Relationship between Aldosterone and Progesterone in the Human Menstrual Cycle

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Context: Aldosterone levels increase during the luteal phase of the menstrual cycle. Prior studies examining relationships between aldosterone and female sex hormones did not control for sodium balance, a major determinant of aldosterone production.

Objectives: The objectives of this study were 1) to compare aldosterone levels between menstrual phases among cycling women in high- and low-sodium balance; and 2) to examine the relationships between aldosterone and female sex hormones in women and the effects of sex hormones on rat zona glomerulosa (ZG) cell aldosterone production *in vitro*.

Subjects/Interventions: Normotensive, premenopausal women were studied in low- and/or high-sodium balance. Urinary aldosterone, basal serum aldosterone, plasma renin activity (PRA), plasma angiotensin II (AngII), and serum aldosterone after AngII infusion were measured. Isolated rat ZG cells were treated with progesterone, estradiol, or both, and aldosterone was measured.

Results: In high-sodium balance, urinary aldosterone, basal serum aldosterone, and serum aldosterone response to infused AngII were significantly greater ($P < 0.05$) in the luteal *vs.* follicular phase. PRA, AngII, and potassium did not differ. Progesterone directly correlated with urinary aldosterone, basal serum aldosterone, and serum aldosterone response to infused AngII. Estradiol did not significantly correlate with aldosterone. In low-sodium balance, no significant differences in aldosterone levels between phases were found. *In vitro*, progesterone increased ZG cell aldosterone production ($P < 0.01$), whereas estradiol had no effect.

Conclusions: In women, urinary and serum aldosterone levels are significantly higher during the luteal phase in high- but not low-sodium balance, whereas PRA and AngII do not differ between phases. Progesterone may directly contribute to increased luteal phase aldosterone production, independent of the renin-angiotensin system. (*J Clin Endocrinol Metab* **91: 3981–3987, 2006**)

A LDOSTERONE LEVELS HAVE been reported to increase during the luteal phase of the human menstrual cycle, a time characterized by increased progesterone and estradiol production. Although progesterone is known to have antimineralocorticoid effects (1–3), it is unclear whether additional mechanisms contribute to increased luteal phase aldosterone production. Most prior studies did not control for (4–12) or document (13) sodium balance, which plays a major role in regulation of aldosterone production via the renin-angiotensin system (RAS). In two prior studies that did account for sodium balance (14, 15), the relationships between aldosterone and the female sex hormones progesterone and estradiol were not investigated.

Progesterone is postulated to mediate the luteal phase increase in aldosterone levels. Because progesterone inhibits aldosterone binding to the mineralocorticoid receptor (1, 3, 16), increased progesterone production during the luteal phase likely leads to compensatory activation of the RAS and thus increased aldosterone production (6, 8, 9, 12, 17). However, it is not known whether additional mechanisms con-

tribute to luteal phase aldosterone increases, independent of the RAS. We examined the mechanisms by which luteal phase aldosterone levels increase among women in sodium balance, because differences in sodium balance independently influence RAS hormone levels. Furthermore, we investigated the role of estradiol in the luteal phase aldosterone increase, which has not been previously reported.

The purpose of this study was to compare aldosterone levels during the follicular and luteal phases of the menstrual cycle among women in documented sodium balance, at baseline and in response to angiotensin II (AngII) infusion. We also sought to investigate the relationships between aldosterone and the female sex hormones progesterone and estradiol among women in sodium balance. Last, we aimed to explore additional mechanisms by which female sex hormones may modulate aldosterone production by determining whether direct administration of progesterone or estradiol to isolated rat zona glomerulosa (ZG) cells influences aldosterone production.

Subjects and Methods

Subjects

Subjects studied as previously described (18, 19) by the international HyperPath (Hypertensive Pathotype) consortium were included in this *post hoc* analysis. Only normotensive, premenopausal women in sodium balance (as described in a subsequent paragraph) were included. Subjects were excluded if they had active medical problems, were pregnant, or were taking exogenous estrogens or progestins.

Normotension was defined as seated systolic blood pressure less than 140 mm Hg and diastolic blood pressure less than 90 mm Hg measured manually with a standard mercury sphygmomanometer on three occasions. Premenopausal status was defined as having regular menstrual cycles or, where menstrual history was not available, age 45 yr or less and serum FSH level less than 20 IU/liter. The luteal phase of the menstrual cycle was defined by a serum progesterone level more than 3 ng/ml.

Protocols

Subjects were admitted to the General Clinical Research Centers (GCRCs) of the Brigham and Women's Hospital, University of Utah Medical Center, or Vanderbilt University. The Institutional Review Boards at each site approved the protocols, and each subject provided written informed consent before enrollment.

As part of the original protocol, subjects were scheduled for study on low-sodium or both low- and high-sodium diets without regard for menstrual cycle phase. For the high-sodium protocol, subjects were placed on an isocaloric diet containing 200 mEq sodium and 100 mEq potassium per day for 7 d. For the low-sodium protocol, subjects were placed on an isocaloric diet containing 10 mEq sodium and 100 mEq potassium per day for 7 d. Urinary sodium, creatinine, and aldosterone excretion were measured in a 24-h urine sample collected at the end of the 7-d period. Subjects were admitted to the GCRC, and after fasting overnight and remaining recumbent for at least 6 h, blood was drawn in the supine position for aldosterone, plasma renin activity (PRA), AngII, potassium, estradiol, and progesterone measurement using an iv catheter. Subjects then received an infusion of AngII-amide (CIBA-Geigy, Summit, NJ) at 3 ng/kg·min for 50 min, delivered by an electronic infusion pump (Baxter Corporation, Deerfield, IL). Serum aldosterone was measured at the end of the infusion. Blood pressure was measured with an automatic indirect recording sphygmomanometer (Dinamap; Critikon, Inc., Tampa, FL) at baseline and then every 2 min during the AngII infusion.

For the high-sodium analysis, data from subjects who successfully achieved high-sodium balance (24-h urine sodium excretion ≥150 and ≤250 mEq) were included. For the low-sodium analysis, data from subjects who successfully achieved low-sodium balance (24-h urine sodium <40 mEq) were included.

Laboratory procedures

Blood samples were collected on ice, spun, and frozen until the time of assay. Urinary aldosterone, serum progesterone, and serum aldosterone were measured by solid phase RIA using the Coat-A-Count procedure [Diagnostic Products Corporation (DPC), Los Angeles, CA]. PRA was measured using the GammaCoat [^{125}I] RIA kit by the RIA of generated angiotensin I (DiaSorin, Stillwater, MN). Plasma samples for AngII were immediately treated with a mix of angiotensinase and angiotensin-converting enzyme inhibitors that included phenylmethylsulfonyl fluoride, phenanthroline, pepstatin, and captopril. Plasma AngII was measured by double-antibody RIA (ALPCO, Windham, NH). FSH was measured by paramagnetic-particle chemiluminescent immunoassay (Beckman Instruments Inc., Chaska, MN). Estradiol was measured using double antibody ^{125}I RIA (DPC). Serum and urinary sodium and serum potassium levels were measured by flame photometry, with lithium as an internal standard (Nova Biomedical, Waltham, MA). Urinary creatinine was measured using the ACE Creatinine Reagent (Alfa Wasserman, West Caldwell, NJ).

In vitro experiments

ZG cells were isolated from intact female 6- to 8-wk-old Wistar rats (Charles River Laboratories, Wilmington, MA). Rats were fed PicoLab Rodent Diet 20 (0.33% sodium; Richmond, IN) for 6 d followed by induction of anesthesia using inhaled isoflurane; they were then killed, and adrenal glands were removed. ZG cells were prepared from the capsular portion of the adrenal as previously described (20). Cells from approximately eight rats were pooled for each study. Briefly, capsules were incubated in collagenase (3.7 mg/ml; Worthington Biochemical Corporation, Freehold, NJ) and DNAase (0.05 mg/ml; Worthington

Biochemical Corporation) for 50 min at 37 C followed by centrifugation. After washing the cell pellets with a modified Krebs-Ringer bicarbonate solution (KRBGA) and determining by microscopic examination that less than 5% of cells were fasciculata cells, cells were resuspended in KRBGA (0.5×10^6 cells/ml). Cells were incubated for 1 h at 37 C in 5% CO_2-95% O_2 with KRBGA containing vehicle, progesterone (50 ng/ml; Sigma-Aldrich, St. Louis, MO), estradiol (80 pg/ml; Sigma-Aldrich), or estradiol (80 pg/ml) plus progesterone (50 ng/ml). These progesterone and estradiol concentrations correspond to peak levels achieved during the rat estrous cycle (21). Incubations were performed in duplicate or triplicate, and the studies were repeated twice. Media from incubates was assayed for aldosterone in duplicate using solid phase RIA (DPC). All experiments were conducted in accord with accepted standards of humane animal care, and experimental procedures were approved by the Institutional Animal Care and Use Committee at Harvard University.

Statistical analysis

Data from the human study are presented as median with interquartile range, and data from the *in vitro* study are presented as mean ± SEM. Comparisons between phases of the menstrual cycle were performed using the Mann-Whitney *U* test. For subjects studied in both high- and low-sodium balance, the Wilcoxon signed ranks test was used to compare parameters in high- *vs.* low-sodium balance. Spearman correlation coefficients were used to examine relationships between sex hormones (progesterone and estradiol) and aldosterone, PRA, and AngII levels. In the *in vitro* study, one-way ANOVA was used to compare treatment groups. A *P* value less than 0.05 was considered statistically significant. Analyses were performed using SPSS version 14.0.2 for Windows (SPSS Inc., Chicago, IL).

Results

Human menstrual cycle

The high-sodium balance group included 27 subjects [median age 30 yr (25–37 yr)], and the low-sodium balance group included 51 subjects [median age 33 yr (27–41 yr)]. Twenty-four subjects were studied in both high- and low-sodium balance. No significant differences in age, race, weight, or blood pressure were found between menstrual phases in either high- or low-sodium balance, except for younger median age ($P = 0.048$) in the low-sodium luteal *vs.* follicular group (Table 1). The majority (80%) of subjects were white.

Consistent with the known effects of sodium balance on the RAS (22), subjects in low- *vs.* high-sodium balance had significantly greater (all $P < 0.001$) 24-h urinary aldosterone [78.8 μg/24 h (29.8–98.0) *vs.* 8.5 μg/24 h (6.8–16.9)], basal serum aldosterone [21.0 ng/dl (8.7–26.1) *vs.* 3.9 ng/dl (2.5–6.0)], serum aldosterone response to infused AngII [increase of 22.8 ng/dl (16.4–37.0) *vs.* 7.9 ng/dl (5.2–11.0)], and PRA [2.8 ng/ml·hr (1.6–4.2) *vs.* 0.4 ng/ml·hr (0.2–0.7)].

As per the prespecified definition of the luteal phase, progesterone levels were significantly greater ($P < 0.001$) in the luteal *vs.* follicular phase in both high-sodium [9.7 ng/ml (6.9–11.4) *vs.* 0.5 ng/ml (0.4–0.6)] and low-sodium [10.1 ng/ml (4.4–13.9) *vs.* 0.5 ng/ml (0.4–0.7)] balance. As expected, estradiol levels were also higher ($P < 0.01$) in the luteal *vs.* follicular phase in both high-sodium [79.5 pg/ml (55.5–119.1) *vs.* 29.4 pg/ml (18.9–48.2)] and low-sodium [63.9 pg/ml (41.6–97.3) *vs.* 37.1 pg/ml (21.8–57.9)] balance.

In high-sodium balance, 24-h urinary aldosterone excretion ($P < 0.05$), basal serum aldosterone levels ($P < 0.05$), and increase in serum aldosterone in response to infused AngII ($P < 0.01$) were all significantly greater in the luteal *vs.*

TABLE 1. Baseline characteristics, according to sodium balance and menstrual phase

	High-sodium		Low-sodium	
	Follicular (n = 17)	Luteal (n = 10)	Follicular (n = 38)	Luteal (n = 13)
Age (yr)	32 [27–37]	25 [22–44]	34 [28–42]	30 [22–36]
Race				
White [n (%)]	15 (88)	6 (60)	30 (79)	10 (77)
Black [n (%)]	1 (6)	3 (30)	6 (16)	2 (15)
Asian [n (%)]	1 (6)	1 (10)	2 (5)	1 (8)
Weight (kg)	62.5 [55.1–69.1]	62.9 [59.0–69.5]	65.5 [58.4–74.8]	61.6 [57.0–63.5]
SBP (mm Hg)	101 [98–112]	109 [100–117]	105 [98–111]	101 [94–109]
DBP (mm Hg)	63 [58–70]	69 [57–72]	62 [58–68]	60 [56–67]

Data are presented as median [interquartile range] or n (%). SBP, Systolic blood pressure; DBP, diastolic blood pressure.

follicular phase, but there were no significant differences between menstrual phases in 24-h urinary sodium excretion, PRA, or plasma AngII levels (Fig. 1). In low-sodium balance, there were no significant differences in luteal *vs.* follicular phase urinary aldosterone excretion, basal serum aldosterone levels, serum aldosterone response to infused AngII, urinary sodium excretion, PRA, or plasma AngII levels (Fig. 2). In both high- and low-sodium balance, there were no differences between phases in serum potassium levels, urinary creatinine, or basal and AngII-stimulated blood pressures (data not shown).

In high-sodium balance (where differences in aldosterone levels between menstrual phases were observed), progesterone was directly correlated with 24-h urinary aldosterone excretion (r = 0.646, P < 0.001), basal serum aldosterone (r = 0.363, P = 0.06), AngII-stimulated serum aldosterone (r = 0.631, P < 0.001), and an increase in serum aldosterone in response to infused AngII (r = 0.53, P = 0.004). There were no significant correlations between progesterone and either PRA or AngII. There were no significant correlations between estradiol and aldosterone levels (basal or stimulated). In low-sodium balance, there were no significant correlations between progesterone and aldosterone levels (basal or stimulated).
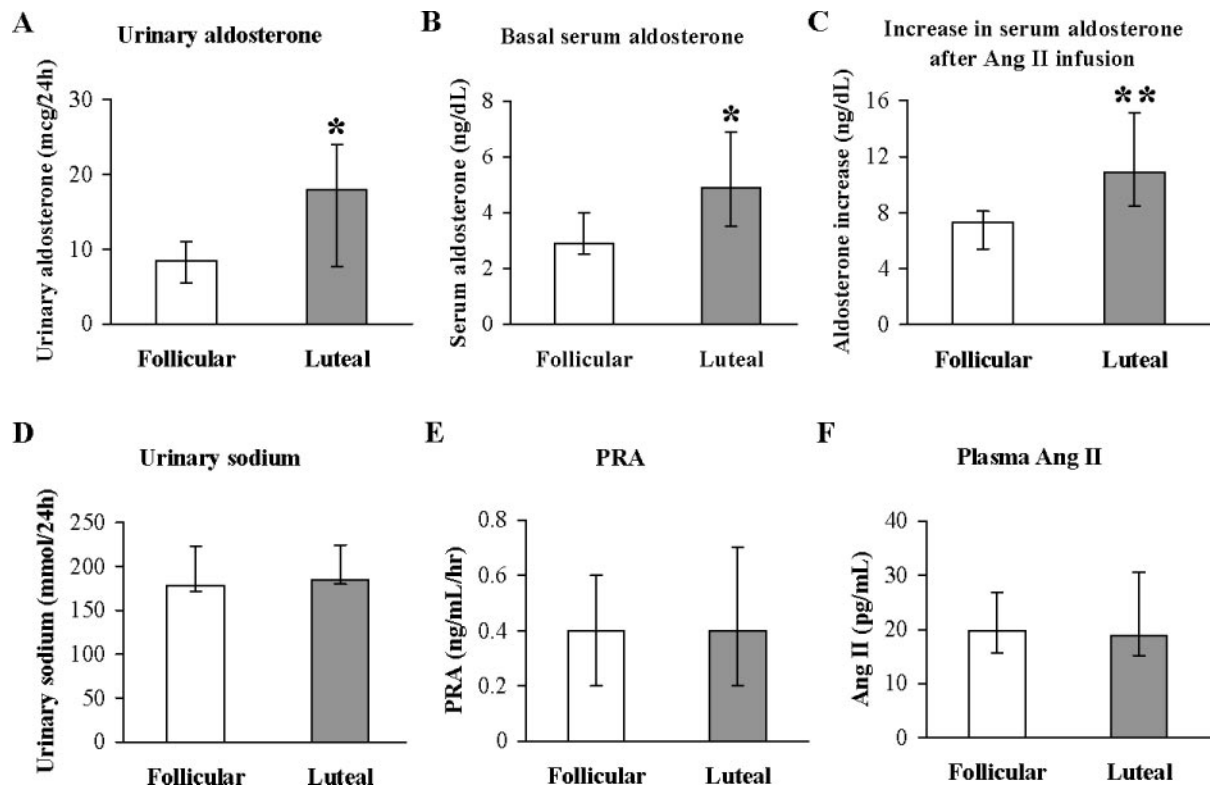

FIG. 1. Follicular (*white bars*) *vs.* luteal (*gray bars*) 24-h urinary aldosterone levels (A), basal serum aldosterone levels (B), increase in serum aldosterone in response to infused AngII (C), 24-h urinary sodium levels (D), PRA (E), and plasma AngII levels (F) under conditions of high-sodium balance. Data are shown as median with interquartile range. Comparisons are between the follicular phase and the luteal phase. *, P < 0.05; **, P < 0.01. Twenty-four-hour urinary aldosterone excretion, basal serum aldosterone levels, and increase in serum aldosterone in response to infused AngII were all significantly greater in the luteal *vs.* follicular phase, but there were no significant differences between menstrual phases in urinary sodium excretion, PRA, or plasma AngII. Conversion factors for Système Internationale units: urinary aldosterone (nanomoles per day), 2.77; serum aldosterone (nanomoles per liter), 0.0277; PRA (nanograms per liter per second), 0.2778.
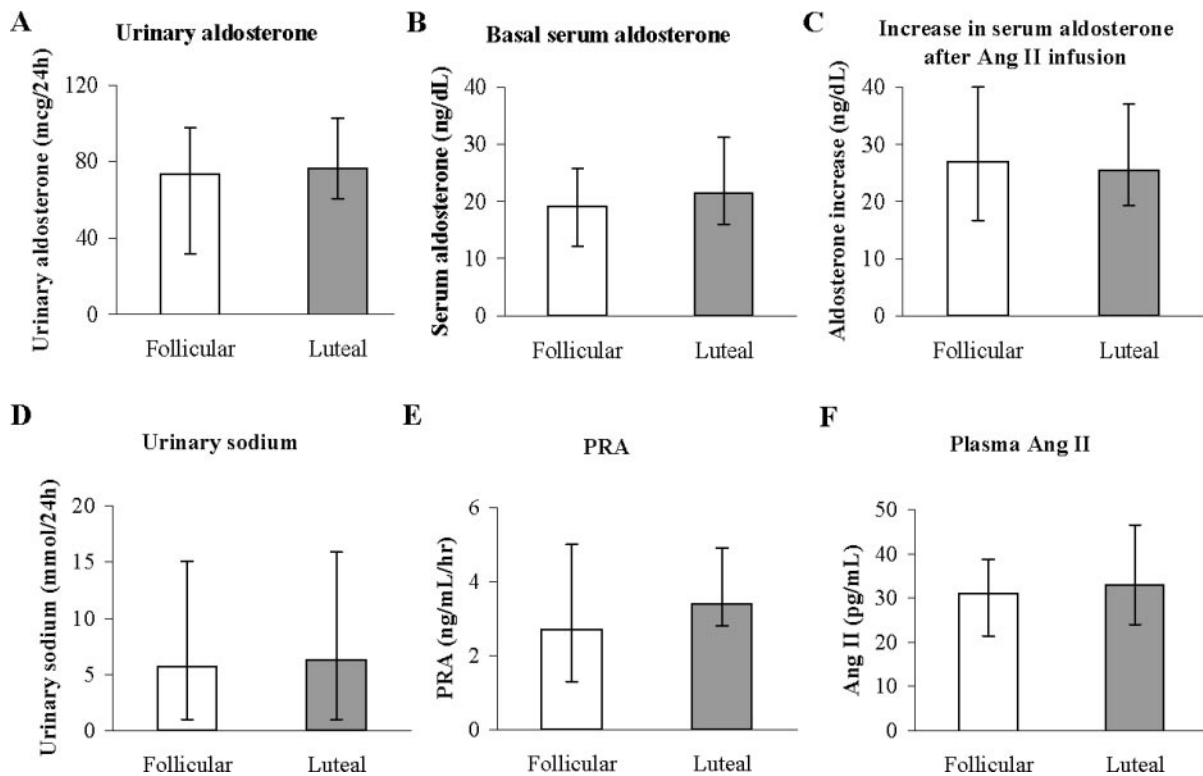
FIG. 2. Follicular (*white bars*) *vs.* luteal (*gray bars*) 24-h urinary aldosterone levels (A), basal serum aldosterone levels (B), increase in serum aldosterone in response to infused AngII (C), 24-h urinary sodium levels (D), PRA (E), and plasma AngII levels (F) under conditions of low-sodium balance. Data are shown as median with interquartile range. There were no significant differences between the follicular phase and the luteal phase in any of these parameters under conditions of low-sodium balance. Conversion factors for Système Internationale units: urinary aldosterone (nanomoles per day), 2.77; serum aldosterone (nanomoles per liter), 0.0277; PRA (nanograms per liter per second), 0.2778.

In vitro aldosterone production

Incubating isolated ZG cells with progesterone significantly increased aldosterone production compared with incubation with vehicle alone (Fig. 3). Estradiol had no effect on aldosterone production, alone or in combination with progesterone.

Discussion

We have demonstrated that urinary and serum aldosterone levels as well as the increase in serum aldosterone in
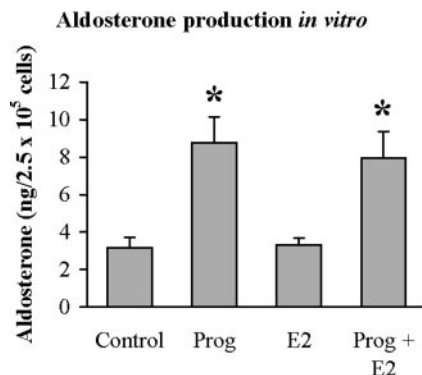

FIG. 3. Aldosterone production *in vitro* by isolated ZG cells incubated for 1 h with vehicle (Control), 50 ng/ml progesterone (Prog), 80 pg/ml estradiol (E2), or the combination (Prog + E2). Data are shown as mean ± SEM, n = 8 per treatment. *, $P < 0.01$ *vs.* control and E2 treatments. There was no significant difference between Prog and Prog + E2 treatments.

response to infused AngII are significantly greater in the luteal *vs.* follicular phase among women in high-sodium balance. Progesterone, but not estradiol, directly correlated with aldosterone levels under these conditions. In contrast, we found no differences in aldosterone levels between menstrual phases among women in low-sodium balance. Because we could not attribute the luteal phase aldosterone increase in high-sodium balance to increased circulating levels of PRA or AngII, we investigated *in vitro* whether progesterone or estradiol might directly influence aldosterone production. Addition of progesterone to isolated rat ZG cells caused a 2.8-fold increase in aldosterone production, whereas addition of estradiol had no effect. These data suggest that progesterone may directly influence adrenal aldosterone production and that this may be one mechanism underlying increased aldosterone production during physiological high-progesterone states.

Previous studies reported higher serum or urinary aldosterone levels during the luteal phase, but sodium intake was either not controlled (4–12) or not documented (13) in the majority of these studies. Since sodium intake, via modulation of the RAS, is a primary determinant of aldosterone production (22), it is difficult to interpret the findings of these earlier studies, because they may have been confounded by changes in sodium intake over the course of the menstrual cycle. Indeed, luteal phase salt restriction is often advised to ameliorate premenstrual symptoms (23–25), and a reduction in dietary sodium intake premenstrually could potentially

increase luteal phase aldosterone levels. Consistent with our findings, two prior studies that controlled for sodium intake demonstrated increased luteal phase serum aldosterone levels in high-sodium balance (14, 15), but these studies did not explore the relationships between aldosterone levels and concentrations of progesterone or estradiol. Our study extends the findings of these prior studies in several ways. Most importantly, unlike these prior studies, we examined the relationships between both progesterone and estradiol concentrations and aldosterone levels. In addition, we report increased luteal phase urinary aldosterone excretion, which represents an integrated summation of aldosterone production over a 24-h period and is a more reliable indicator of overall aldosterone production. Finally, we rigorously controlled not only for sodium balance but also for prolonged supine posture before blood sampling to minimize confounding influences on RAS hormone levels.

In contrast to these two studies (14, 15) as well as many prior studies that did not control for sodium balance (4, 8, 10–13), we did not find concurrent increases in PRA associated with increased luteal phase aldosterone levels. Therefore, our study is unique in that we observed PRA-independent luteal phase increases in aldosterone after rigorously controlling for both sodium balance and recumbent posture, which can confound measurement of PRA. We also demonstrated no difference between menstrual phases in plasma AngII levels, further supporting RAS-independent stimulation of aldosterone production during the luteal phase.

One prior study examined menstrual phase variation in aldosterone levels among subjects assigned to a low-sodium diet, and no differences in aldosterone levels between phases were found (14). In this study, however, the luteal *vs.* follicular group had a significantly higher mean urinary sodium excretion rate, which may have suppressed the RAS and prevented detection of a hormonally mediated aldosterone increase in the luteal group. In the current study, which was not confounded by differences in sodium balance, we found no significant differences in aldosterone levels between menstrual phases in low-sodium balance. Because sodium restriction is such a potent stimulus for activation of the RAS (22), any additional stimulation by progesterone or estradiol during the luteal phase is likely negligible under conditions of low-sodium balance.

Previous studies that accounted for dietary sodium intake did not investigate the relationships between progesterone, estradiol, and aldosterone levels (14, 15). Because both estradiol and progesterone increase during the luteal phase, both could potentially contribute to luteal phase increases in aldosterone. We think it unlikely that estradiol mediates this effect for several reasons. First, although oral estrogen use has been associated with changes in aldosterone plasma protein binding, these changes are not seen during the menstrual cycle (26). Similarly, increases in the AngII precursor angiotensinogen are seen with oral estrogen use (27) but not during the menstrual cycle (8, 27), and plasma AngII levels were not higher during the luteal phase in the current study. Second, estradiol levels did not correlate with aldosterone levels in our study. Lastly, estradiol did not increase aldosterone production by isolated ZG cells *in vitro* in the current study.

Increased luteal phase aldosterone levels are thought to result from increased progesterone, which has known antimineralocorticoid effects (1, 2, 3, 16). However, the relationships between aldosterone levels and concentrations of progesterone and estradiol during the menstrual cycle have not been previously examined among women in sodium balance. Progesterone administration to young men on a fixed-sodium diet led to natriuresis and increases in aldosterone, PRA, and AngII, and these changes were not observed when synthetic progestogens without antimineralocorticoid properties were administered (2). A small study that did not control for sodium intake found that urinary aldosterone excretion increased during the second half of the menstrual cycle only in women whose progesterone levels also increased (8). Our study is the first to demonstrate a direct correlation between progesterone and aldosterone levels among subjects in sodium balance. There are several possible mechanisms by which progesterone could influence aldosterone production. Progesterone is a competitive inhibitor of aldosterone at the mineralocorticoid receptor and has natriuretic properties in humans (1, 2) and rats (3). Therefore, progesterone-induced natriuresis likely leads to compensatory activation of the RAS during the luteal phase (6, 8, 9, 12, 17). However, if this were the only mechanism at work, PRA and AngII levels would be expected to increase concurrently with aldosterone, which was not seen in our study that was rigorously controlled for sodium balance and recumbent posture. It is also possible that progesterone-induced vasodilation (28) leads to RAS activation. However, as above, PRA and AngII would be expected to increase concurrently with aldosterone levels if this were the primary mechanism at work.

Because our data did not support a RAS-mediated increase in aldosterone during the luteal phase, we examined whether progesterone might directly influence adrenal aldosterone production. We showed that progesterone, at physiological concentrations, directly increases aldosterone production from isolated rat ZG cells. Our results are consistent with two prior studies showing that administration of oral micronized progesterone to women for 4–8 d increased serum aldosterone levels, but did not change PRA (29, 30) or plasma AngII (30). We believe that our results are complimentary to these prior studies, because the effects of exogenous hormone administration and endogenous hormone changes are often distinct. Studies performed over 30 yr ago showed that addition of fixed amounts of progesterone to the media of rat (31) and chicken (32) adrenal glands increased aldosterone production. However, it is not known whether physiological progesterone concentrations can effect these changes, because these studies did not control for or measure the progesterone concentration to which the adrenal tissue was exposed. We used a progesterone concentration known to be achieved *in vivo* during the rat estrous cycle (21) and showed that ZG cell aldosterone production is increased by physiological progesterone concentrations. Furthermore, we used isolated ZG cells instead of whole adrenal tissue. This technique allows for more efficient and controlled diffusion of treatments to the ZG cells and more direct evaluation of the impact of regulatory factors on ZG cell aldosterone production, because the secretory products of neighboring fascicu-

lata and reticularis cells can influence glomerulosa cell al-
dosterone production (33, 34) and can confound results in
experiments using whole adrenal tissue. Importantly, our
study further demonstrated that estradiol, alone or in com-
bination with progesterone, did not influence ZG cell aldo-
sterone production *in vitro*, which has not been previously
demonstrated. Progesterone receptor expression has been
detected in adrenal capsular cells from female mice (35),
raising the possibility that progesterone acts through its re-
ceptor to modulate aldosterone production. Alternatively,
because progesterone is a precursor in the aldosterone bio-
synthetic pathway, increased substrate taken up by the ad-
renal cells may contribute in part to increased luteal phase
aldosterone production. Progesterone may also increase al-
dosterone production in ways that have not yet been eluci-
dated and require further study.

Our findings of increased luteal phase aldosterone levels
in high-sodium balance may be physiologically relevant, be-
cause the average dietary sodium intake in the United States
is approximately 150 mmol/d (36), similar to the urinary
sodium levels achieved in our high-sodium balance study.
The fluid retention that is seen during pregnancy and is
variably observed during the luteal phase (37) is poorly un-
derstood. If progesterone led only to compensatory activa-
tion of the RAS via its antimineralocorticoid effects, then total
body volume would be maintained but would not increase.
However, if progesterone also independently stimulates al-
dosterone production, as our data suggest, then volume re-
tention could occur. Furthermore, differences in progester-
one production and adrenal sensitivity to progesterone could
possibly contribute to variability in the degree of fluid re-
tention observed among different individuals. Although
PRA would be expected to decrease in response to increased
aldosterone production and volume retention, it is possible
that because progesterone simultaneously activates the RAS,
the net effect of progesterone on PRA may be minimal.

Our findings may also shed light on the unexplained dis-
sociation between aldosterone levels and PRA that is well
described during pregnancy (38–40). The disproportionately
greater increase in aldosterone levels relative to PRA during
pregnancy is postulated to result from either increased ad-
renal sensitivity to AngII or nonangiotensin-mediated aldo-
sterone release (39). Our results suggest that increased pro-
gesterone levels could possibly lead to both, because our
study demonstrates enhanced serum aldosterone response to
AngII infusion during the luteal phase as well as direct pro-
gesterone-induced stimulation of ZG cell aldosterone pro-
duction. Further studies are needed to better define the pro-
gesterone-mediated changes in aldosterone production that
occur during pregnancy.

Our study has limitations. Ideally we would have per-
formed paired analyses of the same women in the follicular
and luteal phases in both high- and low-sodium balance, but
arranging four consecutive studies in sodium balance for
each subject would have been logistically difficult. A pro-
spective study confirming our results could be informative.
In addition, it is possible that our sample size limited our
ability to detect menstrual phase differences in aldosterone
levels in low-sodium balance, because progesterone's effect
on aldosterone production under these conditions may be

small relative to the effect of sodium restriction. Because
high-sodium intake reduces PRA to levels close to the lower
limit of detection of our assay, it is possible that small dif-
ferences in PRA between phases may have been undetectable
in high-sodium balance. As this study included only nor-
motensive, premenopausal women who were predomi-
nantly white, the results may not be applicable to hyperten-
sive women or women of other ethnicities.

In conclusion, we have shown that both urinary and serum
aldosterone levels are significantly greater during the luteal
phase among subjects in high- but not low-sodium balance.
In high-sodium balance, aldosterone levels directly corre-
lated with progesterone but not estradiol levels. In contrast
to prior studies (4, 8, 10–15), the increased luteal phase al-
dosterone levels observed in our study were independent of
increases in PRA. Our study provides *in vitro* evidence that
progesterone directly stimulates ZG cell aldosterone pro-
duction. Together these data suggest that progesterone may
directly contribute to increased adrenal aldosterone produc-
tion during the luteal phase, particularly when the RAS is
suppressed by normal or high sodium intake.

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