Effects of menstrual cycle and oral contraceptive use on calf venous compliance

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Meendering, Jessica R., Britta N. Torgrimson, Belinda L. Houghton, John R. Halliwill, and Christopher T. Minson. Effects of menstrual cycle and oral contraceptive use on calf venous compliance. Am J Physiol Heart Circ Physiol 288: H103–H110, 2005. First published September 2, 2004; doi:10.1152/ajpheart.00691.2004.—Numerous studies have shown that the female sex hormones estrogen and progesterone have multiple effects on the vasculature. Thus our goal was to investigate the effects of estrogen and progesterone on calf venous compliance by looking for cyclic changes during the early follicular, ovulatory, and midluteal phases of the menstrual cycle and during high and low hormone phases of oral contraceptive use. Additionally, we wanted to compare the venous compliance of normally menstruating women, oral contraceptive users, and men. We studied eight normally menstruating women (23 ± 1 yr of age) during the early follicular, ovulatory, and midluteal phases of the menstrual cycle. Nine triphasic oral contraceptive users (21 ± 1 yr of age) were studied during weeks of high and low hormone concentrations. Eight men (23 ± 1 yr of age) were studied twice within 2–4 wk. With the use of venous occlusion plethysmography with mercury in-Silastic strain gauges, lower limb venous compliance was measured by inflating a venous collection cuff that was placed on the thigh to 60 mmHg for 8 min and then reducing the pressure to 0 mmHg at a rate of 1 mmHg/s. Venous compliance was calculated as the derivative of the pressure-volume curves. There were no differences between early follicular, ovulatory, and midluteal phases of the menstrual cycle or between high and low hormone phases of oral contraceptive use (P > 0.05). Male venous compliance was significantly greater than in normally menstruating women (P < 0.001) and oral contraceptive users (P < 0.002). These data support a sex difference but also suggest that venous compliance does not change with menstrual cycle phase or during the course of oral contraceptive use.

WHEN HUMANS ASSUME AN UPRIGHT posture, blood progressively pools in the compliant veins of the lower limbs. This action decreases central blood volume and venous return and subsequently elicits orthostatic stress by challenging blood pressure (2, 34). Greater lower limb venous compliance has been linked to orthostatic intolerance, which suggests that greater venous compliance augments venous pooling and therefore elicits greater reductions in venous return and stroke volume. For example, Halliwill et al. (12) report that the cardiovascular responses to orthostatic stress are less when blood pooling within the lower limbs is minimized. Furthermore, greater orthostatic tolerance has been found in subjects with decreased venous compliance compared with subjects having highly compliant veins (21, 39). Although Hernandez et al. (14) found no difference in orthostatic tolerance between subject groups with high and low venous compliance, the subjects with the highest venous compliance showed the earliest increases in heart rate and decreases in stroke volume during lower body negative pressure (15). These data suggest that lower limb venous compliance may have a direct impact on cardiovascular responses to orthostatic stress and possibly orthostatic tolerance. It has been clearly demonstrated that women have less orthostatic tolerance than men (5, 8, 11, 31, 36, 41). Thus Monahan et al. (30) recently hypothesized that women would have greater lower limb venous compliance than men and that this potentially predisposes them to decreased orthostatic tolerance. Surprisingly, the authors found that women have a 48% lower venous compliance than men. However, it is unknown what effect the fluctuating levels of estrogen and progesterone have on venous compliance in women over the courses of the menstrual cycle and oral contraceptive use. Estrogen and progesterone have numerous effects on the vasculature, and thus these hormones may alter venous compliance in women. If venous compliance is affected by endogenous and/or exogenous forms of estrogen and progesterone, the previously identified relationship between the venous compliance of men and women may differ with phases of the menstrual cycle and oral contraceptive use. This potentially means that venous compliance of women during certain hormone profiles may be equal to or greater than that of men; these changes may increase women’s cardiovascular stress and possibly their susceptibility to orthostatic intolerance during certain phases of the menstrual cycle or oral contraceptive use. To date, the effects of the menstrual cycle and oral contraceptive use on orthostatic tolerance are unclear.

Earlier studies provided conflicting results regarding the effects of estrogen and progesterone on the veins (1, 6, 7, 9, 10, 22, 23, 40). The inconsistent results of these studies may be due in part to the use of different experimental approaches employed to study venous compliance or regional differences in response to estrogen and progesterone. Therefore, the purpose of our study was to use a novel approach that overcomes many of the limitations of previous studies to investigate the effects of endogenous and exogenous estrogen and progesterone on venous compliance by looking for cyclic changes during three phases of the menstrual cycle and over the course of oral contraceptive use. Additionally, we wanted to compare venous compliance in normally menstruating women and oral contraceptive users to venous compliance in men. We hypoth-
esized that venous compliance would be greater during the ovulatory phase vs. the early follicular and midluteal phases of the menstrual cycle when estrogen is elevated. Additionally, we hypothesized that venous compliance would be greater during the high vs. the low hormone phase of oral contraceptive use.

**METHODS**

Twenty-five healthy young subjects between the ages of 19 and 32 yr completed the protocol. All subjects were normally active (exercise ~1–3 days/wk for 1 h) nonsmokers who were not taking any medication with the exception of the group of women who were taking oral contraceptives. All subjects kept a log of food and liquid consumption the day before the initial study day and were asked to follow the same regimen before reporting to the laboratory on subsequent study days. All subjects were asked to refrain from consuming any over-the-counter medications and alcoholic beverages within 24 h of reporting to the laboratory. All studies were completed at the same time of day for each individual subject. Female subjects were required to take a pregnancy test and show negative results before the beginning of the protocol on each study day. Approval of this investigation was granted by the Institutional Review Board of the University of Oregon. The protocol was explained to the subjects, and oral as well as written consent were given by each subject before participation.

The subject pool was divided into three groups: normally menstruating women, women taking oral contraceptives, and men. Eight normally menstruating women (6 having never taken oral contraceptives, 1 using no type of oral contraception for 2 yr, and 1 using no type of oral contraception for 4 mo) were studied during three phases of their menstrual cycle. The phases were defined as follows: early follicular (1–3 days postmenstrual), ovulatory (within 48 h of luteinizing hormone surge), and midluteal (8–10 days post-ovulation). The order of experimental study days was counterbalanced.

Within the normally menstruating women, three were studied first during their early follicular phase, three were studied first during their ovulatory phase, and two women were studied first during their midluteal phase. The surge in luteinizing hormone was detected via commercial ovulation prediction kits (Clearblue Easy Ovulation Test Pack; Unipath Diagnostics; Waltham, MA), and all menstrual cycle phases were verified by circulating levels of estradiol and progesterone and changes in morning oral temperature. Both estrogen and progesterone were low during the early follicular phase, estrogen peaked during the ovulatory phase, whereas progesterone was low, and both estrogen and progesterone were elevated during the midluteal phase.

Nine oral contraceptive users, all of whom were taking a combined estrogen and progesterone triphasic oral contraceptive (Ortho Tri-Cyclen; Ortho-McNeil; Raritan, NJ), completed the study. Pills in weeks 1, 2, and 3 contained 0.181, 0.215, and 0.250 mg, respectively, of the postgestational compound norgestimate and equal 0.035 mg concentrations of the estrogenic compound ethinyl estradiol. Week 4 pills contained no norgestimate or ethinyl estradiol and served as placebo pills. All oral contraceptive users were studied twice: once during the high hormone phase (days 5–7 of week 3 of active pills) and once during the low hormone phase (days 5–7 of placebo pills). The order of experimental study days was counterbalanced within the oral contraceptive users. Six women were studied first during their high hormone phase, and three women were studied first during their low hormone phase.

Eight men also completed the protocol. The men were studied twice within a 2- to 4-wk period to simulate the general time frame of all women who completed the study.

**Measurement Techniques**

Venous occlusion plethysmography. Changes in calf volume were measured noninvasively by mercury in-Silastic strain gauges (EC4; D. E. Hokanson; Bellevue, WA). The point of maximal calf circumference was measured and marked to determine appropriate size and placement of the strain gauge. After instrumentation, the strain gauges were calibrated electronically (16). A venous collecting cuff (Delfi Medical Innovations; Vancouver, Canada) was placed 5 cm proximal to the knee and connected via a custom-built pressure regulator to a helium tank to provide external pressure. Venous collecting cuff pressure was measured using a pressure transducer (Stancor; St. Louis, MO) attached to the collecting cuff and positioned in line with the venous collecting cuff and air source. The pressure transducer was calibrated using a mercury manometer (American Diagnostic; Hauppauge, NY).

Heart rate and arterial blood pressure. Heart rate (HR) was determined by electrocardiogram (Quinton Heart Rate Monitor; Bothell, WA). Arterial blood pressure was measured noninvasively from the left arm using an arterial blood pressure cuff positioned over the brachial artery (Dinamap Pro 100; Johnson and Johnson; Arlington, TX).

**Blood samples.** Venous blood samples were collected during early follicular, ovulatory, and midluteal phases in normally menstruating women for measurement of circulating levels of estradiol and progesterone to verify menstrual cycle phase. Venous blood samples were also collected from men on both study days to verify consistent levels of testosterone and dihydrotestosterone. All samples were collected into 8.5-ml plastic collection tubes (BD Vacutainer SST Gel and Clot Activator; Becton Dickinson; Franklin Lakes, NJ) after subjects rested quietly in the supine position for 30 min. Samples were separated via centrifuge at 1,300 g relative centrifugal force for 12 min at 4°C. Samples were stored frozen at −70°C until they were transported to Oregon Medical Laboratories for serum analysis of estradiol, progesterone, testosterone, and dihydrotestosterone, which were measured via radioimmunoassay.

**Protocol**

All subjects were instrumented while in the supine position. To position their nondominant leg above heart level, we placed supportive foam blocks at their ankle and thigh to promote venous drainage. After instrumentation of the venous collecting cuff and mercury in-Silastic strain gauge, subjects rested in the supine position for 30 min. During this time, HR and blood pressure were measured every 10 min. After 30 min of rest, venous blood samples were taken from an antecubital vein for determination of hormone concentrations in normally menstruating women and men. After another rest period, calf venous compliance was measured by the technique developed by Halliwill et al. (13). The venous collecting cuff was inflated to 60 mmHg and held constant for 8 min. During this time, all subjects were instructed to remain relaxed without movement of the lower limbs. After the 8-min inflation period, the collecting cuff pressure was reduced at a rate of 1 mmHg/s from 60 to 0 mmHg while changes in calf volume were recorded. Throughout all studies, the laboratory temperature was maintained between 22 and 24°C.

**Data Analysis**

Data were recorded to a computer at 40 Hz and saved for later analysis (WinDaq; Dataq Instruments; Akron, OH).

To characterize compliance, the relationship between calf volume and cuff pressure was compared as the cuff pressure decreased at a rate of 1 mmHg/s from 60 to 0 mmHg after being held at 60 mmHg for 8 min. This method assumes that cuff pressure is equal to intravenous pressure, which has previously been verified experimentally (13). Pressures <10 mmHg were not included in analyses due to the ambiguity of true venous pressure at low cuff pressures (13). To evaluate individual volume-pressure curves, the data were fit to the quadratic regression model, \( \Delta \text{limb volume} = \beta_0 + \beta_1 \times \text{cuff pressure} + \beta_2 \times (\text{cuff pressure})^2 \). The relationship between volume and pressure depicted by the quadratic regression model is nonlinear.
For a given change in pressure, there are small changes in volume at higher pressures and large changes in volume at lower pressures. Thus compliance is a function of venous pressure. Compliance increases as cuff pressure decreases from 60 to 0 mmHg. Owing to this nonlinear relationship, a single number cannot be used to describe venous compliance. As a result, we calculated compliance as the derivative of the volume-pressure curve. This calculation is used to generate a graphical representation that depicts compliance as a function of pressure (13). The average regression parameters $\beta_1$ and $\beta_2$ from the quadratic regression model were used to determine compliance for each group studied, as venous compliance = $\beta_1 + 2 \times \beta_2 \times$ cuff pressure.

**Statistical Analyses**

Subject characteristics were analyzed between each menstrual cycle phase in normally menstruating women, between high and low hormone phases in oral contraceptive users, and between men on their first and second visits using one-way repeated-measures ANOVA and the Student-Newman-Keuls post hoc test. Early follicular, ovulatory, and midluteal subject characteristics were averaged to determine the mean subject characteristics for normally menstruating women. High and low hormone subject characteristics of oral contraceptive users as well as subject characteristics from visits 1 and 2 in men were also averaged to determine the mean subject characteristics of each group. The group averages were then compared to determine any differences in subject characteristics between normally menstruating women, oral contraceptive users, and men using one-way ANOVA and the Student-Newman-Keuls post hoc test.

Regression coefficients $\beta_0$, $\beta_1$, and $\beta_2$ were compared using multiway ANOVA to examine venous compliance between menstrual cycle phases, between oral contraceptive phases, and between visits in men and to compare the venous compliance of normally menstruating women, oral contraceptive users, and men. When main factor differences were observed, planned contrast tests were performed to determine paired differences across groups using SAS 8.0 software (SAS Institute; Cary, NC). All data are presented as means ± SE. Statistical significance was set at $P < 0.05$.

**RESULTS**

**Subject Characteristics**

Table 1 summarizes the physical characteristics of all subjects. The groups did not differ significantly in age, height, weight, and body mass index. The men tended to be taller and to weigh more than the women, but differences were not significant. Resting systolic blood pressure, diastolic blood pressure, and HR were also similar in all groups. As expected, there were significant differences in the estradiol and progesterone concentrations in the normally menstruating women across the menstrual cycle. Estradiol was significantly higher in the ovulatory and midluteal phases of the menstrual cycle compared with the early follicular phase ($P < 0.002$). Progesterone was significantly higher during the midluteal phase compared with the early follicular and ovulatory phases ($P < 0.001$). There was not a significant difference in the testosterone and dihydrotestosterone concentrations in men on visits 1 and 2 ($P > 0.05$), which shows that the concentrations remained consistent on both visits to the laboratory (Table 1).

**Calf Venous Compliance**

Table 2 presents the progressive increase in $\beta_0$ in normally menstruating women: $\beta_0$ was lowest during the early follicular phase, increased in the ovulatory phase, and greatest during the midluteal phase, where it was significantly greater compared with the early follicular phase ($P < 0.05$). We found that $\beta_0$ was not significantly different between high and low hormone weeks of oral contraceptive use nor between men on their first and second visits ($P > 0.05$). Average values for normally menstruating women, oral contraceptive users, and men also showed no significant differences in $\beta_0$ ($P > 0.05$; Table 3).

Table 2 also displays the regression parameters (means ± SE) $\beta_0$, $B_1$, and $B_2$ for normally menstruating women in the early follicular, ovulatory, and midluteal menstrual cycle phases; high and low hormone phases of oral contraceptive use; and men during visits 1 and 2. Menstrual cycle phase did not significantly alter the venous compliance of normally menstruating women ($P > 0.05$), which suggests that endogenous fluctuations in estrogen and progesterone concentrations do not elicit cyclic changes in venous compliance in women. Early follicular, ovulatory, and midluteal phases showed similar volume-pressure curves and compliance-pressure tracings (Fig. 1). In addition, Table 2 shows that there were no significant cyclic differences in venous compliance in oral contraceptive users during high and low hormone phases of oral contraceptive use ($P > 0.05$). The volume-pressure curves and compliance-pressure tracings were indistinguishable during high and low hormone phases (Fig. 2). Table 2 also shows that no significant difference in venous compliance was determined

### Table 1. Selected subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normally Menstruating Women, phase</th>
<th>Oral Contraception Users, phase</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early Follicular</td>
<td>Ovulatory</td>
<td>Midluteal</td>
</tr>
<tr>
<td>Age, yr</td>
<td>21.75±0.6</td>
<td>20.7±0.4</td>
<td>23.3±1.1</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165.0±2.0</td>
<td>162.8±2.5</td>
<td>171.0±2.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65.58±2.2</td>
<td>65.77±2.1</td>
<td>65.41±2.0</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.1±0.8</td>
<td>24.2±0.7</td>
<td>24.1±0.7</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>106.2±1.8</td>
<td>106.0±2.2</td>
<td>103.7±2.3</td>
</tr>
<tr>
<td>Systolic</td>
<td>64.5±1.5</td>
<td>64.5±1.5</td>
<td>64.5±1.5</td>
</tr>
<tr>
<td>Diastolic</td>
<td>63.9±3.3</td>
<td>65.6±2.3</td>
<td>62.6±2.5</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>35.1±3.8</td>
<td>95.3±11.7*</td>
<td>118.0±20.7*</td>
</tr>
<tr>
<td>Estradiol, pg/ml</td>
<td>0.8±0.1</td>
<td>2.4±0.4</td>
<td>11.9±1.5*</td>
</tr>
<tr>
<td>Progesterone, ng/ml</td>
<td>568.9±59.2</td>
<td>559.5±50.4</td>
<td>-</td>
</tr>
<tr>
<td>Testosterone, ng/dl</td>
<td>605.5±43.2</td>
<td>604.7±81.2</td>
<td>-</td>
</tr>
<tr>
<td>Dihydrotestosterone, pg/ml</td>
<td>59.2±59.2</td>
<td>559.5±50.4</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 8$ normally menstruating women, 9 oral contraceptive users, and 8 men. *$P < 0.002$ vs. early follicular phase; †$P < 0.001$ vs. early follicular and ovulatory phases.
between visits in men \( (P > 0.05) \). The volume-pressure curves and compliance-pressure tracings for men on visits 1 and 2 are nearly identical (Fig. 3).

After determining that there were no significant differences in venous compliance during different phases of the menstrual cycle and oral contraceptive use in women and no significant differences across a similar time period in men, we sought to identify any differences between normally menstruating women, oral contraceptive users, and men. The regression parameters \( \beta_0, \beta_1, \) and \( \beta_2 \) were calculated (means ± SE) for normally menstruating women, oral contraceptive users, and men and are shown in Table 3. Men had significantly greater venous compliance than both normally menstruating women \( (P < 0.001) \) and oral contraceptive users \( (P < 0.002) \). The volume-pressure curves and compliance-pressure tracings visually display this sex difference (Fig. 4). The volume-pressure curve for men shows a greater change in volume for a given change in pressure compared with both normally menstruating women and oral contraceptive users. This difference is also evident in the compliance-pressure tracing, as the slope of this line is much steeper for men than for both groups of women. To quantitatively gauge the magnitude of the sex difference, we compared the calculated compliance of normally menstruating women, oral contraceptive users, and men at an extrapolated intravenous pressure of 0 mmHg. These data show the compliance calculations for normally menstruating women and oral contraceptive users were ~26 and ~19% lower than for men, respectively.

**DISCUSSION**

To our knowledge, this is the first study to investigate the lower limb venous compliance of women during three phases of the menstrual cycle and over the course of oral contraceptive use. Furthermore, we are not aware of any previous work that has compared the lower limb venous compliance between normally menstruating women, oral contraceptive users, and men.

There were three primary findings from the present study. First, the fluctuating levels of estrogen and progesterone that accompany the normal menstrual cycle do not elicit cyclic changes in lower limb venous compliance between the early follicular, ovulatory, and midluteal phases of normally menstruating women. Second, fluctuations in hormone concentrations delivered through oral contraceptives do not cause cyclic changes in lower limb venous compliance between high and low hormone weeks of oral contraceptive use. Third, both normally menstruating women and oral contraceptive users have lower venous compliance than men. Overall, these data suggest that fluctuating levels of endogenous and exogenous estrogen and progesterone do not elicit cyclic changes in lower limb venous compliance. Additionally, our data support a previous study (30) that demonstrated a sex difference in lower limb venous compliance and extends this observation to specifically identify a difference between normally menstruating women and men as well as oral contraceptive users and men.

In contrast with our hypothesis, we found venous compliance of the lower limbs to be consistent on all study days throughout the normal menstrual cycle and oral contraceptive use despite large fluctuations in endogenous and exogenous concentrations of estrogen and progesterone. This finding was surprising in light of previous research in which most \((1, 7, 10, 22, 23, 40)\) but not all \((6)\) studies have shown that variations in exogenous and endogenous forms of the female sex hormones change the distensibility of the veins.

Previous studies reported that venous distensibility changes from the early follicular phase to the midluteal phase of the menstrual cycle. Venous distensibility of the forearm \((7)\) and the fingertip \((22)\) were reported to be higher during the midluteal phase of the menstrual cycle compared with the early follicular phase. In contrast, the venous distensibility of the hand was found to be higher during the follicular phase compared with the luteal phase of the menstrual cycle. (40). Additionally, venous distensibility of the fingertip, forearm, and calf have been shown to be higher during oral contracept-

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**Table 2. Values for pressure-volume regression parameters \( \beta_0, \beta_1, \) and \( \beta_2 \)**

<table>
<thead>
<tr>
<th>Phase</th>
<th>( \beta_0 )</th>
<th>( \beta_1 )</th>
<th>( \beta_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normally menstruating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early follicular</td>
<td>1.071 ± 0.163</td>
<td>0.0699 ± 0.0073</td>
<td>-0.00060 ± 0.0007</td>
</tr>
<tr>
<td>Ovulatory</td>
<td>1.522 ± 0.152</td>
<td>0.0658 ± 0.0080</td>
<td>-0.00075 ± 0.00008</td>
</tr>
<tr>
<td>Midluteal</td>
<td>1.431 ± 0.081*</td>
<td>0.0621 ± 0.0041</td>
<td>-0.00056 ± 0.00001</td>
</tr>
<tr>
<td>Oral contraceptive users</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High hormone</td>
<td>1.427 ± 0.222</td>
<td>0.0729 ± 0.0077</td>
<td>-0.00060 ± 0.00006</td>
</tr>
<tr>
<td>Low hormone</td>
<td>1.582 ± 0.237</td>
<td>0.0718 ± 0.0042</td>
<td>-0.00060 ± 0.00004</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
<td>1.021 ± 0.282</td>
<td>0.0869 ± 0.0061</td>
<td>-0.00070 ± 0.00006</td>
</tr>
<tr>
<td>Visit 2</td>
<td>1.069 ± 0.298</td>
<td>0.0921 ± 0.0130</td>
<td>-0.00070 ± 0.00006</td>
</tr>
</tbody>
</table>

Values are means ± SE. ΔLimb volume = \( \beta_0 + \beta_1 \times \text{cuff pressure} + \beta_2 \times (\text{cuff pressure})^2 \). *\( P < 0.05 \) vs. early follicular phase.

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**Table 3. Average values for pressure-volume regression parameters \( \beta_0, \beta_1, \) and \( \beta_2 \)**

<table>
<thead>
<tr>
<th>Group</th>
<th>( \beta_0 )</th>
<th>( \beta_1 )</th>
<th>( \beta_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normally menstruating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.431 ± 0.081</td>
<td>0.0659 ± 0.0060*</td>
<td></td>
</tr>
<tr>
<td>Oral contraceptive users</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.505 ± 0.203</td>
<td>0.0723 ± 0.0049*</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.045 ± 0.246</td>
<td>0.0895 ± 0.0089</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. ΔLimb volume = \( \beta_0 + \beta_1 \times \text{cuff pressure} + \beta_2 \times (\text{cuff pressure})^2 \). *\( P < 0.02 \) vs. men.
Menstrual Cycle, Oral Contraceptives, and Compliance

We speculate that the inconsistent results of previous studies may be partially caused by regional differences of the vasculature being investigated in response to estrogen and progesterone. That is, the previous studies were conducted on various vascular beds including the calf, forearm, hand, and fingertip, all of which may respond differently to estrogen and progesterone. Moreover, the hand and fingertip contain arteriovenous anastomoses and may not be well suited for accurate measurements of venous compliance. Furthermore, the veins of the upper body are not routinely exposed to the hydrostatic pressure fluctuations of the lower limbs. Therefore, we performed our study on legs, because the venous compliance of this region is most relevant to orthostatic tolerance.

In addition to regional vascular differences, the discordant findings of studies may be due to the use of different experimental approaches. Previous studies used a variety of methods (4, 17, 32, 35, 42) to measure venous distensibility, all of which differ from the method used in the present study. One key limitation to previous methods is the assumption that complete volume changes of the limb are due solely to changes in venous volume and not to filtration of plasma volume via capillary leak (13, 29). The method used in this study does not rely on this assumption. Additionally, most of the previous methods focused on the capacitance of the vessels by considering the volume of a limb at a given collecting cuff pressure. Using these methods, volume-pressure curves are constructed by compiling the capacitance values at various pressures. This method makes data analysis difficult, because it relies on the investigator to interpret the time point at which intravenous pressure has reached cuff pressure and achieved a steady state. This point can be hard to distinguish due to venous wall creep and capillary leak, which increases the chance of error (13). By controlling for these potential limitations, our study may provide greater insight into the role of estrogen and progesterone on venous compliance of the leg.

Although β₀ is a difficult variable to interpret because it is affected by a number of factors, our β₀ findings may shed some light on the conflicting results of previous studies. We found that β₀ increased in normally menstruating women from the early follicular phase to the ovulatory phase and continued to increase from the ovulatory phase to the midluteal phase. Changes in β₀ may reflect alterations in unstressed volume...
and/or the difference in calf volume measured at resting venous pressure and the venous pressure at zero. Moreover, changes in $\beta_0$ may also be due to differences in hysteresis via capillary leak and/or venous wall creep (13). Our $\beta_0$ findings may suggest that endogenous estrogen increases venous wall creep or capillary leak, and that progesterone may have additive effects on this relationship. Importantly, our findings of a change in $\beta_0$ with menstrual cycle phase may help explain previous findings of cyclical changes in venous compliance over the course of the menstrual cycle. That is, it is possible that differences in venous wall creep and/or capillary leak were responsible for the observations of increased venous distensibility with increased estrogen and progesterone in the majority of previous studies. Differences in venous wall creep and/or capillary leak could also account for the greater volume observed at elevated intravascular pressures with previous methods for assessing venous distensibility (4, 17, 32, 35, 42). Thus one could conclude that capacitance is greater when concentrations of the female sex hormones are elevated without a concomitant change in vessel wall compliance as demonstrated in our experiment.

In support of this theory, numerous studies have identified that transcapillary fluid shifts are affected by estrogen and progesterone (33, 37, 38). The decrease in plasma volume often observed during the midluteal phase has been suggested to be due to changes in colloid osmotic pressures and plasma albumin shifts, which increase filtration from the intravascular to the interstitial spaces (33). Thus the shifts in $\beta_0$ that were observed in this study may be due to greater interstitial fluid accumulation when estrogen and progesterone are elevated. Furthermore, estrogen is also known to increase the concentration of the local vasodilator nitric oxide in the endothelium (3, 19). The effects of local vasodilators, such as nitric oxide, could also assist in a proposed increase in interstitial fluid accumulation by increasing the surface area available for diffusion (37). Based on our observations of a shift in $\beta_0$ across the menstrual cycle, it is possible that previous studies mistakenly interpreted their results as greater venous compliance when changes in limb volume may have been due to greater interstitial fluid accumulation via menstrual cycle changes in venous wall creep and/or capillary leak. However, we did not observe a significant difference in $\beta_0$ between the high and low hormone phases of oral contraceptive users. This may be attributed to potential differences between endogenous and exogenous concentrations of estrogen and progesterone on vascular function and regulation (27, 28).

Although cyclic changes in venous compliance were not observed with fluctuating concentrations of endogenous and exogenous estrogen and progesterone throughout the normal menstrual cycle and during the course of oral contraceptive use...
in our study, a difference in venous compliance was found between sexes. Our data support previous work from Monahan et al. (30), who found that women have lower venous compliance than men. It is important to note that this previous study did not control for menstrual cycle phase or oral contraceptive use. Although it was not the focus of their study, Hernandez et al. (14) provided additional evidence for a sex difference in venous compliance. While investigating the effects of age and fitness on venous compliance, it was found that young, unfit women had lower venous compliance than young, unfit men. This sex difference was not observed in the young, fit or older subjects. All female subjects participating in this investigation were studied during the follicular phase of their menstrual cycle.

To quantitatively compare the sex difference in venous compliance found by Monahan et al. and Hernandez et al. and in the present study, we recalculated the data using the method employed in the present study (percent change of compliance found by Monahan et al. and Hernandez et al. and cycle). All female subjects participating in this investigation were the same group of subjects. Last, our sample size was relatively small. However, we performed sample-size calculations and determined that the smallest sample size needed to determine a statistically significant difference in venous compliance (i.e., a change in both $\beta_1$ and $\beta_2$) between normally menstruating women was a minimum of 34 subjects. Owing to the large number of subjects needed to identify a statistical difference in venous compliance, we feel confident that our data accurately convey the lack of a physiologically significant difference between venous compliance across the menstrual cycle phases.

In conclusion, lower limb venous compliance did not change with menstrual cycle phase or across the course of oral contraceptive use. Thus both endogenous and exogenous forms of estrogen and progesterone do not appear to cause cyclic changes in venous compliance in women. Furthermore, normally menstruating women in the early follicular, ovulatory, and midluteal phases of the menstrual cycle as well as oral contraceptive users studied during high and low hormone weeks of oral contraceptive use have consistently lower venous compliance than men. These data provide further evidence to support a sex difference in venous compliance and extend the observation to show that women have lower venous compliance than men in all phases of the menstrual cycle and oral contraceptive use. The specific mechanisms behind a sex difference in lower limb venous compliance remain unidentified and warrant additional investigation.

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