Effects of time of day, gender, and menstrual cycle phase on the human response to a water load

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It has been recognized for over a century that humans excrete larger volumes of urine during the daytime than during the nighttime, and it was later recognized that even with no fluid intake and while remaining in the supine position, morning urine flow increased (1). The day-night difference in urine flow was not convincingly related to differences in free water clearance ($C_{\text{H}_{2}\text{O}}$) but better related to differences in osmotic clearance ($C_{\text{Osm}}$) (1). Thus perturbations that cause increases in $C_{\text{Osm}}$ may be expected to be more effective in the daytime. It follows therefore that the diuretic response to a drink of water, which is characterized almost entirely by increases in $C_{\text{H}_{2}\text{O}}$, was reported to be greater during the daytime than the nighttime (23). However, the activity and state of hydration were not controlled in those studies, and the mechanisms explaining the increased ability to excrete a water load during the daytime were not determined.

In addition to circadian effects on water-load responses, differences according to gender also would seem probable. For instance, women experience higher levels of arginine vasopressin (AVP) at the time of ovulation and lowest levels during onset of menstruation (5). Furthermore, estrogen administration to postmenopausal women is associated with increased levels of plasma AVP (P$_{\text{AVP}}$) compared with no steroid treatment (7, 22) and to medroxyprogesterone (7). Similarly, Davison et al. (2) have reported lowered osmotic thresholds for both thirst and vasopressin release during pregnancy. These findings might suggest a tendency toward free water retention during periods of high estrogen. However, estrogen and estrogen combined with progesterone have been reported to inhibit the antidiuretic effects of vasopressin in the rat (26). Furthermore, both estrogen and progesterone reduce vasopressin-stimulated cAMP in rat and human renal medullary cells (13). Moreover, the effects of the estrogen and progesterone on the inhibition of vasopressin-stimulated cAMP are additive. Thus the effects of gender differences on the ability to conserve water following a drink of water are not easily predictable.

The present studies were conducted therefore to compare the human male and female diuretic responses to a moderate water load during the daytime and nighttime and during the follicular and luteal phases of the menstrual cycle. We hypothesized that the diuretic response to a modest water load, consisting primarily of free water loss, would be greater in women than in men and would be greater during the daytime than in the nighttime. Also, owing to the potential additive effects of progesterone on the estrogen effects, we postulated that the diuretic response would be greater during the luteal phase than the follicular phase.
METHODS

The study protocol was approved by the Human Use Committee at Tripler Army Medical Center. Investigators adhered to the policies for protection of human subjects as prescribed in 45 Code of Federal Regulation part 46: the Federal Policy for the Protection of Human Subjects issued by the Department of Health and Human Services.

Subjects. Seven men and six women voluntarily participated in this study. The average ages and weights of the subjects are shown in Table 1. The subjects were not taking medications and were free from endocrine, renal, or cardiovascular disorders. The female subjects were experiencing natural, regular menstrual cycles. All subjects had been working a regular day-night shift for 4 or more days.

Experimental design. Male subjects participated in two experiments, one in the morning and one in the evening. Female subjects participated in four experiments, one in the morning and one in the evening, once during the follicular phase (days 4–11) and again during the luteal phase (days 19–25) of the menstrual cycle. The morning and evening sessions were separated by a minimum of 36 h, with the morning experiment conducted first in 12 of 19 experiments, and the phases of the menstrual cycle were usually (5 of 6) conducted in the order of follicular phase first. The morning and evening experiments were begun at 0600 and 1800, respectively.

On the day before the experiment heavy exercise was not allowed, and on an evening before a morning experiment subjects refrained from eating, drinking, and smoking after 2200. On the morning of a day when an evening experiment was scheduled, subjects could not exercise all day and could not eat, drink, or smoke after 1000.

At 6:00 (AM and PM) the subjects reported to the laboratory and voided their bladders, and the urine was discarded. Other than for urine collections, the subjects remained seated in the upright position. To obtain a consistent state of hydration, the subjects were fed a meal during the first hour, similar in calorie and fat content for all sessions, and with ~150 ml noncaffeinated fluid. At 7:00 AM and PM the first urine sample was collected, and a 20-gauge, 1-inch catheter (Insyte, Becton Dickinson, Sandy, UT) was inserted into a vein in the arm or hand for the duration of the experiment. At 8:00 AM and PM the second urine sample and a blood sample were collected, and over the next 15 min the subjects consumed a water load of 12 ml/kg lean body mass (LBM); tap water was at room temperature. Blood and urine samples were obtained at 8:30, 9:00, 9:30, 10:00, 10:30, and 11:00 AM and PM following the water load.

At the time of the first experiment and following the initial voiding of the urinary bladder and the meal, the subjects were weighed and total body fat was determined by skin-fold thicknesses measured in triplicate over the triceps, biceps, subscapular, and suprailiac areas with a Lange skin-fold caliper (Cambridge Scientific Industries, Cambridge, MD). The tables used for the calculation of %body fat were provided with the skin-fold calipers and are a modification of those derived by Dowmin and Womersley (4). The average body weights and %body fat of the subjects are summarized in Table 1. The LBM was calculated by subtraction of the body fat from the body weight, and the water load was 12 ml/kg LBM. The water load administered was not recalculated after the first experiment and remained identical between the two experiments for the men and the four experiments for the women. The average volume of the water load for the men and women is summarized in Table 1.

Analyses. Urine and plasma were analyzed for sodium and potassium by ion-specific electrodes and creatinine by the Jaffe reaction (Beckman Astra-4 Autoanalyzer, Brea, CA). Urine and plasma osmolalities were measured by freezing point depression (Advanced Micro-Osmometer, 3MO, Norwood, MA). Creatinine, osmotic, and free water clearances were calculated by conventional formulas. P_{AVP}, was measured by a disequilibrium radioimmunoassay as previously described (8). Four milliliters of plasma were acidified with 0.4 ml of 1 N HCl and frozen until assay. The acidified plasma was extracted using octadecylsilane cartridges (Sep-Pak C18; Waters Associates, Milford, MA). The average recovery of added, unlabeled hormone was 91%. The amount of AVP reported is not corrected for losses due to extraction. The sensitivity of the assay was 0.06 μU/ml plasma. The 125I-AVP (NEN, Boston, MA) and AVP for standards (Sigma, St. Louis, MO) were obtained commercially, and the antibody was prepared in our laboratory and designated AS96 with characteristics previously described (25). The between-assay coefficients of variability (CV) for \( P_{AVP} \) was 8.7%.

Radioimmunoassay kits were used for the assay of serum 17β-estradiol, progesterone, aldosterone, plasma cortisol (Diagnostic Products, Los Angeles, CA), and plasma renin activity (NEN Life Science Products, Boston, MA). These were measured in the first blood sample of each experiment, except the ovarian steroids were not measured in the men. The steroid hormones assays were quality controlled with Lyphochek immunoassay control levels 1, 2, and 3 (Bio-Rad Laboratories, Anaheim, CA), providing control serum at three concentration levels. All samples for estrogen and progesterone were assayed in three or less assays, and no between-assay CV was determined. The between-assay CV for aldosterone was 8.5% for the mid-level control. Plasma renin activity controls were also within acceptable ranges of control (NEN Life Science Products) and the between-assay CV was 12.8%.

Statistics. The data were analyzed by three different two-way analysis of variances; sex and sample time were the two variables. “Sex” was compared three ways: 1) male vs. female follicular phase, 2) male vs. female luteal phase, and 3) female follicular phase vs. the luteal phase. Sample time had 16 periods for urine variables and 14 for plasma variables, with one-half of the sample period representing daytime and the other one-half representing nighttime. Post hoc contrast comparisons were made to assess differences between sexes and phases of the menstrual cycle at specific time periods and for differences over time (JMP version 3.1; SAS Institute, Cary, NC).

RESULTS

The cumulative diuretic response to the moderate water load of 12 ml/kg LBM was approximately three times greater during the daytime than during the nighttime (Fig. 1) in both male and female subjects. The daytime urine flow rates were higher in the women.
during both phases of the menstrual cycle at 60 min after the drink and also at 90 min during the follicular phase. The urine osmolalities were indistinguishable between the sexes and between the different phases of the menstrual cycle during either daytime or nighttime. The volume of urine voided by women over 3 h was about 100% of the water load in both phases of the menstrual cycle and was significantly less in the men, who voided about 70% of the water load. This was significantly reduced to about 30% at night in both sexes, and the reduced urine flow was accompanied by higher urine osmolalities in all experiments at the 60- and 90-min time periods following the drink.

The follicular and luteal phases of the menstrual cycle were characterized by similar serum levels of estrogen and with significantly elevated levels of progesterone in the luteal phase (Table 2). Serum aldosterone levels were elevated in the luteal phase, statistically significant only in the daytime sample, whereas plasma renin activity was significantly elevated during the luteal phase in the nighttime sample. Serum cortisol values revealed the typical circadian rhythm with daytime greater than nighttime, but no differences were evident between sexes or phases of the menstrual cycle. Initial levels of \( P_{AVP} \) taken at 0800 and 2000 before the drink were also not different between sexes or phases of the menstrual cycle, but the morning levels were significantly lower (about 50%) than those in the evening (Fig. 2).

The state of hydration appeared to be similar between the sexes and between the morning and evening experiments. For instance, prior to the drink of water, similar levels of urine osmolality and flow rate were observed during the first 2 h of urine collections (Fig. 1). Similarly, plasma osmolality (\( P_{Osm} \), Fig. 2) and plasma sodium concentrations (\( P_{Na} \), Table 3) were similar between the first morning samples and the corresponding evening samples except for a low \( P_{Osm} \) value.
at time 0 in the female subjects during the luteal phase that was not paralleled by evidence of a significantly lowered PNa levels at the same time. Also, hematocrit (Table 3) was similar between morning and evening experiments except for the luteal phase, where the morning value was higher than the evening value.

The water load decreased POsm (Fig. 2) in both sexes and phases of the menstrual cycle at nighttime but only during the follicular phase during the daytime. The POsm was not different between daytime and nighttime. Reductions in PNa (Table 3) were only significant for female subjects during the luteal phase. Overall, there was a tendency for a more sustained decrease in POsm in the nighttime, evidenced by significant reductions in POsm at 120 min following the drink in all groups.

Plasma potassium concentration (PK) and hematocrit (Table 3) were not affected by the drink of water in any of the experiments.

Despite the similar starting values for POsm before the water loads, PAVP was nearly twofold higher in the evening in both sexes and during both phases of the menstrual cycle. Also, despite no remarkable differences in the immediate decrease in POsm in response to the water load between daytime and evening, the magnitude of the PAVP decrease by 90 min following the water drink was more during the evening than during the morning experiments. Furthermore, PAVP re-

Table 2. Baseline hematocrit and hormonal values before water load on the experimental days and nights in the men and in women in different phases of the menstrual cycle

<table>
<thead>
<tr>
<th>Phase and Time of Day</th>
<th>SCort, ng/dl</th>
<th>PRA, ng ANG I·ml⁻¹·h⁻¹</th>
<th>SAldo, ng/dl</th>
<th>SEst, pg/ml</th>
<th>SProg, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foll/day</td>
<td>8.4 ± 1.4</td>
<td>1.1 ± 0.3</td>
<td>8.1 ± 1.5</td>
<td>51 ± 10</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>Foll/night</td>
<td>5.1 ± 0.9*</td>
<td>0.8 ± 0.1</td>
<td>6.1 ± 1.5</td>
<td>50 ± 16</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>Lut/day</td>
<td>9.9 ± 0.9</td>
<td>1.4 ± 0.2</td>
<td>18.9 ± 2.8†</td>
<td>86 ± 15</td>
<td>8.8 ± 1.9†</td>
</tr>
<tr>
<td>Lut/night</td>
<td>4.1 ± 0.7*</td>
<td>2.0 ± 0.4†</td>
<td>11.2 ± 2.6*</td>
<td>60 ± 8</td>
<td>7.7 ± 1.5†</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>7.7 ± 1.2</td>
<td>1.2 ± 0.2</td>
<td>9.4 ± 2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Night</td>
<td>3.5 ± 0.7*</td>
<td>1.7 ± 0.4</td>
<td>7.8 ± 1.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. SCort, SAldo, SEst, SProg, serum concentrations of cortisol, aldosterone, estrogen, progesterone, respectively; PRA, plasma renin activity; Foll, follicular; Lut, luteal. *P < 0.05 compared with corresponding daytime value. †P < 0.05 compared with corresponding value in the follicular phase of the menstrual cycle.

Fig. 2. Plasma osmolality (POsm) and plasma arginine vasopressin concentration (PAVP) responses to the water drink. All symbols are as described in Fig. 1.
The primary findings in this study are that the diuretic response to a drink of water under similar conditions of hydration, posture, and activity is greater in the morning than in the evening and that during the morning the response is greater in women than in men. The volume of the drink we chose to use was predicated on a desire to achieve a response to a volume that a person would voluntarily consume and would not maximally dilute the urine in all situations. We reasoned that this would allow for detection of differences in the degree of urinary dilution and possibly in vasopressin responses. We chose a volume similar to that used by Geelen et al. (9, 10) because of their previous information on the responses. Note that a 70-kg man would consume 700 ml using the water load of 10 ml/kg. By comparison, with the average body fat we observed in men (19%), the water load in our study would be 680 ml. Our objectives were achieved. In regard to the day-night differences, the degree of urinary dilution was reduced and probably accounts for a majority of the day-night differences in urine flow in response to a drink of water. In regard to the response between males and females, the similarity in urine dilution between sexes, despite the dilution to only 200 mosmol/kg, is striking and will be discussed later.

The mechanisms responsible for the diurnal response to the drink of water clearly involve differences in urine dilution between day and night, with resultant differences in \( C_{U_{\text{H,O}}} \) at 60 and 90 min following the drink in all conditions. We observed a slightly higher plasma concentration of vasopressin at 8 PM than at 8 AM that may have contributed to the reduced evening diuretic response. Although a circadian rhythm of vasopressin in the cerebral spinal fluid has been clearly demonstrated in the conscious cat for instance (16), the rhythm in the circulation is sometimes not detected (16, 18). On the other hand, others have detected a circadian rhythm of vasopressin in the blood with val-
ues rising after 2 PM, reaching peak values between midnight and 6 AM (3, 6, 11). Our study was not intended to further define the circadian rhythm of vasopressin but is compatible with reports of higher levels of vasopressin at nighttime.

Despite the higher starting levels of PAVP during the evening experiments, it is clear that after the drink of water the values of PAVP were similar to the morning values during the luteal phase in the women and in the men, but the diuretic response was invariably reduced at night. This apparent uncoupling is possibly the result of several technical factors. It is important to recognize that the major diuretic response occurred between 30 and 90 min after the drink, and in all groups during the daytime PAVP was low. However, the sensitivity of our assay is 0.06 μU/ml plasma (about 0.15 pg/ml), the ability to distinguish between 0.1 μU/ml from anything lower is impossible, and the accuracy below 0.2 μU/ml is poor. It is probable that the level of PAVP necessary to produce large increases in C\textsubscript{H\textsubscript{2}O} is below the level of detectability for our assay. In most of the daytime experiments, PAVP levels had returned to predrink levels at 180 min after the drink. This was not true for the women in the follicular phase, which remained significantly decreased at all times after the drink. The reasons for this exception are not clear, but the observations seem to be supported by the corresponding low PAVP and slightly decreased urine osmolality measurements also observed in the morning experiments conducted during the follicular phase. However, essentially all PAVP values remained decreased after 60 min following the drink in the evening experiments. The maintained decrease in PAVP in the evening experiments is possibly due to the inability to void the free water, which resulted in a more frequently observed reduction in P\textsubscript{Osm} for the evening experiments.

Geelen et al. (10) conducted hormone measurements in human subjects after 24 h of dehydration and subsequently after a 10 ml/kg water drink. Although they do not report the urinary findings, the reported findings are similar to the evening responses in the present studies. They reported a reduction in PAVP by 3 min after the drink that occurred with no significant de-
increase in $P_{\text{Osm}}$, which they attributed to an oropharyngeal reflex inhibition of AVP. The $P_{\text{AVP}}$ remained decreased for 60 min, whereas the mean $P_{\text{Osm}}$ remained near predrink levels. The prolonged decrease in $P_{\text{AVP}}$ they observed at 1 h was most likely due to the slight, not statistically significant, decrease in mean $P_{\text{Osm}}$ of about 3 mosmol/kgH$_2$O, similar to the nighttime experiments in the present studies. In subsequent studies (9), they demonstrated that an oral isotonic saline drink reduced $P_{\text{AVP}}$ at 3, 9, and 15 min postdrink, but then $P_{\text{AVP}}$ returned to predrink levels by 30 min.

Other mechanisms, independent of differences in vasopressin, may also be partially involved in the day-night differences in the urinary response to a water drink. For instance, the diurnal variation in cortisol may play a permissive role in the day-night differences in basal urinary flow. Others have reported that when rhythmicity of glucocorticoids was removed by the exogenous administration of cortisone, the diurnal pattern of urine flow faded out over a course of several days (17).

The data presented support the hypothesis that women, during the midfollicular and midluteal phases of the menstrual cycle, excrete a larger proportion of a modest water intake over a 3-h period than do men. According to the findings of Forsling et al. (5), these periods of the menstrual cycle should be expected to produce similar basal levels of $P_{\text{AVP}}$ and estradiol as we observed in this study. These levels are higher than during the late luteal or early follicular phases. In subsequent studies in postmenopausal women, $P_{\text{AVP}}$ was found to be increased during periods of estrogen administration and unaffected by medroxyprogesterone alone, and the estrogen-stimulated vasopressin release seemed to be reduced by the administration of medroxyprogesterone (7). Others also observed similar $P_{\text{AVP}}$ levels in women during early- (21) and midfollicular (24) compared with midluteal phases of the menstrual cycle, similar to our findings. Trigoso et al. (24) further noted that the osmotically stimulated vasopressin and thirst were similar between the two phases. However, estrogen administration to postmenopausal women has been shown to increase basal $P_{\text{AVP}}$ despite maintained $P_{\text{Osm}}$ (22). Additionally, estrogen reduced the osmotic threshold for vasopressin release in those studies that prompted the authors to suggest that this mechanism may contribute to water retention during high-estrogen periods. Although they further noted that the increased renal sodium retention was the major contributor to the enhanced fluid retention.

Taken together, the previous reports support the idea that estrogen enhances vasopressin release and could therefore contribute to water retention. However, Wang et al. (27) have demonstrated that the antidiuretic effect of vasopressin is greater in male rats than female rats during periods of high-circulating estradiol. Furthermore, estradiol administration has been shown to attenuate the antidiuretic action of vasopressin in the ovariectomized rat (26). This may be related to an effect of estrogen on the vasopressin renal receptor. For instance, in cell cultures of both human and rat renal medullary cells, the AVP-stimulated cAMP response is reduced in the presence of estradiol and is further inhibited by the progesterone (13).

The present results are therefore compatible with a hypothesis that allows for a possible enhanced vasopressin release or reduced osmotic threshold for vasopressin release in women compared with men, but the ability of this mechanism to promote water retention may be overridden by the reduced renal responsiveness to vasopressin in women. Such a theory would not predict a lower $P_{\text{AVP}}$ in the women in response to the water drink, but it is a possibility that the present data cannot rule this out because of assay sensitivity limitations. That is, the $P_{\text{AVP}}$ in women may have been reduced more than in the men, but our assay could not detect the difference at these low levels. The observations of Hatano et al. (13) suggest the possibility that free water loss could have been greater during the luteal phase of the menstrual cycle because of the additive effects of progesterone. The present experiments support that possibility since the mean peak urine flow (Fig. 1) and $C_{\text{H}_2\text{O}}$ (Table 4) were highest in the luteal phase, when progesterone levels were higher. However, the differences in $C_{\text{H}_2\text{O}}$ between the follicular and luteal phases reached statistical significance only in the nighttime. It is likely that the small number of subjects has lead to a beta error and precluded our ability to clearly define this difference between phases of the menstrual cycle during the daytime.

Another point of consideration regards the question of how the free water losses were generally greater in women than men when the urine osmolality was similar. This is in part due to the dependence of $C_{\text{H}_2\text{O}}$ on $C_{\text{Osm}}$. That is, when urine osmolality is less than plasma osmolality, as in the present study, and both are held constant as $C_{\text{Osm}}$ increases, urine flow increases more rapidly in a straight-line relationship with a slope equal to $C_{\text{Osm}}$/urine flow. This results in a greater difference of urine flow-$C_{\text{Osm}}$, which is equal to $C_{\text{H}_2\text{O}}$ as $C_{\text{Osm}}$ increases. This mathematical explanation ignores effects of tubular flow, which would most likely alter the affects of vasopressin on water reabsorption. Nevertheless, the greater $C_{\text{Osm}}$ consistently and inexplicably observed in female subjects during the daytime responses to the water load most likely contributed somewhat to increase in $C_{\text{H}_2\text{O}}$ observed independent of urinary dilution effects. It should be noted, however, that the poor temporal relationship between $C_{\text{Osm}}$ and $C_{\text{H}_2\text{O}}$ suggests other factors played a major role in the increased $C_{\text{H}_2\text{O}}$ in response to the water load.

Cortisol, renin, and aldosterone were only measured in the initial samples of the experiments and revealed no differences between men and women. There was greater renin activity and aldosterone during the luteal phase than follicular phase that is in agreement with previously well-established observations (12, 14, 22).
The unexplained differences in $C_{Osm}$ and $C_{H2O}$ between men and women indicate a need to measure cortisol, renin, and perhaps aldosterone after a water load in both sexes as well. Furthermore, the issue of whether the reported effects of estrogen and progesterone on vasopressin receptors influence the handling of water needs further experimentation, perhaps with levels of vasopressin held constant. Such experiments could also help define a potential role of reduced cortisol levels at night and the interrelationship with vasopressin contributing to the reduced nighttime diuretic response.

**Perspectives**

The present study demonstrates that women excrete a greater proportion of a drink of water than men during a 3-h period in the daytime. There was no difference between sexes during the nighttime when urine flow was greatly reduced in both sexes and in both phases of the menstrual cycle. These data are significant in evaluating the potential for dehydration in women compared with men. On the one hand, it is well documented that estrogen promotes salt retention and therefore water retention. One may conclude that this reflects an ability to conserve water, but this is not the case. Water "retention" with salt must be distinguished from the rate of water turnover. The present data suggest that in a similar state of hydration and in a similar postprandial state, turnover. The present data suggest that in a similar state of hydration and in a similar postprandial state, turnover. The present data suggest that in a similar state of hydration and in a similar postprandial state, turnover. The present data suggest that in a similar state of hydration and in a similar postprandial state, turnover.

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