Effects of estrogen and progesterone administration on extracellular fluid

Nina S. Stachenfeld and Hugh S. Taylor

Departments of Epidemiology and Public Health, and Obstetrics and Gynecology, Division of Reproductive Endocrinology, Yale University School of Medicine, New Haven, Connecticut 06519

Submitted 24 September 2003; accepted in final form 21 November 2003

Stachenfeld, Nina S., and Hugh S. Taylor. Effects of estrogen and progesterone administration on extracellular fluid. J Appl Physiol 96: 1011–1018, 2004. First published December 5, 2003; 10.1152/japplphysiol.01032.2003.—To determine the effect of estrogen and progesterone on plasma volume (PV) and extracellular fluid volume (ECFV), we suppressed endogenous estrogen and progesterone by using the gonadotropin-releasing hormone (GnRH) antagonist gani-relax acetate in seven healthy women (22 ± 1 yr). Subjects were administered GnRH antagonist for 16 days. Beginning on day 5 of GnRH antagonist administration, subjects were administered estrogen (E2) for 11 days, and beginning on day 12 of GnRH antagonist administration, subjects added progesterone (E2-P4) for 4 days. On days 2, 9, and 16 of GnRH antagonist administration, we estimated ECFV (inulin washout), transcapillary escape rate of albumin (TER alb), and PV (Evans blue dye). Plasma E2 concentration increased from 17.9 ± 4.5 ng/ml (GnRH antagonist) to 195.9 ± 60.1 (E2, P < 0.05) to 245.6 ± 62.9 pg/ml (E2-P4, P < 0.05). Compared with GnRH antagonist (1.3 ± 0.5 ng/ml), plasma P4 concentration was unchanged during E2 (0.9 ± 0.3 ng/ml) and increased to 9.4 ± 3.1 ng/ml during E2-P4 (P < 0.05). Both E2 (44.1 ± 3.1 ml/kg) and E2-P4 (47.7 ± 2.8 ml/kg) increased PV compared with GnRH antagonist (42.8 ± 1.3 ml/kg, P < 0.05). Within-subjects TER alb was a strong negative predictor of PV (mean r = 0.92 ± 0.03, P < 0.05), and TER alb was lowest during E2-P4 (5.7 ± 0.5, 4.10 ± 1.1, and 2.8 ± 0.9%/h, P < 0.05, for GnRH antagonist, E2, and E2-P4, respectively). ECFV was reduced during E2 (227 ± 31 ml/kg, P < 0.05) compared with both GnRH antagonist (291 ± 37 ml/kg) and E2-P4 (283 ± 19 ml/kg). Thus the percentage of extracellular fluid in the plasma compartment increased to 21.0% (P < 0.05) during E2 compared with GnRH antagonist (16.1%) and E2-P4 (17.2%) administration. Thus E2 increased PV via actions on the capillary endothelium to lower TER alb and favor intravascular water retention, whereas during E2-P4 PV increased via the combined responses of ECFV expansion and lower TER alb.

extracellular fluid regulation; gonadotropin-releasing hormone antagonist; gani-relax acetate; blood volume; plasma volume

Body fluids are stored in two compartments: inside the cells (intracellular ~60%) and outside the cells (extracellular ~40%). The extracellular compartment is further subdivided into the intravascular and interstitial fluid compartments, which make up ~20 and 80%, respectively. Plasma proteins diffuse slowly across the capillary endothelium, so changes in plasma protein content will change the fluid distribution of the extracellular compartment. For example, an increase in plasma proteins will cause an increase in plasma oncotic pressure and a selective expansion of the plasma volume (PV), whereas plasma protein escape will cause a decrease in plasma oncotic pressure and an increase in interstitial fluid. Thus, when there is an acute PV loss, transcapillary fluid dynamics, or Starling forces, adjust to favor plasma proteins and fluid movement out of interstitial and into vascular compartments, thereby selectively increasing PV. When the PV loss is due to total body water loss, the kidneys also act to retain sodium and water to restore total extracellular fluid volume (ECFV). Sodium and other electrolytes do not act as effective osmotic agents because they freely diffuse across capillary endothelium, so changes in the total ECFV are related to changes in sodium content.

Estrogen and progesterone can have profound effects on PV (31, 32, 34, 37) and capillary fluid dynamics (26, 33, 41–43). PV is highest during the preovulatory phase of the menstrual cycle, when estrogen levels are rising (37). However, relative to the early follicular phase, PV falls by as much as 8% during the midluteal phase, when both estrogen and progesterone are elevated (34). Estrogen administration increases PV (31, 32, 34) and prevents PV contraction during long-term bed rest (11). The last trimester of pregnancy is characterized by a rapid rise in estrogen, which coincides with increases in PV and interstitial fluid (17). Estrogen and progesterone increase renal sodium reabsorption (32, 34), so the PV increases may be a function of ECFV increases. On the other hand, the estrogen- and progesterone-related PV changes may be due to adjustments in extracellular fluid (ECF) distribution between the plasma and the interstitial fluid compartments related to changes in the Starling forces (26, 33, 41–43). The midluteal phase is characterized by the attainment of peak progesterone levels, accompanied by a fall in PV. This PV fall occurs in the face of little or no change in sodium and water retention, so the PV change is likely a function of changes in colloid osmotic pressure and plasma albumin shifts into the interstitial space (26). During the midluteal phase, PV and total circulating proteins decrease by as much as 220 ml (7%) and 12 g (7%), respectively, compared with the early follicular phase (34).

Although both estrogen and progesterone effects on PV and Starling forces that regulate fluid movement across intravascular and interstitial compartments have been measured, there have been no studies directly assessing estrogen and progesterone effects on ECFV distribution. The purpose of the present investigation was to determine estrogen and progesterone effects on ECFV and ECF distribution as well as plasma protein shifts across the capillaries. We hypothesized that estrogen administration would lead to PV increases proportional to any ECFV increases, so that ECFV distribution would be unchanged, despite a PV expansion. We further hypothesized that, when progesterone was administered along with estrogen, PV would fall due to fluid and protein shifts into the interstitial space, with very little or no effect on total ECFV.

Address for reprint requests and other correspondence: N. S. Stachenfeld, The John B. Pierce Laboratory, 290 Congress Ave., New Haven, CT 06519 (E-mail: nstach@jbpierce.org).
METHODS

We recruited seven healthy, nonsmoking women with no contraindications to gonadotropin-releasing hormone (GnRH) antagonist (see [GnRH antagonist (ganirelax-acetate)]) or reproductive hormone administration to participate in these experiments. All subjects were interviewed to obtain their medical history. In addition, subjects provided written confirmation of a negative Papanicolaou smear and normal physical examination within 1 yr of being admitted to the study. They gave written, informed consent to participate in the study, which had prior approval by the Human Investigation Committee of Yale University School of Medicine.

Experimental design. To suppress reproductive function for the duration of the study, subjects received the GnRH antagonist ganirelix acetate (250 μg/day, Antagon, Organon, West Orange, NJ) each day for 16 days (Fig. 1). Beginning on day 5 of GnRH antagonist administration, the women received 11 days of estrogen administration, the women added 200 mg/day of oral progesterone for 4 days. Experimental protocols were performed after 48 h of GnRH antagonist administration, on day 9 [GnRH antagonist with estrogen (E2)], and again on day 16 of administration [GnRH antagonist with estrogen and progesterone (E2-P4)]. This design permitted within-subject comparisons concerning estrogen and progesterone effects on changes in ECF distribution and changes in total ECFV. This hormone suppression, add-back design eliminated other potential confounders, such as GnRH and the gonadotropins (follicle-stimulating hormone, luteinizing hormone), thus providing a direct assessment of the estrogen and progesterone influences on ECFV and PV.

GnRH antagonist (ganirelax-acetate). Ganirelix acetate is a synthetic decapeptide with high antagonistic activity against naturally occurring GnRH. Ganirelix acetate is derived from native GnRH with substitutions at positions 1, 2, 3, 6, 8, and 10. When ganirelix acetate is given in therapeutic doses, it acts by competitively blocking the GnRH receptors on the pituitary gonadotroph and subsequent transductions pathway. It induces a rapid, reversible suppression of gonadotropin secretion (23, 24). In young, cycling women, continued administration of ganirelix acetate leads to suppression of estrogen and progesterone to postmenopausal levels. These decreases occur after 36–48 h of administration, and the suppression of the hypothalamic-pituitary-ovarian axis is reversed on cessation of drug therapy (23, 24).

The GnRH antagonist administration began 9–10 days after the subject’s luteinizing hormone peak. This peak precedes ovulation, usually day 12–14 of a 28-day menstrual cycle (see Fig. 1), and was determined individually by the use of ovulation prediction kits (Ovu-Quick, Quidel, San Diego, CA). Some of the subjects were already taking oral contraceptives (n = 4), so they ceased taking the pills and began GnRH antagonist administration on day 21 of the pill cycle. The subjects self-administered daily subcutaneous injections of the GnRH antagonist after training by qualified medical personnel. This method of GnRH antagonist administration was chosen because it is easily discontinued in the event of uncomfortable side effects, such as headaches or “hot flashes,” and the suppression of the hypothalamic-pituitary-ovarian axis is reversed on cessation of drug therapy.

Adding estradiol and progesterone treatment to GnRH antagonist treatment. For estrogen treatment, subjects received 17β-estradiol administered by using two transdermal patches delivering 0.1 mg/day each (Vivelle, CIBA Pharmaceuticals, Summit, NJ). Progesterone was administered orally, with 100-mg doses each morning and evening (200 mg/day, Prometrium, Solvay Pharmaceuticals, Marietta, GA).

Experimental protocol. For each experiment, subjects arrived at the laboratory at ~7:00 AM, after having eaten a light (~300 kcal) breakfast, having drunk 10 ml/kg body wt of water, and having refrained from alcohol, caffeinated beverages, and food for the previous 12 h. On reporting to the laboratory, subjects voided their bladders and were weighed to the nearest 10 g on a beam balance. Subjects were then seated in a semirecumbent position for a 60-min control period in an environmental chamber (27°C, 30% relative humidity) to ensure a steady state in PV and constituents. During this control period, a 22-gauge Teflon intravenous catheter was placed in an antecubital or forearm vein in each arm with a heparin block (20 U/ml) to maintain catheter patency. A blood pressure cuff was positioned for automatic readings by a sphygmomanometer (Colin Medical Instruments, Komaki, Japan) to monitor changes in blood pressure. A three-lead electrocardiogram (Colin Medical Instruments) was used to monitor heart rate. At the end of the control period, a baseline blood sample was taken, immediately followed by simultaneous infusions of Evans blue dye (New World Trading, DuBarry, FL) and inulin (Cypress Pharmaceutical, Carlsbad, CA).

ECFV was determined by using the inulin dilution technique (19). ECFV determination involved an injection of a volume of a 10% inulin solution containing 60 mg inulin/kg body wt in an arm vein followed by a series of blood samples. The injection was given over ~4.5 min, and blood was drawn before injection and at 5, 7, 10, 15, 30, 45, 60, 75, 90, 105, 120, 150, and 180 min after the start of the injection. The subject did not change posture throughout the control period or the inulin measurement to ensure that ECFV was stable. ECFV is calculated by the rate of clearance of inulin from the plasma. This method is valid with inulin because it is distributed slowly and only throughout the extracellular space, the kidneys excrete 95–100% of the infused inulin via glomerular filtration, and the tracer concentration becomes well mixed in the plasma. ECFV was calculated by using the methods described by Ladegaard-Pedersen (19) using a semilogarithmic plot of the clearance of the tracer from the plasma over 180 min.

Transcapillary escape rate of albumin (TER alb) and PV were determined by using an injection of a body weight-standardized volume of Evans blue dye (0.20 ml/kg) into an arm vein and taking blood samples for determination of dilution. The dye itself attaches rapidly to plasma albumin and, therefore, becomes evenly mixed. TER alb was determined from Evans blue dye washout over 1 h after dye injection and is calculated from the initial slope of the exponential curve fit through the optical density of postdye injection plasma samples at 620 nm (15). Absolute PV was measured after complete mixing of Evans blue dye (10 min). PV was determined from the...
product of the concentration and the volume of dye injected divided by the concentration in plasma, taking into account 1.5% lost from the circulation within the first 10 min. The Evans blue dye is prebound to plasma by premixing ~1 ml of Evans blue dye (depending on body weight) with ~9 ml of whole blood, creating a fixed-binding ratio of 1:1 dye to albumin molecule. The Evans blue dye injection began simultaneously with the inulin infusion from the other catheter.

Urine was collected before the infusions, at the end of the inulin infusion, and at the end of the protocol for determination of renal function. The volume of urine excreted was replaced with an equal volume of tap water after subtracting the amount of fluid infused. Blood pressure and heart rate were recorded every 30 min throughout the protocol.

All blood samples were analyzed for hematocrit, hemoglobin, and total protein. Plasma osmolality, plasma concentrations ofalbumin (P_{Alb}) and creatinine, and serum concentrations of sodium (S_{Na}) and potassium (S_{K}) were determined every 30 min during the infusion. Plasma samples at baseline were analyzed for plasma concentration of estrogen (P_{E2}), progesterone (P_{P4}), aldosterone (P_{Ald}), and atrial natriuretic peptide (P_{ANP}), and plasma renin activity (PRA). Volume, osmolality (U_{Osm}), and Na^+ and K^+ and creatinine concentrations were measured from all urine samples.

Blood samples analysis. Blood samples were separated into aliquots. One aliquot was immediately analyzed for hemoglobin and hematocrit in triplicate by cyanomethemoglobin and microcentrifuge, respectively. A second aliquot was transferred to a heparinized tube to be analyzed for plasma osmolality, P_{Alb}, and plasma creatinine concentration. A third aliquot, for the determination of S_{Na}, and S_{K}, was placed into a tube without anticoagulant. The remaining aliquots were placed in tubes containing EDTA for analysis of P_{Ald}, P_{ANP}, P_{E2}, P_{P4}, and PRA. The tubes were centrifuged at 4°C, and the plasma was taken off. After centrifugation, the plasma samples were frozen immediately at −70°C until analysis. Plasma and urinary concentrations of Na^+ and K^+ were measured by flame photometry (Instrumentation Laboratory model 943, Lexington, MA). Plasma osmolality and U_{Osm} were measured by freezing-point depression (Advanced Instruments Laboratory model 943, Lexington, MA). Plasma osmolality and U_{Osm} of Na^+ and K^+ were determined every 30 min during the infusion.

Calculations. Body water regulation was determined through the assessment of the renal clearance of free water, osmolalities, and sodium. The following equations were used to calculate renal function: Glomerular filtration rate (GFR) was estimated from creatinine clearance, urine Na^+ excretion (U_{Na,V}) was calculated as the product of urinary Na^+ concentration and volume (U_{V}; ml/min); fractional excretion of Na^+ = (U_{Na} × U_{Na,V}/GFR) × [Na^+]), where U_{Na} is the urinary concentration of Na^+, and [Na^+] equals the Donnan factor for cations (i.e., 0.95) times the S_{Na}; and the fractional excretion of water, which is equal to (U_{H2O}/GFR) × 100.

Statistics. Data are expressed as means ± SE. The variables over time (GnRH antagonist alone tests, hormone intervention tests) were analyzed by conditions (E2, E2-P4 vs. GnRH antagonist alone) using ANOVA for repeated measures. When significant differences were found, orthogonal contrasts tested differences between specific means related to the hypothesis of interest. Differences were considered statistically significant when P < 0.05 (SPSS, Chicago, IL).

Sample size calculation. Expected PV differences between the GnRH antagonist alone and estrogen treatments were derived from responses to hormone administration seen in our laboratory (32). We found that estrogen administration increased PV by 405 ml with an estimated pooled standard deviation for the group of 141 ml (32).

The desired statistical test is two-sided at an alpha level of 0.05 with 80% power to detect a difference. Based on our previous work, 80% power is sufficient to detect a significant alteration in PV. For a two-sided test, Z_{0.05} = 1.96, and for 80% power, Z_{0.8} = 0.84. The formula for calculating sample size (N) for continuous response variables is

\[ N = 2\left(\frac{Z_{\alpha} + Z_{\beta}}{\delta}\right)^2 \]

When the values are substituted, the calculated sample size is six subjects.

RESULTS

During GnRH antagonist alone treatment, one of seven subjects reported occasional vasomotor symptoms (hot flashes), which were relieved with estrogen administration. Subjects reported no other adverse effects due to the GnRH antagonist or hormone administration, and side effects did not cause any of the subjects to leave the study.

Subjects’ body weight was unaffected by either E2 or E2-P4 compared with GnRH antagonist alone (Table 1). Preinjection P_{E2} increased during E2 (P < 0.05) with no change in P_{P4}. During E2-P4, P_{E2} and P_{P4} increased over both GnRH antagonist alone and were also greater than E2 (Table 1). Thus the relative ratio of plasma concentrations of progesterone to estrogen (P_{P4}/P_{E2}) was reduced during E2 relative to the GnRH antagonist alone and E2-P4, but P_{P4}/P_{E2} was similar between GnRH antagonist alone and E2-P4 (Table 1, P < 0.05).

Estrogen administration and E2-P4 increased PV over GnRH antagonist alone (Fig. 2 and Table 2, P < 0.05). Relative to GnRH antagonist alone, E2 lowered total ECFV by 19.2%, although E2-P4 did not change ECFV significantly (2.5%). Thus the percentage of ECF in the intravascular space during

Table 1. Baseline subject characteristics

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Body weight, kg</th>
<th>P_{E2}, pg/ml</th>
<th>P_{P4}, ng/ml</th>
<th>P_{E2}, pg/ml</th>
<th>P_{P4}, pg/ml</th>
<th>MAP</th>
<th>SBP</th>
<th>DBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>22±1</td>
<td>162±3</td>
<td>67.2±4.7</td>
<td>17.9±4.5</td>
<td>1.3±0.5</td>
<td>34.4±6.5</td>
<td>90±5</td>
<td>130±6</td>
<td>70±5</td>
<td></td>
</tr>
<tr>
<td>67.6±4.9</td>
<td>0.9±0.3</td>
<td>195.9±60.1*</td>
<td>69.2±24</td>
<td>131±5</td>
<td>72±4</td>
<td>6.2±2.9</td>
<td>86.3</td>
<td>127±4</td>
<td>69.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Baseline subject characteristics are of plasma concentrations of estrogen (P_{E2}), progesterone (P_{P4}), aldosterone (P_{Ald}), and atrial natriuretic peptide (P_{ANP}), plasma renin activity (PRA), mean arterial (MAP), and systolic (SBP) and diastolic (DBP) blood pressure. GnRH, gonadotropin-releasing hormone. *Significantly different from GnRH antagonist. †Significantly different from estrogen treatment. Differences were considered statistically significant at P < 0.05.
E2 was increased (from ~16 to ~21%, P < 0.05, Table 2), whereas E2-P4 had no significant effect on PV/ECFV (Table 2). TERab and P[ab] were reduced during both E2 and E2-P4 compared with GnRH antagonist alone (Table 2, P < 0.05), and the reduction in TERab was linearly related to increases in PV within each subject (Fig. 3, P < 0.05). The relationship between PV and plasma albumin content was also linear in all of the subjects (Fig. 3, P < 0.05).

Baseline renal excretory variables were unaffected by E2 or E2-P4 (Table 3). There was no effect of hormone treatment on water or sodium regulation during the ECFV measurement (Table 3). Neither PRA nor P[ANP] was affected by E2 or E2-P4, although P[Ald] was increased during E2 over GnRH antagonist alone and was greater during E2-P4 compared with the other two experimental days (Table 1, P < 0.05). PV was linearly related to P[Ald] in five of the seven subjects (Fig. 4, P < 0.05).

DISCUSSION

GnRH antagonist administration reduced plasma 17β-estradiol concentration and P[17β] by 48 h in healthy young women to postmenopausal levels. Four days of transdermal estradiol administration concomitant with the GnRH antagonist increased P[17β] by ~12-fold. Combined E2-P4 administration increased P[17β] levels by 19-fold and increased P[P] by ~7-fold compared with GnRH antagonist alone. This hormone suppression, add-back design eliminated other potential confounders such as GnRH and gonadotropins (follicle-stimulating hormone, luteinizing hormone), thus providing a direct assessment of the estrogen and progestrone influences on ECFV and PV. Moreover, P[17β] and P[P] were similar to the concentrations over the course of the menstrual cycle and thus are physiologically relevant. Despite lower ECFV, PV increased during E2 relative to GnRH antagonist alone. Surprisingly, E2-P4 caused the greatest increases in PV, which also restored ECFV to levels similar to those during GnRH antagonist administration alone. The increases in PV under both E2 and E2-P4 conditions were strongly related to changes in TERab, which decreased in direct proportion to the changes in PV within subjects across the three treatments. Thus the primary change in PV during E2, with or without P4, was a function of lower transcapillary escape of albumin and the resulting increase in plasma albumin content and plasma oncotic pressure. The E2-related PV expansion did not require a concomitant increase in total ECFV. However, the increase in PV during E2-P4 was due to the combined mechanisms of lower TERab and whole body sodium and water maintenance.

One somewhat surprising outcome was that P[17β] continued to increase between the E2 and the E2-P4 conditions. The 17β-estradiol has a short half-life (~12–14 h) and typically reaches steady-state levels in the blood within 24 h after the estrogen patch is applied. Thus the continued use of the estrogen patches should have maintained a steady level of P[17β] rather than increased it. The assay that we used to analyze P[17β] had 12% cross-reactivity with estrone, so we analyzed the plasma estrone concentration in a separate assay (Diagnostic Systems, Webster, TX) to ensure that the increase in P[17β] that we measured in our assay was not due to our measuring of changes in plasma estrone levels. Estrone was similar between the E2 and the E2-P4 treatments (37.2 ± 9.5, 90.4 ± 19.4, and 97.4 ± 9.1 pg/ml, for the GnRH antagonist alone, E2, and E2-P4 treatments, respectively). Thus we suspect that the explanation for the greater P[17β] during E2-P4 was due to normal low-level conversion of progesterone to 17β-estradiol (27).

The changes in PV during E2 were related to changes in ECFV distribution, rather than changes in total ECFV. Despite significant ECFV contraction during E2, PV increased by 3%, and the percentage of PV in the ECFV was drastically increased (~21%) compared with GnRH antagonist alone (~16%). Moreover, within each subject, TERab explained 81–99% of the variance in PV across all three hormonal treatments, indicating that slower TERab and thus the greater plasma protein content served to maintain fluid in the intravascular space, despite a significant fall in overall ECFV. These findings support earlier studies in our laboratory, which have consistently reported PV increases (3–7%) in association with high P[17β], even in the absence of fluid retention (6, 34, 35).

Moreover, in an earlier study in which we used ANP infusions to stimulate changes in transcapillary fluid dynamics, we found that E2 did not alter either plasma or interstitial oncotic pres-

Table 2. Extracellular fluid characteristics

<table>
<thead>
<tr>
<th></th>
<th>GnRH Antagonist</th>
<th>Estrogen</th>
<th>Estrogen-Progestrone</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(17β), mol/kgH2O</td>
<td>281 ± 1</td>
<td>280 ± 1</td>
<td>278 ± 1*</td>
</tr>
<tr>
<td>SNa, meq/l</td>
<td>137.0 ± 0.8</td>
<td>136.2 ± 1.1</td>
<td>136.5 ± 0.7</td>
</tr>
<tr>
<td>SCl, meq/l</td>
<td>3.68 ± 0.08</td>
<td>3.68 ± 0.09</td>
<td>3.76 ± 0.05</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>38.5 ± 0.5</td>
<td>38.2 ± 1.0</td>
<td>36.7 ± 1.1*†</td>
</tr>
<tr>
<td>Hemoglobin, g</td>
<td>11.7 ± 0.2</td>
<td>11.7 ± 0.3</td>
<td>11.0 ± 0.4*†</td>
</tr>
<tr>
<td>Blood volume, ml/kg</td>
<td>64.4 ± 2.0</td>
<td>65.7 ± 4.3*</td>
<td>69.8 ± 4.0*†</td>
</tr>
<tr>
<td>PV, ml</td>
<td>2.84 ± 1.48</td>
<td>2.93 ± 1.70*</td>
<td>3.13 ± 1.75*†</td>
</tr>
<tr>
<td>ECFV, ml/kg</td>
<td>291.0 ± 37.1</td>
<td>226.5 ± 31.3*</td>
<td>283.1 ± 19.2*</td>
</tr>
<tr>
<td>PV/ECFV, %</td>
<td>16.1 ± 2.2</td>
<td>21.0 ± 2.0*</td>
<td>17.2 ± 1.4*†</td>
</tr>
<tr>
<td>P[17β], g/dl</td>
<td>4.1 ± 0.0</td>
<td>4.2 ± 0.1</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>Pab content, g/kg</td>
<td>1.77 ± 0.07</td>
<td>1.84 ± 0.13*</td>
<td>1.99 ± 0.15*†</td>
</tr>
<tr>
<td>TERab, %/h</td>
<td>5.7 ± 0.5</td>
<td>4.1 ± 1.1*</td>
<td>2.8 ± 0.9*†</td>
</tr>
</tbody>
</table>

Values are means ± SE; Extracellular fluid characteristics are shown during GnRH antagonist administration alone, with estrogen, and with estrogen-progestrone treatment. Extracellular fluid volume (ECFV), plasma osmolality (P(17β)), plasma volume (PV), plasma albumin concentration (P[A]), plasma albumin (Pab) content, transcapillary escape rate of albumin (TERab), and serum concentrations of sodium (SNa) and potassium (SCl) are shown. *Significantly different from GnRH antagonist. †Significantly different from estrogen treatment. Differences were considered statistically significant at P < 0.05.
sure, although E2 did attenuate the ANP-induced PV loss while increasing capillary filtration coefficient (33). Thus PV was maintained despite losses in total body water (and likely total ECFV) due to the ANP infusion, which would be consistent with our present findings. Taken together, the data are consistent with the hypothesis that E2 affects Starling forces, such as capillary filtration, to favor fluid retention in the plasma.

Our present findings do not support our original hypothesis that changes in PV would be proportional to changes in ECFV. Our findings indicate that, over the course of E2 treatment, ECFV decreased, although there was no change in body weight maintained despite losses in total body water (and likely total ECFV) due to the ANP infusion, which would be consistent with the hypothesis that E2 affects Starling forces, such as capillary filtration, to favor fluid retention in the plasma.

In contrast to the luteal phase in which the increase in plasma aldosterone (P4) occurred as soon as the subjects began taking progesterone, midluteal phase of the menstrual cycle is usually associated with lower PV (6, 34). For example, the increase in endogenous progesterone during the midluteal phase of the menstrual cycle is usually associated with PV contraction (6, 34). The cause for these inconsistencies may be due to the length of time of progesterone exposure. In contrast to the luteal phase in which the increase in plasma progesterone takes place over the course of ~7–10 days, our progesterone administration took place over the course of 4 days, and the increase in P4 occurred as soon as the subjects began taking progesterone. Thus any PV contraction seen in the luteal phase may be due to higher levels of progesterone over a longer period of time. The increase in PV during E2-P4 compared with GnRH antagonist alone, or E2-P4 with progesterone, was consistent with our hypothesis that E2 affects Starling forces, such as capillary filtration, to favor fluid retention in the plasma.

Table 3. Renal characteristics

<table>
<thead>
<tr>
<th>GFR, ml/min</th>
<th>GnRH Antagonist</th>
<th>Estrogen</th>
<th>Estrogen-Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>94±13</td>
<td>94±13</td>
<td>89±8</td>
</tr>
<tr>
<td>180</td>
<td>89±11</td>
<td>100±10</td>
<td>97±8</td>
</tr>
<tr>
<td>240</td>
<td>94±9</td>
<td>103±7</td>
<td>94±6</td>
</tr>
<tr>
<td>Uv, ml/min</td>
<td>1.3±0.4</td>
<td>2.9±1.2</td>
<td>4.6±0.9</td>
</tr>
<tr>
<td>0</td>
<td>1.9±0.9</td>
<td>2.2±0.6</td>
<td>2.4±0.8</td>
</tr>
<tr>
<td>240</td>
<td>2.9±0.5</td>
<td>3.6±0.5</td>
<td>2.6±0.8</td>
</tr>
<tr>
<td>Uosm, ml/min</td>
<td>346±79</td>
<td>310±71</td>
<td>183±80</td>
</tr>
<tr>
<td>0</td>
<td>313±59</td>
<td>286±41</td>
<td>313±79</td>
</tr>
<tr>
<td>240</td>
<td>245±46</td>
<td>198±30</td>
<td>328±72</td>
</tr>
<tr>
<td>Fe%, %</td>
<td>0.5±0.1</td>
<td>1.4±0.5</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>0</td>
<td>0.9±0.2</td>
<td>1.2±0.3</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>240</td>
<td>1.02±0.2</td>
<td>1.6±0.5</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>Fri, %</td>
<td>1.2±0.3</td>
<td>2.6±0.9</td>
<td>5.1±1.0</td>
</tr>
<tr>
<td>0</td>
<td>2.1±0.5</td>
<td>2.1±0.4</td>
<td>2.0±0.5</td>
</tr>
<tr>
<td>240</td>
<td>3.0±0.4</td>
<td>3.6±0.6</td>
<td>2.5±0.6</td>
</tr>
<tr>
<td>Uv, meq</td>
<td>0.5±0.1</td>
<td>1.5±0.6</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>0</td>
<td>4.2±0.9</td>
<td>5.7±1.7</td>
<td>6.3±1.9</td>
</tr>
<tr>
<td>240</td>
<td>2.3±0.4</td>
<td>3.7±1.2</td>
<td>2.9±1.9</td>
</tr>
<tr>
<td>UNa, meq</td>
<td>2.2±0.7</td>
<td>7.3±2.4</td>
<td>2.5±0.6</td>
</tr>
<tr>
<td>0</td>
<td>23.6±4.6</td>
<td>28.3±5.5</td>
<td>24.1±6.2</td>
</tr>
<tr>
<td>240</td>
<td>8.6±1.7</td>
<td>13.0±3.6</td>
<td>9.7±2.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Renal parameters are shown during GnRH antagonist administration alone, with estrogen, and with estrogen-progesterone treatment. Glomerular filtration rate (GFR, estimated from creatinine clearance), urine excretion (U), urine osmolality (Uosm), fractional sodium excretion (Fri), fractional water excretion (Fri), and potassium (Uk) and sodium (UNa) excretion are shown.

over the 4 days of E2. Unfortunately, we did not control or measure diet or physical activity in the subjects, so we cannot definitively state whether there was a change in total body water. However, the baseline renal measurements do not suggest active sodium or water loss during E2.

In contrast to E2, both ECFV and PV increased during E2-P4, compared with GnRH antagonist alone, so overall ECFV distribution was not affected, i.e., ~17% of ECFV was in the intravascular space (Table 2). As described in the previous paragraph, within each subject, TERalb explained a large percentage of the variance in PV across treatments, indicating that slower TERalb and thus greater plasma protein content, was a primary mechanism for E2-P4-related PV expansion. The E2-P4-related PV expansion is consistent with many earlier investigations because high levels of plasma progesterone typically have been associated with lower PV (6, 34). For example, the increase in endogenous progesterone during the midluteal phase of the menstrual cycle is usually associated with PV contraction (6, 34). The cause for these inconsistencies may be due to the length of time of progesterone exposure. In contrast to the luteal phase in which the increase in plasma progesterone takes place over the course of ~7–10 days, our progesterone administration took place over the course of 4 days, and the increase in P4 occurred as soon as the subjects began taking progesterone. Thus any PV contraction seen in
the earlier studies may only be a function of longer term progesterone exposure. Moreover, our progesterone administration took place while other potential confounders, such as follicle-stimulating and luteinizing hormones, were suppressed. Thus our design in the present study may have provided a more direct examination of immediate, direct progesterone effects on PV.

Our findings in the present investigation are complicated by the increase in \( P_{\text{Pl,4}} \) as well as by the length of time of estrogen administration, over the course of E2-P4, both of which may have contributed to the PV expansion during E2-P4. The \( P_{\text{Pl,4}} / P_{\text{Pl,1}} \) as well as the absolute level of plasma estrogen, in the present study was similar to that seen during the luteal phase of a normal menstrual cycle (6, 34, 38). In addition, the \( P_{\text{Pl,4}} / P_{\text{Pl,1}} \) was similar for the GnRH antagonist alone (73.6) and E2-P4 (60.3) treatments. Thus it is possible that the increase in PV during E2-P4 was the result of immediate, direct progesterone effects rather than the increase in estrogen. Conversely, it is possible that the PV expansion during E2-P4 was the result of the longer exposure to estrogen relative to the estrogen alone trial. Clearly, experiments using progesterone treatment alone as well as those in which the time on estrogen are similar would be needed to definitively determine the impact of direct effect of progesterone on PV.

A series of studies examining changes in the Starling forces associated with estrogen and progesterone secretion demonstrated that the ratio of plasma vs. interstitial oncotic pressures is reduced when estrogen and progesterone are elevated, such as during the midluteal phase of the menstrual cycle (26), during in vitro fertilization (41), and during oral contraceptive administration (42). A reduction in this plasma vs. interstitial oncotic pressure ratio would favor ECF movement out of the capillaries in part of the body, there can be compensation in other areas of the body so that overall changes in ECF distribution remain static (2, 3, 12). Although \( \text{TER}_{\text{abh}} \) used in our investigation is not a direct measurement of changes in oncotic pressure, it is a whole body estimate of protein or albumin escape and so may be a better predictor of changes in ECF distribution.

ECFV may have been preserved during E2-P4 due to progesterone-related increases in aldosterone and subsequent increases in sodium retention that may have taken place over the 4 days of progesterone treatment. The increase in aldosterone associated with high levels of plasma progesterone is a common finding (4, 6, 9, 32, 34, 36) and is likely due to both central and peripheral mechanisms. Progesterone, through competitive binding to aldosterone receptor sites in the periphery, induces a transient natriuresis (22). However, significant sodium losses rarely follow rises in progesterone because the sodium loss is buffered by increases in aldosterone (25). Progesterone administration to ovariectomized rats stimulates both basal and ANG II-stimulated aldosterone secretion and PRA through mechanisms similar to those of sodium restriction (4). For example, aldosterone increased during progesterone administration due to activity of the late biosynthetic pathway of aldosterone (assessed by aldosterone synhase conversion of corticosterone to aldosterone) vs. activity of the early biosynthetic pathway (assessed by changes in pregnenolone) in the rat (4). This mechanism was similar to the mechanism for aldosterone increases in earlier experiments during sodium restriction, also in the rat (1). The linear relationship between PV and \( P_{\text{Ald}} \) supports the conjecture that increases in PV and ECFV during estrogen and progesterone administration are related to changes in aldosterone and subsequent sodium retention in the present study.

Although our findings clearly demonstrate roles for both estrogen and progesterone in the regulation of body fluid distribution, they do not elucidate the mechanism for the changes in PV and ECFV. We can speculate that the vasodilatory properties of estrogen, thought to be mediated primarily via nitric oxide (13, 16, 39, 44), may be an important mechanism for the expansion of PV simply by increasing the surface area of capillary filtration (13, 20, 29). Alternatively, estrogen may act on blood-borne substances, such as ANP (5, 21, 28), to alter fluid shifts out of the plasma compartment (33). On the other hand, the estrogen-mediated increase in flow-mediated vasodilation in the brachial artery is obliterated in the presence of progesterone, and a significant negative correlation between progesterone and vasodilation exists in women during the luteal phase of the menstrual cycle (10). Hashimoto et al. (14) also found that the estrogen-associated increases in brachial artery vasodilation were attenuated during the luteal phase. Thus the estrogen-related changes in endothelial function are at least to some extent antagonized by progesterone (10, 14).

This study is the first to isolate estrogen effects on body fluid distribution in young women by suppressing reproductive function and adding back estrogen and progesterone in controlled doses. This study was an attempt to explain earlier data, which indicated an estrogen-related increase in PV in the absence of overall fluid retention, and indicates that the primary cause for the increase in PV during short-term estrogen administration is a change in ECFV distribution. In fact, PV increases, despite a significant fall in ECFV. When progesterone was present, ECFV was maintained at a level similar to that of GnRH antagonist alone, whereas PV increased. These changes in PV were directly associated with changes in \( \text{TER}_{\text{abh}} \) under both hormonal conditions. The changes in ECFV distribution may be related to sex hormone effects on the actions of
blood-borne substances on vessels that alter Starling forces. Finally, future studies of measurements of PV and ECFV during the menstrual cycle are needed because \( P_{(R)} / P_{(L)} \) changes constantly, and our study was only able to examine body fluid distribution under static conditions. Moreover, future studies should include measurements of changes in ECFV and PV during progesterone administration, without concomitant estrogen administration, and should also include simultaneous measurements of PV, ECFV, TER, and Starling forces, such as capillary filtration coefficient and plasma and tissue oncotic pressures.

**Perspectives.** As new information from long-term trials regarding the health benefits of estrogen and progesterone become available (18, 30), careful evaluation of the physiological effects of these hormones on regulatory systems takes on even greater importance. Our research design enables us to transiently suppress reproductive function in young, healthy women and isolate the effects of estrogen and progesterone. Moreover, the levels of estrogen and progesterone in the plasma attained in this study were similar to those during various phases of the menstrual cycle and thus are physiologically relevant. Changes in the distribution of body fluids in response to changes in estrogen and progesterone play a role in the etiology of a number of acute and chronic conditions. For example, men and women differ in their susceptibility to a number of illnesses related to body fluid regulation, such as hypertension, orthostatic hypotension, and compromised renal function. Our findings are also relevant to acute conditions, such as postoperative or postexercise hyponatremia and associated neurological involvement. Finally, the design in this study enabled a closer look at the body fluid responses to rapid, short-term increases in progesterone. As in vitro fertilization becomes more common, women are more at risk for conditions such as ovarian hyperstimulation syndrome during rapid fluctuations in both estrogen and progesterone. This syndrome, which can be fatal, is related not only to extremely high rates of body fluid retention but also to greater accumulation of this fluid in the interstitial space.

**ACKNOWLEDGMENTS**

We gratefully acknowledge the technical support of Cheryl Leone, Jodi Crimmins, Jennifer Fawcett, Wendy Calzone, and Lauren Gulka, and the cooperation of the volunteer subjects.

**GRANTS**

This work was supported by National Heart, Lung, and Blood Institute Grants RO1 HL-62240-01A1 and RO1 HL-071159-01A1.

**REFERENCES**


