30 minutes, and later transferred to Oregon Clinical and Translational Research Institute (OCTRI, Portland, OR) for analysis. Analyses of the samples included measuring blood hormone levels of estrogen and progesterone.

**Statistical Methods**

Subject demographic information was compared between the progesterone group (subjects who went through the progesterone-first study design) and estradiol group (those who received estradiol-first during the study protocol) using two-sample homoscedastic t-tests at baseline. Within- and between-subject comparisons, based on group membership and hormone administration conditions, were made using a mixed model (also called a multilevel model, hierarchical linear model, or random coefficients analysis). This model was chosen because the data include multiple observations for each subject. Multilevel models appropriately account for the resulting dependence between observations that are nested or clustered within subjects. The benefit of the multilevel approach is that it uses a single model for both between-subjects and within-subjects factors, to reduce the chance of biased estimates and inflated Type I error rates (which can be commonly found in ANOVA and ANCOVA analysis approaches). We fit our mixed models with SAS PROC MIXED version 9.2 (SAS Institute, 2009) using Restricted
Maximum Likelihood (REML) estimation for parameter estimation. REML is an efficient algorithm and reduces likelihood of biased variance estimates, which may occur with a full information maximum likelihood approach. Statistical significance was defined as $\alpha=0.05$. All data are expressed as means ± standard error.

**Results**

**Subject Characteristics**

Baseline characteristics for both study groups are displayed in Table 1. Between groups, subjects did not differ in age, height, weight, body mass index, baseline brachial artery diameter, baseline heart rate or baseline blood pressures.

Within each group (those who received progesterone versus estradiol first during the study protocol), there were no significant differences in height, weight, BMI, heart rate, or diastolic blood pressure across the three trial days (See Table 1 for baseline data). However, within the estradiol group, systolic blood pressure was significantly higher during the hormone suppression condition, as compared to the estradiol-first ($p=0.014$) and combined hormone ($p=0.006$) conditions. Mean arterial pressure was also significantly higher in the hormone suppression condition, as compared to the combined hormone condition ($p=0.014$). No blood pressure differences were found within the progesterone group across study days. Additionally, within each group there were no differences in baseline brachial artery diameters across trial days. See Tables 2 and 3 for blood pressure and heart rate data across trial days.

Endogenous estradiol and progesterone levels were suppressed during Trial 1 (hormone suppression condition) in both groups (Tables 2 & 3). In both groups there was an increase in estradiol levels between Trial 1 and Trial 2 (progesterone-first, $p=0.007$; estradiol-first, $p<0.001$), although the small increase in estradiol in the progesterone-first condition would not
indicate any physiological significance. Progesterone levels remained consistent between Trial 1 and Trial 2 in the estradiol-first group. However, as expected, we saw a rise in progesterone levels in the progesterone-first condition (p=0.002) between Trial 1 and Trial 2. This elevated level of progesterone remained stable within the progesterone group for the combined hormone condition (still significantly different from hormone suppression, p<0.001). Within the progesterone group, estradiol levels rose significantly in the combined hormone condition, and were significantly different from both hormone suppression (p=0.003) and progesterone-first (p=0.004) conditions. Within the estradiol group, we saw a decrease in estradiol levels from estradiol-first to the combined hormone condition (p=0.009), yet they were still elevated from hormone suppression (p<0.001). Between-group analyses of hormone levels indicate that estradiol and progesterone levels did not vary between groups during hormone suppression or combined hormone conditions. As designed, estradiol was significantly higher during the estradiol-first condition than during hormone suppression (p<0.001), and progesterone was significantly higher during the progesterone-first condition (p<0.001).

**Endothelium-Dependent Vasodilation**

Endothelial function characteristics are displayed in Tables 2 and 3, and in Figure 2. There were no differences in FMD within, or between, the progesterone group and estradiol group during the hormone suppression and combined hormone conditions. In the estradiol group, FMD was higher in the estradiol-first condition than in the hormone suppression (p=0.037) and combined hormone conditions (p=0.001, see Figure 2A). In the progesterone group, there was a trend toward a decreased FMD in the progesterone-first condition as compared to the hormone suppression and combined hormone conditions (See Figure 2B), yet no statistically significant differences were found (p>0.05). A sample size analysis, using a desired significance level of
α=0.05 and a power of 80%, indicated that 135 subjects would be required to achieve a statistically significant difference between the hormone suppression and progesterone-first conditions. However, FMD in the progesterone-first condition was significantly lower than in the estradiol-first condition (p=0.038).

Endothelium-Independent Vasodilation

There were no significant differences in nitroglycerine-mediated vasodilation (GTN%) within or between the progesterone or estradiol groups across the different hormone conditions (p>0.05, Tables 2 & 3).

Follow-up Study

In the follow-up study, the administration of 0.2 mg of estradiol (double estradiol group) increased serum estrogen levels in the additional subjects as compared to the original study, and estradiol levels were significantly higher in the double estradiol group during the combined hormone condition (133.7±23.5 pg/ml) compared to the single estradiol group (p=0.021). FMD values during the combined hormone conditions between the double estradiol group and the single-dose of estradiol group displayed the same trend, indicating that the lower serum levels of estradiol in the single-dose study did not change the FMD response in Trial 3 (See Table 4).

Discussion

The goal of this study was to explore the effects of progesterone on endothelial function. In contrast to our hypotheses, we found that combined administration of estradiol and progesterone decreased endothelial function, measured via FMD, in comparison to the increase in FMD with estradiol-only administration. There was no statistically significant decrease in
Progesterone antagonizes estrogen in FMD

FMD with unopposed progesterone administration compared to endogenous sex hormone suppression or combined progesterone and estradiol conditions, although we did observe a trend. These findings suggest that oral progesterone alone does not profoundly reduce endothelial function, yet antagonizes estradiol’s beneficial effects.

Changes in endothelial function in response to various exogenous progestogens have produced mixed results in previous studies. Our lab has previously shown a negative impact of desogestrel, levonorgestrel, and MPA (all combined with varying doses of ethinyl estradiol) on FMD levels (25, 26, 47). Lizarelli et al. (22) also discovered similar responses in FMD in young women, showing that levonorgestrel (when combined with ethinyl estradiol) and depot medroxyprogesterone acetate decrease FMD, compared to age-matched controls. Alternatively, our lab found an increase in FMD upon administration of transvaginal etonorgestrel (48), and oral drospirenone when combined with ethinyl estradiol (24), compared to their respective placebo weeks. We suspect the specific form of progestogen may cause the varying effects on the cardiovascular system (38), and may be related to variable effects of hormones on NO production, and possibly endothelin-1 (25).

It has been shown that the FMD response after a five-minute occlusion is predominately mediated by the release of NO (9, 12, 30). Increased endothelial function that is documented with estrogen administration is largely attributed to increased eNOS expression and NO production (33, 42, 44, 50). Some progestins, such as drospirenone, also seem to increase the synthesis of NO in endothelial cells, as reported by Simoncini et al. (40), and lead to enhanced vasodilation. This may account for the increase in FMD our lab has found during combined drospirenone and ethinyl estradiol administration (24). MPA’s deleterious effects on endothelial function may relate to its vasoconstricting properties, caused by a decrease in NO release (37), or an increase in production in endothelin-1 (25).
Natural progesterone appears to stimulate NO synthesis through transcriptional and nontranscriptional pathways, both in human endothelial cells and in ovariectomized rat abdominal aortas (41). Progesterone has also shown several other beneficial effects on the vasculature. It protects against atherosclerosis by inhibiting smooth muscle proliferation in human and rat aortic smooth muscle cells (19, 20), which may be mediated via a p53-dependent pathway (15). In ovariectomized rats with pulmonary hypertension, treatment with progesterone decreased the severity of the hypertension, decreased vascular remodeling, and decreased mortality (45). We hypothesized that these improvements in vascular function would also extend to increases in FMD in our young healthy women.

Contrary to our results, Gerhard et al. (11) showed an increase in FMD with both estradiol-only and estradiol-progesterone administration in postmenopausal women. However, the dose and route of administration of progesterone in our study (200 mg orally) differed from the Gerhard et al. study (300 mg vaginally), suggesting that the difference in responses may be due to the dose or the route of administration of progesterone. When taken orally, progesterone undergoes first pass metabolism in the liver. The route of administration, and subsequent change in chemical structure, may alter which receptors are activated at the target organs, and potentially could cause the effects to be more closely akin to progestin-like responses. We have previously observed this phenomenon in women using MPA, in which the oral form of MPA antagonized estrogen’s effects (25), but estrogen administration resulted in improvements in endothelial function in women taking the intramuscular injection form of MPA (46).

Arterial smooth muscle walls contain progesterone receptors (19, 20, 38). A study conducted by Toth et al. (49) on human umbilical vein endothelial cells discovered that progesterone specifically acts on progesterone receptor-A, and implied that other progestins, such as MPA, may not act on this specific receptor. Future research on progesterone receptors...
may help us to solve the mystery on why different progestogen administrations result in variable
(positive and negative) effects.

Negative effects of progestogens on FMD have also been found in postmenopausal
women. Faludi et al (10) showed that norethisterone acetate administration decreased FMD in
comparison to unopposed estradiol. The Women’s Health Initiative trials (2, 23, 36) ended the
conjugated estrogens-MPA study arm early due to the number of increased cardiovascular events
observed when compared to the estrogen-only and placebo arms. These studies suggest that
progestogens may antagonize estradiol’s effects in the postmenopausal woman as well.

However, similar to our results, Honisett et al. (14) found that unopposed oral progesterone did
not decrease FMD in comparison to baseline measurements in postmenopausal women, showing
that progesterone-alone does not decrease endothelial function in comparison to the menopausal
hormone state.

The effects on FMD from exogenous hormone administration in the current study seem
to mimic FMD changes that have been found with endogenous estrogen and progesterone
fluctuations during the menstrual cycle (15,51). The design of our study, by continuing estradiol
supplementation while adding progesterone (during Trial 3), allows interpretation that the high
level of progesterone during the mid-luteal phase of the menstrual cycle may be antagonizing
estradiol’s effects on the vasculature, possibly accounting for the decrease in FMD observed
during this phase. However, as pointed out above, differences in oral administration versus
naturally occurring, or delivered via an alternative route, make this conclusion tenuous.

Study Limitations. One limitation in our study was the lower than expected levels of
serum estradiol in response to the 0.1 mg transdermal dose observed in Trial 3 of the estradiol-
first group. We did conduct a follow-up study where we administered 0.2 mg of estradiol (double
estradiol group) in place of the dose used in the original study. As shown in the results, serum
progesterone antagonizes estrogen in FMD

Estrogen levels in these subjects were increased from the original study, and no longer decreased between Trial 2 and Trial 3. Furthermore, similar FMD values were observed during the combined hormone conditions of the double and single-dose estradiol groups.

An additional limitation of our study was that through the random assignment process, our data approached a significant difference in BMI between the progesterone-first and estradiol-first groups (p=0.07). As shown in previous studies, a larger BMI is associated with a lower FMD value (32). The intent of this study was to have no differences between the two groups at baseline, yet through random assignment, there was a trend of the progesterone-first group to have a slightly higher BMI. However, all women fell within a normal range of BMI, and there were no differences in FMD values or blood pressure between the two groups at baseline, which leads us to conclude that the near-significant difference in BMI did not translate into FMD results, and have no effect on interpretation of the results of this study.

Lastly, FMD tended to be lower when women were administered progesterone-alone, as compared to hormone suppression or combined hormone conditions. However, the decrease was not statistically significant. As discussed above, we would need to study a large number of women in order to achieve statistical significance, which simply is not feasible due to the cost of the GnRHa injections and the time required to study that large of a sample size. It is not clear how important this trend toward a lower FMD is, as the difference was only approximately 1%.

**Conclusions and Perspectives.** In this study, we verified that the administration of unopposed estradiol improves endothelial function, as measured by FMD. This increase in endothelial function is abolished when progesterone is added to the regimen, indicating that oral progesterone antagonizes estradiol’s effects on the endothelium in young healthy women.

Ongoing studies, such as the Kronos Early Estrogen Prevention Study (KEEPS)(13,14), are currently using oral progesterone in menopausal supplementation protocols (i.e. 200 mg oral
micronized progesterone for 12 days each month) in hopes of having more beneficial outcomes
than the WHI studies did with MPA administration (28). Our hypothesis of progesterone eliciting
differing vascular responses than previously found with MPA administration did not transpire
exactly as we expected. Although progesterone-alone did not decrease FMD values, it did
antagonize estrogen’s effects on the FMD. Therefore, further research is needed to expand our
basic understanding of the specific effects of progesterone on the vasculature, and whether these
changes translate to differences in cardiovascular risk and outcomes.

Disclosures: N/A

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27. Meyer B, Mortl D, Strecker K, Hulsmann M, Kulemann V, Neunteufl T,


48. Torgrimson BN, Meendering JR, Miller NP, Kaplan PF, Minson CT. 


52. Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Mori TA, Green D. 

53. Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. 
Figure 1. Study design. Subjects are tested under hormone suppression, progesterone-first or estrogen-first, and combined progesterone-estrogen conditions. Testing occurred on day 4, 7, and 10. GnRHa (hormone suppression via gonadotropin release hormone antagonist), P₄ (progesterone), E₂ (estradiol).

Figure 2. FMD and hormone condition. A. FMD was higher in the estrogen-first (GnRHa+E₂) condition than in the suppressed hormone state (GnRHa, p=0.037) and combined hormones condition (GnRHa+P₄+E₂, p=0.001). B. No statistical differences in FMD were found between hormone suppression (GnRHa), progesterone-first (GnRHa+P₄), and combined progesterone-estrogen conditions (GnRHa+P₄+E₂), p>0.05, although there was a trend toward a decreased FMD in the progesterone-first condition.
Table 1. *Baseline characteristics in the progesterone group and estradiol group*

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<th>Progesterone Group</th>
<th>Estradiol Group</th>
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<td>Weight (kg)</td>
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<td>Heart Rate (bpm)</td>
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<td>Mean Arterial</td>
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Values are in mean±standard error; progesterone group (n = 8) and estradiol group (n = 9)
Table 2. *Endothelial function and subject characteristics in the estradiol group*

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<tr>
<td>Shear Rate (AUC) (10³)</td>
<td>17.30±2.89</td>
<td>21.06±3.77</td>
<td>16.49±2.16</td>
<td></td>
</tr>
<tr>
<td>GTN %</td>
<td>19.33±2.13</td>
<td>19.12±2.31</td>
<td>18.42±1.86</td>
<td></td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>2.0±0.1</td>
<td>1.9±0.2</td>
<td>5.7±0.6 ‡</td>
<td></td>
</tr>
<tr>
<td>Estrogen (pg/ml)</td>
<td>16.3±1.5 *</td>
<td>122.2±19.9 †</td>
<td>67.7±8.1</td>
<td></td>
</tr>
<tr>
<td>Heart Rate</td>
<td>60±5</td>
<td>59±4</td>
<td>62±4</td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>113±2 *</td>
<td>109±2</td>
<td>108±2</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>71±2</td>
<td>70±2</td>
<td>68±2</td>
<td></td>
</tr>
<tr>
<td>Mean BP</td>
<td>85±2 †</td>
<td>83±2</td>
<td>82±2</td>
<td></td>
</tr>
</tbody>
</table>

Values are in mean ± SE; estradiol group (n = 9); GTN, nitroglycerine administration, AUC, area under the curve.

* Significantly different from Trial 2 and Trial 3
† Significantly different from Trial 3
‡ Significantly different from Trial 1 and Trial 2
Table 3. *Endothelial function and subject characteristics in the progesterone group*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial 1 Hormone Suppression</th>
<th>Trial 2 Progesterone-first</th>
<th>Trial 3 Combined Hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Diameter (mm)</td>
<td>3.17±0.18</td>
<td>3.23±0.20</td>
<td>3.21±0.24</td>
</tr>
<tr>
<td>Peak Diameter (mm)</td>
<td>3.41±0.18</td>
<td>3.44±0.22</td>
<td>3.43±0.23</td>
</tr>
<tr>
<td>Shear Rate (AUC) (10³)</td>
<td>21.99±3.64</td>
<td>22.86±4.32</td>
<td>18.20±1.77</td>
</tr>
<tr>
<td>GTN %</td>
<td>18.63±1.31</td>
<td>18.27±2.26</td>
<td>17.19±1.53</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>1.9±0.1 *</td>
<td>5.4±0.7</td>
<td>6.2±0.7</td>
</tr>
<tr>
<td>Estrogen (pg/ml)</td>
<td>13.0±1.3 *</td>
<td>17.2±1.3 †</td>
<td>81.4±15.3</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>57±3</td>
<td>59±3</td>
<td>58±3</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>110±2</td>
<td>111±1</td>
<td>109±2</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>70±2</td>
<td>69±2</td>
<td>69±1</td>
</tr>
<tr>
<td>Mean BP</td>
<td>84±2</td>
<td>83±2</td>
<td>82±1</td>
</tr>
</tbody>
</table>

Values are in mean ± SE; progesterone group (n = 8); GTN, nitroglycerine administration, AUC, area under the curve.

* Significantly different from Trial 2 and Trial 3
† Significantly different from Trial 3
Table 4. *Endothelial function characteristics in the double-estradiol group*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hormone Suppression</th>
<th>Combined Hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Diameter (mm)</td>
<td>3.10±0.12</td>
<td>3.11±0.12</td>
</tr>
<tr>
<td>Peak Diameter (mm)</td>
<td>3.34±0.14</td>
<td>3.35±0.12</td>
</tr>
<tr>
<td>FMD %</td>
<td>7.62±0.78</td>
<td>7.68±1.18</td>
</tr>
<tr>
<td>Shear Rate (AUC) (10³)</td>
<td>26.23±4.45</td>
<td>23.61±3.31</td>
</tr>
<tr>
<td>GTN %</td>
<td>21.34±1.95</td>
<td>21.22±0.32</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>1.7±0.3</td>
<td>4.8±0.7</td>
</tr>
<tr>
<td>Estrogen (pg/ml)</td>
<td>11.7±2.4</td>
<td>114±17.2</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>59±1</td>
<td>61±1</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>108±2</td>
<td>108±2</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>69±1</td>
<td>63±3</td>
</tr>
<tr>
<td>Mean BP</td>
<td>82±1</td>
<td>78±2</td>
</tr>
</tbody>
</table>

Values are in mean ± SE; double-estradiol group (n = 4); GTN, nitroglycerine administration, AUC, area under the curve.
<table>
<thead>
<tr>
<th>Study Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td>GnRHa</td>
<td>GnRHa+P_4</td>
<td>GnRHa+P_4+E_2</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td>GnRHa</td>
<td>GnRHa+E_2</td>
<td>GnRHa+P_4+E_2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A. FMD Percentage vs. Hormonal Condition
   Estradiol-First Group

B. FMD Percentage vs. Hormonal Condition
   Progesterone-First Group