Effects of oral contraceptives on body fluid regulation

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Stachenfeld, Nina S., Celso Silva, David L. Keefe, Cheryl A. Kokoszka, and Ethan R. Nadel. Effects of oral contraceptives on body fluid regulation. *J. Appl. Physiol.* 87(3): 1016–1025, 1999.—To test the hypothesis that estrogen reduces the operating point for osmoregulation of arginine vasopressin (AVP), thirst, and body water balance, we studied nine women (25 ± 1 yr) during 150 min of dehydrating exercise followed by 180 min of ad libitum rehydration. Subjects were tested six different times, during the early-follicular (twice) and midluteal (twice) menstrual phases and after 4 wk of combined [estradiol-norethindrone (progestin), OC E + P] and 4 wk of norethindrone (progestin only, OC P) oral contraceptive administration, in a randomized crossover design. Basal plasma osmolality (P_{osm}) was lower in the luteal phase (281 ± 1 mosmol/kgH$_2$O, combined means, $P < 0.05$), OC E + P (281 ± 1 mosmol/kgH$_2$O, $P < 0.05$), and OC P (282 ± 1 mosmol/kgH$_2$O, $P < 0.05$) than in the follicular phase (286 ± 1 mosmol/kgH$_2$O, combined means). High plasma estradiol concentration lowered the P_{osm} threshold for AVP release during the luteal phase and during OC E + P [x-intercepts, 282 ± 2, 278 ± 2, 276 ± 2, and 280 ± 2 mosmol/kgH$_2$O, for follicular, luteal (combined means), OC E + P, and OC P, respectively; $P < 0.05$, luteal phase and OC E + P vs. follicular phase] during exercise dehydration, and 17β-estradiol administration lowered the P_{osm} threshold for thirst stimulation [x-intercepts, 280 ± 2, 279 ± 2, 276 ± 2, and 280 ± 2 mosmol/kgH$_2$O for follicular, luteal, OC E + P, and OC P, respectively; $P < 0.05$, OC E + P vs. follicular phase], without affecting body fluid balance. When plasma 17β-estradiol concentration was high, P_{osm} was low throughout rest, exercise, and rehydration, but plasma arginine vasopressin concentration, thirst, and body fluid retention were unchanged, indicating a lowering of the osmotic operating point for body fluid regulation.

estrogen; progesterone; rehydration; osmolality; arginine vasopressin

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ESTROGEN ADMINISTRATION can lead to significant body fluid retention (19) and, in very high doses, hypertension (14). Although the mechanism underlying the estrogen-mediated body fluid retention is unclear, a number of studies have demonstrated that the osmotic thirst and arginine vasopressin (AVP) responses to hypertonicity occur earlier with elevations in estrogen and progesterone, such as during the luteal phase of the menstrual cycle (18, 26, 27) and during pregnancy (8). Using hypertonic saline infusion followed by water loading, Vokes et al. (27) demonstrated a downward resetting of the osmoreceptors during the luteal phase. In addition, we recently demonstrated a reduction in the osmotic threshold for AVP release during hypertonic saline infusion in postmenopausal women who were taking estrogen (19), and the greater AVP response was associated with fluid retention.

Although it seems clear that elevations in estrogen, with and without elevations in progesterone, alter osmotic regulation of AVP (18, 26, 27) and thirst sensitivity (8, 27), the specific estrogen effects on body fluid regulation after body water loss are not known. Addressing the question of estrogen effects during dehydration as opposed to hypertonic saline infusion is important, because hypertonic saline infusion increases plasma osmolality (P_{osm}) and volume (PV), whereas dehydration increases P_{osm} while it reduces total body water and PV. The AVP-P_{osm} and thirst-P_{osm} relationships are shifted with differing volume status (15), so an evaluation of the fluid regulation systems while PV is reduced and the body is actively retaining fluid is necessary to fully understand the effects of estrogen on these systems. These differences in PV status during hypertonic saline infusion and dehydration may exaggerate AVP and thirst responses to osmotic stimulation during dehydration but may also have particular relevance during subsequent rehydration, inasmuch as they could alter the compartmentalization of ingested fluid. Alterations in the compartmentalization of ingested fluid have important implications for physical performance, because changes in body water storage will influence fluid maintenance during exertion and fluid restoration during recovery from exertion in environmentally stressful conditions.

Therefore, to determine estrogen effects on the body water regulation system, we administered oral contraceptives to young women and then evaluated their responses to progressive, exercise-induced dehydration and a subsequent rehydration period. Combined oral contraceptive agents deliver pharmacological levels of estrogens that exhibit 6–10 times the estrogenic activity provided by endogenous, circulating estrogens. In contrast, progestin-only pills contain no estrogen, and the unopposed progestin tends to downregulate estrogen receptors. Thus these two oral contraceptive preparations differ significantly in their estrogenic activity, providing the appropriate conditions in which to isolate estrogen effects on body fluid regulation. We hypoth-

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esized that oral contraceptive pills containing estrogen would reduce the threshold for osmotic AVP and thirst increases to progressive, exercise-induced dehydration to a greater degree than a progestin-only pill. In addition, we hypothesized that fluid intake and renal water retention would also be increased and lead to greater water retention during combined oral contraceptive treatment. Finally, consideration of the PV and arterial pressure control of Na^+ excretion is essential for a complete evaluation of body fluid regulation, so we also determined the effects of our oral contraceptive regimen on Na^+ regulation and the Na^+-regulating hormones.

METHODS

Study design. Subjects were nine healthy, nonsmoking women (age 25 ± 1 yr, range 22–31 yr) with no contraindications to oral contraceptive use. All subjects were interviewed about their medical history, had medical and gynecological examinations, and provided written confirmation of a negative Papanicolaou smear within 1 yr of being admitted to the study. During the month (early-follicular phase) preceding the first dehydration experiment, resting PV was determined with Evans blue dye dilution (see below) and peak O_2 consumption was determined from an incremental cycle ergometer test with use of an automated metabolic cart (Sensor Medics, Yorba Linda, CA).

Each woman participated in two series of experiments (Fig. 1), each consisting of two baseline dehydration tests (4 total) and one dehydration test while taking each type of oral contraceptive (2 total). Estrogen and progesterone vary across the menstrual cycle, so the study design employed two dehydration baseline studies conducted in the early-follicular phase, 2–4 days after the beginning of menstrual bleeding (low estrogen and progesterone), one for each pill treatment, and two conducted in the midluteal phase, 7–9 days after the luteinizing hormone peak (high estrogen and progesterone), determined individually by the use of ovulation prediction kits (OvuQuick, Quidel, San Diego, CA). After completing the first baseline dehydration tests, the subjects again performed dehydration protocols after 4 wk of continuous combined (estrogen-progestin, OC E + P) or progestin-only oral contraceptive treatment (random assignment, double blind, OC P). After completing the first dehydration testing series and after a 4-wk "washout" period, the subjects crossed over to the other pill treatment.

During OC E + P treatment, subjects received 0.035 mg of ethinyl estradiol and 1 mg of the progestin norethindrone daily. During OC P treatment, subjects received 1 mg/day of the progestin norethindrone. To verify phase of the menstrual cycle and compliance to the pill regimen, plasma levels of estrogen and progesterone were assessed from the preexercise blood sample before the dehydration protocol was undertaken.

Dehydration experiments. Volunteers arrived at the laboratory between 7 and 8 AM, after having eaten only a prescribed low-fat breakfast (~300 kcal). The subjects refrained from alcohol and caffeine for 12 h before the experiment. Blood volumes were not manipulated before any of the experiments, although subjects prehydrated by drinking 7 ml/kg body wt of tap water at home before arrival at the laboratory. On arriving at the laboratory, each subject gave a baseline urine sample, was weighed to the nearest 10 g on a beam balance, and then sat on the contour chair of a semirecumbent cycle ergometer in the test chamber (27°C, 30% relative humidity) for 60 min of control rest. During the control period, an indwelling catheter (21 gauge) was inserted into an arm vein, and electrodes and a blood pressure cuff were placed. Subjects were semirecumbent during placement of the catheter and were seated for 60 min before sampling to ensure a steady state in PV and constituents. Resting blood pressure (Colin Medical Instruments, Komaki, Japan) and heart rate (electrocardiogram) were recorded at the end of the 60-min control period. At the end of the control period, a blood sample (20 ml) was drawn and urine was collected. Hydration state was assessed from the specific gravity of the preexercise urine sample (mean = 1.002 ± 0.001).

After the control period the chamber temperature was increased to 36°C and the subjects began pedaling at an intensity corresponding to 50% maximal power output. The exercise duration was 150 min, with 5-min rest periods every 25 min, during which time they received no fluids. Blood samples (10–20 ml) were drawn and body weight was measured immediately before the rest periods at 60, 120, and 150 min during exercise. On the basis of previous experience in our laboratory, we expected a weight loss of 2.0–2.5% of preexercise body weight. To accurately determine weight loss, we previously determined the saturated weight of the shorts and a jog-bra worn during exercise (0.250 kg) and subtracted this weight from the final exercise weight. Heart rate was monitored throughout exercise to ensure subject safety. A urine sample was collected at the end of exercise, and then the chamber temperature was reduced to 27°C for the 3.5-h recovery period.
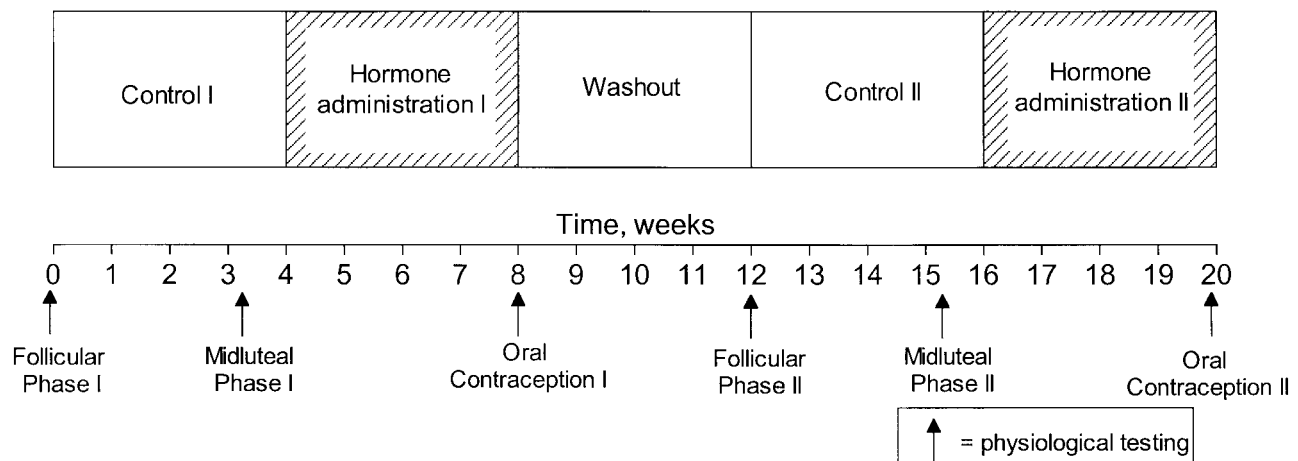

Fig. 1. Time line for sex hormone administration.

For sweat collection during exercise, sealed absorbent patches (Pacific Biometrics, Seattle, WA) were placed on the thigh, forearm, chest, back, and forehead for 20- to 25-min periods. The sweat patch consisted of 4.7×3.1-cm filter paper, sealed and affixed to the skin with Tegaderm. The skin areas used for the patch were cleaned with deionized water before placement of the patch and wiped with a clean dry towel. Local sweat rate was determined by each patch weight increase (to 0.0001 g) from the dry weight per minute on the skin. After sweat was collected and the sweat patch was weighed, the sweat-soaked patches were transferred to plastic screw-capped bottles. The fluid in the patches was collected by centrifugation with use of nylon Microfuge centrifuge filter tubes and analyzed for Na^+ and K^+ concentrations.

After dehydration, each subject rested for 30 min in a contour chair without access to fluids to allow the body fluid compartments to stabilize, then drank water ad libitum for 180 min. Blood was sampled just before drinking (*time 0*, 10 ml) and at 15 (10 ml), 30, 60, 120, and 180 min of rehydration (20 ml each sample). Urine samples were collected and body weight was measured every 60 min of rehydration. The total blood drawn during each experiment was ~180 ml, which is too small to have any independent effect on any of the measured variables.

All blood samples were analyzed for hematocrit (Hct), concentrations of Hb ([Hb]) and total protein ([TP]), P_{osm}, plasma concentrations of creatinine, glucose, urea, and AVP ($P_{[AVP]}$), and serum concentrations of Na^+ ($S_{[Na^+]}$) and K^+ ($S_{[K^+]}$). Plasma renin activity (PRA) and concentrations of aldosterone ($P_{[ald]}$) and atrial natriuretic peptide ($P_{[ANP]}$) were analyzed from the control sample, from samples taken at the end of exercise, and from samples taken at 0, 60, 120, and 180 min of rehydration. The control blood samples were also analyzed for 17β-estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$).

Blood and urine analysis. An aliquot (1 ml) was removed for immediate assessment of Hct, [Hb], and [TP] in triplicate by microhematocrit, cyanomethemoglobin, and refractometry, respectively. A second aliquot was transferred to a heparinized tube, and a third aliquot was placed into a tube without anticoagulant for the determination of $S_{[Na^+]}$ and $S_{[K^+]}$. All other aliquots were placed in chilled tubes containing EDTA. The tubes were immediately centrifuged at 4°C, and the plasma taken off the heparinized sample was analyzed for creatinine and aldosterone. The samples containing EDTA were analyzed for $P_{[AVP]}$, $P_{[ANP]}$, and PRA. These centrifuged samples were frozen immediately and stored at −80°C until analysis. All urine samples were analyzed for volume, osmolality, and creatinine concentration.

Plasma, sweat, and urine Na^+ and K^+ were measured by flame photometry (model 943, Instrumentation Laboratory). P_{osm} and urine osmolality were assessed by freezing-point depression (model 3DII, Advanced Instruments). Plasma and urine creatinine, plasma glucose, and urea concentrations were determined by colorimetric assay (Sigma Diagnostic Products). $P_{[AVP]}$, $P_{[ald]}$, $P_{[ANP]}$, $P_{[E_2]}$, $P_{[P_4]}$, and PRA were measured by RIA. Intra- and interassay coefficients of variation for the midrange standard were as follows: 6.0 and 3.4% (Immuno Biological Laboratories, Hamburg, Germany) for $P_{[AVP]}$ (4.52 pg/ml), 3.4 and 3.6% (Diagnostic Products, Los Angeles, CA) for $P_{[ald]}$ (132 pg/ml), 5.1 and 5.2% (Diasorin, Stillwater, MN) for $P_{[ANP]}$ (63.3 pg/ml), 3.7 and 4.0% (Diagnostic Products) for $P_{[E_2]}$ (64.3 pg/ml), 2.1 and 2.5% (Diagnostic Products) for $P_{[P_4]}$ (3.7 pg/ml), and 2.3 and 2.9% (Diasorin) for PRA (4.5 ng·ml ANG^{-1}·h^{-1}). The assay for AVP has a sensitivity of 0.8 pg/ml, which is necessary to detect small, but important, changes in this hormone.

Blood volume. Absolute blood volume was measured by dilution of a known amount of Evans blue dye. This technique involves injection of an accurately determined volume of dye (by weight, since the specific density is 1.0) into an arm vein and taking blood samples for determination of dilution after complete mixing (10, 20, and 30 min). PV was determined from the product of the concentration and volume of dye injected divided by the concentration in plasma after mixing, with 1.5% lost from the circulation within the first 10 min taken into account. Blood volume was calculated from PV and Hct concentration corrected for peripheral sampling (9).

Thirst ratings. We assessed thirst perception by asking the subject to make a mark on a line rating scale in response to the question, "How thirsty do you feel now?" The line is 175 mm long and is marked "not at all" on one end and "extremely thirsty" at the 125-mm point. We told subjects that they could mark beyond the "extremely thirsty" point if they wished and they could even have extended the line if they felt it was necessary. This method was developed by Marks et al. (11) and has been used with great success in the evaluation of several sensory systems. We have found an extraordinarily good relationship between the perception of thirst and P_{osm} during hypertonic saline infusion and dehydration in young subjects (20, 25).

Calculations. Total water loss due to dehydration was determined from body weight loss during exercise. Net fluid gain during rehydration was calculated by subtracting total urine loss from water intake, with the assumption that respiratory and sweat losses were negligible in the 27°C recovery condition. Changes in PV were estimated from changes in Hct and [Hb] from the control (preexercise) sample according to the equation

$$\%\Delta PV = 100\{[(Hb_b)/(Hb_a)]$$
$$\cdot [(1 - Hct_a \cdot 10^{-2})/[(1 - Hct_b \cdot 10^{-2})]\} - 100$$

in which subscripts a and b denote measurements at *time a* and control, respectively.

Fractional excretions of water (FE_{H_2O}) and Na^+ (FE_{Na^+}) were calculated from the following equations

$$FE_{H_2O} = (U_V/GFR) \cdot 100$$

$$FE_{Na^+} = (U_V \cdot U_{[Na^+]}/GFR \cdot [Na^+]_f) \cdot 100$$

$$[Na^+]_f = \text{the Donnan factor for cations } (0.95) \cdot S_{[Na^+]}$$

in which the subscript f is glomerular filtrate, U_v is urine flow rate, $U_{[Na^+]}$ is Na^+ concentration in urine, and $S_{[Na^+]}$ is $S_{[Na^+]}$ in protein-free solution (meq/kgH_2O). Glomerular filtration rate (GFR) was estimated from creatinine clearance.

Electrolyte losses in sweat and urine during dehydration were calculated by multiplying the volume of water loss in each fluid by the concentration of the electrolyte within the fluid. Whole body sweat electrolyte concentration was calculated from sweat rate, local electrolyte concentration, and body surface area using the following equation (24)

$$[E]_m = (0.07[E]_{fh}SR_{fh} + 0.36[E]_{tr}SR_{tr} + 0.13[E]_{fa}SR_{fa}$$
$$+ 0.32[E]_{th}SR_{th}/0.07SR_{fh} + 0.36SR_{tr} + 0.13SR_{fa} + 0.32SR_{th})$$

where the subscripts m, fh, tr, fa, and th are whole body mean, forehead, trunk, forearm, and thigh, respectively, [E] is electrolyte concentration (Na^+ or K^+, meq/l), SR is local sweat rate (mg·min^{-1}·cm^{-2}), and the constants 0.07, 0.36, 0.13, and 0.32 represent the percent distribution of body surface in the head, trunk, arms, and legs, respectively. Total electrolyte

loss from sweat was calculated by multiplying $[E]_m$ by total body sweat loss, calculated from the change in body weight during exercise. Electrolyte losses during rehydration were calculated by multiplying the volume of water loss by the concentration of electrolytes in the urine.

Statistics. Separate repeated-measures ANOVA models were performed to test differences in the dependent variables due to menstrual phase and OC E + P or OC P administration. Bonferroni's *t*-test was used to correct for multiple comparisons where appropriate. Pearson's product moment correlation was used to assess the relationship of $P_{[AVP]}$ as a function of P_{osm} on individual data during exercise, and the abscissal intercepts defined the "theoretical osmotic threshold" for AVP release (8). We used repeated-measures ANOVA models, followed by Bonferroni's *t*-test, to test differences in the abscissal intercepts and slopes due to menstrual phase or oral contraceptive treatment (4, 8). On the basis of an α-level of 0.05 and a sample size of 8, our β-level (power) was ≥0.80 for detecting effect sizes of 2.0 pg/ml, 0.67 ml/min, 2.0 ng·ml ANG^{-1}·h^{-1}, 40 pg/ml, 10 pg/ml, and 3.0 meq for $P_{[AVP]}$, renal free water clearance, PRA, $P_{[ald]}$, $P_{[ANP]}$, and renal Na$^+$ excretion, respectively (4, 7, 8, 28). Data were analyzed using BMDP statistical software (BMDP Statistical Software, Los Angeles, CA) and expressed as means ± SE.

RESULTS

Combined oral contraceptive administration caused severe nausea in one woman, and she did not complete dehydration testing while on this pill, so all her control data for OC E + P have also been excluded. This analysis compares the dehydration test responses of nine women on OC P with their two control tests and eight women on OC E + P with their control tests.

Subject characteristics. The subjects were 25 ± 1 yr (range 20–34 yr), weighed 62.5 ± 3.6 kg, and were 164 ± 3 cm tall. Their mean blood volume was 66.4 ± 2.0 ml/kg, mean PV was 2,780 ± 124 ml, and mean peak O$_2$ consumption was 30.6 ± 2.4 ml·kg^{-1}·min^{-1}.

Baseline (preexercise). Preexercise body weight was similar for both phases of the menstrual cycle and oral contraceptive administration (Table 1). The $P_{[E_2]}$ and $P_{[P_4]}$ values in Table 1 demonstrate that the subjects were tested in the early-follicular and midluteal phases of the menstrual cycle during both trials. Finally, oral contraceptive administration suppressed the endogenous production of 17β-estradiol and progesterone (Table 1).

Preexercise P_{osm} was lower in the luteal phase and after 1 mo of OC E + P and OC P than in the follicular phase (Fig. 2; $P < 0.05$), although $P_{[AVP]}$ and thirst were unaffected by phase of the menstrual cycle or by oral contraceptive administration (Table 2). Plasma glucose and urea concentrations were unaffected by menstrual phase or either oral contraceptive pill, but $S_{[Na^+]}$ was lower [138 ± 0.5, 136 ± 0.4, 136.2 ± 0.6, and 136.6 ± 0.3 meq/l for follicular and luteal phases (combined means), OC E + P, and OC P, respectively], suggesting that the lower P_{osm} (in the luteal phase and with oral contraceptives) was a function of lower $S_{[Na^+]}$. Changes in Hct and [Hb] indicated an estimated (calculated) contraction of PV compared with the follicular phase (Table 1). There was no effect of menstrual phase or oral contraceptive treatment on plasma protein concentration (6.7, 6.8, 6.7, and 6.8 g/l for follicular and luteal phases, OC E + P, and OC P, respectively). Preexercise PRA was greater in both luteal phase tests than in the follicular phase tests and during OC E + P, and $P_{[ald]}$ was increased in the luteal phase tests compared with the follicular phase tests (Table 3; $P < 0.05$). In contrast, $P_{[ANP]}$ was greater at baseline in the follicular phase tests than in the luteal phase and during OC P, and $P_{[ANP]}$ was greater during OC E + P than in the luteal phase test (Table 3; $P < 0.05$). Preexercise U_v, urine osmolality, GFR, and renal electrolyte excretion were similar within subjects before each exercise test.

Preexercise heart rate and blood pressure were similar at baseline and dehydration within the follicular and luteal phase tests, so the combined mean of the two series is given for the baseline values and for the dehydration tests. Baseline heart rate and mean blood pressure were unaffected by menstrual phase, averaging 78 ± 4 beats/min and 85 ± 2 mmHg during the

Table 1. *Subject characteristics and changes in osmotic AVP and thirst regulation*

	Follicular Phase (n = 8)	Midluteal Phase (n = 8)	OC E + P (n = 8)	Follicular Phase (n = 9)	Midluteal Phase (n = 9)	OC P (n = 9)
Body wt, kg	61.4 ± 4.1	61.8 ± 4.1	61.6 ± 3.8	60.7 ± 3.7	61.1 ± 3.4	60.0 ± 3.5
PV, ml	2780 ± 124					
$P_{[E_2]}$, pg/ml	27.3 ± 5.6 (12.3–40.8)	105.1 ± 26.2 (63.6–189.6)	<12.0	26.1 ± 6.7 (13.1–36.2)	146.7 ± 38.3 (61.1–222.0)	25.1 ± 5.3 (6.4–26.7)
$P_{[P_4]}$, ng/ml	1.3 ± 0.6 (0.3–2.2)	8.7 ± 3.1 (5.2–19.1)	<0.02	0.49 ± 1.0 (0.4–0.8)	9.8 ± 2.2 (5.2–18.3)	<0.02
P_{osm}-$P_{[AVP]}$ slope, pg·ml^{-1}·mosmol^{-1}	0.47 ± 0.11	0.51 ± 0.18	0.49 ± 0.12	0.49 ± 0.14	0.55 ± 0.17	0.46 ± 0.14
P_{osm}-$P_{[AVP]}$ x-intercept, mosmol/kg H$_2$O	282 ± 1	278 ± 1*	276 ± 2†	283 ± 1	279 ± 1*	280 ± 2
P_{osm}-thirst slope, mm/mosmol	13.7 ± 3.5	14.0 ± 2.7	13.3 ± 3.7	12.8 ± 1.7	12.9 ± 2.9	13.7 ± 2.1
P_{osm}-thirst x-intercept, mm	280 ± 3	278 ± 2	276 ± 2†	280 ± 1	279 ± 2	280 ± 2
PV change, %		−8.4 ± 2.5	3.2 ± 2.1		−7.5 ± 2.7	−2.3 ± 2.5

Values are means ± SE; ranges are in parentheses. Resting plasma volume (PV) was measured on a separate day in the follicular phase. Preexercise body weight and plasma concentrations of endogenous 17β-estradiol ($P_{[E_2]}$) and progesterone ($P_{[P_4]}$) were measured in early-follicular and midluteal phases and during administration of combined [estradiol + progestin (norethindrone), OC E + P] and progestin (norethindrone)-only (OC P) oral contraceptive pills. Slopes and abscissal intercepts of individual subject's plasma arginine vasopressin concentration ($P_{[AVP]}$)-plasma osmolality (P_{osm}) and thirst-P_{osm} relationships during dehydration in early-follicular and midluteal phases and OC E + P and OC P are shown. Percent change in PV relative to the follicular phase was estimated from changes in preexercise hematocrit and Hb. * $P < 0.05$, follicular vs. midluteal phase; † $P < 0.05$, follicular phase vs. OC E + P.
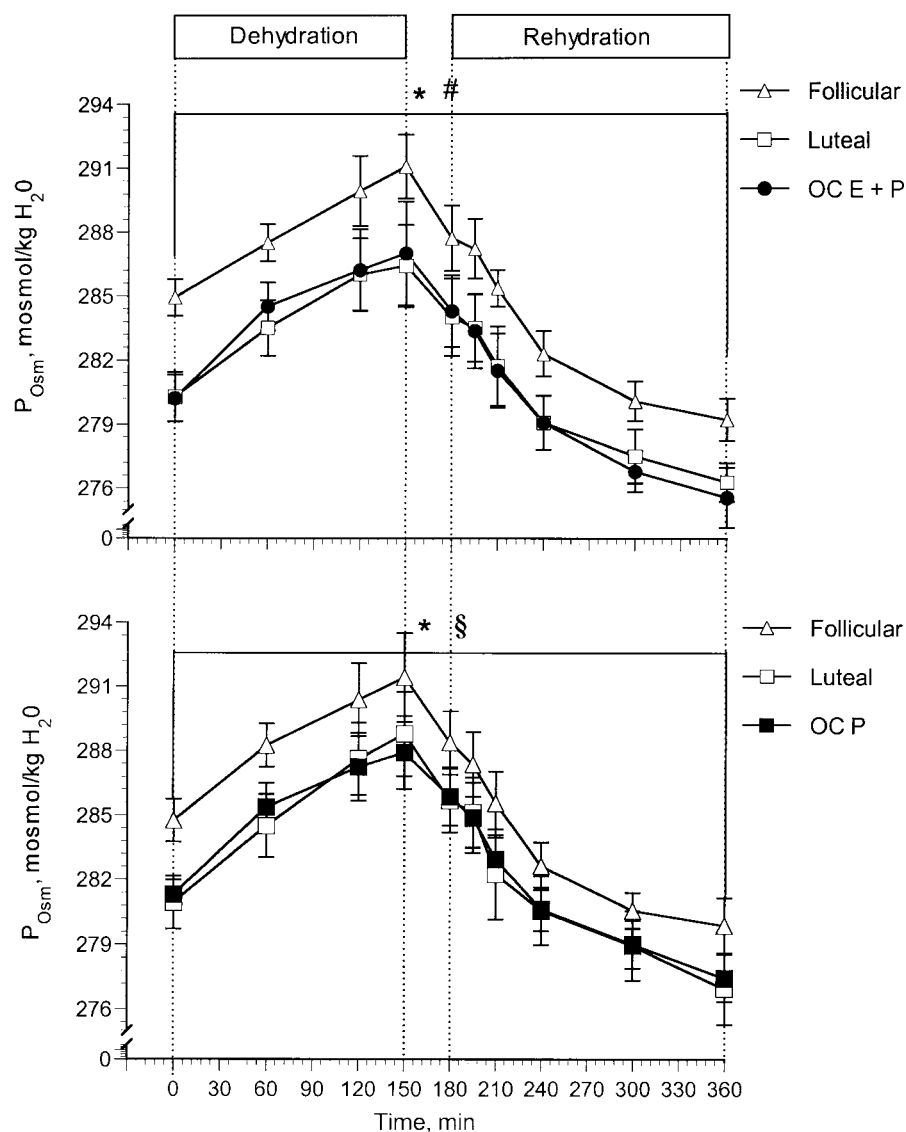
Fig. 2. Plasma osmolality (P_{osm}) at rest and in response to dehydrating exercise and 180 min of ad libitum rehydration in follicular and luteal phases and during combined estradiol-progestin (norethindrone, OC E + P, $n = 8$) and progestin (norethindrone)-only oral contraception administration (OC P, $n = 9$). $*P < 0.05$, follicular vs. luteal phase. $^{\#}P < 0.05$, follicular phase vs. OC E + P. $^{\S}P < 0.05$, follicular phase vs. OC P. Values are means ± SE.

follicular phase and 78 ± 5 beats/min and 82 ± 2 mmHg during the luteal phase. These cardiovascular variables were also unchanged by oral contraceptive treatment, averaging 78 ± 3 beats/min and 83 ± 1 mmHg and 81 ± 2 beats/min and 81 ± 2 mmHg during OC E + P and OC P, respectively.

Exercise responses. The subjects lost similar body weight (and percent body weight) at the end of 150 min of exercise during the follicular (1.4 ± 0.1 kg, 2.3%) and luteal (1.4 ± 0.1 kg, 2.2%) phase tests and during OC E + P (1.3 ± 0.2 kg, 2.3%). The same was true for the follicular (1.4 ± 0.1 kg, 2.3%) and luteal (1.4 ± 0.1 kg, 2.4%) phase tests compared with OC P (1.3 ± 0.1 kg, 2.2%). Heart rate increased to similar levels during dehydrating exercise in the follicular (145 ± 6 beats/min) and luteal (141 ± 5 beats/min) phase tests and during the OC P test (141 ± 7 beats/min), but this increase was attenuated during the OC E + P test (135 ± 6 beats/min, $P < 0.05$). Mean blood pressure did not change during dehydration in any of the experimental conditions.

Exercise increased P_{osm} and $P_{[AVP]}$ and decreased PV similarly during the follicular and luteal phases and during OC E + P and OC P (Fig. 2, Table 2). Linear regression analysis of the individual subjects' data during dehydration indicated significant correlations between $P_{[AVP]}$ and P_{osm} (mean $r = 0.88 ± 0.03$). The abscissal intercepts of the linear $P_{[AVP]}$-P_{osm} relationship, or "theoretical osmotic threshold" for AVP release, was significantly lower in the midluteal phase and with OC E + P than in the follicular phase (Table 1, $P < 0.05$). The slopes of this relationship, however, were unaffected by menstrual phase or oral contraceptive use. Figure 3 shows the downward shift in the linear $P_{[AVP]}$-P_{osm} relationships during dehydrating exercise when $P_{[E_2]}$ and $P_{[P_4]}$ were increased in the luteal phase and during OC E + P. The data in Table 2 indicate that thirst increased similarly during dehydration in all conditions. Linear regression analysis of the individual subjects' P_{osm} and thirst responses indicated significant correlations (mean $r = 0.90 ± 0.03$). Osmotic thirst

Table 2. *Plasma AVP concentrations, subjective thirst responses, and PV changes*

	Preexercise (0 min)	End Exercise (150 min)	Rehydration			
			0 min	60 min	120 min	180 min
P$_{[AVP]}$, pg/ml			*OC E+P*			
Follicular	1.3 ± 0.2	4.0 ± 0.8	3.3 ± 0.9	1.7 ± 0.4	1.6 ± 0.3	1.6 ± 0.3
Luteal	1.2 ± 0.2	3.8 ± 0.7	3.0 ± 0.7	1.5 ± 0.4	1.3 ± 0.3	1.5 ± 0.4
OC E + P	1.6 ± 0.3	3.1 ± 0.4	3.1 ± 0.4	2.7 ± 0.7	1.9 ± 0.4	2.3 ± 0.4
Thirst, mm						
Follicular	18 ± 9	101 ± 10	100 ± 10	21 ± 8	24 ± 11	13 ± 5
Luteal	29 ± 11	111 ± 11	97 ± 12	12 ± 5	23 ± 8	7 ± 3
OC E + P	29 ± 10	94 ± 13	101 ± 12	19 ± 6	22 ± 8	17 ± 6
PV, % change						
Follicular		-8.6 ± 1.3	-2.6 ± 1.6	1.3 ± 1.6	1.2 ± 1.7	2.5 ± 1.8
Luteal		-9.5 ± 2.6	-3.3 ± 2.0	0.2 ± 1.4	0.7 ± 1.6	0.5 ± 1.5
OC E + P		-7.9 ± 1.2	-0.5 ± 1.2	1.9 ± 1.3	3.6 ± 1.0	5.1 ± 1.7
P$_{[AVP]}$, pg/ml			*OC P*			
Follicular	1.2 ± 0.4	3.7 ± 1.0	2.5 ± 0.5	1.8 ± 0.6	1.8 ± 0.6	1.6 ± 0.4
Luteal	1.1 ± 0.3	4.8 ± 1.4	2.3 ± 0.6	2.0 ± 0.5	1.9 ± 0.6	1.9 ± 0.6
OC P	1.0 ± 0.2	4.0 ± 1.2	2.7 ± 0.7	1.8 ± 0.7	2.2 ± 0.7	1.5 ± 0.4
Thirst, mm						
Follicular	20 ± 5	97 ± 12	90 ± 12	17 ± 6	12 ± 4	9 ± 4
Luteal	28 ± 9	97 ± 6	98 ± 11	31 ± 10	21 ± 6	25 ± 10
OC P	24 ± 8	97 ± 9	108 ± 6	19 ± 9	19 ± 9	18 ± 9
PV, % change						
Follicular		-7.5 ± 1.2	0.0 ± 1.4	2.3 ± 1.1	3.1 ± 1.1	5.0 ± 0.7
Luteal		-7.4 ± 1.0	0.1 ± 1.1	3.2 ± 0.1	0.8 ± 1.2	1.6 ± 1.1
OC P		-6.5 ± 1.0	0.4 ± 0.9	4.7 ± 1.4	4.5 ± 1.3	5.2 ± 1.6

Values are means $\pm$ SE. Anginine vasopressin plasma concentration (P$_{[AVP]}$), cognitive thirst ratings, and plasma volume (PV) (estimated percent change from preexercise value) were measured at rest and in response to dehydrating exercise and 180 min of ad libitum rehydration in follicular and luteal phases and during OC E + P ($n = 8$) and OC P ($n = 9$).

stimulation was unaffected by menstrual phase, but OC E + P led to a fall in the abscissal intercept of this relationship (Table 1).

PRA, P$_{[ald]}$, and P$_{[ANP]}$ increased during exercise in all conditions, with luteal phase values for P$_{[ald]}$ remaining above the follicular phase, OC E + P, and OC P (Table 3; $P < 0.05$). Sweat Na$^+$ loss was greatest during exercise in the follicular phase tests (56.3 ± 7.0 and 59.4 ± 9.2 meq, $P < 0.05$) but was similar between the luteal phase tests (45.2 ± 9.1 and 46.5 ± 7.8 meq) compared

Table 3. *Plasma concentrations of Na$^+$-regulating hormones*

	Preexercise (0 min)	End Exercise (150 min)	Rehydration			
			0 min	60 min	120 min	180 min
PRA, ng·ml ANG^{-1}·h^{-1}			*OC E+P*			
Follicular	0.7 ± 0.1[a]	3.4 ± 1.2[a]	1.5 ± 0.4[a]	0.9 ± 0.2[a,b]	0.8 ± 0.1[a,b]	0.8 ± 0.2[a,b]
Luteal	1.5 ± 0.3[c]	6.5 ± 2.2[c]	3.1 ± 0.8	2.0 ± 0.5	1.9 ± 0.5	1.7 ± 0.5
OC E + P	1.1 ± 0.2	3.6 ± 0.8	2.4 ± 0.6	2.0 ± 0.6	2.2 ± 0.7	1.5 ± 0.3
P$_{[ald]}$, pg/ml						
Follicular	72 ± 12[a]	247 ± 67[a]	131 ± 27[a]	94 ± 19[a]	73 ± 14[a]	56 ± 11[a]
Luteal	168 ± 20	405 ± 45[c]	240 ± 38[c]	155 ± 27	122 ± 22	123 ± 22[c]
OC E + P	131 ± 32	235 ± 26	104 ± 17	98 ± 17	87 ± 16	58 ± 11
P$_{[ANP]}$, pg/ml						
Follicular	52.9 ± 6.8[a]	111.5 ± 14.8[a]	69.1 ± 9.2[a]	39.7 ± 5.0	36.3 ± 4.4	33.3 ± 5.2
Luteal	33.0 ± 5.1[c]	91.8 ± 13.0[c]	56.1 ± 9.8	31.9 ± 3.7	30.6 ± 3.8	33.7 ± 5.7
OC E + P	45.8 ± 4.5	113.0 ± 17.5	56.4 ± 5.4	42.2 ± 6.4	40.8 ± 5.8	41.1 ± 4.7
PRA, ng·ml ANG^{-1}·h^{-1}			*OC P*			
Follicular	0.8 ± 0.2[a]	3.2 ± 1.2[a]	1.4 ± 0.4[a]	1.0 ± 0.2[a]	0.9 ± 0.2[a]	0.8 ± 0.2[a]
Luteal	1.7 ± 0.3	7.0 ± 1.6	4.3 ± 1.0	2.8 ± 0.2	2.7 ± 0.5	2.0 ± 0.4
OC P	1.2 ± 0.2	4.0 ± 1.0	2.6 ± 0.7	1.7 ± 0.4	1.5 ± 0.3	1.4 ± 0.2
P$_{[ald]}$, pg/ml						
Follicular	88 ± 19[a]	184 ± 41[a]	98 ± 28[a]	75 ± 15[a]	57 ± 12[a]	38 ± 7[a]
Luteal	162 ± 22	500 ± 47[d]	343 ± 57[d]	220 ± 31[d]	171 ± 24[d]	131 ± 18[d]
OC P	123 ± 40	285 ± 41	169 ± 44	114 ± 32	78 ± 20	77 ± 22
P$_{[ANP]}$, pg/ml						
Follicular	55.8 ± 10.3[a,e]	117.6 ± 25.0[a,e]	61.4 ± 8.8[a,e]	42.7 ± 5.7	40.0 ± 4.3	39.2 ± 4.1
Luteal	37.8 ± 6.0	86.3 ± 13.6	50.7 ± 8.0	31.2 ± 3.0	29.6 ± 2.3	29.6 ± 3.0
OC P	38.9 ± 2.8	86.9 ± 12.1	41.3 ± 3.2	36.7 ± 2.0	33.8 ± 2.3	34.2 ± 2.5

Values are means $\pm$ SE. Plasma renin activity (PRA) and plasma aldosterone (P$_{[ald]}$) and atrial natriuretic peptide concentrations (P$_{[ANP]}$) were measured at rest and in response to dehydrating exercise and 180 min of ad libitum rehydration in the follicular and luteal phases and during OC E + P ($n = 8$) and OC P ($n = 9$). [a] $P < 0.05$, follicular vs. luteal phase; [b] $P < 0.05$, follicular phase vs. OC E + P; [c] $P < 0.05$, luteal phase vs. OC E + P; [d] $P < 0.05$, luteal phase vs. OC P; [e] $P < 0.05$, follicular phase vs. OC P.
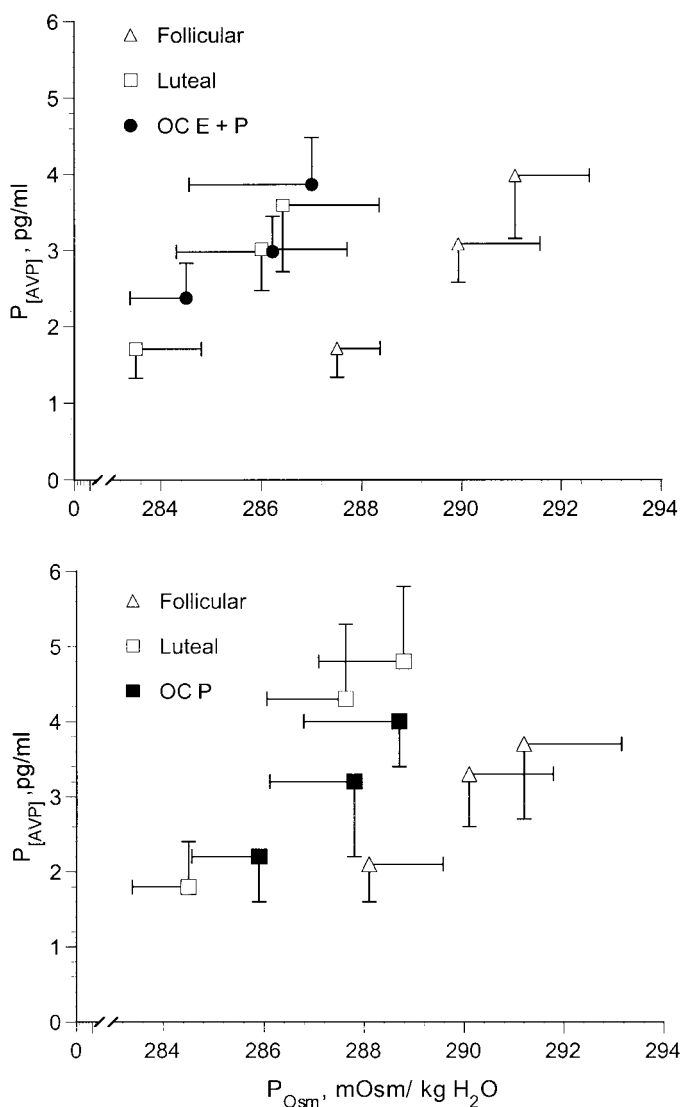
Fig. 3. Mean plasma arginine vasopressin concentration ($P_{[AVP]}$) responses to increases in P_{osm} during dehydration in follicular and luteal phases and during OC E + P ($n = 8$) and OC P ($n = 9$). Values are means ± SE.

with the OC E + P (47.1 ± 10.7 meq) or OC P (46.7 ± 8.8 meq) tests. Sweat K^+ loss was unaffected by menstrual phase or oral contraception administration. Renal Na^+ excretion increased during exercise in all conditions, and this increase was greatest during the follicular phase tests [12.2 ± 2.6, 8.0 ± 1.8, 7.4 ± 1.6, and 8.5 ± 3.3 meq for follicular and luteal phases (combined means), OC E + P, and OC P, respectively, $P < 0.05$].

Rehydration. Ad libitum fluid intake was similar by the end of the 180 min of rehydration on all six experimental test days. At 180 min of ad libitum drinking, subjects had restored 41 ± 5 and 40 ± 10% (follicular phase), 42 ± 7 and 39 ± 6% (luteal phase), 38 ± 11% (OC E + P), and 39 ± 7% (OC P) of body weight that was lost during dehydration. P_{osm} was higher throughout the rehydration period in the follicular phase than in the luteal phase, OC E + P, and OC P tests (Fig. 2; $P < 0.05$), although $P_{[AVP]}$ was similar

during all rehydration tests. For the entire rehydration period, PRA was lower during the follicular phase tests than during the luteal phase tests, and $P_{[ald]}$ was significantly greater in the luteal phase tests than in the follicular phase and the OC P test (Table 3, $P < 0.05$).

During rehydration, neither renal function nor electrolyte excretion was affected by menstrual phase or oral contraceptive administration, and overall fluid balance (i.e., fluid intake − urine output) was unaffected by either phase of the menstrual cycle or oral contraceptive administration (Fig. 4). Heart rate recovered to similar levels during rehydration in the follicular (75 ± 4 beats/min) and luteal (79 ± 4 beats/min) phase tests and during the OC E + P (78 ± 4 beats/min) and OC P (83 ± 4 beats/min) tests. Mean blood pressure remained unchanged throughout rehydration (78 ± 2, 79 ± 4, 77 ± 2, and 79 ± 2 mmHg for follicular and luteal phases, OC E + P, and OC P, respectively).

DISCUSSION

Our major finding was that administration of oral contraceptive pills containing estrogen increased osmotically induced AVP and thirst stimulation during dehydration in young, healthy women, although there were no changes in body fluid regulation during dehydration or subsequent ad libitum rehydration. These findings indicate that the shift in osmotic regulation of AVP and thirst represents a shift in body water regulation to a lower P_{osm} operating point. These data extend to young women our earlier findings in postmenopausal women, in whom estrogen administration reduced the P_{osm} threshold for AVP release during hypertonicity (19), although with an important difference. In postmenopausal women, 17β-estradiol administration reduced the P_{osm} threshold for AVP release during hypertonicity but also increased water retention and, therefore, did not indicate a shift in the operating point for body fluid regulation. In contrast, estradiol administration to the young women in our present investigation reduced P_{osm} but did not affect $P_{[AVP]}$, thirst ratings, or body water loss. After dehydration the subjects restored body water to the reduced, preexercise levels of P_{osm} during ad libitum rehydration, indicating a shift in the operating point for body fluid volume and composition with increased blood levels of estrogen. During dehydrating exercise, Na^+ excretion was lower during the luteal phase and OC E + P and OC P than during the follicular phase. However, although $P_{[ald]}$ and PRA were greater at rest and during rehydration in the luteal phase, neither the estrogen nor the progestin (norethindrone) in oral contraceptives stimulated the renin-angiotensin-aldosterone system or increased Na^+ retention or blood pressure.

Vokes et al. (27) used hypertonic saline infusion and water loading to stimulate and suppress the osmoreceptors, respectively, and demonstrated a resetting of osmoreceptor thresholds for AVP and thirst in the luteal phase of the menstrual cycle. Our findings support those of Vokes et al. and others (18, 26), indicating that AVP secretion persists at lower P_{osm}
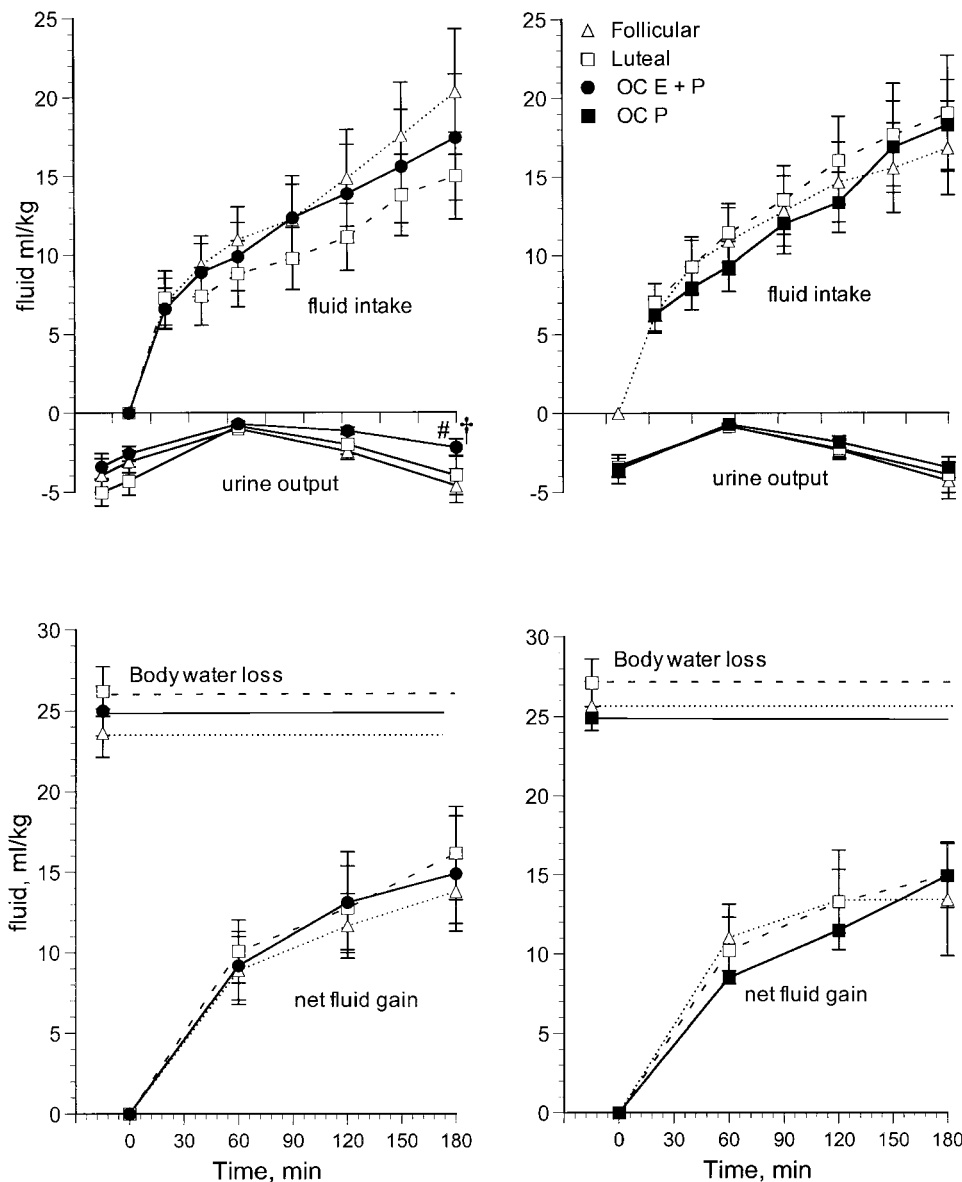
Fig. 4. Body fluid balance after dehydrating exercise and during 180 min of ad libitum rehydration in the follicular and luteal phases and during OC E + P ($n = 8$) and OC P ($n = 9$). #$P < 0.05$, follicular phase vs. OC E + P. †$P < 0.05$, luteal phase vs. OC E + P. Values are means ± SE.

during the luteal phase, thus causing a reduction in renal free water excretion and maintenance of this lower plasma tonicity. Estrogen and progesterone are elevated in the midluteal phase, so these studies did not determine whether the changes in osmoregulation were due to estrogen or progesterone effects. The data in our investigation extend these earlier findings and suggest that the shift in osmoregulation is due to the estrogen component of the oral contraceptive pill, because this shift did not occur during administration of progestin (norethindrone) only, which not only contains no estradiol, but downregulates estrogen receptors (17). Furthermore, progestin does not have a strong impact on estrogenic activity when administered with estradiol because of weak binding of progestins to estrogen receptors (17).

Estrogen readily crosses the blood-brain barrier and can likely modulate osmotic AVP and thirst regulation via its action within the central nervous system. Studies in lower animals have demonstrated that estrogen acts directly on estrogen-binding neurons in the hypothalamus (1, 2, 5, 16), thereby affecting synthesis and release of AVP. Estradiol receptors have been identified in the nuclei of neurophysin- and AVP-producing cells in the mouse supraoptic nucleus (16), and osmotic stimulation of vasopressinergic neuronal activity is upregulated by estrogen in the supraoptic nucleus of brain slices of ovariectomized rats (2). Estrogen may also modulate hypothalamic AVP release indirectly through catecholaminergic (10) and/or angiotensinergic (23) neurons, which bind estrogen and project to the paraventricular and supraoptic nuclei. Using [³H]estradiol, Heritage et al. (10) identified estradiol-binding sites in the nuclei of catecholamine neuronal systems, as well as the presence of catecholamine nerve terminals surrounding estradiol target sites in the paraventricular and supraoptic nuclei. Crowley et al. (6) noted parallel changes in brain norepinephrine and AVP in normally cycling rats and that ovarian steroids modulated norepinephrine turnover in the paraventricular

nucleus, indicating that estrogen may act on the osmoregulatory system through catecholamines. There also is evidence for cholinergic and angiotensinergic innervation of vasopressinergic cells in the paraventricular and supraoptic nuclei, both of which are modulated by sex steroids (23).

Conversely, peripheral mechanisms for the estrogen effect on osmotic stimulation of AVP are unlikely to play a role in the response. For example, PV reduction, such as that during the midluteal phase, could have contributed to the lower P_{osm} threshold for AVP release, because PV is a potent AVP stimulus. However, this mechanism seems unlikely, because there was no change in PV during OC E + P relative to the follicular phase. In addition, the luteal phase PV contraction was not associated with a great enough fall in PV ($<10\%$) to stimulate AVP (15). ANP has also been shown to suppress the osmotically induced rise in AVP (3), but the follicular phase and OC E + P were associated with greater $P_{[ANP]}$, although with different osmotic AVP response.

Blood volume and arterial pressure also play important roles in body fluid regulation, primarily by modulating Na^+ excretion. Previously, oral contraceptives containing high doses of estrogen (2 mg/day) led to hypertension and greater plasma angiotensinogen levels, although with only small elevations in plasma renin or aldosterone levels (14). The estrogen dose in our study did not increase blood pressure or cause consistent elevations in PRA and aldosterone, and norethindrone (the progestin in OC P), a progestational derivative of testosterone without antimineralocorticoid properties, also had no effect on PV. Nonetheless, our data confirm earlier findings demonstrating PV contraction during the midluteal phase of the menstrual cycle during rest, exercise, and heat exposure (21, 22), as well as large elevations in the sodium-regulating hormones (12). During the luteal phase a progesterone-mediated inhibition of aldosterone-dependent Na^+ reabsorption at distal sites in the nephron produces a transient natriuresis (13) and a compensatory stimulation of the renin-aldosterone system (12). The renin and aldosterone stimulation is a component of a system evolved to maintain blood pressure and plasma water and Na^+ content during the luteal phase progesterone peak, although clearly this system is not involved during OC E + P or OC P administration.

We found that oral contraceptive pills containing estradiol led to a lower osmotic operating point for body fluid regulation, similar to that found during the luteal phase. These data suggest that estradiol has the primary effects on body fluid regulation during oral contraceptive administration and indicate that the progestins in oral contraceptives do not have a major effect on osmotic regulation of AVP and thirst. However, more research is needed to determine possible effects of elevations in endogenous progesterone on osmoregulation and the other components of body fluid regulation, such as fluid distribution and Na^+ regulation.

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